6. MULTİDİSİPLİNER KANSER ARAŞTIRMA KONGRESİ

27-30 Ekim 2016 - KONYA

Selçuk Üniversitesi
Süleyman Demirel
Kültür Merkezi

KONGRE KİTABI

6.

MULTİDİSİPLİNER KANSER ARAŞTIRMA KONGRESİ

27 - 30 Ekim 2016

KONYA

ORGANİZASYON

ONURSAL BAŞKAN

Prof. Dr. Mustafa ŞAHİN
(Selçuk Üniversitesi Rektörü – Rector of Selçuk University)

KONGRE BAŞKANI

Prof. Dr. Engin ULUKAYA
(MOKAD Yönetim Kurulu Başkanı – Chair of MOKAD Steering Committee)

KONGRE EŞ BAŞKANLARI

Prof. Dr. Ali ÜNLÜ
Assoc. Prof. Dr. Hüsamettin VATANSEV

KONGRE DÜZENLEME KURULU

Engin ULUKAYA
Konstantinos DIMAS
Jose M. PADRON
Bernd GRONER
Serdar KARAKURT
Hasan KORKAYA
Leena LATONEN
Arzu YILMAZTEPE ORAL
Bahadır ÖZTÜRK
Andreas G. Tzakos
Ali ÜNLÜ
Hüsamettin VATANSEV

MOKAD YÖNETİM KURULU

Engin ULUKAYA
Serap ÇELİKLER
Arzu YILMAZTEPE ORAL
Türkkan EVRENSEL
Egemen DERE

BİLİMSEL KURUL

Orhan ADALI
Hasan AKÇA
Sila APPAK BASKOY
Emel ARINÇ
Ferda ARI
Elif İlkay ARMUTAK
Rengül Çetin ATALAY
Ceyda Açılan AYHAN
Yusuf BARAN
Serap ÇELİKLER
Süleyman DAŞDAĞ
Egemen DERE
Konstantinos DIMAS
Selvi DURMUŞ
Serdar DURDAĞI
Özcan EREL
Devrim GÖZÜAÇIK
Fatih GÜLTEKİN
Mehmet GÜRBİLEK
Lülüfer Tamer GÜMÜŞ
Saadet GÜMÜŞLÜ
Mustafa GÜZEL
Abdurrahman KAPCAL
Serdar KARAKURT
Kati KIVINUMMI
Aslı KOÇ
Abdurrahim KOÇYİĞİT
Hasan KORKAYA
Işıl Aksan KURNAZ
Nurgün KÜÇÜKBOYACI
Özgür KÜTÜK
Leena LATONEN
Inga MARIJANOVIC
Serpil OĞUZTÜZÜN
Ali OMAY
Pınar ONGANER
Bahadır ÖZTÜRK
Süray PEHLİVANOĞLU
Pekka RUUSUVUORI
Alaattin ŞEN
Önder ŞİRİKÇİ
Andreas TZAKOS
Engin ULUKAYA
Ali ÜNLÜ
Hüsamettin VATANSEV
İlhan YAYLIM
Azmi YERLİKAYA
Sema YILMAZ
Veysel Turan YILMAZ
Doğan YÜCEL
Ümit ZEYBEK

İÇİNDEKİLER

ÖNSÖZ

Değerli Temel Kanser Araştırmacıları,

2002 yılından bu yana hem ulusal (MOKAD) hem de uluslar arası (EORTC, EACR) ölçekte düzenlediğimiz temel kanser araştırma toplantılarından birini daha 6. Multidisipliner Kanser Araştırma Kongresi adı altında KONYA'da SELÇUK ÜNİVERSİTESİ REKTÖRLÜĞÜ himayesinde organize etmenin gurur ve heyecanı içindeyiz. 27-30 Ekim 2016 tarihlerinde düzenlenecek kongrede geniş yelpazede oturumlar (uzmanına danış, satellit sempozyumlar, sözlü ve poster bildiriler, paneller, konferanslar/keynote lectures, ve proje pazarı) yer alacaktır. Çoğunlukla yurtiçi konuşmacılardan oluşacak programda ayrıca yurtdışı konuşmacılarımız da olacaktır. Kongrenin hemen önünde 25-26 Ekim 2016 tarihlerinde bir çalıştay da düzenlenecektir. İlk gün Amerika'dan solid tümörlerdeki ilk kanser kök hücre çalışmasının yayınlandığı grupta çalışmış olan Hasan Korkaya'nın açılış konferansının takiben MOKAD Onursal Üye paneli yer alacaktır. Panelde Mehmet Öztürk ve Emin Kansu hocalarımız yer alacaktır. İkinci günün tamamı yurtdışı konuşmacılarımıza ve kısa konuşmalara (proffered papers) ayrılmıştır. Simultane tercüme yapılacaktır. Üçüncü gün yurtiçi konuşmacılarımız ve sözlü bildirilere yer verilecektir. Sosyal program (Sema gösterisi) yer alacaktır. Son gün sözlü bildirileri takiben proje pazarı oturumu yapılacaktır. Proje pazarında araştırmacılar ihtiyaçlarına çözümler bulacaktır.

Geleneksel olarak MOKAD kongreleri kar amacı gütmeyen toplantılar olduğundan öğrenci kayıt ücretleri uygun tutulmaya çalışılmıştır. Ayrıca, her zaman olduğu gibi gene olabildiğince çok sayıda burs olanağı MOKAD tarafından sağlanacaktır. Çeşitli kategorilerde ödüllere (MOKAD temel kanser bilimcisi ödülü, Genç temel kanser araştırmacısı ödülleri, MOKAD özel ödülü, poster ödülleri, sözel bildiri ödülü) her zaman olduğu gibi bu kongrede de yer verilecektir.

Kanser araştırmaya gönül vermiş tüm çalışanları Mevlana'nın şehri KONYA'da bilgilerini güncelleyebilecekleri, yeni işbirlikleri kurabilecekleri ve zengin sosyal programla yorgunluk atabilecekleri bu kongremize bekliyoruz.

Kansersiz bir dünya için çalışma andıyla!..

Prof. Dr. Engin ULUKAYA
Kongre Başkanı

BİLİMSEL PROGRAM

27 Ekim 2016 **Perşembe**

09:00 – 16:00 **Kanserde Veri Madenciliği Çalıştayı (data mining)**

16:30 – 17:00 Opening Ceremony

17:00 – 17:40 Advances and Clinical Applications in Cancer Stem Cell Research
Hasan KORKAYA

MOKAD Onursal Üye Paneli

17:40 – 18:10 Kanser Tedavisinde Yeni Yaklaşım : Precision Medicine
Emin KANSU

18:10 – 18:40 Kanser Hücrelerinde Ölümsüzlük
Mehmet ÖZTÜRK

28 Ekim 2016 **Cuma**

PROFFERED PAPERS

08:30 – 08:50 PI3K Sinyal Yolağın Meme Kanserin Tanı ve Tedavisindeki Önemi
Ebubekir DİRİCAN

08:50 – 09:10 Antikanser Etkili İndol Karboksilik Asit Türevleri: Sentezleri ve DNA Kesim Aktiviteleri
Bircan ÇEKEN TOPTANCI

09:10 – 09:30 Kanser ve Diyabet Gelişiminin Kritik Noktası: İnsülin Reseptör Substrat Proteinleri
Gökhan GÖRGİŞEN

09:30 – 09:50	Evrimsel Bir Süreç Olarak Kanser **Sibel KÜÇÜKYILDIRIM**
09:50 – 10:10	MikroRNA'lar ve Kanser **Zekiye ALTUN**
10:10 – 10:30	Natural Small-Molecules Obtained From Lichens as a Novel Source of Anti-Angiogenic Agents **Mehmet VAROL**
10:30 – 10:45	*Kahve Molası*
	FINNISH PANEL
10:45 – 11:15	Androgen Receptor Pathway in Prostate Cancer **Leena LATONEN**
11:15 – 11:45	Novel Gene Fusions in Prostate Cancer **Kati KIVINUMMI**
11:45 – 12:15	3D-reconstruction and feature based analysis of prostate cancer histology **Pekka RUUSUVUORI**
12:15 – 12:45	A Constructive Debate on Synthetic and Naturally Occurring Anticarcinogen Compounds **Serdar KARAKURT**
12:45 – 14:00	*Öğle Yemeği*
	EUROPEAN PANEL
14:00 – 14:25	Resistance of Cancer Cells to Targeted Therapies **Bernd GRONER**
14:25 – 14:50	Discovery and Development of Glabrescione B (GlaB) for the Therapy of Hedgehog-Dependent Tumors **Mattia MORI**
14:50 – 15:15	Biology and Importance of Angiogenesis in Cancer **Sila APPAK BASKOY**
15:15 – 15:30	*Kahve Molası*

BALKAN PANEL

15:30 – 15:50 Targeted Drug Delivery in Cancer Treatment
Andreas TZAKOS

15:50 – 16:10 Sigma Ligands as Targeted Anticancer Therapeutics
Konstantinos DIMAS

16:10 – 16:30 Targeting Cancer Stem Cells
Inga MARIJANOVIC

16:30 – 16:50 Tartışma

16:50 – 18:00 Sözlü Bildiriler

19:30 Gala Yemeği - Cumhuriyet Balosu

29 Ekim 2016 **Cumartesi**

09:00 – 10:00 Sözlü Bildiriler

OTURUM 1

10:00 – 10:25 Tümör Heterojenitesi ve Moleküler Yansımaları
Safiye AKTAŞ

10:25 – 10:50 Warburg Etkisinin Ötesi: Kanser metabolik bir hastalık olarak düşünülebilir mi?
Mustafa GÜZEL

10:50 – 11:15 *Kahve Molası & Poster Ziyaretleri*

11:15 – 11:40 Yeni PARP-1 Inhibitorlerinin Kucuk Molekul Veri Bankalarinin Sanal Taramalari ile Kesfi: In Siliko ve In Vitro Calismalari
Serdar DURDAĞI

11:40 – 12:05 Preclinical assessment of a novel palladium-based compound as an anti-cancer drug candidate
Selvi DURMUŞ

12:05 – 13:30 *Öğle Yemeği*

OTURUM 2

13:30 – 13:55 Beyin tümörlerive ETS faktörleri
Işıl AKSAN KURNAZ

13:55 – 14:20 NFkB tarafından transkripsiyonu indüklenen miRNA KHDAK invazyonununun regule eder
Hakan AKÇA

14:20 – 14:45 Kanserde ilaç dirençliliğinde GST enzimlerinin rolü
Serpil OĞUZTÜZÜN

UYDU SEMPOZYUMU \ *BİO-RAD*

14:45 – 15:15 Yeni Nesil ddPCR' in (Droplet Digital™ PCR) Sivi Biyopsilerde, Kanser Teshis, Tani ve Gozleminde Kullanimi

15:15 – 16:15 Kahve Molası & Poster Ziyaretleri

19:00 *Sema Gösterisi*

30 Ekim 2016 **Pazar**

09:30 – 11:30 Sözlü Bildiriler

11:30 – 11:45 *Kahve Molası*

11:45 – 12:15 **KONFERANS**
Kanser Tedavisi İçin Tasarlanan Hedeflendirilmiş İlaç Taşıyıcı Sistemler
Gülay BÜYÜKKÖROĞLU

12:15 – 12:45 **CLOSING LECTURE**
Evaluation of Biomarkers in Breast Cancer
Ayşegül ŞAHİN

12:45 – 13:15 Kapanış & Ödül Töreni

SÖZLÜ SUNUM PROGRAMI

28 Ekim 2016 **Cuma**

Ana Salon

16:50 - 17:00	Therapeutic Effect Of Cancer Drugs on MFE-319 Endometrial Carcinoma Cell Line	Işıl Aydemir
17:00 - 17:10	Cytotoxic, Genotoxic, Apoptotic and Reactive Oxygen Generating Effects of Carvacrol on Human Fibroblast (WS-1) and Gastric Adenocarcinoma (AGS) Cells	Ayşe Güneş Bayır
17:10 - 17:20	Effects of fisetin on glioma cell proliferation and apoptosis	Pinar Öztopcu Vatan
17:20 - 17:30	Prediction of endocrine therapy response and resistance in breast cancer cells by exploiting the mitochondria and estrogen receptor status	Bahriye Karakas
17:30 - 17:40	Inhibition of O6-metilguanine-DNA metiltransferase (MGMT) activity enhances Melphalan cytotoxicity in Multiple Myeloma	Dilara Akcora Yildiz
17:40 - 17:50	Cytotoxic, Genotoxic and Apoptotic Effects of Curcumin in Different Cell Lines	Eray Metin Guler
17:50 - 18:00	Cytotoxic, genotoxic and apoptotic activities of olive leaf and sumac extracts on cancer and healthy cells	Hümeyra Nur Kaleli

Malazgirt Salonu

16:50 - 17:00	Investigate The Antitumor Effects of Çemen Extract	Şerife Çinar
17:00 - 17:10	The Detection of Curcumins' Antitumoral Effects via Argyrophilic Nucleolar Organizing Region–Associated Protein Synthesis in Mice with Ehrlich's Ascitic Carcinoma	Mehtap Nisari
17:10 - 17:20	The using of AgNOR parameters for discrimination of benign and malign breast lesion	Mehmet Köksal

17:20 - 17:30	In vitro antioxidant properties and the evaluation of antiproliferative and apoptotic activities on HeLa, MCF-7, OE-33 and HepG2 cell lines of the extracts from medicinal plant *Genista lydia var. lydia* (Fabaceae)	Burcu Tongul
17:30 - 17:40	Characterization of Novel Wnt/β-catenin Pathway Targets	İzzet Akiva
17:40 - 17:50	The Real Time Monitorization of the Cytotoxic Effects of Vanadium Pentaokside on Different Cancer Cell Lines.	Mükerrem Betül Yerer Aycan
17:50 - 18:00	Are there possible associations between MnSOD and GPx1 gene variants for laryngeal cancer risk or disease progression?	Cihan Coşkun

Çanakkale Salonu

16:50 - 17:00	Effects Of Different Diet Types On DNA Damage And Inflammation In Breast Cancer	İlker Çoban
17:00 - 17:10	Dietary calorie restriction alters oxidative stress biomarkers in MMTV-TGF-alpha mice	Munevver Burcu Cicekdal
17:10 - 17:20	Antiproliferative and apoptotic effects of Thymol (*Thyme vulgaris*), a novel monoterpene phenol, in the PC-3 and DU-145 human prostate cancer cell lines	Hulya Elbe
17:20 - 17:30	Dynamic Assessment of Antiproliferative and Antimigratory Activities of the Natural Small-Molecule Alectoronic Acid by Using Real Time Cell Analyzers (RTCA)	Mehmet Varol
17:30 - 17:40	Anti-cancer effect of urfa pistachio (*pistacia vera*) green hull extract on colon adenocarcinoma cells	İsmail Koyuncu
17:40 - 17:50	In silico identification of compounds with selective action on epithelial or mesenchymal tumor cells	Seçil Demirkol
17:50 - 18:00	The expression level of NF-kB/p65 increases in bortezomib-resistant multiple myeloma cell lines	Mehtap Tarhan

29 Ekim 2016 **Cumartesi**

Ana Salon

09:00 - 09:10	The Effect of Metal Chelators on Breast Cancer Stem Cells	Ufuk Özer
09:10 - 09:20	Maternal microchimeric cells turn into cancer stem cells?	Osman Demirhan
09:20 - 09:30	The In Vitro Effect of Sanguinarine Differs on Neuroblastoma Stem Cells Based on the Serum	Ayşe Pınar Erçetin Özdemir
09:30 - 09:40	Cytotoxic Synergy Between Tingenin B and Paclitaxel Against Breast Cancer Stem Cells: Induction of Mitochondria Dependent Apoptosis	Arzu Yilmaztepe Oral
09:40 - 09:50	The Plant-Derived Triterpenoid Pristimerin is a Potent Anticancer Agent Due to Its Cytotoxic Activity on Breast Cancer in vitro and in vivo	Buse Cevatemre
09:50 - 10:00	Negative Effects of Myeloma Cells on Senescent Mesenchymal Stromal Cells Anti-Tumour Paracrine Activity	Servet Özcan

Malazgirt Salonu

09:00 - 09:10	AKT/PKB-mediated Phosphorylation of Twist1 is Essential for Tumor Growth and Metastasis	Suray Pehlivanoglu
09:10 - 09:20	Prostate Cancer and Ranolazine as Ion Channel Blockers	Seyhan Altun
09:20 - 09:30	Novel Function of Elf3: A Potential Regulator of MET	Burcu Şengez
09:30 - 09:40	Targeting of $Na_V1.5$ channel in metastatic breast cancer models *in vitro* and *in vivo* mice as a novel therapy	Mumin Alper Erdogan
09:40 - 09:50	TGF-ß Receptor I/II Signaling at Primary Cilia Membrane is Regulated by Ceramide to Modulate Cell Migration	Salih Gencer

Çanakkale Salonu

09:00 - 09:10	Differential co-expression network in ovarian cancer: Prognostic and therapeutic targets	Esra Gov
09:10 - 09:20	The Role of Biomarkers in The Follow-Up of Patients with Malignant Mesothelioma	Adnan Ayhancı
09:20 - 09:30	Synthesis, characterization and DNA interation of novel platinum(II) complexes containing substitutedbenzimidazole ligands	Semra Utku
09:30 - 09:40	The Effects Of Aceytl-L Carnitine On Cisplatin And Radiation Induced Apoptosis Treatment On Medullablastoma Cells	Ayca Pamukoglu Kaynar

30 Ekim 2016 **Pazar**

Ana Salon

09:30 - 09:40	Investigation of anti cancer properties of new sulphonamide derivative showed carbonic anhydrase-IX enzyme inhibitor feature	İsmail Koyuncu
09:40 - 09:50	The Effect of the *Rosmarinus Officinalis* on Temozolomide Resistant Glioblastoma (U87 MG) Cells	Damla Meryem Özdemir
09:50 - 10:00	Anticancer Effects on Human Leukemia HL-60 Cell Line and Molecular Docking Studies of Novel 2,5-Disubstituted-Benzoxazole Derivatives	Emine Oksuzoglu
10:00 - 10:10	Effects of Embelin on Breast Cancer Cell Proliferation and Apoptosis; Comparing With Docataxel and Tamoxifen	Gülsüm Tekin
10:10 - 10:20	3,4-Bis(3'-indolyl)-1,2,5-oxadiazoles, analogues of marine alkaloid nortopsentin: Synthesis and antiproliferative activity	Fatih Sevgi
10:20 - 10:30	Bioassay-guided isolation and cytotoxic effects of extract and chemical constituents of *Chrysophthalmum montanum* (DC.) Boiss. of Turkish origin	Fatma Ayaz
10:30 - 10:40	Ankaferd Blood Stopper Induces DNA Damage, Apoptosis and Cytotoxic Activity by Generating Reactive Oxygen Species in Melanoma Cells in Vitro	Eray Metin Güler

10:40 - 10:50	Evaluating the Cancer Therapeutic Potential of Supramolecular Calix[4]aren Nanofibers	Pembegül Uyar Arpacı
10:50 - 11:00	Evaluation of the effects of thymoquinone to dynamic thiol-disulfide homeostasis during total body irradiation in rats	Cigdem Damla Deniz
11:00 - 11:10	A Relationship Between Molecular Structure and Anticancer Activity/Cytotoxicity for Poly(maleic anhydride-co-vinyl acetate)/Drugs Conjugates with Gemcitabine, Cyterabin and Methotrexate Drugs	Gülderen Karakuş
11:10 - 11:20	Does MW radiation affect gene expression, apoptotic level and cell cycle progression of human SH-SY5Y neuroblastoma cells?	Handan Kayhan

Malazgirt Salonu

09:30 - 09:40	CTLA-4 Gene +49 A/G Polymorphism in Prostate Cancer Patients	Songül Budak Diler
09:40 - 09:50	Demonstration of the effectiveness of neoadjuvant chemotherapy and radiotherapy by 18F-FDG PET-CT and MRI in patients with colorectal cancer	Serdar Savaş Gül
09:50 - 10:00	Factors Effecting Mortality in Patients Operated Due to Gastric Carcinoma	Selami Ilgaz Kayılıoğlu
10:00 - 10:10	Effects of Meal Frequency and Calorie Restriction on Metabolism in Rats	Hasan Basri Savas
10:10 - 10:20	BRCA2 and RAD51 GENE EXPRESSION ANALYSIS OF CMTs	Özge Özmen
10:20 - 10:30	Fluorescence microscopic detection of some terminal sugar moieties on the cell surface of human thyroid carcinoma cell lines	Serap Sancar Baş
10:30 - 10:40	Near-Infrared Fluorescent Carbon Nanotube Based Sensors for In Vitro and In Vivo Cancer Applications	Fatih Sen
10:40 - 10:50	Sex Hormone Dependent Toxicity of Ochratoxin A in the Kidney of Female Rats	Mehmet Akif Kılıç
10:50 - 11:00	p-Coumaric Acid Attenuate on Cisplatin-Induced Oxidative Stress in Rat's Heart	Fazile Nur Ekinci Akdemir

11:00 - 11:10	Flow Cytometric Assesment Of the Rosa canina Extracts on Different Cancer Types	Kağan Kılınç
11:10 - 11:20	Fhit deficiency drives neoplastic initiation and progression	Bahadır Batar

Çanakkale Salonu

09:30 - 09:40	HDAC inhibitors, MS-275 and Salermide, potentiates the anticancer effect of EF24 in human pancreatic cancer cells	Atiye Seda Yar Sağlam
09:40 - 09:50	Does Chemotherapeutics Contribute to DNMT1 Expression Level in Colorectal Cancer Cells?	Nuray Varol
09:50 - 10:00	Investigating the role of alternative polyadenylation in lung squamous cell carcinoma	Hilal Kazan
10:00 - 10:10	The Role of REL proto-oncogene in Follicular Lymphoma Development	Esra Baytak
10:10 - 10:20	Achievement of Betulinic acid on EGFR initiated signalling	Asuman Demiroglu Zergeroglu
10:20 - 10:30	Characterization of protein-protein interactions between of the MO25α and CCM3 scaffold signal transducers and the STK25 protein kinase	Can Ali Ağca
10:30 - 10:40	The role of YAP1 in prostate cancer tumorigenesis	Filiz Kisaayak Collak

KONUŞMACI ÖZETLERİ

ADVANCES AND CLINICAL APPLICATIONS IN CANCER STEM CELL RESEARCH

Hasan Korkaya

Georgia Cancer Cente, Augusta University

Metastatic disease, the end stage of tumor progression is the major cause of cancer-related death. It is widely accepted that malignant cell plasticity between the epithelial-mesenchymal-transition (EMT) and mesenchymal-epithelial-transition (MET) is required for metastasis to occur. However, the investigation of this process is hampered by the complexity of tumor microenvironment that also contribute to the tumor plasticity. Our studies suggest that early molecular crosstalk between tumor cells and the host immune system during the process of metastasis determines the outcome of the disease. Chronic inflammation has been recognized as a risk factor contributing to the etiology of many malignancies. Accumulating evidence suggests that tumor-infiltrated immune cells (mainly of myeloid origin) differentiate into cells that promote tumor growth and invasion in addition to their immunosuppressive role. We therefore show that myeloid derived suppressor cells (MDSCs) regulate tumor plasticity as well as help generate the permissive microenvironment in distant organs for successful metastasis. In our studies, we show that monocytic MDSCs (mMDSCs) infiltrated at the tumor invasive front facilitate dissemination of tumor cells from the primary site by inducing an EMT/cancer stem cell (CSC) phenotype. In contrast, granulocytic MDSCs (gMDSCs) infiltrated in distant organs promote metastatic growth by reverting the tumor cells back to the MET phenotype promoting tumor cell proliferation. Although both mMDSCs and gMDSCs have been shown to suppress anti-tumor immune responses, our preliminary studies suggest that these cells also play distinct roles in spatiotemporal tumor plasticity between EMT/CSC-MET in the primary site and in distant organs.

In summary, our studies propose a novel mechanism by which the spatiotemporal tumor plasticity is distinctly regulated by mMDSCs and gMDSCs in the primary tumor and in distant organs respectively. We predict these studies will determine the link between immune system and cancer progression, and in so doing it will identify alternative molecular targets that are critical in the metastatic process.

KANSER TEDAVİSİNDE GELECEĞE YÖNELİK YENİ YAKLAŞIM : *"PRECISION MEDICINE"*

Emin Kansu

H.Ü. Kanser Enstitüsü Emekli Öğretim Üyesi

Kanser tedavisinde uzun yıllar cerrahi, kemoterapi ve radyoterapi yöntemleri kullanılmış ve son 25 yıl içinde biyolojik ajanlar (biyoterapi) da terapötik alanda önem kazanmıştır. Tüm bu yaklaşımlara rağmen kanser hastalarında rölapsın izlenmesi, mortalitenin azaltılması ve uzun süreli sağkalım oranlarında istenen başarılara ulaşılamamıştır. İnsan genomunun 2001 yılında haritalanması sonrasında sağlıklı ve hastalıklı bireylerin genomları sekanslanmaya başlanmış (whole genome sequencing) ve özellikle kanserli hastalarda çok önemli veriler elde edilmiştir
(*Cancer Genome Atlas*). Bu alanda ilk ele alınan akciğer kanserinde *driver* ve *passenger* mutasyonlar çalışılarak "*aynı hastada gelişen bir akciğer kanserinin kendi içinde bile çok fazla sayıda farklı gen mutasyonları*"nın varlığı belirlenmiştir. ABD'de NCI Direktörü Dr.Harold Varmus yaptığı bir konuşmada kanserin *çok kompleks bir hastalık* olduğunu ve her bir kanserin *kendi içinde 100'den fazla farklı* kanser türü olduğunu ifade etmiştir.
Bu verilere dayanarak, uzun yıllar kanser tedavisinde istenen başarının elde edilememesinin nedeni olarak her bir hastada genomik profilin iyi bilinmemesi konusu giderek önem kazanmıştır. Bir hastaya kanser tanısı konulduktan sonra "*tümör dokusunun histopatolojik, immünopatolojik ve genomik profil"* çalışmalarının yapılması gerekmektedir. Sonuçta, tümör hücrelerinin genomik yapısı, varsa mutasyonlarının belirlenmesi ve farklı hücre türlerinin gösterilmesi (*heterojenite)* tedavinin rasyonel planlanmasına imkan sağlayacaktır. Gelecekte bu yeni perspektif yardımıyla genomik profil tayinleri sonucu belirlenen kanser hastasının genom yapısı çok daha duyarlı ve doğru (*Precision*) prensipleri temel alan terapötik planlama yapılmış olacaktır (*Precision Medicine). "Precision Medicine"* terimi son yıllarda kullanılmaya başlanan ve temel bilimlerde olan gelişmelerin en etkin ve duyarlı şekilde hasta tedavisine çevrilmesi *(*translation)'ni ifade etmektedir. "*Precision Medicine*" teriminin henüz tam Türkçe karşılığı yerleşmiş değildir.
Günümüzde, kanser ve diabet başta olmak üzere birçok hastalık için koruyucu yaklaşımlar veya kesin tedavi yöntemleri henüz tanımlanmış değildir. Çok sayıda hastalık için prevantif ve etyolojiye yönelik çalışmalar ile moleküler biyolojik ve çevresel araştırmalara ihtiyaç vardır. "*Precision Medicine*" hastalıkların korunması ve tedavilerinde geleceğin çok ümit veren yeni bir yaklaşım modelidir. Bu model, bireylerin genlerini, çevrelerini ve yaşam şekillerindeki değişkenleri dikkate alarak hastanın tedavilerini şekillendirmektedir. "*Precision Medicine*" henüz hasta tedavilerinde günlük uygulamaya geçmiş değildir. "*Precision Medicine*" biyomedikal temel araştırmaları klinik uygulamalar ile entegre edilmesine imkan veren ve bireysel (*personalized*) hasta uygulamasına imkan sağlayan yaklaşımları içeren bir terim olarak kullanılmaktadır. Son yıllarda, insan hastalık genlerinin haritaları "*genome sequencing= genom sekanslama*" teknikleri ile klinisyenlerin yararlanabilecekleri formatlarda ve giderek azalan maliyetlerde kısıtlı da olsa temin

edilmeye başlanmıştır. "*Precision Medicine*"ın gelecekte sağlık hizmetlerinin ve daha kesin tedavilerin planlanmasında büyük önem taşıyacak bir model olması beklenmektedir. Ancak, bu model araştırma merkezleri, üniversite, farmasötik sektör ve kamu kaynaklarının yakın işbirliği (network) içinde çalışmasını da gerekli kılmaktadır.

Gelecekte onkoloji alanında, "*Precision Medicine*" yaklaşımı, bireysel genomik, biyoinformatik, ileri moleküler biyolojik teknikler ve yeni oluşturulacak stratejiler yardımıyla kanser tedavileri çok daha duyarlı (Targeted Therapy/Personalized Medicine), yüksek oranda şifa ve sağkalım elde edebileceğimiz innovatif bir yaklaşım olacaktır. Bu yeni yaklaşım modeli ile uzun vadede ülkelerin ilaç harcamalarında farmako-ekonomik olarak önemli tasarruflar sağlanacağı da öngörülmektedir. ABD-NIH Direktörü Dr. Francis Collins 17 Eylül 2015 tarihinde "*NIH Working Group Advisory Committee*" nin önerileri doğrultusunda 2016 yılı ve sonrası için "*Precision Medicine*" projesinin ilk kohort'larını çalışmak amacıyla hızla alt yapı çalışmalarını programa almış bulunmaktadır.

KANSER HÜCRELERİNDE ÖLÜMSÜZLÜK

Mehmet Öztürk

Dokuz Eylül Üniversitesiİzmir Uluslararası Biyotıp ve Genom Enstitüsü İzmir, Türkiye

Hücresel anlamda "ölümsüzlük", hücrelerin çoğalmaya uygun koşullarda sürekli çoğalabilme özelliğini tanımlar. Bu terim, "hücre yaşlanması" ya da "senesans" olarak tanımladığımız sınırlı sayıda çoğalma özelliğinin karşıtı olarak türetilmiştir. Söz konusu sınırlama, özellikle somatik hücrelerde, kromozom telomerlerinin çoğalma sırasında gittikçe kısalmasına bağlıdır. Bunun nedeni ise, bu tür hücrelerde telomer DNAsının sentezi için gerekli olan Telomeraz Revers Transkriptaz (TERT) enzimi sentezinin baskılanmış olmasıdır. Normal kök hücreler bu enzimi gerekli düzeylerde ifade edebilen özelliğe sahiptirler. Kanser hücrelerinin büyük çoğunluğu ise telomerlerini kısaltmadan çoğalabilme özelliği gösterirler. Bunun başlıca nedeni, somatik hücrelerin kanserleşme sırasında yeniden TERT sentezleyebilme özelliğini kazanmalarıdır. En son bulgulara göre, bir çok kanser türünde bunun nedeni TERT promotorunda oluşan özgün mutasyonlardır. Ancak, TERT ifadesinin ve telomer kısalmasını önlemenin alternatif yolların da mevcut olduğu bilinmektedir. Ölümsüzlük kanser hücrelerinin en ortak özelliklerinden birisidir. Mevcut bilgilere göre, somatik hücrelerde gözlenen senesansın ana nedeni, telomer kısalmasından kaynaklanan DNA hasar sinyallerinin "Retinoblastoma" ve "p53" sinyal yolaklarını uyarması ve bunun sonucu olarak hücre çoğalmasının sürekli olarak durmasıdır. Kanserleşme sırasında, kanser öncesi hücrelerin senesansa girmeleri, ancak söz konusu yolaklarda yer alan genlerde oluşan mutasyonlar neticesinde bu denetimden kurtulmaları, ikinci bir aşamada ise TERT ifadesini yeniden kazanarak sürekli çoğalabilen hücreler haline gelmeleri söz konusu olabilir. Ancak, önce TERT aktivitesinin yeniden kazanılması, sonra senesans denetim mekanizmalarının bozulması senaryosu da bazı kanserlerde söz konusu olabilir. İkinci senaryoyu destekleyen en önemli bulgu, TERT ifade eden hücrelerde, telomer kısalmasına gerek kalmaksızın, DNA hasarı sonucunda da senesansın gelişebilmesidir. Telomerden bağımsız bu tür senesans yanıtı, birkaç gün içinde gerçekleşen hızlandırılmış bir stres yanıtıdır ki burada TERT'den daha çok retinoblastoma ve p53 sinyal yolağı genleri öne çıkmaktadır. İster telomer bağımlı ister bağımsız olsun, senesansa giren hücrelerde özgün metabolik ve morfolojik değişimler olur. Ölümsüz ya da çoğalmakta olan hücreler aynı özellikleri göstermedikleri için, senesans ve ölümsüzlük durumlarında hücrelerin epigenetik programları ve gen ifade profilleri büyük farklılıklar gösterir. Son yıllardaki araştırmalarımızda "karaciğer kanserlerind"e tüm genom üzerinden gerçekleştirdiğimiz gen ifade analizleri, bu kanserlerin patojenezi, tanısı ve tedavisi konularında önemli ipuçları elde etmemizi sağladı. Konuşmamızda bu çalışmalardan seçilen örnekler üzerinden, kanserde hücre ölümsüzlüğünün farklı yönleri tartışmaya açılacaktır.

THE IMPORTANCE OF PI3K SIGNALING PATHWAY IN BREAST CANCER DIAGNOSIS AND TREATMENT

Ebubekir Dirican

Marmara University, School of Medicine Department of Medical Biology, Istanbul, Turkey

Breast cancer (BCa) is the most frequent malignancy among women with an estimated 1.67 million new cases diagnosed each year worldwide. Phosphoinositide 3-kinase (PI3K) signaling pathway has a important role in the cellular processes such as cell survival, growth, division and motility. The PI3K/AKT signaling pathway is frequently activated in human cancers. PI3K also phosphorylation of PIP2 to PIP3 promotes the phosphorylation and activation of AKT. Constitutive activation of the PI3K/AKT pathway occurs in more than 50% of human BCa, most commonly through mutational activation of the PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase-Catalytic Subunit Alpha gene), mutational activation or amplification of AKT1, AKT2, or AKT3, or functional loss of the lipid phosphatase PTEN, whose function counteracts that of PI3K. In present study, correlations between PI3K mutations and their clinicopathological parameters on BCa will be described. Especially, PIK3CA mutations which have been localized mostly on exon 9 and 20 hot spots are detected 25-40% in BCa. PIK3CA mutations have been intensely investigated because of the possibility for therapeutic interventions against activated PI3K. This relatively high frequency can offer an advantage for choosing the best treatment options for BCa. PIK3CA mutations may be used as biomarkers and have been major focus of drug development in cancer with the first clinical trials of PI3K pathway inhibitors currently in progress. Screening of PIK3CA gene mutations might be useful genetic tests for targeted therapeutics or diagnosis. This study offers evidence that personalizing treatment of BCa to PI3K inhibitor therapy may benefit from an analysis of PI3K signaling pathways and will help to introduce new clinical applications in the near future.

Keywords: Breast cancer, PI3K, PIK3CA, Mutation, p110α

ANTİKANSER ETKİLİ İNDOL KARBOKSİLİK ASİT TÜREVLERİ: SENTEZLERİ VE DNA KESİM AKTİVİTELERİ

Bircan Çeken Toptancı

Dicle Üniversitesi, Fen Fakültesi, Kimya Bölümü, 21280 Diyarbakır, Türkiye

Giriş: Küçük moleküllerin DNA'ya bağlanmaları geniş ölçüde çalışılmıştır. DNA küçük oluğuna bağlanma, interkalasyon ve elektrostatik etkileşim veya bunların kombinasyonu ile DNA'ya bağlandığı bilinen birçok molekül mevcuttur.[1] DNA'nın küçük moleküller ile bu kovalent olmayan etkileşimi, birçok hastalığa karşı yeni ve etkili ilaç geliştirilmesine olanak sağlar.

Bu çalışmada Duocarmycin A ve SA antibiyotiklerinin yapısında bulunan, indol karboksilik asit türevleri sentezlenerek, bakır varlığında ve yokluğunda, DNA kesim etkileri incelendi.

Yöntem: (+)-Duocarmycin A ve SA antibiyotiklerinin yapısında bulunan C-5 karbonunda tersiyer amino fonksiyonal gruba sahip suda çözünen indol-2-karboksilik asit hidroklorür türevi sentezlendi.[2] Sentezlenen ligandların DNA kesim etkileri M13+ plazmid DNA (3.2 kb) ve Calf Thymus DNA (8-15 kb) kullanılarak Agaroz Jel Elektroforezi tekniği ile incelendi.

Bulgular: Sentezlenen ligantların konsantrasyona ve zamana bağlı olarak DNA kesimine sebep olduğu, bakır varlığında DNA kesiminin arttığı tespit edildi. DNA kesiminde reaktif oksijen türlerinin etkisini araştırmak için reaksiyonlar histidin, tiyoüre, TEMPO ve DMSO varlığında tekrarlandığında[3] ve bu radikal söndürücülerin DNA kesimini inhibe ettiği gözlemlendi.

Sonuç: Bu çalışmada sentezlenen (+)-Duocarmycin antibiyotiği türevlerinin bakır varlığında ve yokluğunda DNA kesimine sebep olduğu ayrıca bakır varlığında kesimin büyük ölçüde arttığı gözlemlenmiştir. C-5 pozisyonunda dimetilaminoetoksi grubu ve C-6 pozisyonunda metoksi grubu içeren, indol karboksilik asidin hem bakır varlığında hem de bakır yokluğunda DNA kesiminde diğer ligantlara oranla daha etkili olduğu elektroforez sonuçlarından belirlenmiştir.

Bazı kanser türlerinde, tümör hücrelerinde bakır miktarının sağlıklı bireylerle karşılaştırıldığında 2-3 kat daha fazla olması sonucun önemini göstermektedir. Cu^{2+} varlığında ve Cu^{2+} yokluğunda DNA kesiminde değişik spesifik radikal söndürücüler ile reaktif oksijen türlerinin etkisi çalışıldığında plazmid DNA'nın büyük ölçüde korunduğu gözlemlenmiştir. Bu sonuç sentezlenen indol karboksilik asitlerin serbest radikaller oluşturarak DNA kesimini gerçekleştirdiğini göstermektedir.

Bu çalışma Türkiye Bilimsel ve Teknolojik Araştırma Kurumu tarafından 109T788 proje numarası ile desleklenmiştir. Desteklerinden dolayı TÜBİTAK'a teşekkür ederiz.

CRITICAL NODE IN DEVELOPING DIABETES AND CANCER: INSULIN RECEPTOR SUBSTRATE (IRS) PROTEINS

Gokhan Gorgisen

Yuzuncu Yil University, Faculty of Medicine, Department of Medical Biology

IRS proteins are large cytoplasmic docking protein family that plays important roles in transducing signals from transmembrane receptors upon growth factors stimulations. IRS proteins are the primary mediators of insulin-regulated glucose metabolism and mitogenesis in most cell types. IRS1 and IRS2 are widely expressed in human tissues; therefore, most of the studies have been focused on these proteins and their functions.

Ligand-phosphorylated insulin receptor (IR) activates insulin receptor substrate proteins that lead to the activation of two main signaling pathways: AKT/protein kinase B pathway, which is responsible for most of the metabolic actions of insulin, and the Ras–mitogen-activated protein kinase pathway, which regulates expression of some genes and cooperates with the PI3K pathway to control cell growth and differentiation. In addition to insulin receptor, IRS1/2 proteins also interact with the hormone/growth factor receptors such as the prolactin, growth hormone, leptin, vascular endothelial growth factor, TrkB, ALK and members of the integrin receptors. Since the common feature of these receptors is the induction of proliferation, survival, and cell migration it is suggested that IRS may play important role in development of cancer and metastasis.

IRS function is regulated by post-translational modifications. Although tyrosine phosphorylation of IRS promotes insulin signaling, its Ser/Thr phoshorylation generally inhibits the insulin signaling by inducing degradation and dissociation of IRS from the insulin receptor and inhibition of its tyrosine phosphorylation under physiological conditions. Furthermore, this mechanism can be utilized by inducers of insulin resistance under pathological conditions. To date there are significant amounts of studies that have shown the metabolic effects of IRS1 Ser/Thr phosphorylation in insulin resistance, but there have been limited studies of IRS activation and Ser/Thr phoshorylation levels and their effects on tumorigenesis. This talk focuses on the importance of Ser/Thr phosphorylation of IRS proteins and their effects on developing cancer and diabetes.

EVRİMSEL BİR SÜREÇ OLARAK KANSER

Sibel Küçükyıldırım

Hacettepe Üniversitesi, Moleküler Biyoloji Anabilim Dalı

Kanser, adaptif evrimsel bir değişiklikten çok düzensiz gelişimin somut bir örneğidir ve çoğu zaman hücre doğumu-ölümünün düzenlenmesinde ortaya çıkan bir hata olarak tanımlanır. Bu görüş, temelde, kanseri doğal seçilimin bir mekanizması olarak ele almaya karşıt gözükmektedir. Ancak, bir karakter (özellik) zamansal ya da mekansal bir ölçekte işlevini yerine getiremiyor gibi görünse de, farklı bir ölcekte "etkin işleyiş" sağlayabilir (Hausman 2012). Nitekim, çok hücreli organizmaların –az ya da çok- hiyerarşik şekildeki organizasyonu göz önüne alındığında bu bizim için şaşırtıcı değildir.

Kanserin evrimsel bir mekanizma olarak ele alınabileceği fikri, ilk defa, kanserli hücre populasyonu ile evrimleşen bir mikroorganizma populasyonunu karşılaştıran Gause (1966) tarafından ortaya atılmıştır. Nowell (1976) ve Cairns de (1975, 1978) benzer şekilde, kanserin, bölünen hücre populasyonları arasında gelişen rekabet olarak doğal seçilimin bir mekanizması gibi düşünülebileceğini tartışmışlardır. Başlangıçta az sayıda bilim insanı tarafından ilgi gören bu fikir, bugün, matematiksel modelleme, bilgisayar simülasyonu, model sistemlerde deneysel evrim ve yeni nesil yüksek çözünürlüğe sahip dizileme yöntemlerini kullanan aktif bir araştırma alanına dönüşmüştür (Gerlinger et al. 2012; Navin et al. 2011; Yachida et al. 2010).

Bir tümör, genetik ve epigenetik olarak değişikliğe uğramış hücrelerin oluşturduğu bir mozaiğe benzetilebilir. Bu hücreler yayılmak ya da diğer organları işgal etmek için birlikte calışsalar da, alan, kaynaklar ya da bağışıklık sisteminden kaçmak için rekabet ederler. Bir neoplastik hücrenin uyum başarısı hücre ve mikroçevresinde (ekoloji) bulunan diğer faktörlerin karşılıklı etkileşimiyle şekillendirilir. Bu faktörler, kanseri önlemeye ve tedavisine aracılık eden etkenleri de kapsar. Tümör hücresinin/hücrelerinin sahip olduğu somatik değişiklikler bu hücrelerin uyum başarısı üzerinde farklı kalıtsal etkilere sahip olabilirler ve bu sayede mutant klonlar gelişebilir. Oluşan mutant klonlar genetik sürüklenme ve seçilimin etkisiyle, organizma üzerinde herhangi bir negatif etki oluşturmaksızın, varlıklarını sürdürürler. Klonal evrim genellikle artmış proliferasyon ve sağkalımı seçer ve bu özellik invazyona, metastaza ve tedaviye dirence neden olabilir. Güncel araştırmalar, 1966`da ilk defa Gause tarafından öne sürülen kanserin evrimsel bir sistem olarak tanımlanması fikrini geniş ölçüde desteklemektedir. 1966`dan beri, araştırmacılar çok sayıda farklı neoplazm tipinde klonal gelişmeyi ve genetik heterojeniteyi tanımlamışlardır.

Ancak, karsinogenezde evrimsel biyoloji yaklaşımının fayda sağlayabileceği çok sayıda fırsat hala keşfedilmeyi beklemektedir. Örneğin, "Bir neoplazmda genetik ve epigenetik değişikliklerin oluşma hızı nedir? Bu hızı nasıl değiştirebiliriz? Klonlar nasıl gelişirler ve biz bu gelişmeyi kontrol altında tutmak için neler yapabiliriz? Farklı genetik/epigenetik değişiklerin tümörün göreceli uyum başarısına etkisi nedir? Uyguladığımız tedavi yaklaşımlarının seçilimsel etkileri nelerdir?" gibi soruları cevaplandırmak neoplastik gelişimi durdurmaya ve tedavinin etkilerini ölçmemize olanak sağlayacaktır.

MİKRORNALAR VE KANSER

Zekiye S. ALTUN

Dokuz Eylül Üniversitesi, Onkoloji Enstitüsü, Temel Onkoloji AD, İzmir

MikroRNAlar (miRNA) kısa kodlamayan RNA'lar olup gen ekspresyonunu mRNA translasyon inhibisyonu ya da mRNA stabilitesini azaltarak düzenlerler. miRNA'ların bozulmuş regülasyonu birçok kanser tipinde önemlidir. miRNAlar tümörün başlaması, progresyonu ve disseminasyonuna neden olan proliferasyon, diferansiasyon, migrasyon ve apoptoz gibi hücresel yolları etkileyerek değişik aşamalarda etkili olan gen ekspresyonlarını regüle etme özelliğine sahiptir. Spesifik miRNA'lar onkojenik ya da tümör supresif mRNA'ları baskılayarak onkojenik ya da tümör süpresif etki ortaya çıkması konusunda birbirleriyle yarışabilirler. Ayrıca miRNA'lar kanser-immün sistem etkileşimi, stromal hücre etkileşimleri, onkovirüsler ve tedaviye duyarlılık gibi tümörü modifiye eden ekstrensek faktörleri de düzenleyebilmektedirler. Bu işlemler arasında ki dengeye bağlı olarak spesifik miRNAların net onkojenik ya da tümör supresif etkileri şekillenmektedir.

Serbest dolaşan miRNAlar (cfmiRNA) elde etme ve depolama koşullarına dayanıklı olmaları, ayrıca sadece plazmada değil diğer tüm vücut sıvılarında da tesbit edilebilmeleri nedeni ile mükemmel bir biyobelirteç özelliği sergilemektedir. Bu nedenle serbest dolaşan miRNAlar kanser taraması, prognoz ve anti-kanser tedavilerin monitorizasyonunda ümit vadetmektedir. Yine plazmada bu cfmiRNAların primer tümör ile benzer düzeyde bulunduğu da belirtilmektedir. Son zamanlarda ilginç bir gelişme de viral ve bitki miRNAlarının da (xenomir) sağlıklı kişilerin plazmasında bulunduğu derin sekanslama teknikleri ile gösterilmiştir. XenomiRler, insan vücudunda bulunması gerekmeyen ve insan genomu tarafından kodlanmayan miRNAlar olup, dolaşımda bulunan şekillerine cfxeno-miRNA denmekte olup insan mRNA'larını hedefleyerek etkili olabilecekleri belirtilmektedir. Ayrıca, besinlerin sindirimi ile alınan eksojen miRNAların insan mRNAlarını nasıl düzenlediği de anlaşılmaya çalışılmaktadır. Eksojen miRNA'ların ya da xenomiRlerin kanserin başlama ve ilerlemesinde etkili olduğu ve plazmada bulunduğuna dair deliller bulunmakta ve bunların sadece kanserin erken tarama programında değil aynı zamanda hastaların prognozunda kullanılabilirliği araştırılmaktadır.

Karsinogenez, mikroçevre ilişkisi ve hücre ölüm yollarının düzenlenmesi gibi kanserin değişik aşamalarından itibaren miRNA'ların önemli fonksiyonları olduğu belirtilmektedir. Ayrıca miRNA'lar birbirleriyle yarışarak değişik kanserlerde farklı rollerin ortaya çıkmasına neden olabilmektedir. Let-7 miRNA, RAS onkogenini baskılayarak tümör supresör olarak çalışmakta ve onkogenez açısından RAS-MAPK yolağını hedefleyen miRNAların önemli olduğu belirtilmektedir. miR-152, hücre proliferasyonu, migrasyon ve invazyonu regüle ederek kanserde tumör supresör miRNA olarak görev aldığı belirtilmektedir. mir-197 ise bazı tümörlerde upregüle bazılarında ise downregüle olduğu gösterilmiş tümör hücresinin proliferasyonu, apoptoz ve metastaz basamaklarında önemli ve hücrelerinin yayılımını arttırıcı ve kanserde ilaç direncine neden olan bir miRNA olup iyi bir tedavi hedefi olarak öne sürülmektedir.

miRNA'ların fonksiyonlarının ve hedef genlerinin araştırılması ile kanser başta olmak üzere değişik hastalıklarda tedavi hedefleri ortaya konmaya devam edecektir. Özellikle hücre kökenli olmayan ve değişik vücut sıvı kaynaklarında bulunabilen serbest dolaşan miRNA'ların ve exomiR'lerin araştırılması kanserde gelecek bakış açımızı değiştirebilecektir.

NATURAL SMALL-MOLECULES OBTAINED FROM LICHENS AS A NOVEL SOURCE OF ANTI - ANGIOGENIC AGENTS

Mehmet VAROL [1,2]

[1] *Department of Biology, Faculty of Science, Anadolu University, Yunusemre Campus, 26470, Eskişehir, TURKEY.*
[2] *Department of Molecular Biology and Genetics, Faculty of Science, Mugla Sitki Kocman University, Kotekli Campus, 48000, Mugla, TURKEY.*

It is well known that natural products as the lead compounds in drug discovery have a great importance to cure many diseases including cancer and the other angiogenesis-dependent syndromes. Although detection methods of the pharmacologically active compounds are costly and/or time-consuming processes due to the complex compound contents of highly structured-organisms, lichens provide a lot of advantages through their exclusive biological and chemical structures. The biological structure of lichens is characterized as a sophisticated symbiotic system composed by a photobiont and a mycobiont with a tight metabolic relationship between these partners. Thence, lichens can produce more than 1000 unique secondary metabolites which manifest a wide variety of biological and pharmacological activities including anti-proliferative, anticancer, anti-migratory, anti-inflammatory and anti-angiogenic properties. On the other hand, researchers have a methodological opportunity to separate the natural small-molecules of lichens in pure forms by using simple isolation methods because every lichen species produces only a few exclusive lichen acids. Among these advantages of lichens, it was noticed that the lichen-derived natural small-molecules have a great potential to influence on angiogenesis and angiogenesis-related cellular events. The promotion of cancer is tightly linked to angiogenesis, occurred as a complex process that includes tumor-mediated stimulation of endothelial cells, degradation of basement membrane of existing blood vessels, migration, proliferation and adhesion of endothelial cells, and organization of endothelial cells into new capillary vessel branches. Targeting angiogenesis provides a treatment opportunity on a wide range types of cancers, and many natural products that inhibit angiogenesis also indicates other anticancer activities including the inhibition of tumor progression and reduction the risk of metastasis. To the best of our knowledge, the first study about the anti-angiogenic activities of lichen acids have lighted up the anti-angiogenic activity of olivetoric acid isolated from the acetone extract of the lichen *Pseudevernia furfuracea* (var. ceratea). After this study, more comprehensive studies have been performed by using other lichen acids including the chiral forms of usnic acid, vulpinic acid, secalonic acid-D, etc. The published papers showed that lichen acids have a great potential to be anti-angiogenic agents with low toxicities and significant inhibition of angiogenesis, angiogenesis-related molecules and cellular signaling cascades including the inhibition of hypoxia-inducible factor 1-alpha (HIF1α), vascular endothelial growth factor (VEGF) and regulation of Akt/mTOR/p70S6K and ERK1/2 pathways. We therefore would like to draw your attention to lichen derived small-molecules as the new source of anti-angiogenic agents.

ANDROGEN RECEPTOR PATHWAY IN PROSTATE CANCER

Leena Latonen

BioMediTech and Prostate Cancer Research Center, Finland

Prostate cancer is a major health care problem being the most common male malignancy in Western countries, and the second most common cause of cancer death among men. Currently, there is no curative treatment for advanced prostate cancer. Prostate cancer is dependent on androgens, which mediate their effect through androgen receptor (AR). Identifying androgen- and AR-regulated events relevant for prostate cancer aims to find novel diagnostic and prognostic markers, and targets for new cancer therapies. The current challenge lies in understanding the molecular events turning primary prostate cancer into CRPC.

In the laboratory of prof. Tapio Visakorpi we have studied the role of AR-mediated effects in prostate cancer by developing cell line models and using clinical samples from prostate cancer patients. The molecular mechanisms of elevated AR levels and activity, and the effects of these in chromatin binding of AR and AR target gene expression has been studied. Based on these studies, we have identified several interesting AR target genes and studied their effects in more detail. For example, miR-32, a microRNA upregulated in CRPC, is provides a survival advantage to prostate cancer cells and is able promote proliferative changes in prostate epithelium *in vivo*.

Recently, we have performed a state-of-the-art large-scale, quantitative proteomics analysis by mass spectrophotometry from a series of clinical prostate cancer samples. By combining this data with RNA expression data from the same tumors, this unique dataset allows us, for the first time, to explore the proteins that are quantitatively dysregulated in prostate cancer at the protein expression level. Several interesting and novel events occurring during prostate cancer development and progression have been found in this study. These findings will be discussed in the presentation.

NOVEL GENE FUSIONS IN PROSTATE CANCER

Kati Kivinummi

University of Tampere, BioMediTech, Computational Biology & Molecular Biology of Prostate Cancer, Tampere, Finland

Genomic rearrangement are known to play a major role in the onset of prostate cancer (PC), and roughly half of PCs harbor gene fusions that juxtapose ETS-transcription factors (ERG, ETV1, ETV4, ETV5, ELK4 or FLI1) with androgen regulated promoters. The most common such chromosomal rearrangement is the promoter of TMPRSS2 withERG.
In a recent studies of ours, we have identified two novel 3'-fusion-partners: SKI like proto-oncogene (SKIL) andhes family bHLH transcription factor 6 (HES6)that both do not belong to the ETS-family. Although HES6 fusion was present in only one sample, it's overexpression phenotype has been reported earlier leading to highly aggressive neuroendocrine type of PC. In our PC cohort HES6 was found to be fused withDOT1L leading to it's overexpression without upstream activator, ASCL1. We also showed that this tumor originally carried TMPRSS2:ERG fusion and that HES6 overexpression induced androgen independent growth in LNCaP-cells.
Different from HES6-fusion, recurrent SKIL fusions were all mutually exclusive with all known ETSalterations and thus formed a new PC subtype. These novel rearrangements juxtapose SKIL (known inhibitor of SMAD) with androgen regulated promoters (TMPRSS2, MIPOL1, SLC45A3, MIPEP and ACPP). Genetic alteration (gene amplification) of SKIL have earlier been reported in other cancers, but no gene fusions of SKIL have been earlier reported, nor it's role in PC has been earlier studied. To determine whether SKIL plays a significant role in PC, we knocked SKIL down and observed reduced cell growth, invasiveness and colony formation. Oppositely when SKIL was overexpressed in normal prostate epithelial cells, greater invasive potential was detected. These findings support that SKIL has also an oncogenic role in PC, forms a new PC subtype and might be an important new molecular target for personalized therapy in PC for the future.

3D-RECONSTRUCTION AND FEATURE BASED ANALYSIS OF PROSTATE CANCER HISTOLOGY

Pekka Ruusuvuori

BioMediTech, University of Tampere, Tampere, Finland

Digital pathology has been rapidly expanding into a routine practice, which has enabled the development of image analysis tools for quantification of histological images. We develop methods for extracting information from scanned whole slide images to facilitate cancer research. In particular, we are interested in quantitative analysis of spatial heterogeneity of cancerous tissue, especially in the context of prostate cancer. Prostate cancer is multifocal in nature, and histological grading is the key clinical prognostic factor. In order to build non-subjective histological analysis tools, and to model the multifocality of prostate cancer within the organ, we use automated image analysis and machine learning. We use high-resolution digital whole slide images of serial sections of H&E stained tissue, enabling to use data from whole organ, and moreover, enabling to model the 3D structure of the organ based on the histological sections. We use automated image analysis for extracting hundreds of local descriptors capturing the characteristics of each spatial location. The descriptors include several common image morphology, texture and intensity features as well as features specifically engineered for characterizing the spatial context of the region. We then use these descriptors for building a discriminative model for normal and cancerous tissue, as well as for separating spatial locations within prostate. Specifically, we use machine learning for building a classifier, providing a subset of informative features related to spatial heterogeneity of the tissue. Our analysis pipeline is generic, allowing the use of different stainings, such as H&E or immunostaining, making the method applicable in various applications. For example, we have used similar approach in detection of breast cancer tissue from scanned whole slide images of lymph node sections. Our hypothesis is that methods for quantitative analysis of histological images will lead to increased knowledge of the histological changes in cancer tissue. Our aim is to histologically model prostate cancer in 3D, and in the future, combine genomic measurements in the three-dimensional spatial context.

A CONSTRUCTIVE DEBATE ON SYNTHETIC AND NATURALLY OCCURRING ANTICARCINOGENIC COMPOUNDS

Serdar Karakurt

Selcuk University, Faculty of Science, Department of Biochemistry, Konya, Turkey

Cancer is the one of the dangerous diseases resulted with death in the world. Thereby, many studies have been focus on to find out curative compounds against several cancers. The main and incredible source of medicinal compounds are plants and microorganisms. Alkaloids, phenolics, flavonoids have been proved for their anticarcinogenic effects on many cancer types including prostate, colon, breast and cervical cancers. Quercetin, tannic acid and rutin are the important members of those groups. Tannic acid has been demonstrated for its anticarcinogenic properties against human prostate cancer with IC_{50} of 25 µM. beside this, it inhibited migration, colony formation and invasion properties of On the other hand, these compounds have crucial obstacles for human cancer treatment due to less solubility in physiological conditions, low stability and random distribution in tissues. Therefore synthesis of synthetic analogs of those phenolic and flavonoids gain importance. Calixarenes are synthetic compounds which increased solubility and stability of the naturally occurring compounds. With 3 nm gold nanoparticles, *p*-sulfocalix[4]arenes increased cytotoxicity of quercetin 52-fold and made it soluble in water. Beside this, this synthetic carrier causes active transport of quercetin into the cell that formerly passively diffused from cell membrane. Hence, combination of naturally anticarcinogenic compounds and their synthetic counterparts seems a good strategy for the treatment of cancer.

DISCOVERY AND DEVELOPMENT OF GLABRESCIONE B (GLAB) FOR THE THERAPY OF HEDGEHOG-DEPENDENT TUMORS

Mattia Mori

Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy

Hedgehog (Hh) signaling is essential for tissue development and stemness. Activating germline or somatic mutations of genes encoding Hh pathway components are found in human and murine basal cell carcinoma (BCC) and medulloblastoma (MB), while uncontrolled Hh signaling has been reported to drive tumor progression in several cancers, including lung, breast, stomach, pancreas and hematopoietic malignancies. For this reason, the development of Hh inhibitors is eliciting great interest in drug discovery.[1]
Here, we set up a structure-based strategy, boosted by computational studies, to clarify the structural requirements of the pathway effector Gli1 for binding to DNA and to identify novel Hh inhibitors. Glabrescione B (GlaB), an isoflavone naturally found in the seeds of *Derris glabrescens* (Leguminosae), [2,3] emerged as valuable Gli1 antagonist that binds Gli1 zinc-finger and interferes with its interaction to DNA. The direct interaction between Gli1ZF and GlaB predicted by molecular modeling was subsequently confirmed by NMR spectroscopy and site-directed mutagenesis. Remarkably, GlaB inhibited the growth of Hedgehog-dependent MB and BCC cells *in vitro* and *in vivo* (30 and 15 mg/kg, respectively), as well as the self-renewal ability and clonogenicity of MB cancer stem cells. Preclinical development of GlaB is running with very positive outcomes. GlaB has a satisfactory pharmacokinetics for oral administration, and a very low toxicity up to 100 mg/kg dose.

BIOLOGY AND IMPORTANCE OF ANGIOGENESIS IN CANCER

Sila Appak Baskoy

Signal Transduction and Growth Control (A100) German Cancer Research Center (DKFZ) Im Neuenheimer Feld, Heidelberg, Germany

The concept of 'angiogenic switch' is postulated that tumors are unable to grow beyond 1-2mm^3 without a functional vasculature as they become nutrient deprived and hypoxic. These starving tumor cells start secreting molecules to recruit new blood vessels from the host tissue and blocking these molecules (pro-angiogenic factors) which, in turn, leads to the development of anticancer drugs. Among these molecules, vascular endothelial growth factor and vascular destabilizing molecule Angiopoietin-2 are the major angiogenic molecules. The Angiopoietin growth factors and their endothelial Tie receptor are essential for blood and lymphatic development. While tumors need blood vessels, it is not well identified why most of these tumors lack intratumoral lymphatic vessels. In this talk, firsty, I would like to introduce the importance of angiogenesis and lyphangiogenesis. The second part will focus on important ligands and receptors and finally in the last part the importance of endothelial cells and related molecules in cancer therapy will be discussed. Since tumor angiogenesis and developmental angiogenesis have similar properties, different embryonic and postnatal models to decipfer the important pathways of vascularization will also be mentioned.

SIGMA LIGANDS AS TARGETED ANTICANCER THERAPEUTICS

Konstantinos Dimas

Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Biopolis, 41110, Larissa, Greece

Sigma receptors, a relatively novel group of receptors widespread in the central nervous system [CNS] [1] and in multiple peripheral tissues [2], are divided into two subtypes, sigma-1 (s1R) and sigma-2 (s2R) receptor [3] that are distinguished based on their different ligand selectivity patterns and molecular weights [1]. Selective sigma ligands (agonists and antagonists) have been shown to specifically label tumor sites, induce cancer cells to undergo apoptosis and inhibit tumor growth [4]. However the mechanisms of action underlying the anticancer activity of sigma ligands and their signaling pathways are reported to be highly dependent both on the type of the ligand and the type of the tumor [5] they target even though they may share similarities in their receptor binding properties. Aim of this work is to study the expression of sigma ligands and their relation to pancreatic cancer development, their potential as drugs against this cancer using patient derived animal cancer models and to detect common features of the mechanism of action that ligands of the same selectivity may share.The expression of the sigma receptors was examined in cancer and normal tissue derived from different patients with pancreatic or colorectal cancer, pancreatic cancer cell lines ($AsPC_1$, $BxPC_3$, MiaPaca 2) and primary pancreatic cancer cell lines (021013 Attached, 021013 Floating). Pancreatic cell lines ($AsPC_1$, $BxPC_3$) and primary cell lines (021013 Attached, 021013 Floating) were treated with known chemotherapeutic drugs and multiple sigma ligands (agonists and antagonists). The antiproliferative effect of these compounds was studied with In vitro Cancer Screen assay (SRB assay).

Results: Expression of sigma 1 and sigma 2 receptors was observed in all cancer cell lines and tumor tissues. Sigma 2 receptor is highly expressed in cancer compared to adjacent normal tissue. In addition, sigma 2 receptor seems to be overexpressed in cancer compared to sigma 1 receptor. Amongst the sigma ligands that so far have been tested, PB28 and Siramesine found to exhibit the best anticancer activity. Studies to evaluate the potency of those ligands either as single agents or in combination with established drugs in human-to-mouse models of cancer are ongoing. The results from these ongoing in our lab studies as well as the recent advances in the field will be the subject of this lecture.

TARGETING CANCER STEM CELLS

Inga Marijanovic

Division of Molecular Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

Cancer stem cells (CSCs) play an important role in tumor formation, metastasis and tumor relapse. Therapies based on radiation and chemical agents that target proliferating cells, affect most of the tumor tissue, but miss the source of the tumor - CSCs. Their resistance to therapy is due to special characteristics like quiescence, increased drug-efflux ability, increased DNA repair ability and increased resistance to apoptosis. There are multiple strategies being conceived with the specific aim to destroy CSCs together with their niche. These strategies include targeting of specific surface markers, modulation of signaling pathways, adjustment of the microenvironment signals, inhibition of drug-efflux pumps, manipulation of miRNA expression and induction of apoptosis and differentiation. The last approach of differentiation and death is used to develop the therapy targeting osteosarcoma CSCs. Osteosarcoma, the most common type of solid bone cancer, is hierarchically organized and sustained by a subpopulation of CSCs that can generate the full repertoire of tumor cells. It is believed that osteosarcoma arises from mesenchymal stem cells (MSCs) or osteoprogenitor cells due to a disruption of osteoblast differentiation pathway. Therefore, osteosarcoma CSCs probably originate from MSC-derived cell types. We hypothesize that osteosarcoma CSCs would loose their stem-like properties that make them resistant to therapy, after the treatment with bone morphogenetic protein 2 (BMP2). BMP2 is a well-known and well-characterized protein that can differentiate MSCs into osteoblast lineage. To test the hypothesis, we treat CD133+ sarcospheres derived from tumor tissue with adenovirus expressing BMP2 protein. After treatment, we assess growth and disruption of sphere formation, gene expression analyses of stem- and bone-marker genes and sensitivity to conventional chemotherapy. If BMP2 treatment overcomes the differentiation block of the osteoblast lineage, it could eliminate CSC population and turn them into the bulk of tumor tissue that is sensitive to conventional treatment.

TÜMÖR HETEROJENITESI VE MOLEKÜLER YANSIMALARI

Safiye Aktaş

Dokuz Eylül üniversitesi Onkoloji Enstitüsü, Temel Onkoloji Anabilim Dalı, İzmir

Kanser tek hücreden gelişir. Ancak proliferasyon sürecinde eklenen mutasyonlarla zamanla kanser dokusu içinde farklı özelliklerle hücre popülasyonları gelişir. Bu duruma heterojenite denir. Heterojenite intratümöral yani aynı tümör dokusu içinde farklı karakterlerde hücrelerin bulunması, ya da intertümöral, aynı tümörün farklı bölgelerdeki oluşumlarının örneğin lenf düğümü ya da uzak organ metastazlarında tümör dokularının primer bölgedeki tümörle farklı özellikler göstermesi şeklinde karşımıza çıkabilir.

Heterojenite morfolojik,f izyolojik, genetik olabilir. En sık proliferasyon yeteneği, anjiogenez yeteneği ve hücre yüzey reseptörlerinin durumu heterojenite gösterebilir. Bu durum tümör içinde farklı hücrelerin tedaviye farklı yanıt vermesi ile karşımıza çıkabilir. Tedaviye yanıtsızlık, ilaç direnci, hedefe yönelik tedaviler sonrası, rekürrenslerden heterojenite sorumlu tutulmaktadır. Metastaz yapan hücrelerin primer tümör içinde invazyon metastaz yeteneği kazanmış farklı moleküler özelliklerde hücreler olduğu düşünülmektedir. Farklı metaztaz bölgelerindeki hücreler de birbirinden farklı olabilmektedir.

Heterojeniteyi kanser kök hücre bakış açısı ile yorumlamak olasıdır. Kanser kök hücre hipotezine göre kanser dokusu içinde az sayıda hücre kök hücre karakterindedir ve kanser bu hücrelerden değişik diferansiyasyonlarda hücrelerin çoğalması ile oluşur. Bu hücreler arasında prolifere olması duran ve ölüme giden hücreler de bulunmaktadır.

Tümör heterojenitesi tümör mikroçevresi ile de iletişim içindedir. Heterojeniteyi mikro çevrenin indüklemesi olasıdır. Ya da tersi heterojen farklı karakterde tümör hücreleri farklı stroma oluşturabilir.

Tümörde heterojenite araştırmak için normal doku olmadığından emin olduğumuz farklı tümör bölgelerini mikrodiseksiyonla ayırarak bu dokularda karşılaştırmalı analizler yapılır. Bunlar arasında mikrosatellitlere bakmak, bilinen kromozomal aberasyonlar için FISH yapmak, o kanserde bilinen mutasyonlara yönelik dizi analizi yapmak, hematolojik malignitelerde immunglobulin ve T hücre reseptör analizi yapmak vardır.

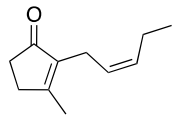
Tümör heterojenitesinin klinik yansımaları büyük önem taşımaktadır. Üzerinde önemle çalışılması gereken bir konudur. Tümör içindeki farklı moleküler özelliklere sahip hücrelerin birbirleri ile biyolojik etkileşimleri üzerinde çalışılmamış bir konu olarak karşımızdadır. Yeni kuşak dizileme sistemlerinin tümör heterojenitesini saptamadaki yeri güncel bir konudur. Artan teknolojiler ile kanser dokusunda saptanabilen moleküler bilgiyi yorumlamak yeni önemli bir sorun olarak ortaya çıkmıştır.

NOVEL ANTI-CANCER DRUG DISCOVERY AND DEVELOPMENT STUDIES

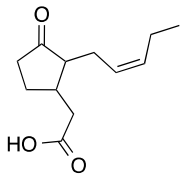
Mustafa Güzel

İstanbul Medipol Üniversitesi, Uluslararası Tıp Fakültesi, Tıbbi Farmakoloji AnaBilimDalı, Kavacık, İstanbul

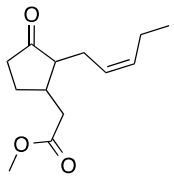
Cancer is one of the well-known illnesses leading to death. One way to inhibit the metabolism of cancer cells is to inhibit Hekzokinaz 2 (HK-2) enzyme. HK-2 has been studied in the field of cancer metabolism and obtained some beneficial and hopeful results. It has been also confirmed that HK-2 enzyme is expressed 10-15 times more in cancer cells than normal cells. Inhibition of HK-2 enzyme will prevent cancer cells from nutrition and it is expected that speeding of cancer cells will be slowed down.
It is known that methyl jasmonate is an HK-2 inhibitor. To develop and to use those inhibitors actively, which have high selectivity for HK-2 enzyme and high bioactivity, have an enourmous importance for cancer patients. That is why the novel methyl jasmonate analogs have high potential to become drug candidates.



Cis-Jasmon



Jasmonik Asit



Metil Jasmonat

Cis-jasmon, Jasmonic acid and Methyl jasmonate are cyclopentanones that are fatty acid derivatives. Jasmonates are plant stress hormones and we worked with a range of concentrations based on the plasma concentrations achieved upon administration of a well-studied plant stress hormone, salicylic acid [1]. Jasmonates induced suppression of cell proliferation and death in a variety of cancer cell lines and cytotoxicity to cervical cancer cells with almost no effect on normal primary human kerati- nocytes [2].
As a result of our research, we concluded the fact that although methyl jasmonate is long-known natural component. It has not studied enough as an anti cancer agent.
In our research, we aim to synthesize novel methyl jasmonate analogs. In accordance with our research we plan to do spectroscopic analysis of synthesized molecules and than in vitro studies.

This project (215S890) is funded by TUBITAK. We kindly appreciate for their support.

HIGH THROUGHPUT VIRTUAL SCREENING OF LARGE DATABASES FOR THE DISCOVERY OF NOVEL PARP-1 INHIBITORS

Serdar Durdagi

Department of Biophysics, School of Medicine, Bahcesehir University, Istanbul, Turkey

Poly(ADP-ribose) polymerase-1 (PARP-1) enzyme has critical roles in DNA replication repair
and recombination thus their inhibitors play an important role in the cancer therapy. In this study, structure-based virtual screening was carried out for an available small molecules database. More than 250.000 ligands from Otava database were screened at the binding side of the PARP-1 using high throughput virtual screening (HTVS) techniques. Filtered structures based on predicted binding energy results were then used in more sophisticated molecular docking simulations (i.e., Glide/SP, Glide/XP, Induced Fit Docking- IFD, and Quantum Mechanics Polarized Ligand Docking- QPLD). Potent high binding affinity compounds that are predicted by molecular simulations were then tested by in vitro methods. Computationally proposed compounds as PARP-1 inhibitors were confirmed by in vitro studies. The molecular mechanism analysis, Free Energy Perturbation calculations using long multiple molecular dynamics (MD) simulations for the discovered compounds which showed high binding affinity against PARP-1 enzyme, as well as structure-based pharmacophore development (E-pharmacophore) studies (Figure 1) were also studied.[1,2]

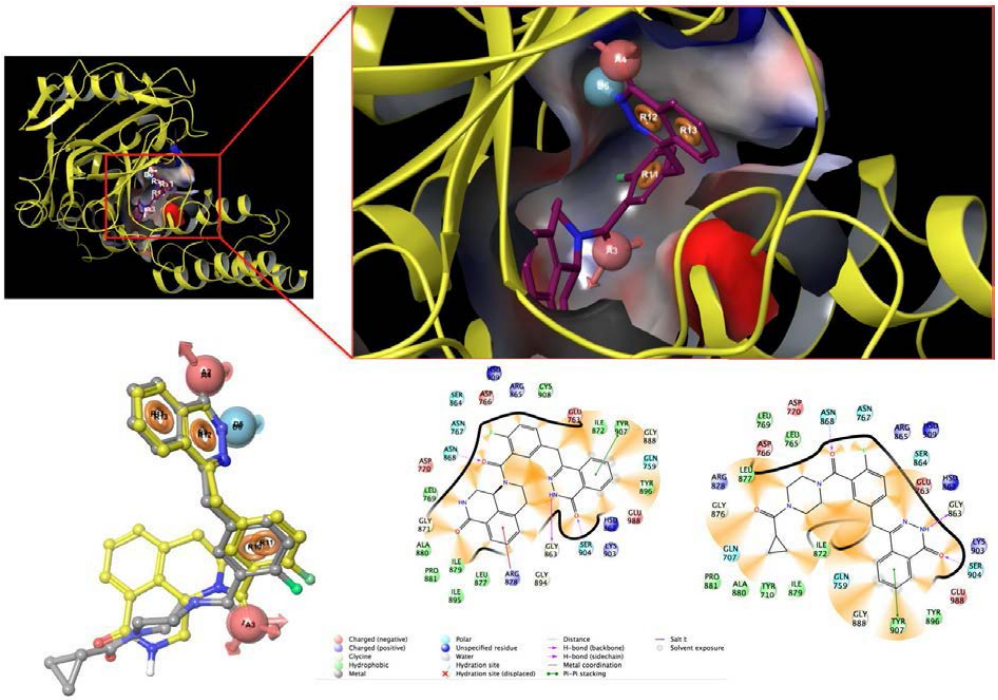


Figure 1. E-pharmacophore modeling resulted 6-sited AAADRR hypothesis as top-scored hypothesis for both known PARP-1 inhibitors CHEMBL2322618 and olaparib.

BRAIN TUMORS AND ETS FACTORS

Işıl Aksan Kurnaz[1,2]

[1]*Gebze Technical University, Department of Molecular Biology and Genetics, Gebze, Kocaeli*
[2]*Gebze Technical University, Institute of Biotechnology, Gebze, Kocaeli*

Background: ETS superfamily of transcription factors are implicated in a wide range of functions in almost all tissues, from angiogenesis to hematopoiesis, from tumorigenesis to metastasis, branching morphogenesis to name a few. They are known to be regulated by mitogen-dependent MAPKs, as well as other signaling pathways. Work from our lab and others have since shown that Elk-1 is important for survival of neurons, as well asproliferation of brain tumors, however the effect of and interaction with other ETS factors, particularly the Pea3 subfamily, has been unclear.
Objective: The aim of this study was to investigate the molecular mechanisms underlying the role of Elk-1 in survival and proliferation of brain tumors, as well as its crosstalk with members of the Pea3 subfamily.
Material and Method: A combination of microarray, promoter analyses and luciferase assays, as well as real-time PCR and brain tumor initiating cell assays have been used to underpin the role of ETS proteins in brain tumors.
Results: Microarray analysis of Elk-1 overexpression in SH-SY5Y cell lines have shown that a number of apoptosis and autophagy-related genes were regulated by Elk-1, in addition to components of hypoxia signaling pathway. More interestingly, however, quite a number of organogenesis- and stem cell-related genes were found to be regulated in response to Elk-1. On the other hand, many axon outgrowth and guidance-related genes as well as endocytic vesicle pathway elements were regulated in response to members of the Pea3 subfamily, namely Pea3, Erm and Er81 proteins. Both TCF subfamily member Elk-1 and the Pea3 subfamily was observed to regulate cell cycle and apoptosis pathways, although not exactly the same genes. Moreover Elk-1 in particular was found to regulate mitotic kinases, in addition to interacting with them. Furthermore, all ETS proteins investigated were found to regulate other ETS proteins.
Conclusion: In line with our findings, we propose that these ETS proteins are engaged in a close cross-regulatory interaction parallel to their critical functions in cells, so as to achieve as optimal redundancy of the system as possible. Such an auto-regulatory circuit among other ETS proteins, and the impact of this circuit to progression of the tumor, should be investigated further.

NF-KB TARAFINDAN TRANSKRİPSİYONU INDÜKLENEN MIRNA KHDAK İNVAZYONUNU REGÜLE EDER

Hakan Akça

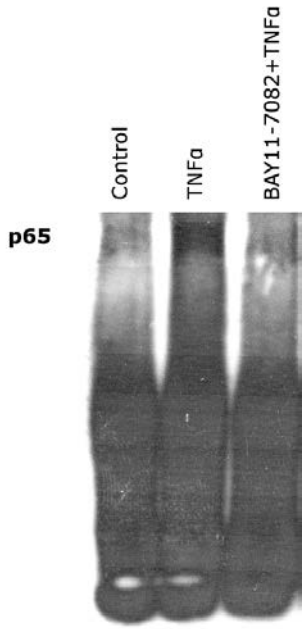
Pamukkale Üniversitesi, Tıp Fakültesi, Tıbbi Biyoloji Anabilim Dalı, Denizli, Türkiye

Akciğer kanseri, kanser ilişkili ölümler arasında önde gelmektedir. Dünya genelinde akciğer kanserinde gözüken sağ kalım oranı %10-15'tir. Bunun sebebi akciğer kanseri tümörüne sahip hastalarda metastazın ve lezyonların teşhisinin geç olmasıdır. Güncel Dünya Sağlık Örgütü (WHO) sınıflandırmasına göre; akciğer kanserinin bir alt türü olarak küçük hücreli dışı akciğer karsinomu (KHDAK), akciğer kanseri vakalarının yaklaşık olarak %70-80'ini oluşturduğu belirtilmektedir. Kanser ilişkili ölümlerde akciğer kanserinin başta gelmesinin sebebi; akciğer kanseri vakalarının büyük çoğunluğunu oluşturan KHDAK'nin yüksek invazyon ve metastaz yapabilmelerinden kaynaklanmaktadır. Bu yetenekleri, hücrede çeşitli sinyal yolaklarının sürekli aktif olmasından kaynaklanmaktadır. Bu yolaklardan en önemlisi çoğu kanserde sürekli aktif olan PI3K(Fosfatidilinositol-3-OH kinaz)/Akt yolağıdır. PI3K/Akt yolağının aktivasyonu ile STAT3, Ap-1 ve Nükleer Faktör kappa B (NF-kB) gibi bazı transkripsiyon faktörleri aktive edilerek, invazyon, metastaz, hücre çoğalması, farklılaşma ve apoptozdan kaçış gibi birçok hücresel aktivite indüklenmektedir. Son 10 yılda ortaya çıkan ve hücre de gen düzenlenmesinin master düzenleyicileri olan MikroRNA'lar (miRNA), transkripsiyon faktörleri tarafından uyarılarak genleri mRNA düzeyinde negatif yönde düzenleyerek birçok hücresel aktivitenin aktivasyonuna veya inhibisyonuna neden olmaktadırlar.

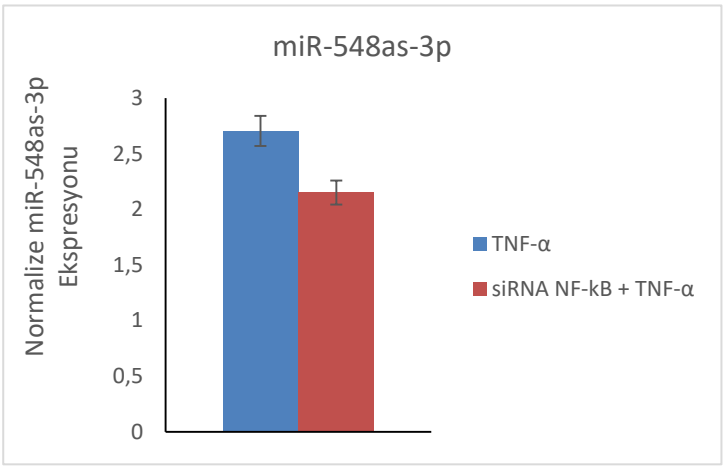
Günümüze kadar NF-kB tarafından düzenlenerek çeşitli biyolojik aktivitelerde rol alan bazı miRNA'lar tespit edilmiştir. Ancak, kanser ilişkili ölümlerin gerçekleşmesinde önemli bir adım olan invazyonun indüklenmesine, NF-kB tarafından düzenlenmesi sonucu neden olan miRNA'lar hakkında literatürdeki bilgi yeterli değildir. Bu bilgiler doğrultusunda, KHDAK hücrelerinde sürekli aktif olan PI3K/Akt/NF-kB yolağında NF-kB tarafından bazı miRNA'ların transkripsiyonel aktivasyonu arttırarak hücresel invazyonu indüklendiğini hipotez ettik. Dolayısı ile NF-kB bir transkripsiyonel faktör olarak bazı miRNA genlerinin promotör bölgelerine bağlanarak transkripsiyonunu indükleyebilir.

KHDAK hücrelerinde NF-kB aktivasyonunu arttırmak için, PI3K'ı aktive eden TNF-α kullanıldı. TNF-α muamelesi ile NF-kB aktivasyonu arttırılmış (Şekil 1) olan H1299 KHDAK hücre hatlarında ChIP-Sekans yöntemi kullanılarak p65 (NF-kB)'in hücre genomundaki bağlanma bölgeleri tespit edildi. Belirlenen kromozom bağlanma bölgelerinin herhangi bir miRNA'nın promotör bölgesinde olup olmadığı tespit edildi. Bu bölgelerin, olası NF-kB (p65) bağlanma domaini içerip içermedikleri biyoenformatik araçlar kullanılarak teyit edildi. Bu analizler sonucunda, olası miRNA'lar seçilerek, bu miRNA'ların NF-kB (p65) tarafından indüklenip indüklenmediğini tespit etmek amacıyla, TNF-α ile muamele edilmiş ve siRNA NF-kB (p65) transfekte edilmiş olan H1299 hücrelerinde ki transkripsiyon seviyeleri tespit edildi. TNF-α ile muamele edilen hücrelerde transkripsiyon seviyelerinin arttığı, siRNA NF-kB ile transfekte edilen hücrelerde ise

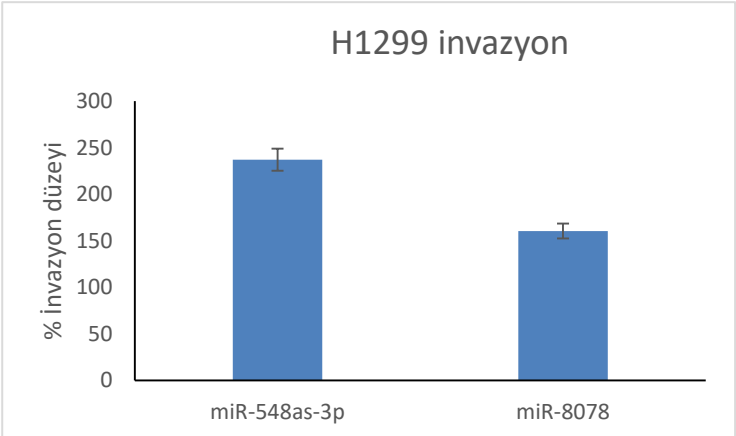
transkripsiyon seviyesinin azaldığı miRNA'lar seçildi (Şekil 2). Bu miRNA'ların hücresel invazyona olan etkisini tespit etmek amacıyla, H1299 hücrelerine mimik miRNA olarak transfekte edilerek gerçekleştirilen invazyon chamber deneyinde, miR-8078 ve miR-548as-3p'nin invazyonu büyük ölçüde arttırdığı tespit edildi (Şekil 3). Sonuç olarak, miR-548as-3p ve miR-8078'in NF-kB tarafından transkripsiyonel olarak aktivasyonu artarak KHDAK invazyonunu düzenlediği ilk kez bulgularımızla gösterilmiştir.



Şekil 1. TNF-alfa ve spesifik NF-kB inhibitörü BAY11-7082 muamelesi sonucunda H1299 hücrelerindeki NF-kB aktivasyonunu gösteren EMSA jel görüntüsü.



Şekil 2. miR-548as-3p'nin H1299 hücrelerinde NF-kB'nin aktivasyonu ve inhibisyonu sonucu ekspresyon seviyesi.



Şekil 3. H1299 hücrelerinde mimik miR-548as-3p ve miR-8078'in invazyona olan etkisi.

KANSERDE İLAÇ DİRENÇLİLİĞİNDE GST ENZİMLERİNİN ROLÜ

Serpil Oğuztüzün

Kırıkkale Üniversitesi Fen Edebiyat Fakültesi Biyoloji Bölümü, Kırıkkale/Türkiye

Günümüzde kansere karşı kemoteröpatik olarak kullanılan ilaçların çoğu, tümör hücrelerine sitotoksik etki göstererek onların büyüme ve çoğalmalarını önlerler. Antikanser ilaçlarının tedavideki etkinliğini kısıtlayan önemli faktörlerden biride, tümör hücrelerinin kemotörapatik ajanlara karşı, bazı kanser türlerinde kendiliğinden, bazılarında ise kemoterapiden sonra geliştirdikleri direnç mekanizmalarıdır. Tümör hücrelerinin gösterdiği bu ilaçlara karşı direnç, ilaçların hücre dışına atılmasını sağlayan membran proteinlerinin ekspresyonunun bir sonucudur ve ilaçların hücre içindeki konsantrasyonlarının düşmesine neden olmaktadır. Bu membran proteinlerinden en önemli üyelerinden biride ABC (ATP-binding cassette) Taşıyıcı proteinleridir. Bu duruma çoklu ilaç direnci [multiple drug resistance (MDR)] denilmektedir. Kanser tedavisinde başarıya ulaşmak için genellikle birden fazla anti-kanser ilaç uygulamasına gidilmektedir. Son yıllarda yapılan çalışmalarda ilaç metabolizmasındaki değişiklikler, açıklanan bu ilaç dirençlilik proteinlerinin yanında diğer hücre içi proteinlerinde etkin olabileceğini göstermiştir. Ayrıca, alkilleyici özellikteki kanser ilaçlarına gelişen dirençte, hücre içi glutatyon (GSH) ve glutatyon S- konjugatlarının seviyelerinin artmasının rolünün olduğu bildirilmiştir. Glutatyon S-transferazlar (GST), endojen ve ekzojen kaynaklı, elektrofilik ve hidrofobik bileşiklerin glutatyon ile konjugasyonunu sağlayarak, genellikle daha kolay atılabilen ve daha az toksik metabolitlere dönüşümünü katalizleyen II. Faz detoksifikasyon enzim ailesidir. Anti-kanser ajanın tümör hücresine girmesiyle birlikte hücre içinde GSH düzeyinde ve GST enziminin ifadesinde artış olmaktadır ve ilacın hücre içinde uzun süre kalması bu nedenle zorlaşmaktadır. Bununla birlikte, enzime paralel olarak bu dışarı pompalama proteinlerinin [Multi Drug Resistance Proteins (MRPs)] ifadesinde de artış gözlenmektedir. Son yıllarda yapılan çalışmalar, tümör hücrelerin de GSH'ın yüksek düzeylere ulaşmasının ve GST'nin aşırı ekspresyonunun MDR gelişimini ile paralel geliştiği yönündedir.

Anahtar Kelimeler: Glutatyon S-Transferaz, Kemoterapi, İlaç dirençliliği

TARGETED DRUG DELIVERY SYSTEMS: DESIGNED FOR CANCER TREATMENT

Gülay Büyükköroğlu

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey

Cancer among the most serious health problems recently is characterized by uncontrolled cell growth leading to cancer mass or tumor formation and is caused by damage or mutations in the genetic material of the cells due to environmental or inherited factors. While surgery and radiotherapy are the most effective and valuable treatments for local and non-metastatic cancers, anti-cancer drugs such as chemotherapeutic agents, hormones and biological molecules are the current choice for the treatment of metastatic cancers. Majority of these anti-cancer drugs used in conventional cancer treatment show cytotoxic effects towards both cancer and normal cells, due to their non-selective tissue distributions. Therefore, in order to reduce its toxicity and side effects, there is a need to develop targeted drug delivery systems. Thus, in order to achieve sufficient concentrations of these drugs in the desired tissues, they must be administered frequently and in high dosages.

Chemotherapeutic agents are also associated with problems of low water-solubility, instability, rapid metabolism and quick and non-selective drug distributions. For these reasons, conventional chemotherapeutic agents are being replaced with those packaged in smart drug delivery systems. Delivery of drugs to desired locations is achieved with controlled drug delivery systems having high targeting capabilities. An effective solution to inhibit these limitations is to deliver cancer drugs within biocompatible nanocarriers. Simple nanocarriers span diverse materials such as magnetic or colloidal metals, silica, carbon nanotubes, liposomes, dendrimers, polymeric micelles, polymeric conjugates, polymeric and lipidic nanoparticles and use for passive and active targeted cancer therapy. The unique properties of nanocarriers, such as large surface-to-volume ratio, small size, the ability to encapsulate various drugs, multivalent surface modification with targeting ligands and tunable surface chemistry, give them many advantages over their bulk counterparts.

The novel targeted therapies with these delivery systems cause to block biologic transduction pathways and/or specific cancer proteins to induce the death of cancer cells by means of apoptosis and stimulation of the immune system, or specifically deliver chemotherapeutic agents to cancer cells, minimizing the undesirable side effects.

SÖZLÜ BİLDİRİ SUNUMLARI

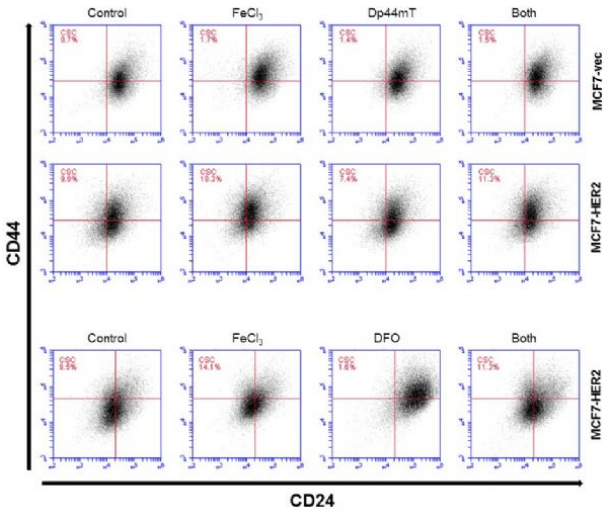
S-001 - THE EFFECT OF METAL CHELATORS ON BREAST CANCER STEM CELLS

Özer Ufuk[1,2]

[1]*Moleküler Biyoloji ve Genetik Bölümü, Fen Fakültesi, Dicle Üniversitesi, Diyarbakır, Türkiye*
[2]*Center For Colon Cancer Research, University Of South Carolina, Columbia, Sc, USA*

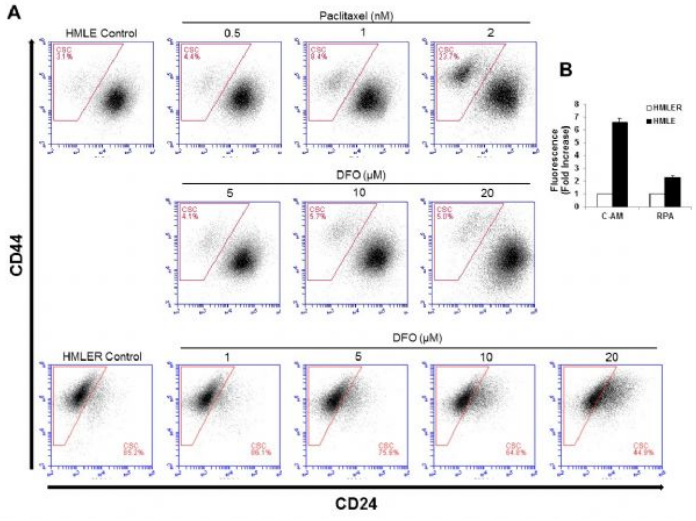
Background and Aim: Neoplastic cells need essential metals such as iron and copper for cellular functions and rapid growth. In breast cancer cells, human epidermal growth factor receptor 2 (HER2) is overexpressed around 30% with poor prognosis and this results in elevated the proportion of cancer stem cells (CSCs) that are in charge of cancer recurrence. Metal chelation and changing their redox cycle in favor of oxidative stress may be a critical to make these cells vulnerable to cell death. Investigating whether metal chelation alters HER2-induced CSC population may provide a new tools for breast cancer therapy.
Material and Methods: MCF7-HER2, overexpressing HER2, and MCF7-vec control cells were used to evaluate the effect of HER2. Also, we have used other breast cancer cell lines; HCC1954, MDA-MB-435 and Hs578T in order to substantiate our results. DFO and Dp44mT were used as metal chelators. ROS production, iron levels and CSC survival in response to chelators were detected by flow cytometry and cell viability was measured by MTT assay.
Results: MCF7-HER2 cells require iron more than their vector counterparts and HER2-increased CSCs are vulnerable to iron chelation. Additionally, this sensitivity of CSCs to iron reduction is obviously indicated in other breast cancer cell lines. Finally, the concept is also shown in neoplastically transformed breast cancer cell line, HMLER. ROS levels were relatively increased by Dp44mT in the cells and this was reversed by combination of iron while copper combination further induced ROS. Parallel changes were observed in the inhibition of cell growth by Dp44mT and this was partially rescued by NAC supplement.
Discussion and Conclusion: Altogether, this study demonstrates that iron depletion causes toxicity for CSCs. Dp44mT depletes iron and binds copper to form redox active complex that leads to oxidative stress. This dual cytotoxic cases are significant for survival of cancer cells.
Keywords: Breast cancer, Cancer stem cells, DFO, Dp44mT, HER2

Effects of iron and iron chelators on the proportion of CSCs



Cells were grown ± 20 µM FeCl3 and its combinations with 2 nM Dp44mT for 3 days, and 10 µM DFO for 5 days. They are stained with CD44-FITC and CD24-PE antibodies and then flow cytometry assay was done. Results represent 3 separately repeated experiments

Effects of paclitaxel and DFO on CSC population in HMLE and HMLER cells.



A. HMLE cells were treated with paclitaxel (0.5, 1 and 2 nM) and DFO (5, 10 and 20 µM) for 4 days and recovered with fresh media for another 4 days. HMLER cells were treated with DFO (1, 5, 10 and 20 µM) as the same way and then flow cytometry assay was done. Results represents 3 separately repeated experiments. B. Basal iron levels were measured with C-AM and RPA staining followed flow cytometry assay. Bars represent fold increase of the mean fluorescence ± SEM from 3 experiments.

S-002 - MATERNAL MICROCHIMERIC CELLS TURN INTO CANCER STEM CELLS?

Demirhan Osman[1], Taştemir Deniz[2], Yalav Orçun[3], Bakan Ergin Melek[4], Çetinel Nesrin[1]

[1]*Department of Medical Biology and Genetics,Faculty of Medicine, Çukurova University, Balcali-Adana/Turkey.*
[2]*the Department of General Surgery, Faculty of Medicine, Çukurova University, Balcali-Adana/Turkey.*
[3]*Department of Pathology, Faculty of Medicine, Çukurova University, Balcali-Adana/Turkey.*
[4]*Vocational School of Health Services, Adıyaman University, Adıyaman, Turkey.*

Background: We are only beginning to understand the role that fetal-maternal microchimeric cells (F-MMcCs) play in cancer. Although their function is not yet fully known, it would be really cool to understand functionally what the F-MMcCs may be doing in a normal healthy pregnancy and postpartum or in the offspring. Whether, F-MMcCs have a beneficial or detrimental effect partially depends on what kind of cells it develops into, but more so on how the mother's childreen responds to having those extra cells around. The phenomenon of feta-maternal microchimerism inspires numerous questions. We aimed to evaluate the possible roles of MMcCs in sarcoma cancer by FISH.
Materials and Methods: We report a case of UPS in a 73-year-old male, with STS, enlarging mass in the left breast with a history of one year. There was a firm and rounded edge mass, about 7x7 cm diameter, behind the nipple areola complex in the left breast. Right breast and right axillary were completely normal. Breast ultrasound revealed solid hypoechoic mass including central necrosiss in left breast, bilateral axilla are reported as normal and computer tomography indicated 5x8 cm mass with high peripheral vasculer and apperriance of hypodense necrotic at the center. A small piece of the tumor sample and the peripheral blood sample were obtained for genetic studies. The FISH and standard cytogenetic techniques were used for the cancer tissue and blood tissue to detect the MMCs, respectively.
Results: We found the MMcCs in 18% of sarcoma tissue-cells and in 0.2% of the blood tissue-cells. There was a significant difference in the frequencies of McCs between the tumoral tissue and the blood tissue ($p<0.0001$).
Conclusion: The available informations suggests that there is two basic possibilities, which covers pathogenic or beneficial Mc. We focused on do Mc play any role in carcinogenesis.
Keywords: Maternal Microchimeric Cells, Sarcoma Cancer, The FISH And Standard Cytogenetic Techniques

S-003 - THE IN VITRO EFFECT OF SANGUINARINE DIFFERS ON NEUROBLASTOMA STEM CELLS BASED ON THE SERUM

Erçetin Özdemir Ayşe Pınar[1], Aktas Safiye[1], Yavuz Busra[2], Ozturk Tuba[2], Kaman Damla[2], Sulu Melik[2], Altun Zekiye[1], Cecen Emre[3], Olgun Nur[4]

[1]*Basic Oncology Department, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey*
[2]*Biology Department, Faculty of Science, Ege University, Izmir, Turkey*
[3]*Pediatric Oncology Department, Adnan Menderes University, Aydin, Turkey*
[4]*Pediatric Oncology Depatment, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey*

Neuroblastoma (NB) is the most common extracranial solid cancer in childhood. Cancer stem cells (CSCs) are thought to be associated with micrometastasis, cause of cancer,drug resistance and recurrences. Recent studies showed that sanguinarine (Sng) could used as an anti-cancer due to its apoptosis inducing mechanism. FBS is used as a common supplement in most of the in vitro studies however there are other factors interacting with tumor cells and CSCs in in vivo conditions. Thus, the aim of this study was to evaluate the effect of Sng on NB CSCs in different culture conditious.

CD133+ cancer stem cells were isolated from Kelly(N-myc+) NB cells with magnetic beads. Normal serum (NS) was obtained from peripheral blood sample of a healthy donor.CSCs were incubated in four different subgroups such as; RPMI+FBS, RPMI+NS, RPMI+NS+Sng and RPMI+FBS+Sng. After 24 hours Annexin V staining was performed to evaluate apoptosis and CD133 positivity was analyzed by flow cytometry to evaluate CSCs differentiation.

Late apoptotic CSCs were two times more in FBS+Sng medium than medium containing NS. Also,in medium containing NS has lower CD133 positivity.In other words,medium containing NS cells was more differentiated in comparison with other conditions. According to our results, the type of serum used in in vitro experiments affect apoptotis and differentiation of CSCs induced by Sng. In addition, our study showed that Sng induces apoptosis in NB CSCs. We suggest that different serum conditions should be included in studies evaluating the effect of an anti-cancer agent.

Keywords: Neuroblastoma, Cancer Stem Cells, Sanguinarine, Serum

S-004 - CYTOTOXIC SYNERGY BETWEEN TINGENIN B AND PACLITAXEL AGAINST BREAST CANCER STEM CELLS: INDUCTION OF MITOCHONDRIA DEPENDENT APOPTOSIS

Yilmaztepe Oral Arzu[1], Cevatemre Buse[2], Karakas Didem[2], Aztopal Nazlian[2], Ulukaya Engin[1]

[1]*Department of Clinical Biochemistry, Faculty of Medicine, Uludag University, Bursa, Turkey*
[2]*Department of Biology, Faculty of Arts and Sciences, Uludag University, Bursa, Turkey*

Introduction: Despite the advances in chemotherapy regimens, the outcome of patients with breast cancer is not satisfactory. In recent years, this failure attributed to cancer stem cells (CSCs) as they show and/or gain resistance to therapies. Thus, compounds that target CSCs are urgently needed. The aim of this study is to investigate the cytotoxic activity of tingenin b (or 22β-hydroxytingenone, a quinone-methide triterpenoid structurally related to tingenone) in combination with paclitaxel against breast CSCs.
Material&Methods: The anti-growth activity was investigated by the ATP assay. Mode of cell death was evaluated using fluorescence microscopy (Hoechst 33342+Propidium iodide staining), western blotting (apoptosis related markers) and flow cytometry (annexin v staining, determination of caspase 3/7 activity, mitochondrial membrane potential, BCL-2 and PI3K expressions).
Results: It has been found that combination of tingenin b and paclitaxel enhanced the cytotoxic activity and apoptotic cell death at 72 h, compared to single use of each agent in MCF-7s (cancer stem cell enriched population). Apoptosis was evident by the presence of pyknotic nuclei, annexin v staining positivity, increased caspase 3/7 activity, Bcl-2 inactivation, cleavage of PARP and increased expression of Bax. The PI3K/AKT pathway was also found to be inhibited by this combinatorial treatment. In addition, the stemness factor, Oct4, was also found to be decreased.
Discussion: The combination of tingenin b and paclitaxel exerted a promising cytotoxic and apoptotic effect on cancer stem cells of breast cancer. Therefore, the application of this combination may be regarded as a novel and effective approach for due to its cytotoxic activity and apoptosis inducing effect against breast CSC although in vivo experiments are required for the proof-of concept.
Keywords: Cytotoxic, Synergy, Between

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S-005 - THE PLANT-DERIVED TRITERPENOID PRISTIMERIN IS A POTENT ANTICANCER AGENT DUE TO ITS CYTOTOXIC ACTIVITY ON BREAST CANCER IN VITRO AND IN VIVO

Cevatemre Buse[1], Erkısa Merve[1], Aztopal Nazlihan[1], Karakaş Didem[1], Alper Pınar[1], Tsimplouli Chrisiida[2], Sereti Evangelia[2], Dimas Konstantinos[2], Ikitimur Armutak Elif I.[3], Gurel Gurevin Ebru[4], Uvez Ayca[3], Mori Mattia[5]

[1]*Uludag University, Faculty of Arts and Sciences, Department of Biology, Bursa, Turkey*
[2]*Department of Pharmacology, Faculty of Medicine, University of Thessaly, Larissa, Greece*
[3]*Department of Histology and Embryology, Faculty of Veterinary Medicine, Istanbul University, 34320, Istanbul-Turkey*
[4]*Department of Biology, Faculty of Science, Istanbul University, 34134, Istanbul-Turkey*
[5]*Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, viale Regina Elena 291, 00161 Roma, Italy*

Introduction: Several natural products have been suggested as effective agents for breast cancer and given the important role of CSCs (Cancer Stem Cells) in breast tumorigenesis and progression, it is worth investigating the effects of pristimerin on CSCs (MCF-7s) and their parental cell line MCF-7 and also MDA-MB-231.
Material and Methods: The anti-growth activity of pristimerin against MCF-7 and MCF-7s (cancer stem cell enriched population) cells was investigated by the ATP assay and xCELLigence System. Mode of cell death was evaluated using TEM and fluorescence microscopy (Hoechst 33342+Propidium iodide staining), western blotting (autophagy, apoptosis and ER-stress realted markers) and flow cytometry (annexin v staining, caspase 3/7 activity, BCL-2 and PI3K expression).
Results: Pristimerin decreased the cell viability in a dose dependent manner in breast cancer cells. In addition, as expected, MCF-7s cells were less sensitive to pristimerin (IC50 values were found to be 0.75 µM, 1.75 µM and 0.38 µM for MCF-7, MCF-7s and MDA-MB-231 respectively). Pristimerin also inhibited sphere formation at lower doses (<1.56 µM). Apoptosis was induced in MCF-7 and MCF-7s cells which was evidenced by pyknotic nuclei, annexin v staining, caspase 3/7 activation, BCL-2 dephosphorylation and cleavage of PARP. In addition, regarding the extensive cytoplasmic vacuolation in both cells, we suggest that these cells may be dying via autophagy. However, analysis of the expressions of autophagy related proteins (p62 and LC3-II) revealed a process in which autophagic flux was blocked rather than being stimulated. Furthermore, apoptotic cell death was found to harbor endoplasmic reticulum stress and unfolded protein response (UPR) in breast cancer cells. Lastly, pristimerin inhibited the growth of MCF-7 and MDA-MB-231 xenografts. In these tumors, Pristimerin reduced the expression of Akt and PCNA. Besides, the cleavage of PARP, and levels of PTEN, active caspase 3 and/or 7, LC3B, TUNEL stainings were found to be increased.

Discussion: Collectively, pristimerin exerted both in vitro and in vivo cytotoxic and anti-growth effects on breast cancer cells. Our observations identified a mechanism by which pristimerin functions as an anticancer agent.

Keywords: Plant, Derived, Triterpenoid

S-006 - AKT/PKB-MEDIATED PHOSPHORYLATION OF TWIST1 IS ESSENTIAL FOR TUMOR GROWTH AND METASTASIS

Pehlivanoglu Suray[1], Gorgisen Gokhan[2], Kahraman Dirice Sevim[3], Ozes Osman Nidai[4]

[1]*Necmettin Erbakan University, Faculty of Science, Department of Molecular Biology and Genetics, Konya, Turkey.*
[2]*Yuzuncu Yil University, Faculty of Medicine, Department of Medical Biology, Van, Turkey.*
[3]*Harvard Medical School, Joslin Diabetes Center, Section on Islet Cell and Regenerative Biology, Boston, United States.*
[4]*Akdeniz University, Faculty of Medicine, Department of Medical Biology and Genetics, Antalya, Turkey.*

Introduction: Cancer cells show epithelial-mesenchymal transition (EMT) during cell migration, and invasion. Within this period it has been shown that the conserved basic helix-loop-helix transcription factor Twist1 plays pivotal role during EMT. The molecular mechanism responsible for Twist1 function is not fully understood. In this context, we aimed to clarify the activation mechanism of Twist1.
Material and Methods: In our study, we have made use of 293T and MDA-MB-231 cell lines. Twist1 cDNA was cloned into the pcDNA3.1 expression vector. Site-directed mutagenesis is used to generate A and E mutations in S42,T121, and S123 residues of Twist1. The interaction between AKT/PKB and Twist1 was shown by IP and in vitro kinase assays. DNA binding properties of the Twist1 was analyzed by using EMSA method. The EMT marker expression levels, the proliferation, and the migration rates of 293T cells that expressed wild type, A, and E mutants of Twist1 were determined.
Results: Here we show that Twist1 binds to and phosphorylated by AKT/PKB at S42,T121 and S123. While conversion of S42,T121 and S123 to phosphorylation-mimicking glutamic acids created active Twist1, Alanin mutants of the same sites diminished the DNA-binding and transctivating functions of Twist1. In line with this, Glutamic Acids mutants suppressed the expression of E-Cadherin, whose expression is negatively regulated by active Twist1, Alanin mutants induced the expression of E-Cadherin. Similarly, we tested the impact of above mentioned mutants on cell migration and proliferation. Our results demonstrated that while Glutamic acid mutants accelerated, Alanin mutants suppressed the migration and proliferation of 293T cells.
Discussion: According to our results, S42,T121, and S123 amino acids are important for the activation of Twist1. Thus, Twist1 and PI3K-AKT/PKB pathway plays an important role in induction of EMT-mediated metastasis. In this context, Twist1 could be evaluated as a new therapeutic molecule in cancer therapy.
Keywords: Twist1, AKT, Metastasis

S-007 - PROSTATE CANCER AND RANOLAZINE AS ION CHANNEL BLOCKERS

Altun Seyhan

Biology of Department, Faculty of Science, University of Istanbul, İstanbul, TURKEY

Introduction: It is known that the incidence of prostate cancer is gradually increasing and the deaths that occur result from metastases as in other types of cancer. Studies have focused on the presence of the excess Na^+ ion channels in the membranes of cells with the metastatic capability and on the drugs/agents blocking these channels. It has been concluded in many studies which have been carried to date that cell movements can be suppressed and metastasis can be decreased by blocking the voltage-gated Na^+channels(VGSC) in cell membranes. One of the VGSC-inhibiting drugs is ranolazine(RNL) which is among the drugs used for angina pectoris. It was aimed to reveal the effect of RNL, a VGSC blocker on metastases*in vivo* and the lateral movement of Mat-LyLu cells *in vitro*, in the Dunning model.

Material and Methods: RNL(1-5µM) was applied to Mat-LyLu cells, and the movements of the cells were examined with the "wound heal" method. Cells($2x10^4$) were inoculated in Copenhagen male rats, and RNL(2.5-5µM/1ml) was systemically administered. The rats were dissected on day 22, and the metastases in the lungs were evaluated in terms of number and size.

Results: It was determined that RNL which had no effect on cell proliferation suppressed the movement of Mat-LyLu cells. As a result of administering RNL, it was observed to decrease both the total number of metastases and the number of small metastases in the lungs of the rats.

Discussion: It has been revealed for the first time that VGSC blocker RNL inhibits the lung metastases of rats with prostate cancer by suppressing the movement of the Mat-LyLu cells and decreases the total tumor burden of the animals. It is necessary to deepen the studies on the effects of RNL that increases survival by decreasing metastasis on human cells/xenograft models to be included in the oncology clinic. Ethical number:2010/116

Keywords: Prostate Cancer, Ranolazine, VGSC, Metastasis

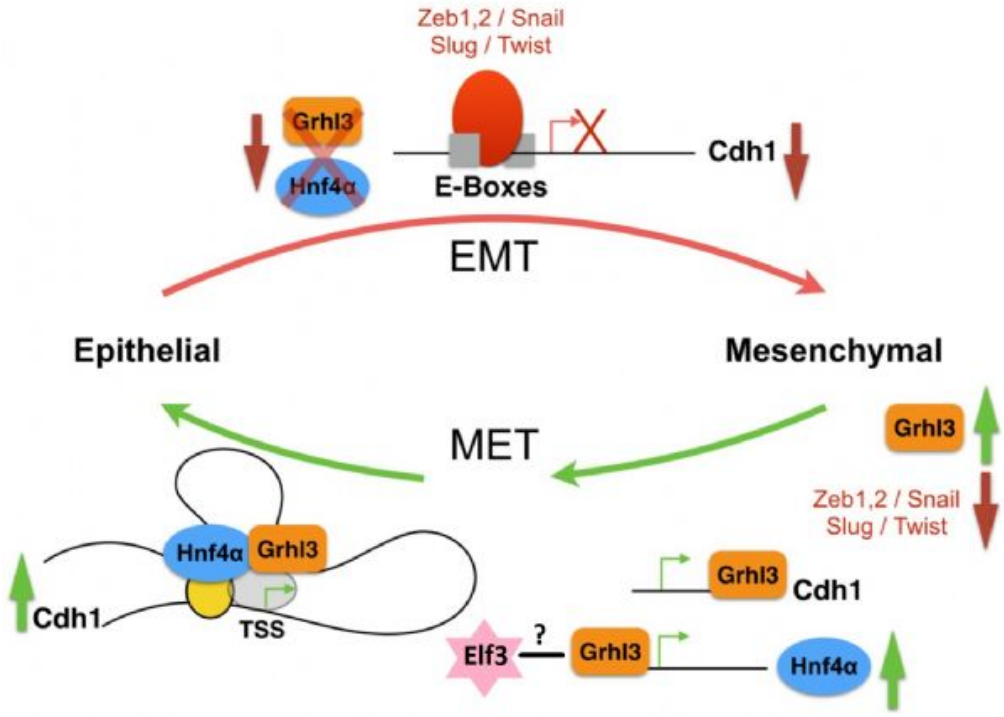
S-008 - NOVEL FUNCTION OF ELF3: A POTENTIAL REGULATOR OF MET

Şengez Burcu, Aygün İlkin, Alotaibi Hani

Izmir International Biomedicine and Genome Institue, Dokuz Eylul University, Izmir, TURKEY

Introduction and Aim: To date, there is a substantial amount of data in the literature analyzing the epithelial to mesenchymal transition (EMT) program both in development and during tumorigenesis, but a similar understanding of the mesenchymal to epithelial (MET) program is still lagging. Similar to the requirement of EMT in tumor progression, MET is also essential for metastasis. Thus, a better understanding of how MET is regulated is of utmost importance for better management of metastatic disease.
Recently, we have identified a feed forward loop composed of the transcription factors Grhl3 and Hnf4α which are essential for the progression of MET (Figure 1). In particular, we became interested in the Ets transcription factor Elf3 as a potential regulator of Grhl3.
Results: Despite the strong association of Elf3 with an epithelial phenotype, the expression levels of Elf3 remained stable during EMT-MET. We also found that silencing of Elf3 resulted in a failure to initiate MET in NMuMG cells. In addition, the levels of Grhl3, Ehf, Cebpa and Hnf4α were siginificantly downregulated in the absence of Elf3, Cdh1 expression was unaffected. To our surprise, E-cadherin was not localized to the plasma membrane, instead, it localized in the cytoplasm, which explains the failure of MET. We also identified that Elf3 could activate the promoter of Grhl3.
Conclusions: Our unpublished data suggest an active role of Elf3 in controlling MET. First, we found that Elf3 is a potential regulator of Grhl3, in several epithelial cell lines as well as during the MET induction in NMuMG cells. Second, our preliminary findings presented here suggest a function of Elf3 in regulating the relocation of E-cadherin to the plasma membrane. We hypothesize that Elf3 accomplishes this function by regulating either members of the post-translational modification pathways, or members of the trafficking pathways to the plasma membrane.
Keywords: Mesenchymal to epithelial transition, Grhl3, Elf3

Figure 1



During EMT activators of E-cad expression are downregulated and transcription at the Cdh1 locus is blocked by at least one of the EMT inducers Zeb1, Zeb2, Snail, Slug and Twist by binding to E-boxes at the promoter. Upon MET induction Grhl3 is binding to sites at Cdh1 and Hnf4α. Subsequent expression of Hnf4α leads to recruitment of Grhl3 and Hnf4α to intronic enhancers that induces DNA looping by interaction of the two factors and at the TSS that involves PolII (grey) and p300 (yellow) (enhancer cooperativity). The assembly and stabilization of the core transcription machinery leads to induction of E-cad expression. Up- and downregulation is indicated by vertical green and red arrows, respectively.

S-009 - TARGETING OF $NA_V1.5$ CHANNEL IN METASTATIC BREAST CANCER MODELS *IN VITRO* AND *IN VIVO* MICE AS A NOVEL THERAPY

Erdogan Mumin Alper[1], Ozpolat Bulent[2]

[1]*Department of Physiology, School of Medicine, Ege University, Izmir, Turkey; Department of Experimental Therapeutics, UT MD Anderson Cancer Center, Houston, TX, USA*
[2]*Department of Experimental Therapeutics, UT MD Anderson Cancer Center, Houston, TX, USA*

Introduction and Aim: Breast cancer (BCa) is the most common cancer among women worldwide. The major reason for patient death is due to metastasis and resistance to current therapies. Thus, the novel targeted therapeutic strategies is urgently needed. Voltage-gated ion channels (VGSC) are a group of ion channels that has been correlated with BCa. Importantly, VGSC activity contributes to many cellular behaviors integral to metastasis. In a recent study, authors determined upregulation of the sodium channel $Na_V1.5$ and its neonatal spliced form ($nNa_V1.5$) in metastatic BCa cells and breast tumors from patients who had a recurrence. The aim of the current study was to reveal molecular mechanisms underlying the effects of $Na_V1.5$ and $nNa_V1.5$ down-regulation on BCa *in vitro* and *in vivo*.
Material and Methods: As *in vitro* experiments, cell proliferation, invasion, apoptosis, cell cycle, western blot, RT-PCR etc. analysis were performed after siRNA treatments in BCa cells. Effects of $Na_V1.5$ siRNA treatments (nanoliposomal) on both tumor growth and metastasis of breast cancer were evaluated by performing xenograft orthotopic breast cancer and lung metastasis models *in vivo*.
Results: Our results showed that expression of $Na_V1.5$ and $nNa_V1.5$ mRNAs are higher in metastatic BCa cells. Specific $Na_V1.5$ siRNA treatments caused a significant reduction in cell proliferation/colony formation/drug resistance/invasion/migration/wound-healing capacity in metastatic BCa cells ($p<0,0001$), but didn't effect normal MCF10A cell proliferation. These siRNAs also increased the level of apoptosis and caused G1-cell cycle arrest in MDA-MB-231 cells ($p<0,0001$). Targeting of these channels inhibited tumor growth, tumor weight and lung metastasis *in vivo* ($p<0,0001$). Additionally, we found that these channels may enhance tumorigenesis and metastasis through the upregulation of pro-tumorigenic and metastatic proteins in BCa.
Conclusion: In conclusion, it was revealed that these channels have an important role in the metastasis and progression of BCa and targeting $Na_V1.5$s by siRNA may be beneficial to BCa patients.
Keywords: Breast Cancer, Metastasis, Nanoliposom, $Na_V1.5$, siRNA

S-010 - TGF-ß RECEPTOR I/II SIGNALING AT PRIMARY CILIA MEMBRANE IS REGULATED BY CERAMIDE TO MODULATE CELL MIGRATION

Gencer Salih[1], Ogretmen Besim[2]

[1]*Department of Molecular Biology and Genetics, Uskudar University, Istanbul, Turkey*
[2]*Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, USA*

Mechanisms that regulate TGF-beta receptor I/II (TßRI/II) trafficking to primary cilia membrane for mediating signal transduction remain unknown. Here, we show that ceramide synthase 4 (CerS4) generated ceramide, bioactive sphingolipid, stabilized Smad7-TßRI association, which then inhibited the trafficking of TßRI/II to primary cilia membrane. Expression of a mutant TßRI, which is resistant to Smad7 binding/inhibition, restored receptor signaling to increase migration in response to CerS4/ceramide induction. Genetic or molecular alterations of CerS4 abundance prevented Smad7-TßRI inhibitory complex, and increased association between Arl6 transporter and TßRI via novel cilia targeting signal (31-ATALQ-35). Mutation of the cilia targeting signal abolished the trafficking of the receptor to the cilia membrane in response to CerS4 knockdown in various cell types. Localization of TßRI/II to primary cilia activated sonic hedgehog (Shh) receptor smoothened (Smo), inducing migration/invasion and liver metastasis both in wild type and CerS4-/- knockout mice in response to endogenous CerS4/ceramide knockdown in 4T1 mammary cancer cells, injected in the mammary pads. Smad7 overexpression or primary cilia inhibition by shRNA-mediated knockdown of intraflagella transport protein 88 (IFT88) prevented TßRI-Smo crosstalk and attenuated liver metastasis of mammary cancer cells stably transfected with shRNA against CerS4/ceramide. Overall, these data define a key mechanism for the regulation of TßRI/II targeting selectively at the primary cilia membrane by CerS4/ceramide-Smad7 inhibitory complex to control Shh-mediated cell migration and invasion without affecting canonical TGF-ß signaling.

Keywords: Metastasis, Primary cilia, TGF-beta, Sonic Hedgehog, Ceramide

S-011 - IN SILICO IDENTIFICATION OF COMPOUNDS WITH SELECTIVE ACTION ON EPITHELIAL OR MESENCHYMAL TUMOR CELLS

Demirkol Seçil, Güre Ali Osmay

Molecular Biology and Genetics, Bilkent University, Ankara, TURKEY

Aim: Although epithelial to mesenchymal transition (EMT) is a biological event applicable to all tumor types studied so far, published gene signatures defining the epithelial or mesenchymal status of cancer cells or tissues have been mostly tissue specific. Recent studies showed that EMT status can be determined with certain gene lists independent of tissue-type. In this study, we aimed to identify drugs with differential effects on mesenchymal, and epithelial groups regardless of the tissue type.
Methods: We used one gene list determining EMT status of cancer cell lines (1), as well as one we generated ourselves to analyze in silico several cell line panels (2,3), and defined the compounds which can selectively inhibit the growth of epithelial and mesenchymal cells.
Results: We thus identified compounds that caused growth inhibition of either epithelial or mesenchymal cells, and observed groups of drugs behaving in a similar pattern. Proteomic analysis of cell lines within each group identified pathways related to sensitivity of drug subgroups. Our analysis revealed that the EMT based classification was also related to the stemness status of cancer cell lines.
Conclusion: Our study demonstrates a novel use for online databases and might help us understand the major mechanisms that should be targeted for both types of cancer cells.
Keywords: Epithelial, Mesenchymal, Transition, Tumor Heterogeneity, Chemotherapy

S-012 - THE EXPRESSION LEVEL OF NF-KB/P65 INCREASES IN BORTEZOMIB-RESISTANT MULTIPLE MYELOMA CELL LINES

Tarhan Mehtap, Öztürk Kamile

Aksaray University, Science and Letter Faculty, Molecular Biology Department, 68011, Aksaray

Background: Multiple myeloma (MM) is a hematologic malignancy characterized by the accumulation of clonal plasma cells in the bone marrow. MM cells often show constitutive expression of nuclear factor kappa B (NF-kB) transcription factors. Bortezomib, the first therapeutic proteasome inhibitor, was approved for the treatment of MM. The primary action of bortezomib is explained by blocking the NF-kB activation pathway. Treatment of MM with bortezomib has greatly improved survival for patients, however relapse due to bortezomib-resistance is inevitable and the disease remains incurable. Although, NF-kB activation is involved in MM pathogenesis, its role in bortezomib-resistance is controversial.
Methods: In this study, we aimed to investigate the effects of NF-kB/p65 and p50 subunits in bortezomib-resistance mechanism of MM cells. We utilized bortezomib-sensitive KMS-28 and bortezomib-resistant KMS-20 human MM cell lines. These cells were treated with different concentration of bortezomib for 12, 24 and 48h. The effects on cell viability of bortezomib were determined using the MTT assay. The expression levels of NF-kB p65 and p50 subunits were determined by real time RT-PCR method.
Results: Bortezomib time and dose dependently reduced cell viability in MM cells. In bortezomib-sensitive KMS-28 cell line, IC50 values were 11.83, 5.30, 3.67 nM at 12, 24 and 48h, respectively. In bortezomib-resistance KMS-20 cell line, IC50 values were 32.06, 15.62, 6.05 nM at 12, 24 and 48h, respectively. In KMS-28 cell line, the expression levels of p65 and p50 did not show any change with dose and time dependent. Moreover, the expression levels of NF-kB/p65 were observed increased in a dose dependent manner in bortezomib resistant KMS-20 cells, whereas no changes were observed in the NF-kB/p50 levels.
Conclusion: Bortezomib-resistance in MM cells can be associated with increasing of NF-kB/p65 expression levels in the high bortezomib concentrations
Keywords: Bortezomib Resistance, Multiple Myeloma, NF-kB Signaling

S-013 - INVESTIGATION OF ANTI CANCER PROPERTIES OF NEW SULPHONAMIDE DERIVATIVE SHOWED CARBONIC ANHYDRASE-IX ENZYME INHIBITOR FEATURE

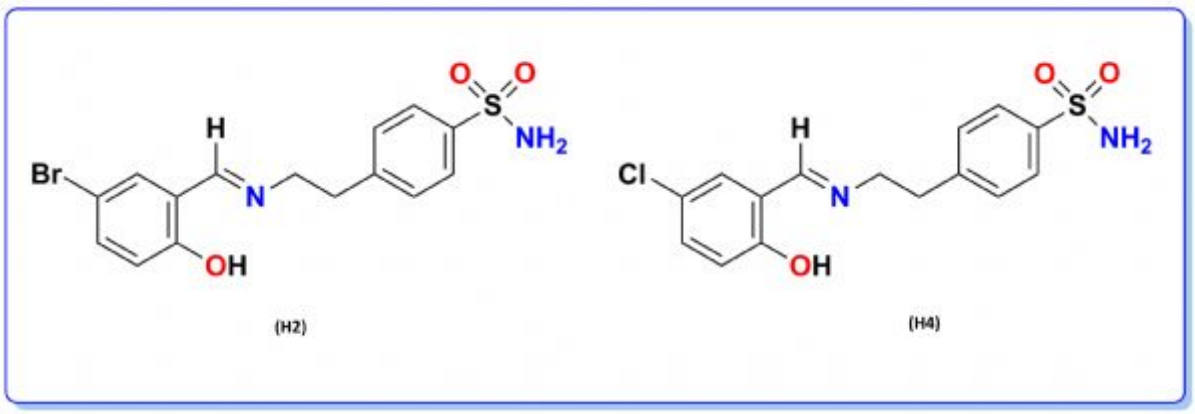
Koyuncu İsmail[1], Yüksekdağ Özgür[2], Koçyiğit Abdürrahim[4], Güler Eray Metin[4], Durgun Mustafa[3], Kirmit Adnan[1], Gönel Ataman[1]

[1]*Harran University, Faculty of Medicine, Department of Medical Biochemistry, Sanlıurfa, Turkey*
[2]*Harran University, Faculty of Arts and Sciences, Department of Biology, Sanlıurfa, Turkey*
[3]*Harran University, Faculty of Arts and Sciences, Department of Chemistry, Sanlıurfa, Turkey*
[4]*Bezmialem Vakif University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, Turkey*

Introduction: Membrane-associated carbonic anhydrase CA-IX is one of the most important enzymes, which is related to tumour metabolism. Especially, CA-IX is an attractive target for cancer therapy. For, while it is over-expressed in a wide variety of solid tumours, CA-IX is expressed in a limited way in normal tissues. Pharmacologic interference of CA-IX catalytic activity has showed that by consequently disrupting pH regulation by cancer cells, CAIX-specific small molecule inhibitors impairs primary tumour growth and metastasis. Recently, CA-IX inhibitors have been proposed as a potential new class of anti-tumour agents.
The aim of this study is to evaluate the anti-tumour activity of CA inhibitors, two newly synthesized aromatic sulphonamides with high affinity for CAIX, 4-(2-((5-bromo-2-hydroxybenzylidene) amino)ethyl)benzenesulfonamide (H2) and 4-(2-((5-chloro-2-hydroxybenzylidene)amino)ethyl) benzenesulfonamide) (H4) and against human tumour cells.
Materials and Methods: The effects of H2 and H4 on cell cyto-toxicity have been evaluated by using CA-IX positive HELA, HT-29 cells and CA IX negative MDA-MB-231 cells. As a normal cell PNT-1A, HEK-293 is used. The effect of sulphonamides on cell viability is determined through WST-1 assay and then IC50 value of each compound is assessed. Apoptosis induction is determined by flow cytometry annexin V analyse, Anti proliferative effects of compounds are determined by using BrdU elisa assay. Intra-cellular accumulation of ROS is determined using the fluorescent probes.
Findings: H2 and H4 could reduce cell cyto-toxicity, proliferation and induce apoptosis in HELA cells. Moreover, all two inhibitors could increase intra-cellular ROS production in the same cells. The two inhibitors do not show any anti-tumour activity in normal PNT-1 cells.
Conclusion: CA inhibition can decrease cell proliferation and induce apoptosis in human tumour cells. The ability of CA inhibitors to increase ROS might trigger cell apoptosis. Activation of this apoptotic cascade is probably mediated by inhibition of the CA IX isoform.

Keywords: Sulphonamide erivatives, Anti-cancer,Apoptosis, Carbonic anhydrase-IX

The molecule structures of sulphonamide compounds



*H2: 4-(2-((5-bromo-2-hydroxybenzylidene) amino)ethyl)benzenesulfonamide and H4: 4-(2-((5-chloro-2-hydroxybenzylidene)amino)ethyl). benzenesulfonamide). *This study supported by Technological and Scientific Research Council of Turkey (TUBITAK Project no: 115Z681).*

S-014 - THE EFFECT OF THE *ROSMARINUS OFFICINALIS* ON TEMOZOLOMIDE RESISTANT GLIOBLASTOMA (U87 MG) CELLS

Özdemir Damla Meryem[1], Göktürk Dilek[2]

[1]*Adana Science and Technology University, Graduate School of Natural and Applied Sciences, Department of Nanotechnology & Engineering Sciences, ADANA, Turkey*
[2]*Adana Science and Technology University, Faculty of Engineering and Natural Sciences, Department of Bioengineering, ADANA, Turkey*

Introduction: Glioblastoma multiforme (GBM) is the most common and aggressive type of the primary brain cancer. Temozolomide is the primary medicine of GBM. But sometimes GBM can gain resistance to temozolomide. In this case some other chemotherapeutics can be used but they have serious side reactions. To avoid this we aimed to investigate the effect of *Rosmarinus Officinalis* (rosemary), an aromatic plant that posses phenolic diterpenes such as carnosol, carnosic acid, rosmarinic acid and effective for various cancer types.
Methods: Studies were carried out with the Glioblastoma (GBM) cell line (U87 MG) and Mouse Embryonic Fibroblast (MEF) cell line. Cells were seeded into 24 well plate and cultured. Rosemary was prepared as tea and was given to cells at various doses (1/1000, 1/100 and 1/75 (v/v)). Cells were incubated for 1 day and cell viability was measured by neutral red assay. Then with optimum dose of rosemary only GBM cells were cultured for 3 and 5 days and cell viability assays were applied.
Results: According to neutral red assay, at increasing concentrations of rosemary, MEF cells proliferated whereas GBM cells couldn't survive. Even 1/1000 (v/v) rosemary increased the viability of healthy cells about 8% and reduced the viability of tumor cells about 23%. The 1/75 (v/v) rosemary concentration which is determined as optimum, increased the viability of healthy MEF cells by nearly 9.5% and reduced the viability of GBM cells by nearly 42%. Also 1/75 (v/v) rosemary concentration reduced the viability of GBM cells in 3 days by nearly 57% and in 5 days by nearly 44%.
Conclusion: The results show that, while rosemary helps to proliferation of healthy cells, it eliminates the tumor cells. It can be said that rosemary has a potential to be cure for temozolomide resistant GBM without damaging the healthy cells.
Keywords: Glioblastoma multiforme, MEF, Neutral Red, *Rosmarinus Officinalis*, Temozolomide

S-015 - SYNTHESIS, CHARACTERIZATION AND DNA INTERATION OF NOVEL PLATINUM(II) COMPLEXES CONTAINING SUBSTITUTEDBENZIMIDAZOLE LIGANDS

Utku Semra[1], Nzeiyama Abdoul[1], Açık Leyla[2], Çelebi Keskin Ayten[3]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mersin University, Mersin, Turkey*
[2]*Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey*
[3]*Department of Bioengineering, Faculty of Engineering, Kırıkkale University, Kırıkkale, Turkey*

Objectives: The fortuitous discovery of cisplatin in 1965, and by the 1978, it had been approved by FDA for treatment of the different types of cancer such as testicular, ovarian, head and neck, colon, bladder, gastric, and lung cancer. However, there are two considerable problems associated with clinical cisplatin usage: intrinsic or acquired resistance and side effects including nephrotoxicity, ototoxicity, nausea and emetogenicity. These have led to the development of cisplatin analogs that would be clinically effective without and/or less toxicity. From this context, we report on the synthesis and spectral characterization of eight new platinum(II) complexes of the type [Pt(L1-L4)2Cl2] C1-C4 and [Pt(L1-L4)2I2] C5-C8 (L1=5(6)-chlorobenzimidazole, L2=5(6)-methylbenzimidazole, L3=5(6)-chloro-2-methylbenzimidazole, L4=5(6)-methyl-2-methylbenzimidazole). The interactions with pBR322 plasmid DNA and inhibition of the BamHI and HindIII restriction enzyme activity through the synthesized complexes were also studied.
Methods: C1-C4 or C5-C8 were prepared by the reaction of the corresponding ligand and K2PtCl4 or K2PtI4 in ethanol/water solution. The plasmid DNA interactions and restriction enzyme activities of them were also investigated using Agarose Gel Electrophoresis method.
Results: An attempts of synthesizing new potent anticancer drugs were done by combining Pt(II) chlorido and iodido compounds with benzimidazole derivatives ligands L1-L4. The description of compounds after the synthesis was assumed by using spectroscopic characterization, pBr322 plasmid DNA interaction and then BamHI and HindIII restriction enzymes. Therefore, looking after plasmid DNA interacting outcomes, synthesized complexes modified the tertiary structure of pBR322 plasmid DNA, and the results showed that the complex C2 was highly active compound regarding to all synthesized complexes.
Conclusion: It was profound that the labile ligands containing chlorido (C1-C4) are more active than those containing iodido (C5-C8). Promising biological activity from synthesized complexes provides useful information for further cytotoxic evaluation including cisplatin resistant cell lines and future platinum-drug design strategies.
Keywords: benzimidazole, platinum complexes, synthesis, gel electrophoresis, cisplatin
Acknowledgements: Financial support was provided by grant the Research Foundation of Mersin University (2015-TP2-1175)

S-016 - ANTICANCER EFFECTS ON HUMAN LEUKEMIA HL-60 CELL LINE AND MOLECULAR DOCKING STUDIES OF NOVEL 2,5-DISUBSTITUTED-BENZOXAZOLE DERIVATIVES

Oksuzoglu Emine[1], Ertan Bolelli Tugba[2], Tarhan Mehtap[1], Ozturk Kamile[1], Yıldız Ilkay[2]

[1]Molecular Biology Division, Department of Biology, Faculty of Science and Letters, Aksaray University, 068100, Aksaray, Turkey
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, Tandogan, 06100, Ankara, Turkey

Introduction and Aim: Cancer is a disease that leads mortality in the worldwide. Recently, many efforts have been made to develop more effective ways of treating cancers and to search for novel chemotherapeutic agents with minimal side effects. Benzoxazoles, which are an important class of heterocyclic compounds that exhibit substantial biological and pharmacological activities. We previously synthesized some novel 2,5-disubstituted benzoxazole derivatives[1,2]. In this study, synthesized derivatives were evaluated from anticancer perspective by using various assays.
Materials and Methods: These compounds were investigated for their antitumor activities against human leukemia HL-60 cell line by using the MTT cell proliferation assay and IC50 values of the compounds were determined. Moreover, molecular docking into active site of the DNA Topo II enzyme was performed on 3QX3.PDB file in order to find out possible mechanism of antitumor effect.
Results: The results showed that some of the 2,5-disubstituted benzoxazoles were found to be more potent antitumor activity against human leukemia HL-60 cells than the well-known anticancer drug etoposide. Moreover, molecular docking studies revealed that active benzoxazoles interacted into an active site of DNA Topo II enzyme with a low binding energies.
Discussion and Conclusions: According to all obtained results showed that active 2,5-disubstituted-benzoxazole derivatives could be potential drug candidates as new antitumor agents and are worthy to carry on the anticancer studies.
Keywords: Anticancer drugs, Antitumor effect, Benzoxazoles, Human leukemia HL-60 cell line, Molecular docking

S-017 - EFFECTS OF EMBELIN ON BREAST CANCER CELL PROLIFERATION AND APOPTOSIS; COMPARING WITH DOCATAXEL AND TAMOXIFEN

Tekin Gülsüm[1], Koçak Fatma Emel[2], Koçak Cengiz[3], Öztürk Bahadır[1]

[1]*Department of Medical Biochemistry, Faculty of Medicine, Selcuk University, Konya,Turkey*
[2]*Department of Medical Biochemistry, Faculty of Medicine, Dumlupinar University, Kutahya, Turkey*
[3]*Department of Medical Pathology, Faculty of Medicine, Dumlupinar University, Kutahya, Turkey*

Introduction: Embelin is a X-linked inhibitör of apoptotisis protein (XIAP) which obtained from Embele ribes plant and shown to exhibit chemopreventive, anti-inflammatory, NF-κB down-regulative and apoptotic activities through an unknown mechanism. In this study, the effect of different concentrations and durations of embelin to cell proliferation was investigated in MCF–7 (ER+, PR+, HER-2-) and MDA MB-231 (ER-, PR-, HER-2-) breast cancer cell lines by comparing with Tamoxifen and Docetaxel.
Material and Method: A real-time cell analyzer (xCELLigence, Roche Diagnostics GmbH, Penzbeerg, Germany) was used to evaluate the effects of different doses of Embelin (12,5-100µM), Tamoxifen (12,5-100µM) and Docetaxel (12,5-100nM) on the proliferation of both cell lines and determined IC50 for each drug. Cell blocks were prepared from cultured cells treated with drugs and formalin-fixed paraffin-embedded breast cancer cells were examined histopathologically using Haematoxylin&Eosin staining method. In addition, the expressions of Ki-67, Bcl-2, BAX, and cyclin-D1 were assessed immunohistochemically. Statistical analysis was performed GraphPad Prism version 6.05 (GraphPad Software, Inc., CA, USA).
Results: Embelin inhibits the proliferation in both cell lines time and dose dependent manner. IC50 for Embelin, Tamoxifen and Docetaxel in MDA MB-231 and MCF-7 cells were 64µM at 40h and 63µM at 66h; 50 µM at 45h and 40 µM at 41h; 32 nM at 60h and 43nM at 40h, respectively. As a results of histopathologic and immunohistochemical analysis, Embelin decreases Ki-67, cyclin-D1 and increases BAX/Bcl-2 ratio in both breast cancer cells. Tamoxifen and Docetaxel effects have been compared with Embelin.
Conclusions: According to results, Embelin is more effective in both cell lines compared with Docetaxel and it also has more potent for MDA MB-231 compared with MCF–7. This knowledge could be beneficial in the development Embelin-based therapies for treating breast cancer.
Keywords: Docetaxel, Embelin, MCF-7, MDA MB-231, Tamoxifen

S-018 - 3,4-BIS(3'-INDOLYL)-1,2,5-OXADIAZOLES, ANALOGUES OF MARINE ALKALOID NORTOPSENTIN: SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY

Sevgi Fatih[1], Tekin Gülsüm[2], Ünlü Ali[2]

[1]*Vocational School of Health Services, Medical Laboratory Program, Selcuk University, Konya, TURKEY*
[2]*Department of Biochemistry, Selcuk University Faculty of Medicine, Konya,TURKEY*

Introduction: Nortopsentins A-C, having a charecteristic 2,4-bis(3'indolyl)imidazole skeleton, were isolated from marine sponge spongsorites ruetzleri, exhibited invitro cytotoxicity against P388 cells (IC50,4.5-20.7µM). Due to their interesting biological activities, various bis(indolyl) derivatives in which the imidazole moiety of nortopsentin was replaced by thiazole,pyrazole, furan,thiophene, etc. rings were designed and synthesized.Our aim is synthesis of new analogues of nortopsentin in which the central imidazole ring is replaced by 1,2,5-oxadiazole (furazan), in order to study how these structural modifications influence biological activity.
Methods: We have developed a new strategy for the synthesis Bisindolyl furazans. Initially indoles react with dichloroglyoxime from electron-rich carbon atom (C3), and thus C-C bound α-dioximes are obtained.Conversion of α-dioximes to the bioactive furazanes achieved by microwave assisted dehydration of dioximes. This method, which was carried out using the closed vessel microwave system, was a technique that was used for the first time in furazan synthesis. A real-time cell analyzer (xCELLigence, Roche Diagnostics) was used to evaluate the effects of different doses of the synthesized compounds on the proliferation of MCF–7 breast cancer cell line.Changes in the number of cells in special cell culture flasks that containing micro-electrodes was observed continuously for every 15 minutes during the 54 hours.
Results: In the present work we have synthesized three novel 3,4-Bis(3'indolyl)furazans from parent novel vic-dioximes. All of newly synthesized compounds characterized in terms of various techniques, such as 1H-NMR, 13C-NMR, FT-IR, LC-MS, and elemental analyses. The analogue with 2-methylindole substituent showed the best antiproliferative activity with IC50 23,7 µM for MCF–7 breast cancer cell line.
Conclusion: We have developed a highly efficient synthesis of 3,4-Bis(3'-indolyl)-1,2,5-oxadiazole, which are analogues of marine bis(indole)alkaloid of nortopsentins. The compounds exhibited mild to good cytotoxic activities against MCF–7 breast cancer cell line. Extensive exploration of structure–activity relationship of this novel 1,2,5-oxadiazole scaffold and its biological target studies are underway.
Keywords: Bisindole, Furazan, Microwave Synthesis, Nortopsentin, Oxadiazoles

S-019 - BIOASSAY-GUİDED ISOLATION AND CYTOTOXIC EFFECTS OF EXTRACT AND CHEMICAL CONSTITUENTS OF *CHRYSOPHTHALMUM MONTANUM* (DC.) BOISS. OF TURKISH ORIGIN

Fatma Ayaz[1], Nurgun Kucukboyaci[1], Nezhun Goren[2], Ihsan Calis[3], Seyma Aydinlik[4], Engin Ulukaya[5], Hayri Duman[6], Sammer Yousuf[7], Muhammad Iqbal Choudhary[7]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey*
[2]*Department of Molecular Biology and Genetics, Faculty of Science and Arts, Yıldız Technical University, Istanbul, Turkey; H. E. J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi, Pakistan*
[3]*Department of Pharmacognosy, Faculty of Pharmacy, Near East University, Nicosia, Turkish Republic of Northern Cyprus*
[4]*Department of Biology, Faculty of Science, Uludag University, Bursa, Turkey*
[5]*Department of Clinical Biochemistry, Faculty of Medicine, Uludag University, Bursa, Turkey*
[6]*Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey*
[7]*H. E. J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi, Pakistan*

Introduction: Asteraceae family is known to have ethnomedicinal uses in cancer related diseases, and mainly investigated on cytotoxic activity. The genus *Chrysophthalmum* Schultz Bip., Asteraceae, comprises three species in Turkey. *C. montanum* (DC.) Boiss., also known “tutça”, “nezle otu”, is used on injured part of the body, against flu and sinusitis. The aim of the present study was to isolate and identify the active natural constituents from the aerial parts of *C. montanum* through bioactivity-guided fractionation.

Material and Methods: Aerial parts of *C. montanum* were extracted with methanol (80%). The methanolic extract was successively partionated with *n*-hexane, chloroform, *n*-butanol, and water. The chloroform extract was subjected to separation and purification by using various chromatographic techniques. The structures of the isolated four compounds were identified by means of spectral methods, such as UV, IR, NMR, X-ray crystallography as well as EI- and HREI-MS. Beside that various cancer cells (MCF-7 and MDA-MB-231 breast; LNCaP and PC3 prostate; A549 and PC3 lung, and HT-29 colon cancer cell lines) were treated with 20 µg/ml (1), (2), (3) and (4) guaianolides. Sulforhodamine B (SRB) assay was perfomed to determine cytotoxicity after 48h treatment.

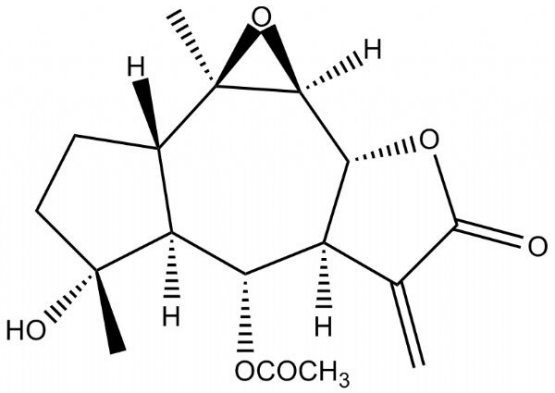
Results: The active chloroform fraction yield four guaianolides (1), (2), (3), and (4). Our data showed that (1), (3) and (4) strongly decreased cell viability compared to (2). On the other hand, our finding led us to consider (2) has a specific and selective effect on LNCAP cells.

Conclusion: This is the first report on the cytotoxic activity of phytochemical constituents of *C. montanum*. Based on these results, (1), (3) and (4) can be regarded as an effective

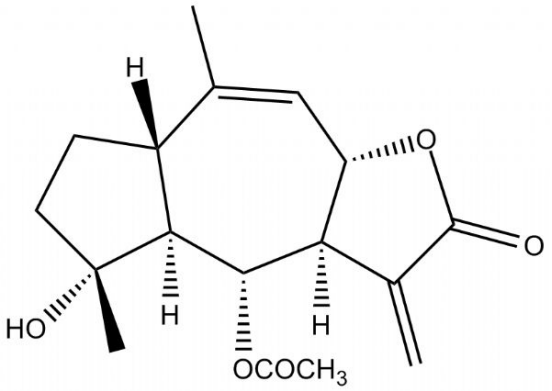
approach for the treatment of cancer cells mentioned above. Therefore, these compounds deserve further attention for the proof of concept in the treatment of various cancer cells.

Keywords: Bioassay-guided, cytotoxic activity, *Chrysophthalmum montanum*, Asteraceae

(1) 6α-acetoxy-4α-hydroxy-9β.10β-epoxy-1βH-guaia-11(13)-en-12.8α-olide



(3) 6α-acetoxy-4α-hydroxy-1βH-guaia-9.11(13)-dien-12.8α-olide



S-020 - ANKAFERD BLOOD STOPPER INDUCES DNA DAMAGE, APOPTOSIS AND CYTOTOXIC ACTIVITY BY GENERATING REACTIVE OXYGEN SPECIES IN MELANOMA CELLS IN VITRO

Kocyigit Abdurrahim, Güler Eray Metin

Bezmialem Vakif University School of Medicine Department of Medical Biochemistry

Introduction: Although, Ankaferd Blood Stopper (ABS) could be utilized successfully as hemostatic agent for the management of clinical hemorrhages, studies demonstrated that it has cytotoxic and apoptotic effects on cells. However, the mechanism(s) of these effect has not been elucidated yet. In this study, cytotoxic, genotoxic, apoptotic and reactive oxygen generating (ROS) activities of ABS were investigated in melanoma cancer and normal cells.
Material and Methods: The cells were incubated with different concentrations of ABS (0.125 to 2 %) for 24 h. The cell viability was assessed based on ATP cell viability assay. Intracellular accumulation of reactive oxygen species (ROS) was determined using the fluorescent probes 2',7'-dichloro-dihydrofluorescein-diacetate (DCFH-DA). DNA damage was evaluated by alkaline single cell gel electrophoresis assay (Comet Assay) and, apoptosis induction was detected by acridine orange (AO) staining method.
Results: Our results demonstrated that ABS increases DNA damage, apoptosis and ROS levels in both melanoma and normal cells in a dose dependent manner, and all of these activities were significantly higher in melanoma cells than in normal cells. There was a statistically significant positive correlation between DNA damage, apoptosis and ROS levels in ABS treated melanoma and normal cells.
Conclusion: Our results revealed that although ABS commonly used as hemostatic agent, it causes DNA damage and apoptosis by generating ROS activity in a dose dependent manner. Further studies are needed to better understand the anticancer potential of this novel hemostatic agent. These results could also contribute to the development of new treatment for cancer.
Keywords: Ankaferd, Apoptosis, Cytotoxicity, DNA Damage, Reactive Oxygen Species

S-021 - EVALUATING THE CANCER THERAPEUTIC POTENTIAL OF SUPRAMOLECULAR CALIX[4]AREN NANOFIBERS

Uyar Arpacı Pembegül[1], Özcan Fatih[2], Ertul Şeref[2]

[1]*Department of Biotechnology, Science Faculty, Selcuk University, Konya, Turkey*
[2]*Department of Chemistry, Science Faculty, Selcuk University, Konya, Turkey*

Introduction: The search for new potent anticancer drugs that can only target cancer cells, rather than affecting normal tissues is very much commendable. Supramolecular approaches have been applied to drug delivery systems and have attracted much attention. Calixarenes are a family of bowl or cone shaped synthetic supramolecular macrocycles, composed of phenol units linked by methylene bridges through and an aldehyde. The search for new potent anticancer drugs that can only target cancer cells, rather than affecting normal tissues is very much commendable. Calixarene is a highly promising candidate in this regard, and could be modified to fabricate nanofibers by electrospinning and appropriately used for targeted chemotherapy.

Methods: Non-polymeric calixarenes nanofibers were obtained from the newly synthesized organic 5,11,17,23-Tetra-tert-butyl-25,27-bis(4-aminomethyl-pyridineamido)-26,28-dihydroxycalix[4]arene(4-AMP) by electrospinning. FT-IR, 1H-NMR and 13C-NMR [3] and SEM analysis were done to characterize newly synthesized 4-AMP. As cancer and healthy in-vitro models, Caco-2 and L-929 cells ($2x10^5$) were cultured on nanofibers, respectively. After 48 h incubation, cell growth/proliferation analysis were done by XTT assay and to evaluate cell morphology and adhesion to nanofiber SEM measurement were done.

Results: A series of experiments were performed for optimizing electrospinning parameters used to fabricate the calixarenes nanofibers. It could be observed that the proliferation rate on nanofibers of L-929 were higher than Caco-2 cells with XTT results. % cell viability of Caco-2 cells for 48 h was less than 10, however L-929 proliferated well (almost 100 %). There were low cell adhesion of Caco-2 when compare to L-929 on calixarenes nanofibers viewed by SEM/EDS.

Discussion and Conclusions: In this work,non-polymeric calixarenes nanofibers was fabricated through using electrospinning techniques and characterized to colon cancer cells. The results reported in this study demonstrated that tumor-preferential in-vitro cytotoxicity of calix[4]aren nanofibers against Caco-2 over L-929 cells present a promising approach for efficient and safe cancer therapy.

Keywords: Calixarenes Nanofibers, Electrospinning, Tumor-Preferential Cytotoxicity

S-022 - EVALUATION OF THE EFFECTS OF THYMOQUINONE TO DYNAMIC THIOL-DISULFIDE HOMEOSTASIS DURING TOTAL BODY IRRADIATION IN RATS

Deniz Cigdem Damla[1], Aktan Meryem[2], Erel Ozcan[3], Gurbilek Mehmet[1], Koc Mehmet[2]

[1]Department of Medical Biochemistry, Meram School of Medicine, Necmettin Erbakan University, Konya, Turkey
[2]Department of Radiation Oncology, Meram School of Medicine, Necmettin Erbakan University, Konya, Turkey
[3]Department of Biochemistry, Faculty of Medicine, Yildirim Beyazit University, Ankara, Turkey

Purpose and Objective(s): Ionizing radiation-induced free radicals causes functional and structural harmful effects. Thiol, an important antioxidant, plays an major role in the eradication of reactive oxygen molecules. Thiol/disulfide homeostasis is a marker for oxidative stress. The objective of this study was to assess the potential effects of Thymoquinone (TQ) to dynamic thiol/disulfide homeostasis of rats received total body irradiation.
Materials and Methods: Twenty-two adult Sprague-Dawley rats were divided into 3 groups. Sham control group (n=6) did not receive thymoquinone or irradiation. Irradiation (IR) group (n=8) received only total body IR of 6 Gy. TQ+IR group (n=8) received IR plus TQ (10 mg/kg, i.p, 30 min before IR). One and a half hour following IR, blood samples were taken. Thiol/ disulphide homeostasis parameters in blood were analysed by a newly established method that measures the exact thiol/ disulphide status in the body. Data analyses were performed using SAS 9.4. The statistical comparison of results has been performed by using Welch's Analysis of variance (ANOVA) and Tukey test.
Results: Native Thiol was highest in Sham-Control group. Native Thiol levels of IR group was significantly lower than Sham-Control group when compared (p=0.03). Total Thiol and Disulfide levels were not found different among groups (p>0,05). Disulfide/Native Thiol Ratio was least in Sham-Control group and Only Disulfide/Native Thiol Ratio of IR group was significantly higher than Sham-Control group (p=0.027). Disulfide/Total Thiol and Native Thiol/Total Thiol Ratios were found significantly different between Sham-Control group and IR group (p=0.007 and p=0.007, respectively).
Conclusion: In TQ+IR group; Disulfide, Native thiol, Total thiol, Disulfide/Native Thiol Ratio, Disulfide/Total Thiol Ratio and Native Thiol/Total Thiol Ratio means were not found significantly different when compared with Sham-Control group. Consequently, the use of TQ before radiation treatment, helps to protect the rats from oxidant side effects of radiation.
Keywords: Disulfide, İrradiation, Thiol, Thymoquinone

S-023 - A RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND ANTICANCER ACTIVITY/CYTOTOXICITY FOR POLY(MALEIC ANHYDRIDE-CO-VINYL ACETATE)/DRUGS CONJUGATES WITH GEMCITABINE, CYTERABIN AND METHOTREXATE DRUGS

Karakuş Gülderen

Cumhuriyet University, Faculty of Pharmacy, Pharmaceutical Chemistry, 58140, Sivas, Turkey

Introduction: Anticancer drugs such as Gemcitabine, Methotrexate and Cyterabin, which are commonly used for treatment of breast cancer, has a limited use due to their short half-life and also they have too many side effects depending on their cytotoxic effects on tissues. The objectives of this study to conjugated the anticancer agents, Gemcitabine Methotrexate, and Cyterabin, to drug carrier poly(maleic anhydride-co-vinyl acetate) (MAVA) copolymer for improve their water solubility; decrease toxic effects; and increase antitumor activity compared to crude drug.
Material and Methods: Structural characterization of the conjugates, MAVA/Gemcitabine, MAVA/Methotrexate and MAVA/Cyterabin, were performed by Fourier Transform Infrared Spectroscopy (FTIR) and Proton Nuclear Magnetic Resonance Spectroscopy (1H-NMR). Anticancer activity of conjugates on MCF-7 cells was determined by XTT assay in comparison with pure drugs, while their toxic effects on L929 cells were determined by XTT assay again in comparison with the pure drug. The results were also analyzed statistically with the Mann-Whitney-U Test.
Results: The synthesized conjugates structurally characterized with successful amidation mechanism and they exhibited good solubility in water.
Killing effects for the highest concentration of Gemcitabine, Methotrexate, and Cyterabin on MCF-7 cells as follows: Cyterabin (70.17%) > MAVA/Methotrexate (65,19%) > MAVA/Cyterabin (60.64%) > Methotrexate (58.43%) > MAVA/Gemcitabine (54.84%) > Gemcitabine (39.45%) ($p<0,05$).
Toxic effects for the highest concentration of Gemcitabine, Methotrexate, and Cyterabin on L929 cell lines (as a function of vitality rate) as follows: MAVA/Cyterabin (100%) > Cyterabin (89.86%) > MAVA/Methotrexate (77,10%) > Methotrexate (75.45%) > MAVA/Gemcitabine (73.03%) > Gemcitabine (62.11%) ($p<0,05$).
Conclusion: Water-soluble MAVA/Gemcitabine, MAVA/Methotrexate, and MAVA/Cyterabin conjugates observed that current anticancer activity was increased as the result of the formation of conjugates, and that also their toxic effect was decreased, compared to the its crude drug. Furthermore conjugates of Cyterabine and Gemcitabine are almost the same anticancer activity because they have very similar molecular structure.
Keywords: Anticancer Activity, Cytotoxicity, Gemsitabine, Metotharexate, Cyterabin

S-024 - THE ROLE OF REL PROTO-ONCOGENE IN FOLLICULAR LYMPHOMA DEVELOPMENT

Baytak Esra[1], Hu Xiaozhou[2], Li Jinnan[3], Okay Kaan[2], Hu Genfu[4], Scuto Anna[5], Zhang Wenyan[3], Küçük Can[1]

[1]İzmir International Biomedicine and Genome Institute (iBG-İzmir), Dokuz Eylul University, İzmir, Turkey; Department of Medical Biology, Faculty of Medicine, Dokuz Eylul University, İzmir, Turkey
[2]İzmir International Biomedicine and Genome Institute (iBG-İzmir), Dokuz Eylul University, İzmir, Turkey
[3]Department of Pathology, West China Hospital of Sichuan University, Chengdu, Guangxi, China
[4]İzmir International Biomedicine and Genome Institute (iBG-İzmir), Dokuz Eylul University, İzmir, Turkey; Department of Clinical Medicine, Guilin Medical University, Guangxi, China
[5]Department of Pathology, City of Hope Medical Center, Duarte, California

Follicular lymphoma (FL) is the second most frequent lymphoma with limited knowledge regarding its etiology. REL, a proto-oncogene located on frequently amplified 2p16.1-p15 locus, has been known to promote tumorigenesis in many cancer types through deregulation of the NF-κB pathway; however, its role in FL pathogenesis has not been addressed.
In this study, we evaluated REL copy number status with q-PCR in FFPE FL tumors. Using the same tumor samples we determined REL mRNA expression with q-RT-PCR and then investigated whether there is any association between REL amplification and mRNA expression, which did not show a notable correlation. REL conserved coding sequence analysis with PCR-Sanger did not reveal any oncogenic mutation in FL tumors. However, REL amplification correlated with B symptoms and high grade disease. Next we ectopically expressed c-REL in a FL cell line, and observed moderate level of positive selection under limiting serum concentrations in support of an oncogenic role.
To sum up, REL may have a marginal role in FL pathobiology, and other genes in 2p16.1-p15 locus may have a more pivotal role.
Keywords: Amplification, FL, NF-κB, Oncogene, REL

S-025 - ACHIEVEMENT OF BETULINIC ACID ON EGFR INITIATED SIGNALLING

Gul Huseyin, Demiroglu Zergeroglu Asuman

Gebze Technical University, Department of Molecular Biology & Genetics, Gebze/Kocaeli

Introduction: It is recognized that unrestrained activation of Epidermal Growth Factor Receptor (EGFR) signalling contributes the progression of cancers including Malignant Mesotheliomas. Betulinic acid (BA) is a plant derived compound, which has an anti-carcinogenic activity mostly associated with apoptosis. The aim of this work is to investigate the effect of BA on EGF induced signal pathways in Malignant Mesothelioma (MM) cells.
Material Methods: MeT-5A (Mesothelial) and SPC212 (MM) cell lines were used as models for treatments. Cell viability was measured by MTS assay; protein phosphorylations and gene expressions were assessed by western blot and RT-qPCR.
Results: BA reduced viability of MM cells in a concentration and time dependent manner. The viability of mesothelial cells were also decreased, but only at high concentrations and late time periods. BA inhibited phosphorylation of *EGFR*, *MAPK/ERK*, *PI3K/AKT* and *STAT* proteins but induced *JNK* and *p38* proteins in MM cells. qRT-PCR analysis revealed that *STAT3* and *STAT5* mRNA levels were down regulated in BA treated cells.
Discussion and Conclusion: The effect of BA on the viability of MM cells is more potent than that of mesothelial cells. This suggests that BA reduces cell viability and it is more cytotoxic to malignant cells than normal cells. Our results corroborate with current reports indicating that BA shows selective cytotoxicity to some tumour cells, but not to normal cells *in vitro*. In addition BA is able to inhibit EGF induced cancer proliferative and survival pathways in MM cells. Thus, according to our results, we propose that BA can be thought of a promising chemotherapeutic agent with potential future use in the treatment of MMs with uncontrolled EGFR signalling.
Keywords: Betulinic acid, EGFR, ERK1/2, AKT, STAT

S-026 - CHARACTERIZATION OF PROTEIN-PROTEIN INTERACTIONS BETWEEN OF THE MO25α AND CCM3 SCAFFOLD SIGNAL TRANSDUCERS AND THE STK25 PROTEIN KINASE

Ağca Can Ali[1], Hergovich Alexander[2]

[1]*Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Bingol University, Bingol, Turkey; UCL Cancer Institute, University College London, WC1E 6BT, London, United Kingdom.*
[2]*UCL Cancer Institute, University College London, WC1E 6BT, London, United Kingdom.*

Backround: The Ste20 (sterile 20) proteins are a large family of serine/treonine kinases, which are involved in a number of biological functions such as the regulation of cell proliferation, programmed cell death (apoptosis) and cell differentiation. In this study, we aimed to expand the horizon of our current understanding of the regulation of the serine/threonine kinase 25 (STK25), a member of the Ste20-like kinase family, by the disease-associated scaffold proteins CCM3 and MO25α.
Material and Methods: To characterize the interactions of STK25 wild-type (wt) and mutant variants with the signal transducers CCM3 or MO25α, human embryonic kidney HEK293 cells were transfected with myc-tagged STK25 versions and HA-tagged CCM3 or MO25α, and their interactions analysed by a series of co-immunoprecopitation experiments.
Results: Our results suggest that STK25 carrying mutations at L386D/A389D/L408D/V409D,L386D/A389D, orA389D display impaired interactions with CCM3. In contrast, the L386D/A389D/L408D/V409D variant of STK25, like STK25(I67A), interacted normally with MO25α, while the STK25(E54A) mutant did not bind to MO25α.
Conclusion: Our findings collectively suggest that two residues of STK25 are essential for the interactions of STK25 with CCM3 and MO25α, respectively. Ala389 of STK25 is required for complex formation with CCM3, while Glu54 of STK25 is central for the association of STK25 with MO25α. Thus, we describe here the identification and characterisation of STK25 mutants that allow the dissection of the importance of protein-protein interactions between STK25 and CCM3 and MO25α, respectively.
Keywords: MO25α, CCM3, STK25, co-immunoprecopitation

S-027 - THE ROLE OF YAP1 IN PROSTATE CANCER TUMORIGENESIS

Kisaayak Collak Filiz[1], Demir Ummuhan[1], Sagır Fatma[1], Ozkanli Seyma[2], Engin Zerk Pınar[2], Kurum Esra[3]

[1]*Istanbul Medeniyet University, Faculty of Engineering and Natural Sciences, Molecular Biology and Genetics Department*
[2]*Istanbul Medeniyet University, Faculty of Medicine, Pathology Department*
[3]*Yale School of Public Health*

Introduction: YAP is a transcriptional co-activator negatively regulated by Hippo Tumor Suppressor Pathway. The pathway inactivates YAP by phosphorylating and increasing its cytoplasmic localization through binding with the 14-3-3 proteins. Deregulation of the pathway has been shown to have a role in tumorigenesis and metastasis including prostate cancer (PCa).
Methods: Immunohistochemical staining of YAP1 and phosphorylated YAP1 (pYAP1) at S127 protein was used to assess the expression of proteins in prostate tumor tissues. The Kruskal Wallis H-test and postdoc pairwise comparison test were used to determine the statistical significance of the data. siRNA oligonucleotides were used to knockdown the expression of YAP1 and their effects on prostate cancer cells were investigated using cell viabilty, proliferation, migration, invasion, clonogenic and anchorage-independent growth assays.
Results: IHC in PCa tissues revealed YAP1 staining intensities were moderate to weak in the nucleus and cytoplasm of the tumor cells, whereas the adjacent normal epithelial showed strong staining. pYAP1 staining was not observed in the nucleus of tumor and normal cells. There was a significant association between YAP1 staining intensity and extraprostatic extension (EPE). In cultured cells, YAP1 expression increased in the whole cell lysates of AR negative (PC3 and DU145) compared to AR positive (LNCaP) cell lines and primary prostate epithelial cells. Treatment of LNCaP and PC3 with YAP1-targeting siRNA oligonucleotides (YAP1 siRNA) significantly reduced their proliferation in vitro. Furthermore, treatment with YAP1 siRNA diminished the clonogenicity and anchorage-independent growth on soft agar and migration and invasion of PC3 cells, suggesting a role of YAP1 in PCa tumorigenesis.
Conclusions: Loss of function experiments in LNCaP and PC3 revealed that YAP1 potentially plays an important role in migration and invasion. Importantly, in vitro results were supported by data from human tumors; clinically high expression of YAP in prostate tumors is correlated with EPE.
Keywords: Hippo Pathway, YAP1, Prostate Cancer, Migration, Invasion

S-028 - NEGATIVE EFFECTS OF MYELOMA CELLS ON SENESCENT MESENCHYMAL STROMAL CELLS ANTI-TUMOUR PARACRINE ACTIVITY

Özcan Servet

Erciyes University Faculty of Science, Biology Department & Genome and Stem Cell Center - GENKÖK

Mesenchymal stromal cells can be found in many organismal tissues and plays an important role in tissue growth and repair. Cellular senescence is a process that results from a variety of stresses that lead to a state of irreversible growth arrest. This encounter is adamant by secreted specific factors that is called senescence associated secretory phenotype (SASP). These secreted factors effect neighboring cells that are sensitive to senescence and prevent them from entering the neoplastic process. SASP factors alert the normal tissue cells to stop supporting neoplastic cells. These factors have positive effects as well as negative effects. It was observed that SASP has a negative effect that accelerates the tumor growth in the late stage of tumorigenesis. Cancer cells are able to misuse SASP elements to survive and grow.
In this study, we cultivated cancer cells in the presence of naive senescent MSC conditioned media (CM) and evaluated their proliferation, DNA damage, apoptosis and senescence. Our findings indicated that senescent secretomes induced apoptosis or senescence on cancer cells. However, this anti-tumor activity became impaired when senescent cells had previous contact (primed) with cancer cells. Conditional media was collected from each group and the secreted proteins were isolated. The isolated proteins were identified by using LC-MS/MS and the data analysis was performed by using PANTHER, DAVID and Ingeniuty Pathway Analysis (IPA).
According to our findings, cancer cells can misuse SASP factors as they induce a change in protein production by interacting with senescent cells. Priming with myeloma cells induced the production of 55 proteins and repressed the expression or secretion of 102 proteins.
Repressed proteins generally belong to networks associated with senescence, apoptosis, catabolic or anabolic processes while expressed ones belong to ECM networks and the promotion of metastasis.
Keywords: Secretome, Proteome, Stem Cell, Senescence

S-029 - DOES MW RADIATION AFFECT GENE EXPRESSION, APOPTOTIC LEVEL AND CELL CYCLE PROGRESSION OF HUMAN SH-SY5Y NEUROBLASTOMA CELLS?

Kayhan Handan[1], Esmekaya Meric Arda[2], Yar Saglam Atiye Seda[3], Canseven Ayse Gulnihal[2], Yagci Munci[1], Seyhan Nesrin[3]

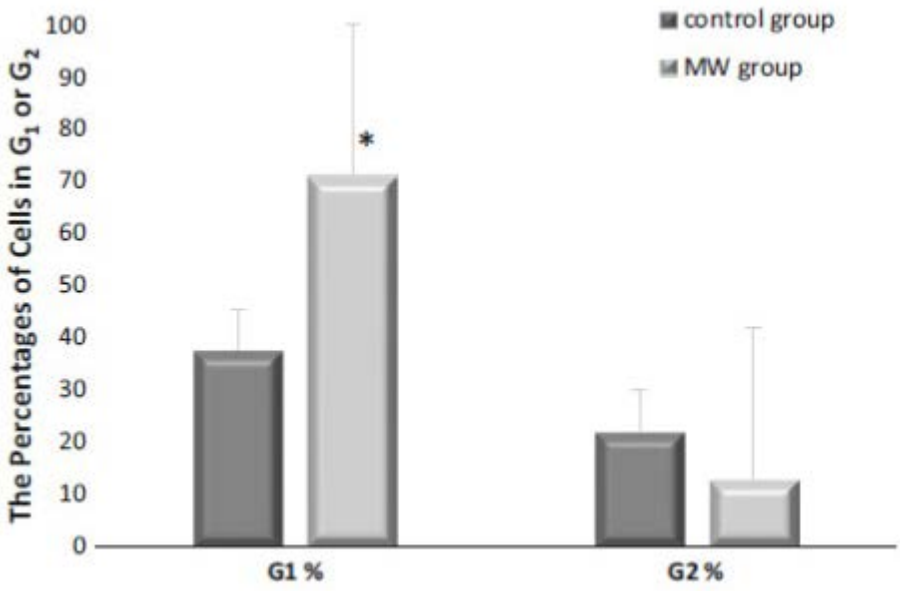
[1]*Gazi University Faculty of Medicine Department of Adult Hematology, Ankara, Turkey*
[2]*Gazi University Faculty of Medicine Department of Biophysics, Ankara, Turkey*
[3]*Gazi University Faculty of Medicine Department of Medical Biology, Ankara, Turkey*

Neuroblastoma (NB) is a cancer that occurs in sympathetic nervous system arising from neuroblasts and nerve tissue of the adrenal gland, neck, chest, or spinal cord. It is an embryonal malignancy and affects infants and children. In this study, we investigated the effects of Microwave (MW) radiation on apoptotic activity, cell viability and cell cycle progression in human SH-SY5Y NB cells which can give information about MW radiation effects on neural cells covering the period from the embryonic stages to infants.

SH-SY5Y NB cells were exposed to 2.1 GHz W-CDMA modulated MW radiation for 24 hours (h) at a Specific Absorption Rate (SAR) of 0.491 W/kg. Control samples were in the same conditions with MW exposed samples but they were not exposed to MW radiation. The apoptotic activity of cells was measured by Annexin-V-FITC and propidium Iodide (PI) staining. Moreover, mRNA levels of proliferative and cell cycle proteins were determined by real time RT-PCR. The change in cell cycle progression was observed by using CycleTest-Plus DNA reagent. No significant change was observed in apoptotic activity of MW exposed cells compared to control cells. The mRNA levels of *c-myc* and *cyclin D1* were significantly reduced in MW group ($p<0.05$). The percentage of MW exposed cells in G1 phase was significantly higher than the percentage of control cells in G1 phase. MW radiation caused cell cycle arrest in G1 phase. These results showed that 2.1 GHz W-CDMA modulated MW radiation did not cause apoptotic cell death but changed cell cycle progression.

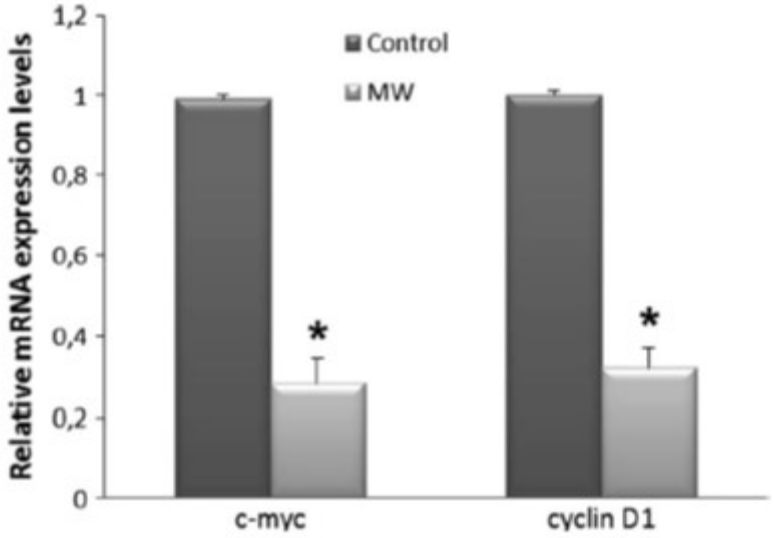
Keywords: Microwave radiation, Neuroblastoma, SH-SY5Y, *c-myc*, *cyclin D1*

Cell cycle results



Cell cycle analysis of RF and Sham groups of neuroblastoma cells. The SH-SY5Y cells with (a) or without synchronization of cell cycle (b). It can be seen that RF group cells were not significantly higher in G1 phase of cell cycle compared to sham (p>0.05).

Quantitative real-time PCR results



*Quantitative real-time PCR results showing c-myc and cyclin D1 mRNA expression levels in SH-SY5Y cells. The bars represent expression levels normalized to ABL as the housekeeping gene and relative to control group. *p< 0.05. mRNA: messenger RNA; PCR: polymerase chain reaction.*

S-030 - INVESTIGATE THE ANTITUMOR EFFECTS OF ÇEMEN EXTRACT

Çinar Şerife[1], Bozkurt Özlem[2], Yılmaz Seher[3], Ertekin Tolga[4], Nisari Mehtap[4], Şeker Karatoprak Gökçe[5], Ülger Harun[4]

[1]*KTO Karatay University, Faculty of Medicine, Department of Anatomy, Konya*
[2]*Haci Bektas Veli Nevsehir University, Semra and Vefa Kucuk Health College, Nevşehir*
[3]*Bozok University, Faculty of Medicine, Department of Anatomy, Yozgat*
[4]*Erciyes University, Faculty of Medicine, Department of Anatomy, Kayseri*
[5]*Erciyes University, Faculty of Pharmacy, Department of Pharmacognosy, Kayseri*

Background and Aim: Currently cancer was identified of one of the leading causes of death. Therefore studies on cancer is increasing day by day. Çemen that was made by mixture of fenugreek, red pepper, garlic, cumin, black pepper, clove, coriander, cinnamon, ginger and pimento. In this study, we investigated to antitumor effect of extract that derived from çemen on Ehrlich ascites tumor carrying Balb/C mice.
Materials and Methods: Çemen extract concentration determined 200-400mg/kg; 250-500 and 1000µg/ml respectively in vivo and in vitro studies. In vitro study, while at the end of 3 and 24 hours cell culture, cells counted, in vivo study tracking weight were performed in animal experiments. In the end of experiment, acid fluid volume and cell number of intraperitoneal fluid were calculated. Metastasis of EAT cells were evaluated histologically on abdominal organs.
Results: Our founding that çemen extract delaying the weight gain due to proliferation of EAT cells. The number of cells in the assits fluid were statistically lower (p=0.041) in the group that given 400mg/kg çemen extract (47.28x106) than control group (67.60x106). After 3 hours cultur period, there were no significant difference between groups in the number of viable cells. After 24 hours cell cultures, the viable cells number were significantly decrease in the treatment groups (5.7±0.2,5.7±0.2and5.6±0.1) when compared to control group (5.9±0.2) (p=0.013). Tissues that taken from abdominal organs on control and treatment groups were evaluated histopathologically. While there was intensive EAT cells adhesion on the tissues that taken from control group, there was reduciton in the treatment groups.
Discussion and Conclusion: As a result, the çemen extract shows antitumor effect on EAT cells. We believe that our studies will be guiding for new studies about çemen and çemen could be adviced as a food because of its anticancer effect.
Keywords: Allium sativum L., Capsicum annuum L., Çemen, Ehrlich ascites tumor, Trigonella foenumgraecum L

S-031 - THE REAL TIME MONITORIZATION OF THE CYTOTOXIC EFFECTS OF VANADIUM PENTAOKSIDE ON DIFFERENT CANCER CELL LINES.

Yerer Aycan Mükerrem Betül, Arslan Ayşe Kübra, Öztürk Ebru, Dokumacı Alim Hüseyin

University of Erciyes, Faculty of Pharmacy, Dept. of Pharmacology, 38039, Kayseri

Introduction: Conventional cancer treatment's relapse rate is often high and since mortality of the patients are high, new anticancer drugs are being sought day by day. Vanadium compounds have various pharmacological effects and the recent evidences reveal that it might be one of the new generation promising metal drugs of the future. In this study, the cytotoxicity of the vanadium compoun V2O5 has been investigated on A549, Colo205 and MCF7 cell lines in a real time manner via Real Time Cell Analyzer.
Materials and Methods: To examine the cytotoxic effects of the compound vanadium pentoxide, on A549, Colo205 and MCF7 cell lines were seeded to plates as 12.500cells/well., and healthy fibroblast cells as 3000cells/well. 6 different doses of vanadium pentoxide (250µM, 200µM, 150µM, 100µM, 50µM, 25µM) were applied to examine the effects to the cell lines and Cell indexes were profiled to evaluate the cytototoxic effect and the IC50 levels were calculated.
Results: IC50 levels were calculated for each cell at 12th and 24th h. For MCF7 The IC50 level for 12th h was 64.14uM, where as it was 118.58uM and 136.9UM for Colo205 and A549cell lines respectively. Our studies revealed that the vanadium compound containing vanadium pentoxide element has been found to reduce cell viability in a dose dependent manner and this is the known first study profiling the real time effects of the compound on cell lines used.
Conclusion: Vanadium pentoxide, coming from a new generation metal based drugs with various pharmacological effect is promising to be one of the promising medicine. These results are further mechanism of action studies can be studied to outline the effectiveness of the compounds on these cell lines. The combination of conventional anticancer drugs can be used to increase the effectiveness and reduce the side effects of these drugs.
Keywords: Vanadium pentaokside, A549, MCF7, Colo 205, Xcelligence

S-032 - THE DETECTION OF CURCUMINS' ANTITUMORAL EFFECTS VIA ARGYROPHILIC NUCLEOLAR ORGANIZING REGION–ASSOCIATED PROTEIN SYNTHESIS IN MICE WITH EHRLICH'S ASCITIC CARCINOMA

Nisari Mehtap[1], Yılmaz Seher[2], Eröz Recep[3], Ertekin Tolga[1], Bircan Duygu[3], Ülger Harun[1]

[1]*Department of Anatomy, Erciyes University School of Medicine, Kayseri, Turkey*
[2]*Department of Anatomy, Bozok University School of Medicine, Yozgat, Turkey*
[3]*Department of Medical Genetics, Düzce University School of Medicine, Düzce, Turkey*

Background: Curcumin is a polyphenol compound that has antioxidant, anticancer, anti-inflammatory, anti-hyperlipidemic and antimicrobial effects. Nucleolar-organizing regions are the sites of the gene on chromosomes. The present study was aimed to show the antitumoral effect of curcumin via AgNOR protein synthesis in Ehrlich's ascitic carcinoma (EAC) bearing mice.
Methods: Twenty three mice with EAC were randomly divided to 3 groups as positive control (n=7), group 2 (n=8) and 3(n=8) treated intraperitoneally with curcumin (25 mg/kg) and (50 mg/kg), respectively. The animals were sacrificed on 16 d, the solid tumors were removed out. Then, total AgNOR area/nuclear area (TAA/NA) and mean AgNOR number were estimated for each mice.
Results: Statistically significant differences were determined among whole groups for TAA/NA ratio (p=0.000), conversly mean AgNOR number (p=0.361). In comparison of two groups; while no difference was determined between control and curcumin (25 mg/kg) groups (p=0.061), the significant differences were detected between control and curcumin (50 mg/kg) groups (p=0.000) and between curcumin (25 mg/kg) and curcumin (50 mg/kg) groups (p=0.000) for TAA/NA ratio. However there was no significant difference for mean AgNOR number in double comparison of the groups.
Conclusion: The current study showed that curcumin has a crucial function against cancer development. Also both AgNOR values may be used as biomarkers for detection of most reliable therapeutic dose selection of cancer treatment.
Keywords: AgNORs, Cancer Treatments, Curcumin, NOR, rDNA

S-033 - THE USING OF AGNOR PARAMETERS FOR DISCRIMINATION OF BENIGN AND MALIGN BREAST LESION

Köksal Mehmet[1], Doğan Serap[1], Eröz Recep[2], Öztürk Figen[1], Öztürk Ahmet[1], Cücer Nurhan[1]

[1]*Erciyes Üniversitesi Kayseri*
[2]*Düzce Üniversitesi Düzce*

Introduction: In the worldwide, the most common type of cancer in women is breast cancer. Therefore the development of early diagnostic tests for breast cancer is very important. Total amount of AgNOR proteins is related to the cell proliferation rate. Thus, we evaluated the potential of the AgNOR parameters for being a useful tool for the diagnostic and prognostic purposes in distinguishing malignant and benign breast lesions.
Materials and Methods: For the comparision of the benign and malignant breast lesions using with AgNOR staining technique, three groups consist of control (n=14), benign (n=18) and malignant (n=28) were included in the study. The AgNOR staining technique was performed for slides of each individual and both mean AgNOR number and total AgNOR area/Nuclear Area (TAA / NA) ratio were evaluated via a special computer program. Fifty nuclei for each individuals were evaluated and mean AgNOR number and TAA/NA were counted and measured.
Results: The mean AgNOR number and TAA/NA were detected as 1.09 ± 0.54 and 2.51 ± 0.11 for control group, respectively. These values were 2.29 ± 1.13 and 4.21 ± 1.07 for benign and 3.03±1.86 and 6.55±2.73 for malign, respectively. According to the data, the differences were statistically significant for TAA/NA values for all comparison combinations between the three groups ($p < 0.001$). A statistically significant difference was detected among control and both benign and malignant group for mean AgNOR area/Nuclear Area ($p < 0.001$). But the difference between benign and malignant group was not significant for mean AgNOR number ($p > 0.05$).
Conclusion: As a result, we thought that the evaluation of TAA/NA rate, when compared with the AgNOR number, to be a more sensitive and useful tool for distinguishing benign and the malignant breast lesions from each other.
Keywords: AgNOR, Brast Cancer, FNAB

Bu çalışma Erciyes Üniversitesi BAP birimi tarafından TDK-2013-4723 koduyla desteklenmiş, üniversite etik kurulu tarafından 2013/193 karar numarası ile onaylanmıştır.

S-034 - IN VITRO ANTIOXIDANT PROPERTIES AND THE EVALUATION OF ANTIPROLIFERATIVE AND APOPTOTIC ACTIVITIES ON HELA, MCF-7, OE-33 AND HEPG2 CELL LINES OF THE EXTRACTS FROM MEDICINAL PLANT *GENISTA LYDIA VAR. LYDIA* (FABACEAE)

Tarhan Leman[1], Tongul Burcu[2], Kavakçıoğlu Berna[1]

[1]*Dokuz Eylül Üniversitesi, Fen Fakültesi, Kimya Bölümü, İzmir*
[2]*Dokuz Eylül Üniversitesi,Fen Bilimleri Enstitüsü Kimya Anabilimdalı, İzmir*

Background and Aim: *Genista lydia* has been used to treat menopausal symptoms, estrogen related diseases as prostate and breast cancers, osteoporosis and cardiovascular diseases in traditional medicine because of its known phytoestrogen content. It was aimed to investigate the antioxidant capacities and also cytotoxic and apoptotic effects of G. lydia extracts on HeLa, MCF-7, OE-33 and HepG2 cell lines.
Materials and Methods : The antioxidant properties were evaluated by measuring OH• and DPPH radical scavenging activity, total phenolic-flavonoid content, reducing power and metal chelating capacity. While colorimetric assay was used to evaluate cytotoxic activity, fluorometric assays were performed to assess the apoptotic activity of the extracts.
Results: The highest OH• and DPPH scavenging activities were found in EA_L and EA_F extracts with the IC_{50} value of 9.75 and 250.00 µg/ml, respectively. While the highest total phenolic content was found in the ChlF extract as 152.36 mg gallate/g, the highest total flavonoid content was observed in WL with 96.92 mg catchein/g. Met/W_L extract was found as the most effective in terms of its reducing power with the EC50 value of 1.51 mg/mL and metal chelating capacity with 8.65%. While the most effective extract on HeLa proliferation was Met/W_L, Met/W_F was the most effective one on MCF-7 and OE-33 cells. Any of the extracts could not show cytotoxic effect on HepG2. The percentage of apoptotic HeLa cell increased from 3.9% to 49.1% in Met/W_L treated group. While this induction was relevant with the loss of $\Delta\psi m$, independent from caspase-3 and 9. Although impressive apoptosis induction could not been observed in MCF-7, the percentage of apoptotic cell death increased from 12.9 to 72.9% in OE-33 treated with Met/$W_F$but caspase-3 and -9 independently.
Discussion and Conclusion: *G. lydia* extracts showed apoptotic activities on HeLa and OE-33 independent from caspase -3 and -9 activities.
Keywords: *Genista Lydia Var. Lydia* (Fabaceae), Antioxidant Activity, Cytotoxicity, Antiproliferative Effect, Apoptosis

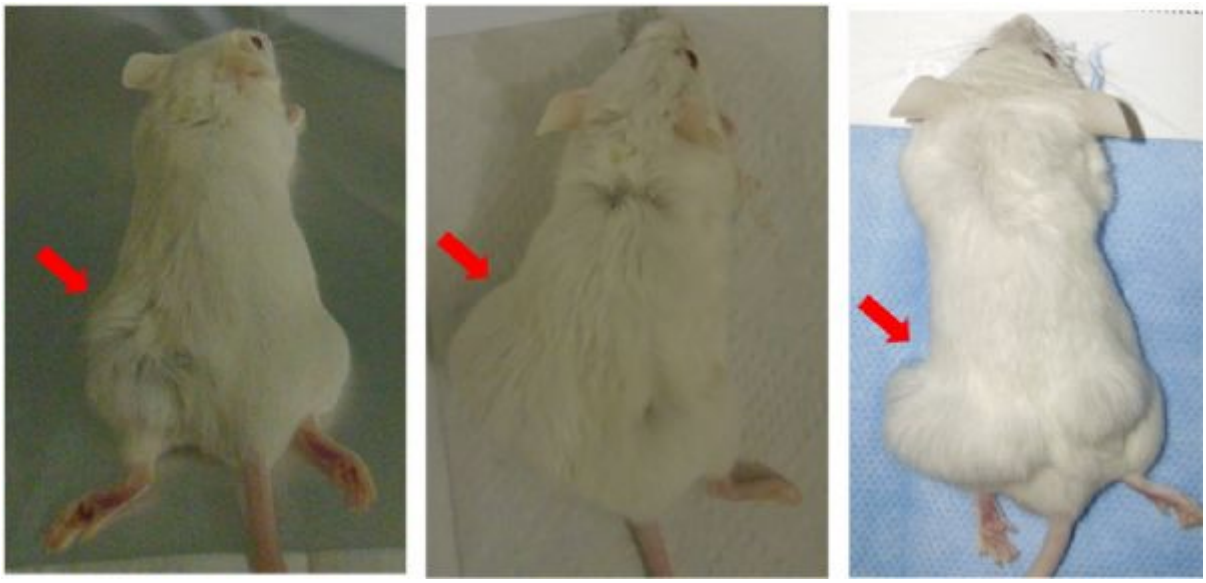
S-035 - CHARACTERIZATION OF NOVEL WNT/β-CATENIN PATHWAY TARGETS

Akiva İzzet, Birgül İyison Necla

Boğaziçi University, Molecular Biology and Genetics department, İstanbul

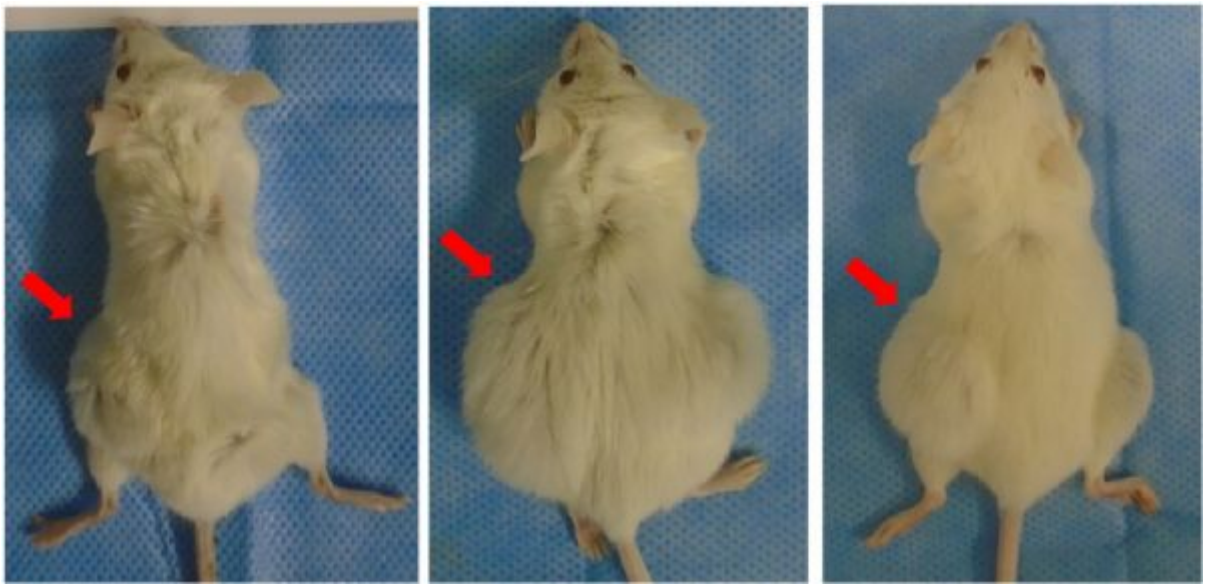
Introduction and Purpose: Wnt/β-catenin signaling pathway is an evolutionary conserved pathway which has important functions in vertebrate development, axis formation, cellular proliferation and morphogenesis. Apart from its roles in various cellular processes, Wnt/β-catenin signaling pathway is also one of the most important intracellular pathways for cancer progression. Previous studies confirmed BRI3 gene to be one of the transcriptional target genes of this pathway and MGAT1 gene was determined to be among the putative target genes. The main purpose of this study is further characterization of these candidate molecules in order to elucidate their biological roles and eventual implications in cancer.
Material and Methods: Yeast-two-Hybrid Assay, Coimmunoprecipitation, Confocal Microscopy, Overexpression studies in Huh7-Hepatocellular Carcinoma cells, Luciferase Reporter Assay, Western Blotting, Quantitative Real-Time-PCR Analysis, Cell Proliferation and Cell Migration Assay, Xenograft Assay in NUDE/SCID mice, RNA-Sequencing.
Results: Functional characterization of novel Wnt/β-catenin pathway targets has been carried out by using various approaches. Among these; cell proliferation and migration assays showed that, Huh7 cells stably expressing each of the BRI3 and MGAT1 genes have greater proliferative and invasive capabilities compared to control Huh7 cells. Furthermore, in vivo xenograft experiments were performed and it was determined that the stable overexpression of both of these genes in Huh7 cell lines lead to tumorigenesis in NUDE/SCID mice.
Discussion and Conclusion: As a result of xenograft assays, BRI3 and MGAT1 are determined to have tumorigenic effect when overexpressed in stable cell lines. RNA-Sequencing is used in order to determine the possible interacting pathways in their cancer initiation process. IFITM3 and MGAT1 proteins were confirmed as novel binding partners for BRI3 by Y2H and Co-IP techniques. BRI3 is upregulated in response to TNF-α treatment and overexpression of BRI3 leads to an increase in NFkB promoter activity. MGAT1 is a putative novel target of Wnt/β-catenin signaling pathway and is upregulated in response to β-catenin activation.
Keywords: Wnt/β-catenin pathway, BRI3, MGAT1, Xenograft, RNA-Sequencing

Xenograft on SCID mice - BRI3 vs control



Xenograft on SCID mice with BRI3 overexpressing cells on the left side and control (GFP) expressing cells on the right side of each mice

Xenograft on SCID mice - MGAT1 vs control



Xenograft on SCID mice with MGAT1 overexpressing cells on the left side and control (GFP) expressing cells on the right side of each mice

S-036 - THE EFFECTS OF ACEYTL-L CARNITINE ON CISPLATIN AND RADIATION INDUCED APOPTOSIS TREATMENT ON MEDULLOBLASTOMA CELLS

Pamukoglu Kaynar Ayca[1], Altun Zekiye[1], Ercetin Ozdemir Ayse Pinar[1], Olgun Yuksel[2], Cetinkaya Oguz[3], Aktas Safiye[1], Olgun Nur[4]

[1]Basic Oncology Department, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey
[2]Otorhinolaryngology Department, Dokuz Eylul University, Izmir, Turkey
[3]Radiation Oncology Department, Dokuz Eylul University, Izmir, Turkey
[4]Pediatric Oncology Department, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey

Aim: Cisplatin and radiotherapy are commonly used regimens in the treatment of pediatric malignant tumors such as medulloblastoma. Acetyl-L-carnitine (ALC) is a natural compound and it has protective effects against CDDP induced toxicities. The effects of ALC on apoptotic cell death mechanism of CDDP and if radiotherapy (RT) added with cisplatin therapy on medulloblastoma cells whether they will change cell death mechanism were aimed.
Methods: HTB-186 medulloblastoma cells were maintained in DMEM containing 5% FBS at 37°C. Cells were incubated with CDDP, RT, ALC and combinations by 24 hours. Cell viability was measured with using WST-1 test. The LD50 doses of 75uM CDDP, 5Gy RT, 25uM ALC and combinations were detected by viability assay. The apoptotic cell death evaluated with Annexin-PI analyzed with Flow Cytometry. Mann-Whithney U test used for statistical evaluation and $p<0.05$ was accepted as a significant level.
Results: Apoptotic cell death was apoptosis 16% in the control group, 77,9% in the 75uM CDDP and 28,95% 5Gy RT group. Apoptotic cell death was apoptosis 15,8% in the 25uM ALC, 48,1% 25uM ALC+75uM CDDP and 35,3% 50uM ALC+5Gy RT group. Apoptotic cell death was apoptosis 86,3% in the 75uM CDDP+5Gy RT and 49 % in the 25uM ALC+75uM CDDP+5Gy RT group.
Conclusion: CDDP and RT apoptotic cell death were increased compared to that in the control group. ALC+CDDP and ALC+RT apoptotic cell death were decreased compared to that in the CDDP and RT group. ALC decreased the apoptotic cell death of cells with CDDP and RT treatment affected in medulloblastoma cells.
Keywords: Medulloblastoma, Cisplatin, Radiotherapy, Acetyl-L-Carnitine, Apoptosis

S-037 - ANTIPROLIFERATIVE AND APOPTOTIC EFFECTS OF THYMOL (*THYME VULGARIS*), A NOVEL MONOTERPENE PHENOL, IN THE PC-3 AND DU-145 HUMAN PROSTATE CANCER CELL LINES

Elbe Hulya[1], Yigitturk Gurkan[2]

[1]*Mugla Sıtkı Kocman University, Faculty of Medicine, Department of Histology and Embryology, Mugla*
[2]*Ege University, Faculty of Medicine, Department of Histology and Embryology, Izmir*

Background: Prostate cancer is one of the most common malignant tumors and the leading cause of cancer related death in men. Many anticancer drugs currently used clinically have been isolated from plant species or are based on such substances. Accumulating data has revealed anticancer activity in plant-derived monoterpenes. Conventional treatment of prostate cancer has been proven to be effective but there many highly undesirable side effects. Thus, an alternative chemotherapy agent is needed that has similar efficacy of conventional chemotherapy with minimal side effects. Thymol (5-methyl-2-isopropylphenol) is an oxygenated aromatic compound from monoterpene group. It is the main constituent of thyme essential oil and shows antioxidant, antiseptic and antiproliferative properties. The aim of this study is to determine the antiproliferative activity and apoptotic effect of thymol on PC-3 and Du-145 human prostate cancer cells.
Methods: PC-3 and DU-145 cell lines were treated with different concentrations of thymol (100, 200, 400, 600, 800 µM) at 24 h, 48h and 72h. The cell viability was investigated by MTT assay and analysis of apoptosis with annexin V assay was determined by the Muse® Cell Analyzer.
Results: The study clearly showed the dose and time-dependent cytotoxic effect of thymol in PC-3 and DU-145 cell lines. The half maximal inhibitory concentration (IC50) values of thymol at 24h, 48h, 72h were 799, 721, 448 µM and 711, 601, 552 µM, respectively. Thymol significantly induced apoptosis in all groups as dose-dependent. Statistical analysis showed significant difference between thymol treated cell lines compared to control ($p<0.001$).
Conclusion: The data in the present study clearly demonstrated that thymol has apoptotic and antiproliferative properties towards PC-3 and DU-145 prostate cancer cell lines. Thymol could have a potential therapeutic significance in treating cancer.
Ethic protocol no: 2016/132.
Keywords: Thymol, Prostate Cancer, Apoptosis, Antiproliferative

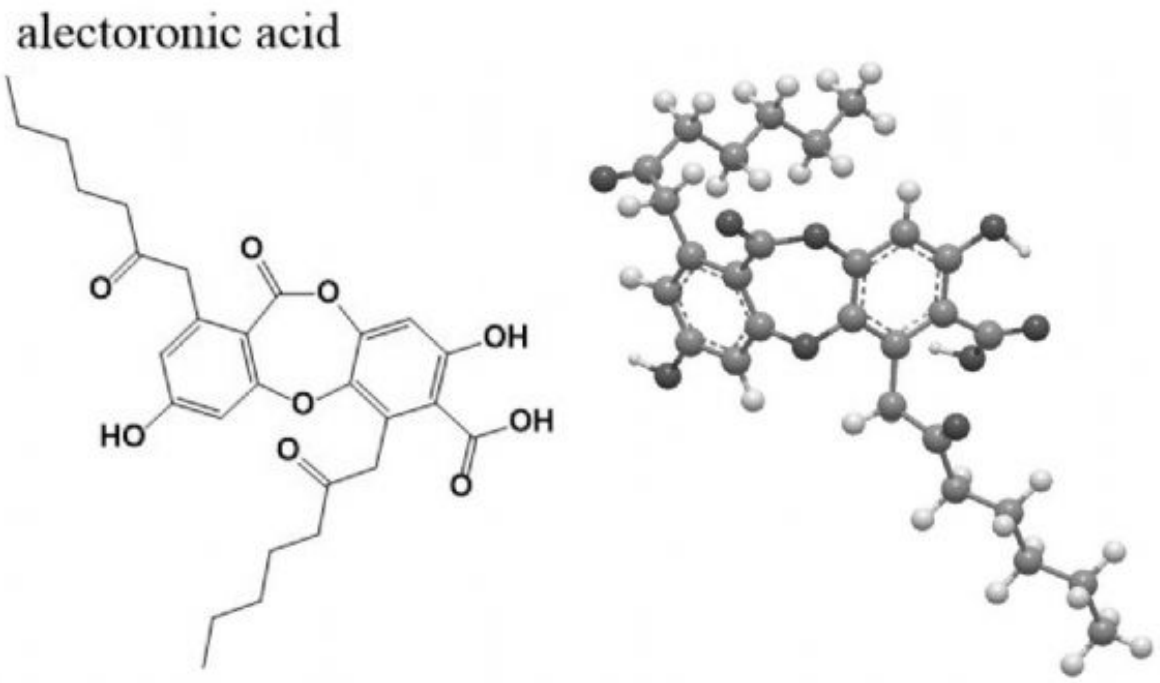
S-038 - DYNAMIC ASSESSMENT OF ANTIPROLIFERATIVE AND ANTIMIGRATORY ACTIVITIES OF THE NATURAL SMALL-MOLECULE ALECTORONIC ACID BY USING REAL TIME CELL ANALYZERS (RTCA)

Varol Mehmet[1], Dikmen Miriş[2]

[1]*Department of Molecular Biology and Genetics, Faculty of Science, Mugla Sitki Kocman University, Kotekli Campus, 48000, Mugla, TURKEY.*
[2]*Department of Pharmacology, Faculty of Pharmacy, Anadolu University, Yunusemre Campus, 26470, Eskişehir, TURKEY.*

Introduction: Many drugs originate from the natural sources, and lichens have a great potential to produce unique compounds which have been found pharmacologically active against many biological targets due to their unique structures as a result of the collective metabolism within their complex symbiotic structures composed by heterotrophic mycobionts and autotrophic photobionts [1].
Material and Methods: The natural small-molecule named “alectoronic acid” was isolated from the Tephromela atra (Fée) and chemical characterization was realized by using 1H-NMR, IR and melting point analyses. The anti-proliferative activity was determined on human endothelial cells (HUVEC), breast carcinoma cell line (T-47D) and cisplatin-resistant breast adenocarcinoma cell line (HCC1428) by using xCELLigence RTCA MP. The antimigratory activity of alectoronic acid, it was previously established as anti-angiogenic compound by using endothelial tube formation assay, was evaluated on HUVECs by performing RTCA DP system.
Results: The obtained results showed that alectoronic acid blocks the proliferation of all of the three cell lines by depending on its increasing concentration and application time, and the proliferation of T-47D cells were more effected than HCC1428. Interestingly, alectoronic acid showed no significant anti-proliferative activity on healthy endothelial cells except the high concentrations such as 200 and 400 µM. The antimigratory activity study by using the non-toxic 25, 50 and 100 µM concentrations indicated that alectoronic acid dramatically inhibits endothelial cell migration in a correlation with angiogenesis study.
Conclusion: Consequently, alectoronic acid might be potential anti-cancer drug ingredient among the anti-angiogenic, anti-migratory and tumor suppressor activities for the prolong tumor control as some of the other lichen substances [1-2].
Keywords: Alectoronic Acid, Angiogenesis, Lichen, Migration, Proliferation.

Chemical structure of Alectoronic Acid



S-039 - ANTI-CANCER EFFECT OF URFA PISTACHIO (*PISTACIA VERA*) GREEN HULL EXTRACT ON COLON ADENOCARCINOMA CELLS

Koyuncu İsmail[1], Koçyiğit Abdurrahim[3], Yüksekdağ Özgür[2], Güler Eray Metin[3], Gönel Ataman[1], Kirmit Adnan[1]

[1]*Harran University, Faculty of Medicine, Department of Medical Biochemistry, Sanlıurfa, Turkey*
[2]*Harran University, Faculty of Arts and Sciences, Department of Biology, Sanlıurfa, Turkey*
[3]*Bezmialem Vakif University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, Turkey*

Introduction: *Pistacia vera* L. is among the top fifty nutrition products that have the highest antioxidant potential due to its rich phenolic compound content and therefore is regarded to be a unique food. Also, it contains bioactive polyphenols such as isoflavones and trans-resveratrols that have anti-cancer potential. In the present study, we have investigated cytotoxic and apoptotic effect of P.vera green hull extract on cancer cells.
Materials and Methods: *P.vera* green hull (skin) were extracted in different solvents, sequentially. The obtained five extracts were analysed for their in vitro anti-cancer properties, using the MTT assay, on five human carcinomas: colon (HT-29, DLD-1), breast (MCF-7, MDA-MB-231), prostate (PC-3), endometrium (ECC-1) and cervix (HeLa) cancer and normal PNT-1A cell lines. The cells were incubated with different doses of extracts (5 to 500 µg/ml) for 24 hours. The cell viability was assessed via MTT assay. Apoptotic effects of hexane extracts on HT-29 cells were analysed by using flow cytometry annexin V analyse. Anti proliferative effects of compounds were determined through BrdU Elisa assay. Intra-cellular accumulation of reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) was determined via using the fluorescent probes. Genotoxicity was evaluated by alkaline single cell gel electrophoresis assay (Comet Assay) methods. The quail / quantitative determination of phenolic compounds in hexane extracts were determined by GC/MS-MS and LC/MS-MS.
Results: The best anti-cancer activity was observed for the hexane (Hex) extract of *P.vera* green hull on HT-29 cell lines. Its hexane (Hex) extract showed a low activity against PNT-1A cancer cell lines. Hexane extract has also shown cytotoxic, genotoxic, apoptotic and ROS generating effects in a dose-dependent manner. However, further studies at molecular level are required to support our findings and to elucidate chemotherapeutic effects of this extract on colon cancer.
Keywords: Pistacia Vera, Colon Cancer, Apoptosis

**This study supported by HÜBAK. (HÜBAK Project no: 15057).*

S-040 - DIFFERENTIAL CO-EXPRESSION NETWORK IN OVARIAN CANCER: PROGNOSTIC AND THERAPEUTIC TARGETS

Gov Esra, Arga Kazım Yalçın

Department of Bioengineering, Marmara University, Istanbul, Turkey

Ovarian cancer is one of the leading causes of death amongst gynaecological disorders. Interactions such as protein-protein, protein-RNA interaction can change with the environmental, genetic perturbations, and disease formation. Integrative differential coexpression analysis have been successfully used to identify active gene module in disease specific conditions. In the present study we analysed six gene expression datasets for serous ovarian cancer by comparing gene expression levels between laser microdissected epithelial carcinoma and healthy ovarian epithelial cells. Afterwards, ovarian cancer differential co-expression network was reconstructed and co-expressed gene modules were defined to identify disease genes and new interactions depend on disease specific gene expression profile. Corresponding largest component included module were investigated. Topological and functional enrichment analysis were performed. Furthermore, prognostic transcriptional regulatory elements (i.e: TFs and miRNAs) of module were investigated. It was identified 698 mutual differential expressed transcripts in six datasets. Topological analyses results show that while this module contained highly connected genes groups (network density: 0.88), this module was destroyed in healthy state (network density: 0.03). Co-expressed 84 prognostic genes which are a group of highly interconnected genes in ovarian cancer were identified. These genes (20%) are related to signalling by GPRC and (10%) metabolism associated pathways and 35 of them cannot be categorized in any biological pathway. As the regulators, the TFs GATA2, YBX1, AR, ETS3 and FOXP3 as well as the miRNAs miR-335-5p, miR-4284, miR-190a-3p, miR-16-5p, and miR-26b-5p dominated the disease-specific sub-network regulating excessive number of co-expressed genes. Our results demonstrate new insights on determine the ovarian cancer related prognostic genes based on differential coexpression analysis, and here we present reciprocal interplay between candidate ovarian cancer genes and transcriptional regulatory dynamics.

Keywords: Differential Coexpression, Prognostic Genes, Ovarian Cancer

S-041 - THE ROLE OF BIOMARKERS IN THE FOLLOW-UP OF PATIENTS WITH MALIGNANT MESOTHELIOMA

Ayhancı Adnan[1], Metintaş Muzaffer[2], Ak Güntülü[2], Yılmaz Şenay[2], Boğar Filiz[2], Teksoy Özgün[1]

[1]Eskişehir Osmangazi University, Faculty of Medicine, Department of Thorax, Eskişehir,Turkey
[2]Eskişehir Osmangazi University Faculty Science and Letters Department of Biology, Eskişehir, Turkey

Malignant mesothelioma (MM) is the primary tumour affecting the mesothelial cell wall that form in the pleura (90%), the peritonea (6-19%) and the pericardia, which is often attributable to asbestos. It is usually an aggressive, incurable type of cancer, the global residence of which keeps increasing. While clinical findings are not restricted to its early symptoms, a large number of patients with malignant mesothelioma are diagnosed late. Unfortunately, chemotherapy is the only choice for an anti-tumour treatment, and the average survival time is about 13 months. Therefore, however difficult it may be, an early diagnosis is a potential key factor to making good progress in treating Malignant Pleural Mesothelioma (MPM). Currently, there have been many commendable attempts to help early diagnosis and/or to provide more effective markers. Recent studies have introduced such promising biomarkers as CERC/M (mesothelin), NERC/M (megakaryocyte-potentiating factor), OSP (osteopontin) and HYA (Hyaluron). It is hoped that osteopontin (OPN) will be detected in early stages of MM. Osteopontin (OPN) is regulated by the protein in the cell-signal paths that mediates between cell-matrix interactions and cell signals by binding to integrin and CD44 receptors, which is also a protein associated with asbestos-based carcinogenesis. It is unfortunate that cases MPM are on the increase in Turkey, whether due to occupational reasons or due to exposure to asbestos and erionite in rural areas. The present study aims to investigate the effects of the difference in the levels of CERC / M, NERC / M, Fibulin 3, Syndecan 1, HYA, OPN and Midkine in the blood samples of patient with MPM, in addition to determining if these markers work in pre-treatment, especially in different responses to the treatment process like complete response, incomplete response, stable disease and progressive disease, and if they can be utilised in assessing the response of tumours.

Keywords: Asbestos, Biomarkers, Fibulin 3, Malignant Pleural Mesothelioma, Mesothelin

S-042 - EFFECTS OF DIFFERENT DIET TYPES ON DNA DAMAGE AND INFLAMMATION IN BREAST CANCER

Çoban İlker[1], Çiçekdal Burcu[2], Güvenç Tuna Bilge[3], Aydın Ahmet[4], Doğan Soner[5]

[1]*Department of Physiology, Yeditepe University Medical Faculty,Istanbul,Turkey*
[2]*Department of Biotechnology, Yeditepe University,Istanbul,Turkey*
[3]*Department of Biophysics, Yeditepe University Medical Faculty,Istanbul,Turkey*
[4]*Department of Toxicology, Yeditepe University Faculty of Pharmacy,Istanbul,Turkey*
[5]*Department of Medical Biology,Yeditepe University Medical Faculty,Istanbul,Turkey*

Aim:Previous studies has reported that obesity has effects on all steps of carcinogenesis including cancer initiation by inducing DNA damage via the obesity induced chronic inflammation. Inflammation induced nuclear kappa B, reactive oxygen species, specific miRNAs andproinflammatory cytokines(PIC)may trigger the emergence of many cancer types including breast cancer(BC).Calorie restriction(CR)is suggested to be effective for the prevention of carcinogen induced or spontaneously emerged mammary tumors in rodents. Although exact mechanisms of CR on BC prevention could not totally be elucidated, the link might be attributed to diet induced changes in oxidative stress status and PIC levels. The aim of this study is to analyze the effect of different diet types on BC development and to understand the link between PIC(IL1-a,IL6,TNFα)levels and BC.Methods: C57/BL6 (MMTV-TGFα+) 10 weeks old mice were divided into 4 groups: Ad-libitum(AL),chronic calorie restriction(CCR),intermittent calorie restriction (ICR-R and ICR-RF). Mice fed with different type of diets for the following 7-8 weeks. 8-OHdG levels (a biomarker for DNA damage) were measured from serum by, 8-OHdG ELISA assay kit, Elabscience and PIC levels were measured from liver homogenizates by Milliplex cytokine assay kit, Merck-Millipore.Results: Our results showed that CR has an impact on 8-OHdG and PIC levels. 8-OHdG levels were significantly decreased(%45) in CCR group compared to AL group. There was no significant difference between ICR groups. IL1-a levels significantly increased in CCR group compared to AL group. CR group had significantly more IL6 levels than AL group. However, TNFα levels of CCR group were not significantly different compared to other groups.Conclusions: We can conclude that CR might prevent or delay the development of BC by lowering 8-OHdG induced DNA damage and changing the expression of PIC.Acknowledgement:This project was supported by the grand from(project no:114S100 and 114S894)Turkish Scientific and Tecnological Research Council(TUBITAK).

Keywords: Breast Cancer, Calorie Restriction, Dna Damage, Inflammation

S-043 - DIETARY CALORIE RESTRICTION ALTERS OXIDATIVE STRESS BIOMARKERS IN MMTV-TGF-ALPHA MICE

Cicekdal Munevver Burcu[1], Hamitoglu Muhammed[2], Coban Ilker[4], Aydin Ahmet[2], Guvenc Tuna Bilge[3], Dogan Soner[1]

[1]*Yeditepe University Faculty of Medicine, Department of Medical Biology, Istanbul*
[2]*Yeditepe University Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul*
[3]*Yeditepe University Faculty of Medicine, Department of Biophysics, Istanbul*
[4]*Yeditepe University Faculty of Medicine, Department of Physiology, Istanbul*

Introduction and Aim: Breast cancer is a major health problem among women in worldwide. It is known that obese women have an increased risk factor for breast cancer. The aim of this study is to understand the effects of different calorie restriction (CR) types on oxidative stress biomarkers in a transgenic breast cancer mouse model.
Material and Methods: C57/BL6 (MMTV-TGF-α+) mice were enrolled in the study at 10 weeks of age into ad libitum-fed (AL), Chronic Caloric Restriction (CCR, %15 CR), and Intermittent Caloric Restriction [ICR, 3 weeks of AL (ICR-R) and 1 week of %60 CR (ICR-RF) in a cyclic periods] groups. Mice were euthanized at 10 (base), 18 or 50 weeks old. To determine the oxidative stress status lipid peroxidation as evidenced by malondialdehyde (MDA) and the status of the antioxidants superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured in erythrocytes and liver by using spectrophotometric methods.
Results: Lipid peroxidation in erythrocytes of AL group was enhanced by age compared to the base ($p<0.05$) while, there was no significant difference due to aging in CCR group. MDA levels of ICR-R group increased at week 18 and decreased at week 50 ($p<0.05$). MDA levels of liver were not different in all groups. GPx levels were higher in ICR-RF group compared to other groups at week 50 ($p<0.05$). SOD levels in erythrocytes was increased in AL group by age ($p<0.05$). CAT levels were decreased in all groups at week 18 compared to base in liver ($p<0.05$), an increase in CAT levels for ICR-R group were determined at week 50 compare to early ages.
Conclusion: These results displayed that CR has an impact on oxidative stress parameters. Moreover, ICR group demonstrated higher protective effect against oxidative stress than CCR group. This project is supported by TÜBİTAK (114S100).
Keywords: Oxidative Stress, Calorie Restriction, Transgenic Mice, Breast Cancer, İntermittent Calorie Restriction

S-044 - EFFECTS OF MEAL FREQUENCY AND CALORIE RESTRICTION ON METABOLISM IN RATS

Savas Hasan Basri, Gultekin Fatih

Department of Medical Biochemistry, Faculty of Medicine, Alanya Alaaddin Keykubat University, Alanya, Antalya, Turkey

Objective: In this project, the effects of meal frequency and caloric restriction on metabolism were examined as a whole.
Materials and Methods: As a result of a one-month pilot study amount of food and meal times was determined. In the main study, Wistar albino, 12 weeks, male, 24 rats were divided into three groups as; Ad libitum control (AL) (n = 8), two meals fed group (TM) (n = 8), two meals fed and 20% calorie restriction of group (TM-CR) (n = 8). All rats were kept individually in cages. According to the results of a pilot study in main study; 20 g/day; 10 g for the morning and evening meals were given to TM group, 16 g/day; for the morning and evening meals were given feed in the form of 8 grams to TM-CR group. All rats finished the nutrition regulation in a healthy way for 20 weeks. At the end of experiment rats were sacrificed. Basic metabolic hormones and enzymes, biochemical parameters were measured with ELISA and spectrophotometric method.
Results: In TM and TM-CR group, according to the AL group; HOMA-IR ratio showed a significantly decreasing ($p < 0.05$). At the beginning of experiments, there were no significant differences each of the three groups of rats body weight ($p > 0.05$), at the end the experiment; there was significant differences among the three groups (AL> TM> TM-CR) ($p < 0.05$).
Conclusions: Significant differences between HOMA-IR and body weight showed that TM and TM-CR nutrition are useful protects against insulin resistance, obesity, diabetes and other related diseases like some cancers.
Keywords: Nutrition, Meal Frequency, Caloric Restriction, Metabolism, İnsulin Resistance, Obesity, Cancer.

This study was supported by Suleyman Demirel University OYP Coordination Unit with ÖYP05333-DR-12 project number and Suleyman Demirel University BAP with 4476-ÖYP-D2-15 project number. Ethical issue: All of the this study's procedures were approved by the Suleyman Demirel University Head of the Local Ethics Committee of Animal Experiments. (Approving date: 21.05.2015, number: 21438139-172).

S-045 - THERAPEUTIC EFFECT OF CANCER DRUGS ON MFE-319 ENDOMETRIAL CARCINOMA CELL LINE

Aydemir Işıl[1], Türköz Uluer Elgin[1], Korkmaz Oya[1], Tuğlu Mehmet İbrahim[1], İnan Sevinç[2]

[1]*Department of Histology and Embryology, Faculty of Medicine, Manisa Celal Bayar University, Manisa, Turkey*
[2]*Department of Histology and Embryology, Faculty of Medicine, İzmir University of Economics, İzmir, Turkey*

Introduction and Aim: Endometrial cancer is the most common carcinoma of the female reproductive tract. Various types of endometrial cancer are affected women such as type I and type II. Type II endometrial tumors are generally more invasive, estrogen receptor and progesterone receptor (ER/PR) negative. MFE-319 endometrial carcinoma cell line is an aggressive form like type II cancer. In our study we aimed to determine the therapeutic effect of metformin, cisplatin and paclitaxel on MFE-319 cell line using MTT and immunocytochemistry assay.
Materials and Methods: MFE-319 cells were seeded in 96-well plate and different dilutions of cancer drugs were applied and MTT assay was used. IC50 doses of metformin, cisplatin and paclitaxel were calculated. For immunocytochemistry, cells were exposed to the IC50 doses of metformin, cisplatin and paclitaxel alone and in combination for 24 h. Then cells were stained with PI3K/akt signal pathway which is critical for cell survival and cell growth markers PI3K, pErk1-2, akt-1, Pakt-1-2-3 and also angiogenic factor VEGF. Immunoreactivities were evaluated H-score and analyzed using One-Way ANOVA test statistically.
Results: Immunoreactivities of PI3K, pErk1-2, akt-1 and Pakt-1-2-3 were higher in metformin application than the other drugs. Cisplatin was decreased the immunoreactivities of PI3K, pErk1-2, akt-1 and Pakt-1-2-3. VEGF staining was the highest in control and was diminished in cisplatin application. It was ascertained that these drugs caused decrease in the immunoreactivities in the following order of potency: cisplatin>paclitaxel>metformin. And also comparison of these drugs showed the same effect in the combination exposes: paclitaxel+cisplatin>metformin+ paclitaxel>metformin+cisplatin.
Conclusion: These results were showed that cisplatin and paclitaxel were more effective than metformin. Cisplatin and paclitaxel can be used in the treatments of the invasive endometrial cancer focused PI3K/akt signal pathway. Metformin needs further studies for its use in the cancer therapies
Keywords: Endometrial Carcinoma, MFE-319, PI3K/akt, Apoptosis

S-046 - CYTOTOXIC, GENOTOXIC, APOPTOTIC AND REACTIVE OXYGEN GENERATING EFFECTS OF CARVACROL ON HUMAN FIBROBLAST (WS-1) AND GASTRIC ADENOCARCINOMA (AGS) CELLS

Güneş Bayır Ayşe[1], Kocyigit Abdurrahim[2], Güler Eray Metin[2]

[1]*Department of Nutrition and Dietetics, Faculty of Health Sciences, Bezmialem Vakif University, Istanbul, Turkey*
[2]*Department of Medical Biochemistry, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey*

Carvacrol is a natural phenolic compound from the plants of Family Lamiaceae. It's some beneficial effects were reported such as antimicrobial, anti-inflammatory, and antioxidant. Additionally, anti-cancer effect was also determined on human colon carcinoma, liver carcinoma, ovarian adenocarcinoma, cervical, breast, lung cancer cells in vitro. Recently, it has been reported that loading of carvacrol in combination with chemotherapy agents into the human serum albumin nanoparticles may treat gastric cancer cells better than single drug loaded nanoparticles. However, anticancer mechanism of carvacrol has not yet been fully elucidated. Thus, the aim of this study was to explore the potential anticancer activity of carvacrol on human AGS cells. The results were statistically compared with human fibroblast (WS-1) cells which have also exposed to 0-600 µM carvacrol.
In both cell cultures carvacrol after 24 h of exposure showed cytotoxic, genotoxic, apoptotic and reactive oxygen species (ROS) generating effects in a dose-dependent manner. Significant differences exist after exposure of carvacrol in both cell cultures in the sense of cell viability, ROS generation, and DNA damage ($p < 0.001$). The results were statistically significant at the all same concentration which was applied to both cell cultures ($p < 0.05$). Interestingly, carvacrol at lower dose (10 µM) effect significantly on the proliferation of WS-1 cells ($p < 0.01$), and decrease the ROS generation ($p < 0.01$) in respect to the control cells. In general, a close negative relationship was found between cell viability and ROS level. In conclusion, carvacrol causes cytotoxic, genotoxic, apoptotic, and ROS generating effects on AGS cells more effectively than the WS-1 cells *via* its pro-oxidant activity.
Keywords: AGS Cells, Apoptosis, Carvacrol, Genotoxicity, WS-1 Cells

S-047 - EFFECTS OF FISETIN ON GLIOMA CELL PROLIFERATION AND APOPTOSIS

Öztopcu Vatan Pinar[1], Pak Fulya[2]

[1]*Department Of Biology, Faculty Of Arts And Sciences, Eskisehir Osmangazi University,Eskisehir,Turkey*
[2]*Graduate School Of Natural And Applied Sciences,Eskisehir Osmangazi University,Eskisehir,Turkey*

Aims: Glioblastoma multiforme (GBM) remains the most aggressive and resistant brain tumor in adults. Besides a limited number of drugs, therapy resistance is the major obstacle for efficient treatment of GBM. Fisetin is a natural flavonoid. In this study the effects of fisetin on the cell morphology, proliferation and the apoptosis on glioma cells were evaluated.
Materials: The cytotoxic and the morphologic effects of fisetin (1 to 500 µM) were examined in T98G human glioma cells by inverted microscope and MTT assay. Carmustine was used as positive control and human bronchial epithelium (BEAS-2B) cells were used to see the morphological and the cytotoxic effects of the fisetin in healthy cells. Alterations on the T98G cell morphology by fisetin treatment were also analyzed by transmission electron microscopy. DNA fragmentation analysis and quantitative real time PCR (QRT-PCR) were used to evaluate the apoptotic effects of the treatment.
Results: The IC50 values of fisetin were determined as 93 and 75 µM for T98G, and 270 and 90 µM for BEAS-2B cells, respectively in 24 and 48 h. For the selected fisetin doses an increased apoptotic cell death on T98G cells when compared to BEAS-2B cells. We observed prominent expression of apoptotic genes CASPASE 3, 9, 8, BAX and decreased expression of BCL-2 and SURVIVIN in T98G cells.
Conclusion: According to the findings of this study, fisetin was found to have more efficient cytotoxic and apoptotic effects in T98G cells than normal cells, depending on the dose and the time. Additional in vivo and in vitro studies will show the place of this chemical in the treatment of glioma in the future.
Funding: This study was supported by Eskisehir Osmangazi University, Scientific Research Projects Committee (Project number: 201319A112).
Keywords: Apoptosis, Cytotoxicity, Fisetin, Glioma, QRT-PCR

S-048 - PREDICTION OF ENDOCRINE THERAPY RESPONSE AND RESISTANCE IN BREAST CANCER CELLS BY EXPLOITING THE MITOCHONDRIA AND ESTROGEN RECEPTOR STATUS

Karakas Bahriye[1], Giray Kurt Asli[2], Temel Sehime Gulsun[3, 4], Gul Ozgur[5], Basaga Huveyda[1], Kutuk Ozgur[2]

[1]*Molecular Biology, Genetics and Bioengineering Program, Sabanci University, Istanbul, Turkey*
[2]*Department of Medical Genetics, Baskent University School of Medicine, Adana Dr. Turgut Noyan Medical and Research Center, Adana, Turkey*
[3]*Department of Histology and Embryology, Near East University School of Medicine, Nicosia, Northern Cyprus*
[4]*Department of Histology and Embryology, Uludag University School of Medicine, Bursa, Turkey*
[5]*Dept. of Genetics and Bioengineering, Bilgi University, Istanbul, Turkey*

Background: Breast cancer is the leading cause of death among women globally. Estrogen receptor status is important prognostic factor and anti-estrogen (endocrine) therapy is the choice of first-line treatment in ER-positive breast cancer cases. Resistance to treatment and tumor recurrence often occur even though targeted therapies exist. Therefore, accurate biomarkers are needed to predict which individuals will respond to endocrine therapy.
Objective: In this study, we aim to explore how estrogen receptors affect mitochondrial cell death priming and endocrine therapy response in breast cancer cells. Methods: We use a novel assay called BH3 profiling to measure how close the mitochondria for the apoptosis. ER status is determined with RT-qPCR and immunoblotting. CellTiter-Glo is used to measure cell viability in response to endocrine therapy. Confocal immunofluorescence microscopy is used to determine localization of ER isoforms.
Results: Differential expression of estrogen receptor isoforms were detected in both RNA and protein level. Endocrine therapy agents have similar EC50 values regardless of ER-α and ER-β expression status. Breast cancer cells have different mitochondrial priming status. Immunofluorescence analysis revealed mitochondrial localization of both receptors in addition to nucleus and cytoplasm.
Conclusion: Our initial results indicate mitochondria might be targeted by estrogens and this point outs the role of estrogen receptors in endocrine therapy response and breast cancer progression. Our work highlights the promising potential of using BH3 profiling assay in prediction of breast cancer endocrine therapy response.
Keywords: Breast Cancer, Estrogen Receptor, Endocrine Therapy, Mitochondria, BH3profiling

This work was supported by grants from TUBITAK (SBAG-113S481), Baskent University Research Fund and The Science Academy.

S-049 - INHIBITION OF O6-METILGUANINE-DNA METILTRANSFERASE (MGMT) ACTIVITY ENHANCES MELPHALAN CYTOTOXICITY IN MULTIPLE MYELOMA

Akcora Yildiz Dilara[1], Özkan Tülin[2], Yükselten Yunus[2], Özkanca Şeyma[2], Beksaç Meral[3], Sunguroğlu Asuman[2]

[1]Department of Biology, Art&Science Faculty, Mehmet Akif Ersoy University, Burdur, TURKEY
[2]Department of Medical Biology, Faculty of Medicine, Ankara University, Ankara, TURKEY
[3]Department of Hematology, Faculty of Medicine, Ankara University, Ankara, TURKEY

Background and Aim: Multiple Myeloma (MM) is the second most prevalent hematologic cancer manifested by proliferation of malignant plasma cells in the bone marrow. Despite introduction of novel agents such as new immunomodulators and proteasome inhibitors MM is still incurable. High-dose melphalan (L-PAM), an alkylating agent, used with stem cell transplant support increases response rates and progression-free survival. Melphalan is known to induce cytotoxicity because of the production of interstrand crosslinks, which are formed through the intermediate production of O6-alkylguanine. O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein, removes alkylating and methylating adducts from O6-guanine in DNA. As increased DNA repair activity has been implicated in protection of cancer cells from apoptosis, in this study we investigated whether pharmocological inhibition of MGMT activity enhances Melphalan cytotoxicity in MGMT proficient MM cells.
Materials and Methods: Protein expression of MGMT was investigated in MM cells by Western blotting and found that RPMI 8226 and NCI H929 cells have MGMT protein expression, wheras U266 cells are MGMT deficient. Then, RPMI 8226 and NCI H929 were incubated with MGMT inhibitor Lomeguatrib alone, Melphalan alone and Lomeguatrib in combination with Melphalan for 48 hr. Cell viability and apoptosis were assessed by MTT and Annexin V assays, respectively. DNA damage levels were examined by alkaline comet assay and immunoblotting of DNA repair proteins and Ƴ-H2AX phosphorylation.
Results: Apoptosis was found to be further increased by combined treatment with Lomeguatrib and Melphalan in MGMT proficient MM cells. In RPMI 8226 cells alkaline comet assay showed increased DNA damage levels and Western blot analysis revealed PARP cleavage, reduction in pCycE1 and DNA repair proteins in addition to an increase in phosphorylation of ϒH2AX levels.
Conclusion: Lomeguatrib seems to enhance Melphalan cytotoxicity in MGMT proficient MM cells by perturbation of DNA repair capacity. This research has been supported by The Scientific and Technological Research Council of Turkey (No:113Z383).
Keywords: MGMT, Melphalan, DNA repair, Apoptosis

S-050 - CYTOTOXIC, GENOTOXIC AND APOPTOTIC EFFECTS OF CURCUMIN IN DIFFERENT CELL LINES

Guler Eray Metin, Kocyigit Abdurrahim

Bezmialem Vakif University School of Medicine Department of Medical Biochemistry

Background: Gliomas are aggressive brain tumors with poor prognosis. Curcumin is one of the phenolic compounds. It has been known that turmeric compounds are used to treatment of many diseases due to its anti-inflammatory, anti-oxidant and anti-cancer properties. Curcumin inhibits the growth of some kinds of tumors. However, the effect of curcumin on cancer senescence is unclear.
Purpose: In this study we performed in vitro experiment to determine prooxidant, cytotoxic, genotoxic, and apoptotic effect of curcumin on different cell lines.
Materials and Methods: Glioma and healthy cell lines were treated with different doses of curcumin and incubated for 24 h. After incubation, genotoxic effect of curcumin was measured by comet assay. Cytotoxic effect was evaluated by ATP cell viability assay. Phenol & flavonoid and antioxidant effects are measured by prooxidant activities. To determine apoptotic effect of curcumin by western blotting and acridine orange staining methods at below the half maximal inhibitory concentrations (IC50) levels. Mitochondrial membrane potential (MMP) methods were performed and observed by using flow cytometer. Intracellular accumulation of reactive oxygen species (ROS) was determined using the fluorescent probes 2', 7'-dichloro-dihydrofluorescein-diacetate.
Results: It was found that curcumin has a remarkable effect on the rate of cancer proliferation. Cytotoxic, genotoxic apoptotic and ROS generating effects in a dose dependent manner of curcumin.. There was a statistically significant negative relationship between cell viability and ROS and, positive correlation between DNA damage, apoptosis and ROS levels. These results revealed that curcumin induced DNA damage and apoptosis by generating much more ROS via its pro-oxidant activity in glioma cells than in normal cells.
Conclusion: Although we found the effect of curcumin on the glioma and healthy cell lines, further studies will be needed to identify the inhibition mechanism of curcumin clearly. Further analyses are needed to understand the cancer inhibition mechanism.
Keywords: Apoptosis, Curcumin, DNA damage, Oxidative stress

S-051 - CYTOTOXIC, GENOTOXIC AND APOPTOTIC ACTIVITIES OF OLIVE LEAF AND SUMAC EXTRACTS ON CANCER AND HEALTHY CELLS

Kaleli Hümeyra Nur, Güler Eray Metin, Koçyiğit Abdurrahim

Department of Medical Biochemistry, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey

Background: Olive leaf and sumac have been known as an antioxidant and anti-cancer agents. The anti-cancer properties are thought to be mediated by phenolic compounds present in olive leaf and sumac. Their effects on the different cells by measuring the level of cytotoxicity, genotoxicity, apoptosis, mitocondrial membrane potential and reactive oxygen species have been studied.
Objective and Purpose: The aim of this study is to investigate antioxidant, cytotoxic, genotoxic and apoptotic effect of olive leaf & sumac extracts on the human brain (C6) adenocarcinoma and human skin fibroblast cell lines.
Material and Methods: Total phenolic, flavonoid content, and antioxidant activities were determined using suitable methods as DPPH, antocyanin and prooxidant activity. C6 and CCD cells were incubated with different doses of olive leaf extract and sumac extract separately. After 24 h incubation of cells cytotoxicity, apoptosis and reactive oxygen species (ROS) generation were analyzed. Apoptotic effects were determined by annexin and mitocondrial membrane potential(MMP) methods. Genotoxicity was evaluated by Comet Assay. Cytotoxicity was analyzed by using ATP cell viability assay. Intracellular accumulation of ROS was determined using the fluorescent probes 2',7'-dichloro-dihydrofluorescein-diacetate.
Results: It was determined that extract have shown antioxidant activity in all tests and that they could be considered as a source of natural antioxidants. Cytotoxic effects were concentration-dose dependent manner. Specifically, apoptotic and genotoxic effect increased at 100 and 200 µg/ml concentrations by 24 hours. Our results shown that olive leaf and sumac extracts had more antiproliferative, genotoxic and apoptotic effects on the human cancer cell line than skin fibroblast normal cell line.
Conclusion: Further studies will be needed to use olive leaf and sumac extracs as phytotherapheutic agents for cancer therapy.
Keywords: Olive Leaf & Sumac Extracts, Antioxidant, Anticancer, Apoptosis, Genotoxicity

S-052 - HDAC INHIBITORS, MS-275 AND SALERMIDE, POTENTIATES THE ANTICANCER EFFECT OF EF24 IN HUMAN PANCREATIC CANCER CELLS

Yar Sağlam Atiye Seda[1], Yılmaz Akın[2], Önen Hacer İlke[1], Alp Ebru[3], Kayhan Handan[4], Ekmekçi Abdullah[1]

[1]Department of Medical Biology and Genetics, Faculty of Medicine, Gazi University, Besevler, Ankara, Turkey
[2]Department of Medical Biology, Faculty of Medicine, Hitit University, Çorum, Turkey
[3]Department of Medical Biology, Faculty of Medicine, Giresun University, Giresun, Turkey
[4]Department of Adult Heamatology, Faculty of Medicine, Gazi University, Besevler, Ankara, Turkey

Histone deacetylases (HDACs) play a major role in the regulation of chromatin structure and gene expression by changing acetylation status of histone and nonhistone proteins. MS-275 (entinostat, MS) is a well-known benzamide-based histone deacetylase inhibitor (HDACI) and Salermide (SAL), a reverse amide compound HDACI, have antiproliferative effects on several human cancer cells. In this study, we aimed to investigate the effects of HDACIs (MS and SAL) alone and/or combined use with EF24 (EF), a novel synthetic curcumin analog, on human pancreatic cancer cell line (BxPC-3). In vitro, BxPC-3 cells were exposed to varying concentrations of MS, SAL with orwithout EF, and their effects on cell viability, acetylated Histone H3 and H4 levels,cytotoxicity, cleaved caspase 3 levels, and cell cycle distribution were measured. The viability of BxPC-3 cells decreased significantly after treatment with EF, MS and SAL treatments. MS and SAL treatment increased the acetylation of histone H3 and H4 in a dose dependent manner. MS and SAL alone or combined with EF were increased the number of cells in G1 phase. In addition, treatment with agents significantly decreased the ratio of cell in G2/M phase. There were significant dose dependent increases at cleaved Caspase 3 levels after MS treatment but not after SAL treatment. Our results showed that HDAC inhibitors (MS and SAL), when combined with EF, may effectively reduce pancreatic cancer cell (BxPC-3) progression and stop the cell cycle at G1 phase. Further molecular analyses are needed to understand the fundamental molecular consequences of HDAC inhibition in pancreas cancer cells.

Keywords: EF24, HDACI, MS-275, Pancreatic Cancer, Salermide

S-053 - DOES CHEMOTHERAPEUTICS CONTRIBUTE TO DNMT1 EXPRESSION LEVEL IN COLORECTAL CANCER CELLS?

Varol Nuray[1], Arıkan Terzi Evrim Suna[2], Soylemez Zafer[1], Yildiz Salina Handan[1], Ozdemir Erdogan Mujgan[2], Solak Mustafa[1]

[1]*Afyon Kocatepe University, Faculty of Medicine, Department of Medical Genetics, Afyonkarahisar*
[2]*Afyon Kocatepe University, Faculty of Medicine, Department of Medical Biology, Afyonkarahisar*

Introduction and Aim: Epigenetic modifications, particularly DNA methylation in selected gene promoters is a pivotal role in the development of colorectal cancer. DNA methylation is considered as one of the most important epigenetic mechanisms and it is catalyzed by DNA methyltransferases (DNMTs). DNMT1 abundance has been frequently seen in colorectal cancers but the reasons are not well understood. We investigated to the effect of chemotherapeutics used in treatment of colorectal cancer on expression of DNMT1 and this effect is achieved over which signalling pathway.
Materials and Methods: Cell proliferation levels in HT29 cells treated with specific inhibitors (LY294002 for Akt1; SB216763 for GSK3β; IWP2 for β-catenin) and chemotherapeutics (oxaliplatin, fluorouracil, irinotecan) were detected by WST1. DNMT1 expression level was determinated by real-time PCR; and protein levels of GSK3β, pGSK3β(Ser9), Akt1, pAkt1(Ser473), β-catenin, pβ-catenin(Ser675) and DNMT1 by western blot.
Results: Our results indicated Akt1 increased the protein level of DNMT1 expression without coordinate transcriptional change via β-catenin pathway. Fluorouracil and irinotecan decreased DNMT1 expression both transcriptional and translational levels but not oxaliplatin. Oxaliplatin increased DNMT1 expression at mRNA and protein levels. This effect is achieved by specific phosphorylation of β-catenin protein.
Conclusion: The results revealed that use of some chemotherapeutic, particularly oxaliplatin, with spesific inhibitors combination led to a reduced DNMT1 expression. Our findings may offer a new approach for determination of molecular effects of β-catenin signal pathway on DNMT1. This may allow us to identify new molecular targets for the treatment of coloreactal cancers. However, the results revealed that some chemotherapeutics may contribute aberration of DNA methylation.
Keywords: Chemotherapeutics, DNMT, Akt1 signalling pathway, β-catenin signalling pathway, Colorectal cancer

S-054 - INVESTIGATING THE ROLE OF ALTERNATIVE POLYADENYLATION IN LUNG SQUAMOUS CELL CARCINOMA

Kazan Hilal

Department of Computer Engineering, Uluslararası Antalya Üniversitesi, Antalya, Turkey

Introduction: Polyadenylation is an RNA processing step that involves the cleavage of pre-mRNA at a poly(A) site and addition of the poly(A) tail. Approximately 70% of human genes contain multiple poly(A) sites and can undergo alternative polyadenylation (APA). APA could lead to the production of mRNA isoforms with variable lengths of 3'UTRs. Gain or loss of cis-regulatory regions in 3'UTR isoforms can alter mRNA stability and translation dramatically. In particular, a strong association has been found between proliferation and 3'UTR shortening through the use of proximal polyA sites. Previous studies that explored the effects of 3'UTR shortening have mainly focused on the regulatory effects of microRNAs (miRNAs); however, it is well known that 3'UTRs also harbor sites for several RNA-binding proteins (RBPs).
Methods: In this study, we developed a computational model that incorporates APA-related changes in both miRNA and RBP sites. To map miRNA sites, we utilized TargetScan predictions as well as Ago-CLIP-derived peaks. For RBP sites, we scanned the 3'UTRs with RNAcompete motifs, and also included CLIP-derived peaks [1, 2]. Then we used a recently proposed approach called DaPARS to infer proximal and distal APA sites from raw RNA-seq datasets for matched tumor-normal samples in lung squamous cell carcinoma.
Results: We identified the RBP and miRNA sites that are lost or gained due to APA and developed a regression model that links these alterations with gene expression changes [3]. Our analysis revealed a strong association between the loss of binding sites and downregulation for a number of RBPs. One of these RBPs is ELAVL1, a well-characterized stabilizing factor. Altogether, these results indicate that future studies of APA must incorporate the regulatory effects of RBPs in addition to miRNAs.
Keywords: post-Transcriptional Regulation, Alternative Polyadenylation, RNA-Binding Proteins, miRNAs, Regression

S-055 - CTLA-4 GENE +49 A/G POLYMORPHISM IN PROSTATE CANCER PATIENTS

Budak Diler Songül

University of Niğde, Faculty of Art and Science, Department of Biotechnology, 51200, Niğde, Turkey.

Background and Aim: Prostate cancer are the most common cancers in Western population and its rate is increasing in the Eastern World. The aim was to evaluate cytotoxic T lymphocyte associated antigen-4 (CTLA-4) gene +49 A/G polymorphisms in prostate cancer patients.
Methods: This study included 119 (68.94±8.38) healthy controls and 62 (72.83±7.60) patients with prostate cancer. The CTLA-4 +49 A/G (rs231775) gene regions were amplified using polymerase chain reaction (PCR), detected by restriction fragment length polymorphism (RFLP).
Results: At the end of our research, we found that the prevalence of genotypes of AA (wild-type), AG (heterozygous mutant) and GG (homozygous mutant) profiles for the CTLA-4 +49 A/G polymorphism were 50%, 45% and 5% respectively in prostate cancer patients, and 56%, 36% and 8% respectively in healthy control groups.
Conclusions: Any association was not found for CTLA-4 +49 A/G polymorphism between prostate cancer patients and the control groups in Turkish population.
Keywords: CTLA-4 +49 A/G polymorphism, Prostate cancer, PCR, RFLP

S-056 - DEMONSTRATION OF THE EFFECTIVENESS OF NEOADJUVANT CHEMOTHERAPY AND RADIOTHERAPY BY 18F-FDG PET-CT AND MRI IN PATIENTS WITH COLORECTAL CANCER

Gül Serdar Savaş[1], Rahatlı Samed[2], Güler Avcı Gülhan[3], Sönmezgöz Fitnet[4], Hasbek Zekiye[5]

[1]*Gaziosmanpaşa University, School of Medicine, Department of Nuclear Medicine, Tokat, Turkey*
[2]*Gaziosmanpaşa University, School of Medicine, Department of Internal Medicine, Tokat, Turkey*
[3]*Gaziosmanpaşa University, School of Medicine, Department of Radiation Oncology, Tokat, Turkey*
[4]*Gaziosmanpaşa University, School of Medicine, Department of Radiology, Tokat, Turkey*
[5]*Cumhuriyet University, School of Medicine, Department of Nuclear Medicine, Sivas, Turkey*

Aim: Colorectal cancer is one of the most common tumors of the gastrointestinal tract. The adjuvant therapy selected according to the stage and location of the disease affects the prognosis. In our study, the effectiveness of preoperative chemotherapy and radiotherapy in patients with colorectal cancer was evaluated by using Fluor-18 Fluorodeoxyglucose Positron Emission-Computerized Tomography (18F-FDG PET-CT) and Magnetic Resonance Imaging (MRI).

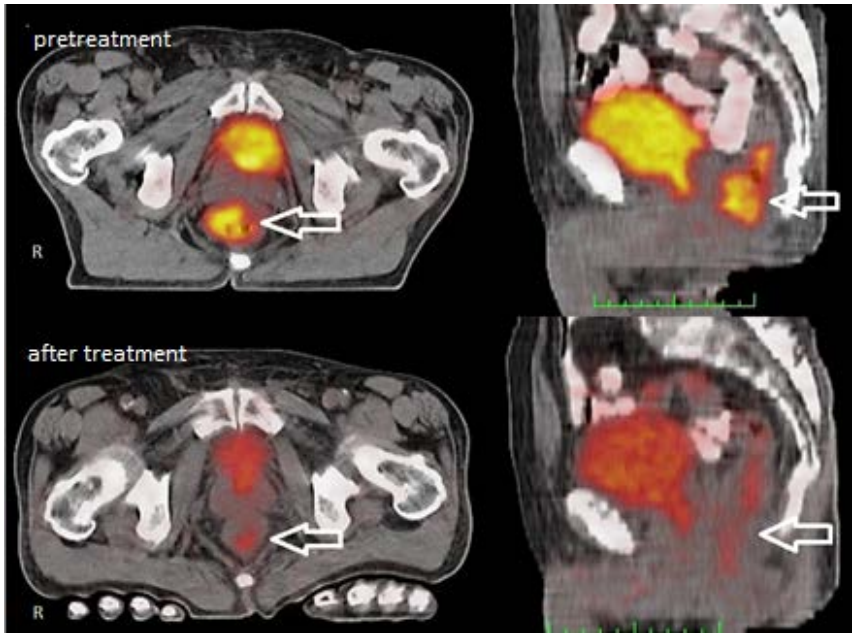
Methods: Twelve patients with a diagnosis of colorectal adenocarcinoma were included in the study (5 females and 7 males; mean age 68.1±11.8 years). Patients were treated with concomitant chemotherapy and radiotherapy before the surgery. Preoperative and postoperative F-18 FDG PET-CT and MRI images were obtained. PET-CT images were used to determine the tumor size, area and volume with the SUVmax and were compared to the tumor size, area and volume on MRI.

Results: Chemotherapy regimen of capacitamine 825mg/m2 two times daily for 5days/week was given to 8patients and 5-Fluouracil 225mg/m2 with an infusion pump for 7days was given to the remaining 4patients. Simultaneous radiotherapy included a total of 45Gy with a fractioned dose of 1.8Gy/day to the areas with standard risk and a total of 50Gy with a fractioned dose of 2Gy/day to the high-risk areas. Preoperative and postoperative F-18 FDG PET-CT and MRI images were obtained (Figure1). Both imaging methods revealed a significant decrease in the tumor size, volume and area after the treatment (Table1).

Conclusion: Effect of perioperative chemotherapy and radiotherapy on survival rate is an important research area in terms of adjuvant therapy with no available clear conclusions. Results of the present study suggest that chemotherapy and radiotherapy given before the surgery is an effective treatment method resulting in a significant decrease in tumor size which was shown in both PET-CT and MR images. In conclusion, F-18 FDG-PET-CT is considered to be a good alternative for conventional MRI.

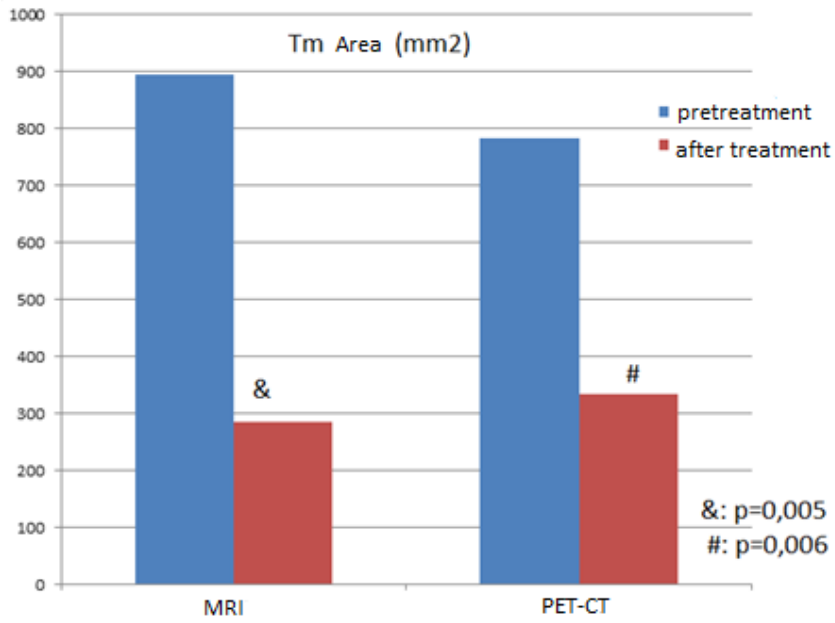
Keywords: Neoadjuvant Chemotherapy, Radiotherapy, 18F-FDG PET-CT, MRI, Colorectal cancer

Figure 1



The decrease in tumor size (white arrow) on transaxial and sagittal F-18 FDG-PET-CT images obtained before and after the surgery in patients with colorectal cancer.

Table 1



Comparison of the decrease in tumor size after the neoadjuvant chemotherapy and radiotherapy by using MRI and PET-CT in patients with colorectal cancer.

S-057 - FACTORS EFFECTING MORTALITY IN PATIENTS OPERATED DUE TO GASTRIC CARCINOMA

Kayılıoğlu Selami Ilgaz, Dinç Tolga, Göktuğ Ufuk Utku, Bostanoğlu Akın, Sonışık Muhittin, Coşkun Faruk

General Surgery, Ankara Numune Research and Training Hospital, Ankara, Turkey

The purpose of this study is to investigate the results of data obtained from the records of patients who were operated due to gastric carcinoma in our hospital, to determine whether the evaluated variables have an effect on mortality. Demographic information (age, gender, contact information, hospital registration, and citizenship number), types of surgery performed (total/subtotal gastrectomy), histopathological diagnosis (tumor size, lymph node calculation and status), pathological stage, serum albumin levels, tumor markers, complete blood count, and survival status of 170 patients who underwent surgery due to gastric carcinoma were observed and recorded. According to these data, metastatic lymph node ratio (MLR), red cell distribution width - platelet ratio (RPR), neutrophil - lymphocyte ratio (NLR), platelet - lymphocyte ratio (PLR), and prognostic nutritional index values were calculated. Results: According to the univariate analysis of the independent variables which effect mortality, NLR, MLR, age, stage, and gender parameters were found statistically significant ($p<0.05$). According to the multivariate analysis (Cox regression analysis), age, the stage of disease, and RPR were found statistically significant. For patients who underwent surgery due to gastric carcinoma, being over the age of 68, RPR rate higher than 0.038, and disease in stage 4 have been determined as prognostic factors which have negative effects on mortality.
Keywords: Gastric Carcinoma, Mortality, Prognostic Factors

Multivariate logistic regression model for the factors effecting general survival (95% CI)

Variables	Odds Ratio	95% CI	p value
Age	1.0475	1.0100 to 1.0864	0.0130*
Stage	2.6539	1.0277 to 6.8532	0.0448*
RPR	4.0480	1.2362 to 13.2555	0.0215*

*RDW: Red cell distribution width, RPR: red cell distribution width - platelet ratio, CI: Confidence Interval *P<0.05 significant.*

S-058 - BRCA2 AND RAD51 GENE EXPRESSION ANALYSIS OF CMTS

Özmen Özge[1], Kul Selim[2], Rişvanlı Ali[3], Kul Oğuz[4]

[1]*Ankara University, Faculty of Veterinary Medicine, Department of Genetics, Ankara, Turkey.*
[2]*Firat University, Faculty of Veterinary Medicine, Department of Animal Breeding, Elazig, Turkey.*
[3]*Firat University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Elazig, Turkey.*
[4]*Kirikkale University, Faculty of Veterinary Medicine, Department of Pathology, Kirikkale, Turkey.*

Tumors of the mammary glands are the most common tumors to affect entire female dogs representing between 50-70% of all tumors types, which is three times higher rate of incidence than humans. No other animal species has such high probability of onset of mammary tumors. In humans, heritable breast cancers have been linked with mutations in the breast cancer susceptibility gene BRCA2. The primary function of BRCA2 is homologous recombination, and its mediates the recruitment of recombinase RAD51 to DNA double-strand breaks; RAD51 recruitment is not only essential for homologous recombination but also responsible for the tumor-suppressive function of this repair process. In this study, BRCA2 and RAD51 mRNA expression was measured in tissue samples of adenomas and adenocarcinomas of the mammary gland by real-time quantitative reverse transcription polymerase chain reaction. Tumoral biopsies taken from the mammary gland regions of 64 canine patients were examined histopathologically and a total of 22 mammary tumors (benign n=10 and malign n= 12) were used for the study. Expression levels in the tumors were normalized to the geometric mean of two housekeeping genes (ATP5B, HPRT) and quantified relative to normal mammary epithelium of the same dog. In adenomas, mRNA expression was reduced for BRCA2 (2/10, 20%) and RAD51 (4/10, 40%). BRCA2 and RAD51 were overexpressed in 9 of 12 (75%) and 10 of 12 (83%) of adenocarcinomas, respectively. The results of this study indicate that BRCA2 and RAD51 genes are overexpressed in malignant canine mammary tumors (CMTs).

Keywords: BRCA2, RAD51, Gene Expression, CMTs

BRCA2 expression in canine mammary tumors

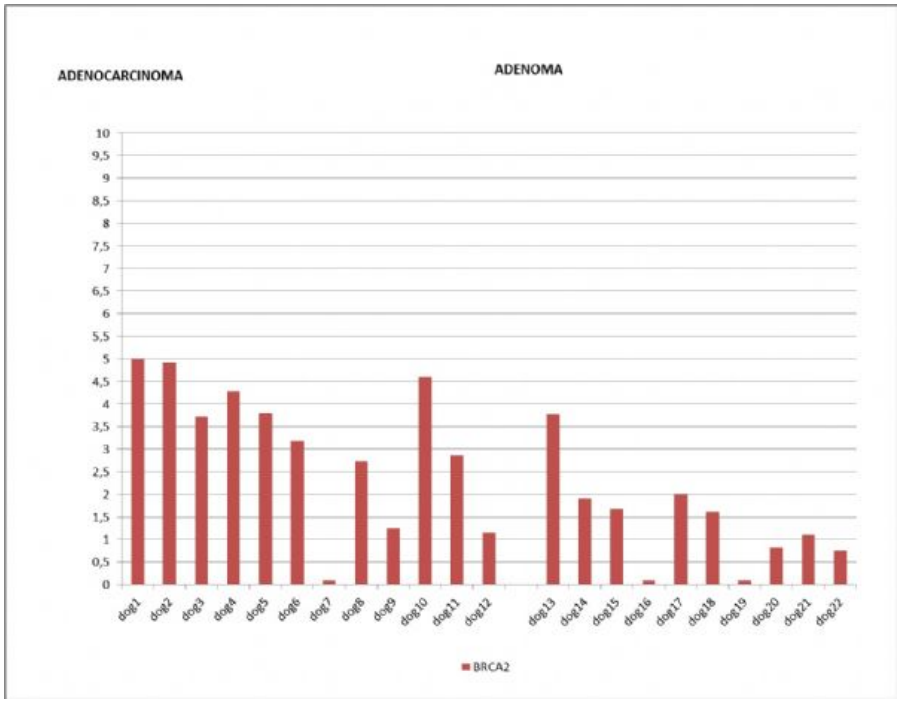


Figure 2. BRCA2 expression in canine mammary tumors. (dogs1-12 adenocarcinoma; dog13-22 adenoma)

RAD51 expression in canine mammary tumors.

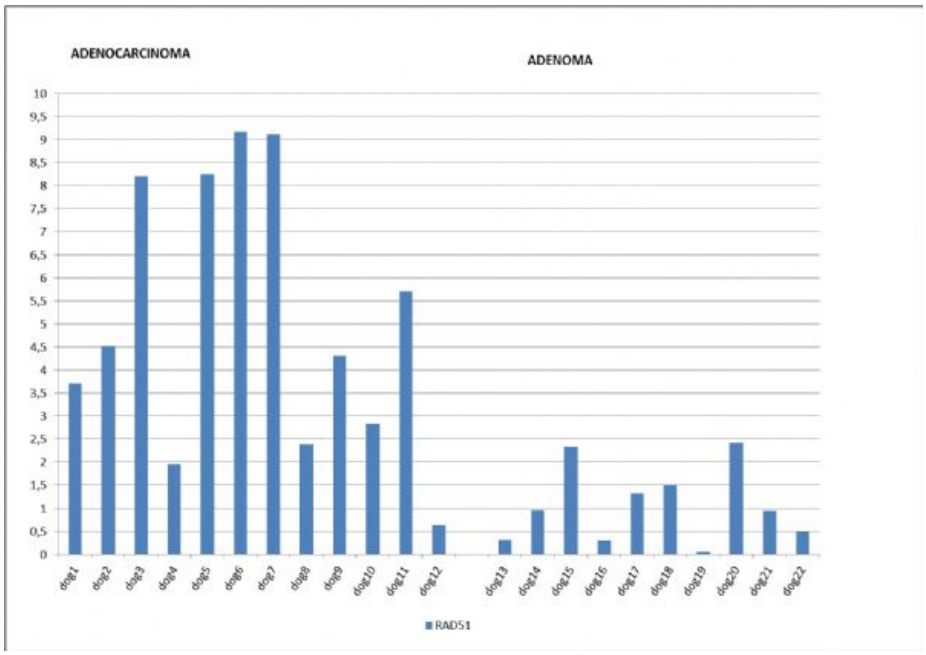


Figure 1. RAD51 expression in canine mammary tumors. (dogs1-12 adenocarcinoma; dog13-22 adenoma).

S-059 - FLUORESCENCE MICROSCOPIC DETECTION OF SOME TERMINAL SUGAR MOIETIES ON THE CELL SURFACE OF HUMAN THYROID CARCINOMA CELL LINES

Sancar Baş Serap[1], Kaptan Engin[1], Sancaklı Aylin[2], Bolkent Şehnaz[1]

[1]*Istanbul University, Faculty of Science, Department of Biology, Section of Molecular Biology Vezneciler 34134 İstanbul/Turkey*
[2]*Istanbul University, Graduate School of Engineering and Science, Department of Biology, Vezneciler, Istanbul/Turkey*

Background: Aberrant glycosylation is a common phenomenon in various pathological processes. Differences in the enzyme expressions induce changes in the composition of membrane glycans conjugated with glycolipids and glycoproteins. Thyroid cancers root from follicular cells of thyroid gland and it is the most common type of endocrine cancers.
Objective: In this study, we aimed to show terminal α-2,3, α-2,6 sialic acid and α-1,6 fucose residues, which are known to be effective in malignancy in several cancer types, on cell surface glycan chains in anaplastic 8505C, follicular FTC-133 and papillary K1 thyroid cell lines.
Material and Methods: The cells were treated with determined doses of the biotinylated lectins; Maackia amurensis lectin-I (MAL-II; Neu5Ac α-2,3Galβ1-4GlcNAc), Sambucus nigra agglutinin (SNA; Neu5Ac α-2,6 GalNAc), Aleuria aurantia lectin (AAL; GlcNAcβ1-4(Fucα-1,6) GlcNAc) for lectin binding assay. The cells were examined under fluorescent microscope after incubation of streptavidin-Texas red. Surface glycosylation patterns of the cell lines were compared with human thyroid follicular epithelial cell line Nthy-ori 3-1 by considering fluorescent density.
Results: We found that α-1,6 fucosylated glycan chains, α-2,3 and α-2,6 sialylated glycan chains were dramatically high on all cells lines we used when compared to human thyroid epithelial cell line Nthy-ori 3-1. MAL-II binding of K1 was lower than those of FTC-133 and 8505C. AAL binding of FTC-133 was lower than those of K1 and 8505C. Also, SNA binding of 8505C was lower than those of FTC-133 and K1.
Discussion: It is suggested that predominantly found α-1,6 fucosylated, α-2,3 and α-2,6 sialylated glycan chains can be indicators of the aberrant glycosylation. Each of the aberrant glycosylation moieties that we detected may be responsible for different tumorigenic and malignant characters.
Conclusion: The cell surface glycosidic properties of thyroid carcinoma cells we detected can be used as a target for developing new strategies in diagnosis and therapy.
Keywords: Aberrant glycosylation, Aleuria aurantia lectin (AAL), Maackia amurensis Lectin (MAL II), Sambucus nigra agglutinin (SNA), Thyroid carcinoma

S-060 - NEAR-INFRARED FLUORESCENT CARBON NANOTUBE BASED SENSORS FOR IN VITRO AND IN VIVO CANCER APPLICATIONS

Sen Fatih

Department of Biochemistry, Dumlupinar University

Since carbon nanotubes exhibit a fluorescent signal in a spectral region where there is minimal interference from biological media, they are particularly attractive for biomedical applications. Even though carbon nanotubes have been used as highly sensitive detectors for cancer applications, their use as in vivo biomarkers requires the simultaneous optimization of various parameters, including biocompatibility, molecular recognition, high fluorescence quantum efficiency and signal transduction. Adrressed herein, a polyethylene glycol ligated copolymer stabilizes near infrared- fluorescent single-walled carbon nanotubes sensors in solution, enabling intravenous injection into mice and the selective detection of local nitric oxide concentration with a detection limit of 1 mM. The half-life for liver retention is 4 h, with sensors clearing the lungs within 2 h after injection, thus avoiding a dominant route of in vivo nanotoxicology. After localization within the liver, it is possible to follow the transient inflammation using nitric oxide as a marker and signalling molecule. Finally, we demonstrate that alginate-encapsulated single-walled carbon nanotubes can function as implantable inflammation sensors for nitric oxide detection, with no intrinsic immune reactivity or other adverse response for more than 400 days.

Keywords: Biosensor, Carbon Nanotube, Infrared, NO

S-061 - SEX HORMONE DEPENDENT TOXICITY OF OCHRATOXIN A IN THE KIDNEY OF FEMALE RATS

Kılıç Mehmet Akif[1], Mor Firdevs[2], Özmen Özlem[3]

[1]*Akdeniz University, Science Faculty, Department of Biology, Antalya, Turkey.*
[2]*Mehmet A. Ersoy Uni., Veter. Faculty, Dept. of Pharm. and Toxicology, Burdur, Turkey*
[3]*Mehmet Akif Ersoy University, Veterinary Faculty, Dept. of Pathology, Burdur, Turkey.*

Introduction and Aim: Ochratoxin A (OTA) could cause pathological lesions, including renal cancers. Female rodents ought to be less susceptible to OTA toxicity. The objective of this study was to deduce the role of sex hormones in the OTA-related pathogenesis in female rat kidneys.
Material and Methods: Female rats, 16-weeks old, were divided into 5 different groups (n=7): intact females (F), ovariectomised (F-OVA), testosterone injected (F-TEST), antitestosterone injected (F-AntiTEST) and ovariectomised and testosterone injected (F-OVA+TEST) and fed with a diet containing 3 ppm of OTA for 9 weeks. Lesions in kidney parts were evaluated and scored in a range 0-3. A score of 3 was used for the most prominent histological change.
Results: In all experimental groups, the outer medulla had the highest karyomegalic cells. Karyomegaly scores of rats were; F-OTA (2.00±00), F-OVA-OTA and F-TEST-OTA (2.28±0.48), F-OVA+TEST-OTA (2.66±0.57) and F-AntiTEST-OTA (1.00±0.00). The results showed that although ovariectomy or testosterone treatment did not significantly change number of karyomegaly in kidneys, testosterone injection to ovariectomised females markedly increased karyomegalic lesions and conversely, antitestosterone injection significantly reduced karyomegalic lesions. The number of apoptotic cells and their localization were also evaluated and the cortex was more affected than other parts. The F-OVA+TEST-OTA had the highest apoptosis score (1.33±0.57) and antitestosterone treatment significantly reduced apoptotic cell death (0.28±0.48). All these findings suggest that testosterone also plays an important role in the OTA-related pathogenesis in female rats.
Conclusion: Although OTA is toxic to all parts of the kidney, 1- the outer medulla seems to be more sensitive to OTA toxicity, 2- testosterone remarkably increase its deleterious effects and 3- testosterone repression in female rats significantly reduces its side effects. We suggest that testosterone is the major player in the OTA-related sex dependent toxicity differences observed in rats.
Akdeniz University Animal Ethics Committee (2011.12.02) and Akdeniz University Research Funding (2012.01.0115.005).
Keywords: Ochratoxin A, Testosterone, Antitestosterone, Kidney, Female rats

S-062 - P-COUMARIC ACID ATTENUATE ON CISPLATIN-INDUCED OXIDATIVE STRESS IN RAT'S HEART

Ekinci Akdemir Fazile Nur[1], Gürsul Cebrail[2], Gülçin İlhami[3], Alvasel Saleh H.[4], Gözcü Dusak Lale[5], Bayır Yasin[5]

[1]*Department of Nutrition and Dietetics, Health School, Ağrı İbrahim Çeçen University, Ağrı, Turkey*
[2]*Department of Physiology, Faculty of Medicine, Erzincan University, Erzincan, Turkey*
[3]*Department of Biochemistry, Faculty of Science, Atatürk University, Erzurum, Turkey*
[4]*Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[5]*5. Department of Biochemistry, Faculty of Pharmacy, Atatürk University, Erzurum, Turkey*

Aim: The healing importance of cisplatin as anticancer treatment is limited via its cardiotoxicity. The most of natural phenolic acids in the protection from many pathological events have been determined in the previous study. In the present study, we were aimed to investigate the protective effect of p-coumaric acid, as a phenolic acid, against cisplatin-induced oxidative damage on the rat's heart.
Matherial and Methods: In our experimental study thirty Sprague-Dawley type adult rats were used. The rats were divided into five groups; control, control+ethanol, cisplatin, p-coumaric acid, and p-coumaric acid+cisplatin. Cisplatin was administered i.p. in a single dose of 10 mg kg-1. p-Coumaric acid was used in a doses of 100 mg kg-1 i.p. for three sequential days. In cisplatin group, rats were sacrificed after for 72 hours administration of cisplatin. At the end of the experiment, rats were sacrificed with high dose anesthetic agent and heart tissues removed quickly. The biochemical measurements were performed in tissue samples.
Results: Pretreatment with p-coumaric acid was improved the tissue content of glutathione level, and superoxide dismutase activities, compared to cisplatin-received rats. Also, tissue MDA decreased following p-coumaric acid pretreatment, compared to cisplatin-treated rats. But this damage was decreased in group of p-coumaric acid treatment.
Conclusions: Our results were indicated that p-coumaric acid can prevent heart tissue against cisplatin-induced oxidative damage. We have belived that p-coumaric acid may have valuable benefits in cancer treatment.
Keywords: Cardiotoxicity, p-coumaric acid, Cisplatin, Oxidative stress

S-063 - FLOW CYTOMETRIC ASSESMENT OF THE ROSA CANINA EXTRACTS ON DIFFERENT CANCER TYPES

Kılınç Kağan[1], Turan İbrahim[1], Demir Selim[2], Burnaz Nesibe Arslan[4], Genç Berna[1], Örem Asım[3], Alver Ahmet[3]

[1]*Gumushane University Faculty of Engineering and Naturel Science Department of Genetic and Bioengineering Gumushane Turkey*
[2]*Karadeniz Technical University Faculty Of Health Sciences Department of Nutrition and Dietetics, Trabzon, Turkey*
[3]*Karadeniz Technical University Faculty of Medicine Department of Medical Biochemistry, Trabzon, Turkey*
[4]*Gumushane University Faculty of Health Department of Nutrition and Dietetic Gumushane Turkey*

Introduction: Rosa canina is the rich including polyphenols, carotenoids, ascorbic acid and fatty acids. Several studies have indicated that Rosa canina hips show antioxidant, anti-inlammatory, anticancer, properties. Rosa canina is also used in the treatment of several illnesses and disfunctions in traditional medicine due to the above mentioned compounds. In this study we aimed to investigating anticanser effect and their apopitotic ways of different Rosa canina extracts.

Marherial and Methods: Rosa canina samples were extracted by using different solvents and solvents plus 0,5% hydrogen chloride. Rosa canina extracts (RCE) analyzed with spectrophotometric and HPLC methods to detect their antioxidant capacity, species of phenolic compounds. We were chose the most appropriate RCE sample for anticancer and flow cytometry analyzes according to their values.This RCE sample extracted with dimethyl sulfoxide (DMSO), DMSO-acetic acide(DMSO-AA) and DMSO-hydrogen chloride (DMSO-HCl).

Human lung carcinoma (A549) and human prostate adenocarcinoma (PC-3) cell lines were used to detect antiproliferative and possible apopitotic effects of RCE on cancer.Flow cytometrically, Cell cycle, Annexin V, mitochondrial membrane potential and caspase 3/7 activites tests carried out on significantly antipolifreative cell lines.

Results: In A549 and PC-3 cell lines, during cell cycle test, RCE with DMSO-AA IC90 value has shown significantly differences according to negative control ($P<0.01$) to keep the cells in G1 phase (G2/M phases in PC-3 cells). RCE with DMSO, DMSO-AA IC90 and DMSO-HCl IC50 also has shown significantly differences ($P<0.01$) during Annexin V and mitochondrial membrane potential tests according to number of necrotic cells. RCE with DMSO-AA was induced the caspase 3/7 activities significantly in A549 line (P0.01) whereas RCE has no effect on PC-3 cell line.

Discussion: It was concluded that Rosa canina may have anticancer activity by different signal ways necrotic, apoptotic and cell cycle mechanisms.

Keywords: Anticancer, Flow cytometry, Dimethyl sulfoxide, Rosa canina

S-064 - FHIT DEFICIENCY DRIVES NEOPLASTIC INITIATION AND PROGRESSION

Batar Bahadır[1*], Karras Jenna R.[1], Schrock Morgan S.[1], Zhang Jie[2], Perle Krista La[3], Druck Tresa[1], Huebner Kay[1]

[1]*Department of Cancer Biology and Genetics, Ohio State University Wexner Medical Center, Columbus, Ohio, USA.*
[2]*Department of Biomedical Informatics, Ohio State University Wexner Medical Center, Columbus, Ohio, USA.*
[3]*Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, Columbus, Ohio, USA.*

**Current address: Department of Medical Biology, Istanbul University, Cerrahpasa Medical School, Istanbul, Turkey*

Background: The Fragile Histidine Triad (FHIT) gene, spans the most active common fragile site, FRA3B, is one of the earliest and frequently altered genes in preneoplasia and cancer. Fhit protein, which is reduced in expression in the majority of human cancers, is a genome 'caretaker' whose loss initiates genome instability in preneoplastic lesions. Our goal was to demonstrate that Fhit deficiency supports tumorigenic initiation and progression.
Material and Methods: We established epithelial cell lines from kidney tissues of Fhit -/- and +/+ mouse pups early after weaning and subjected cell cultures to nutritional and carcinogen stress, to assess the early genetic alterations and functional changes. Through transcriptome profiling and protein expression analysis, we defined alterations in proteins in signal pathways that are frequently altered in cancers.
Results: Fhit-deficient cells exhibited alterations in Trp53/p21 and survivin apoptotic pathways and in expression of proteins involved in the epithelial-to-mesenchymal transition (EMT). Some Fhit-/- cell lines displayed anchorage-independent colony formation and increased invasive capacity in vitro. Furthermore, cells of stressed Fhit-/- cell lines formed subcutaneous and metastatic tumors in nude mice.
Conclusions: Fhit-deficient cells are more susceptible to acquire cancer-promoting mutations. Thus, Fhit loss provides a 'mutator' phenotype. Loss of Fhit leads to survival and selective expansion advantage for transformation and cancer progression.
Keywords: Fhit, Genome İnstability, Cell Transformation

S-065 - ARE THERE POSSIBLE ASSOCIATIONS BETWEEN MNSOD AND GPX1 GENE VARIANTS FOR LARYNGEAL CANCER RISK OR DISEASE PROGRESSION?

Coşkun Cihan[1], Verim Ayşegül[2], Farooqi Ammad Ahmad[3], Turan Saime[4], Mezani Brunilda[4], Küçükhüseyin Özlem[4], Hakan Mehmet Tolgahan[4], Ergen Arzu[4], Yaylım İlhan[4]

[1]*Department of Biochemistry, Haseki Training and Research Hospital, Istanbul, Turkey*
[2]*Otorhinolaryngology Clinic, Haydarpasa Training and Research Hospital, Istanbul, Turkey*
[3]*Laboratory for Translational Oncology and Personalized Medicine, Rashid Latif Medical College, Lahore, Pakistan*
[4]*Department of Molecular Medicine, Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey*

Introduction: Laryngeal squamous cell carcinoma (LSCC) is a multifaceted and genomically complex disease and cellular and preclinical studies have demystified wide ranging molecular mechanisms which underpin its development and progression and resistance against wide ranging molecular therapeutics. Oxidative stress is a widely studied molecular mechanism and reportedly involved in carcinogenesis. Increasingly it is being realized that accumulation of Reactive Oxygen Species (ROS) activates defensive mechanism to counteract oxidative stress induced damage. Manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPx) are important members of defensive machinery. We investigated whether the polymorphisms of MnSOD (Ala-9Val, rs4880) and GPx1 (Pro198Leu, rs1050450) are associated with LSCC and also evaluated possible interactions between these polymorphisms and various lifestyle factors or pathological features of patients.
Material and Method: For this purpose, 67 LSCC patients and 73 healty controls were enrolled. Molecular assessment of MnSOD and GPx1 variants were determined with polymerase chain reaction-restriction fragment length polymorphism techniques.
Result: We found that the frequency of both heterozygous PL genotype and P allele was considerably higher in patients with advanced tumor stage (T3/T4) than in those with early tumor stage (T1/T2) (OR= 5.106; 95% CI=1.372-19.004; p<0.001, OR=5.787; 95% CI =1.564-21.414; p<0.001 respectively). Although the frequency of ValVal/LL combine genotype was significantly decreased (OR=0.204, 95% CI=0.055-0.760; p=0.021), the frequency of ValAla/PL combine genotypes was higher in patients with stage T3/T4 than in those patients with stage T1/T2 (p=0.027).
Conclusion: Consequently, we have concluded that variants of GPx1 and MnSOD should not be considered as a risk factor of LSCC, only may be accepted as a prognostic markers. Use of new technologies such as metabolomics and deep DNA sequencing will prove to be helpful in developing a deeper knowledge related to how cancer cell metabolism adapts and provides a buffer against increased oxidative stress.
Keywords: Larynx cancer, Genotype, MnSOD, GPx1, Polymorphism

POSTER BİLDİRİ SUNUMLARI

P-001 - EVALUATION OF SERUM IMMUNOFIXATION ELECTROPHORESIS RESULTS IN KONYA REGION

Hayırlıoğlu Mustafa Fatih[1], Deniz Çiğdem Damla[1], İyisoy Mehmet Sinan[2], Gürbilek Mehmet[1]

[1]*Necmettin Erbakan University, Meram Medicine Faculty, Department of Biochemistry, Konya*
[2]*Necmettin Erbakan University, Faculty of Medicine, Department of Medical Education and Informatics, Konya*

Introduction: Multiple myeloma (MM) is a malignant plasma-cell disorder caused by an uncontrolled proliferation of monoclonal plasma cells in the bone marrow. MM is characterized by malignant plasma cell hyperplasia, monoclonal immunoglobulin (M protein) in the serum and urine, which can lead to anemia, renal insufficiency, wide-spread bone lesions, hypercalcemia and serious recurrent infections. Multiple myeloma is increasing abnormal plasma cells produce abnormal proteins and that useful not fight infections.
In addition to history taking and physical examination, the diagnostic work-up for multiple myeloma comprises clinical chemistry, cytogenetic analysis of bone marrow, and radiological investigation to detect bone changes. We aimed to evaluate three years IFE results of our laboratory.
Material ve Methods: In this study, serum IFE levels of totally 706 individuals (372 females and 334 males) who admitted to our faculty with different complaints between 01.08.2013 and 01.08.2016 were assayed. Serum ife analysis was performed using Helena SAS-I and SAS-II devices. Statistical analysis was performed using SPSS version 21.0.
Results: We examine serum IFE studies from our laboratory; The number of female patients 372 (52.7%) was higher than the number of male patients 334 (47.3%). The average age of female patients (63,17) was significantly lower than male patients (65,04). The result of the evaluation of 497 patients serum ife results were normal. The remaining 309 patients were seen in the monoclonal band. The most common monoclonal band we identified was IgG kappa with 48%. IgM lambda is a monoclonal gammopathy of rare species with 3%.
Discussion and Decision: The follow cases IFE results were accordance with the literature. Because of its great versatility, potentially high sensitivity, ease to perform and customize, and relatively low cost with no requirement for expensive instrumentation, IFE remains a valuable tool for both clinical diagnostic testing and research.
Keywords: Immunofixation electrophoresis, Konya region, Monoclonal gammopathy

Age and MGUS Type

Type	Mean	N	Std. Deviation
A KAPPA	63,57	21	12,38
A LAMBDA	69,43	14	9,28
G KAPPA	67,56	101	10,18
G LAMBDA	69,13	54	8,67
M KAPPA	71,08	13	8,09
M LAMBDA	67,67	6	8,73
MGUS is not	62,43	497	13,56
Total	64.06	706	12,83

The result of the evaluation of 497 patients serum ife results were normal. The remaining 309 patients were seen in the monoclonal band. The most common monoclonal band we identified was IgG kappa with 48%. IgM lambda is a monoclonal gammopathy of rare species with 3%.

Age and Sex

Sex	Mean	N	Std. Deviation
Female	65,04	334	12,147
Male	63,17	372	13,361
Total	64,06	706	12,826

We examine serum IFE studies from our laboratory; The number of female patients 372 (52.7%) was higher than the number of male patients 334 (47.3%). The average age of female patients (63,17) was significantly lower than male patients (65,04).

P-002 - EFFECT OF MESENCHYMAL STEM CELLS,TUMOR INFILTRATING LYMPHOCYTES AND CANCER STEM CELLS IN NEUROBLASTOMA

Yıldırım Zübeyde, Aktaş Safiye, Altun Zekiye, Serinan Efe, Demir Banu, Erçetin Pınar, Olgun Nur

Institute of Oncology, Dokuz Eylul University, Izmir, Turkey

Introduction: Cancer stem cells are thought to play a central role in tumor initiation, progression and recurrence The aim of this study investigate the interaction between neuroblastoma cancer-initiating cells and the elements of the tumor microenvironment which is tumor infiltrating lymphocytes and mesenchymal stem cells.
Methods: isolated from fresh tissue of neuroblastoma surgical material were perfomed single-cell suspension and cultured. CD133 + stem cells were isolated with magnetic insulation from cultured neuroblastoma cancer cells. by using AIMV migration method proved accessing to the migration of tumor infiltrating lymphocytes from fresh tissue fragments to medium and expanded with GCSF and IL-2. Mesenchymal stem cells were isolated with CD54 +, CD90 +magnetic insulation. The isoleted cells, tumor infiltrating lymphocytes and cisplatin were seeded in multi-well Plates in single quad binary and ternary various combinations. Then the cells viability measured at 24 and 48 hours. Mann Whitney U testi test was used for the comparison of the nonparametric outcomes. $P < 0.05$ was considered statistically significant.
Results: A total of 20 neuroblastoma samples obtained from patients who are 2 to 168 (mean 39) months old were evaluated with cell culture. Tumor infiltrating lymphocytes and mesenchymal stem cells protect the neuroblastoma cells effect of cisplatin was observed. Tumor infiltating lymphocytes has no effect on neuroblastoma stem cells but mesenchymal stem cells protect the neuroblastoma stem cells effect of cisplatin was observed.
Conclusion: In this study, interaction with neuroblastoma cancer stem cells and tumor microenvironment cells was investigated ex vivo for the first time. Protection of mesenchymal stem cells form cytotoxic effects of cisplatin the neuroblastoma stem cells effect of cisplatin was observed.
Keywords: Neuroblastoma, cancer Stem Cells, Mesenchymal Stem Cells

Note: This study was supported by Turkish Pediatric Oncology Group Assosiation

P-003 - IN VITRO EFFECT OF FLUBENDAZOLE ON NEUROBLASTOMA STEM CELLS

Erçetin Özdemir Ayşe Pınar[1], Aktas Safiye[1], Genc Nese[2], Serinan Efe[1], Altun Zekiye[1], Olgun Nur[3]

[1]*Basic Oncology Department, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey*
[2]*Molecular Biology and Genetics Department, Faculty of Basic Sciences, Gebze Technical University, Kocaeli, Turkey*
[3]*Pediatric Oncology Department, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey*

Aim: Despite the development of new treatment options, the prognosis of high-risk neuroblastoma patients remains poor and more than half of NB patients have disease recurrence. Cancer stem cells (CSC) are thought to be responsible for cancer initiation, drug resistance and metastasis so various therapeutic agents have been studied to eliminate this subgroup of cells. Based on this issue we aimed to evaluate the in vitro effect of Flubendazole (FB) on cell proliferation, apoptosis and DNA damage of CD133+ N-myc amplified NB cells.
Methods: Kelly (Nmyc positive) NB cells were cultivated in RPMI medium supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine. CSCs were isolated with ferritin coated CD133 monoclonal antibodies at magnetic field. CSCs were incubated with 300 and 500 mM FB for 24 hours. Flow cytometric analysis of cell proliferation was performed by BrdU, apoptosis levels were determined according to cleaved-PARP and DNA damage levels were detected by H2AX expression.
Results: FB increased anti-apoptotic effect in a dose dependent manner (14,7% and 42,5%). However, DNA damage of cells did not show a significant difference (6,9% and 3%). Cell proliferation was less in 500 mM FB treated group (47,8%) compared to 300 mM FB treated group (29,1%). Cell proliferation of control group was 26,9%.
Conclusion: FB showed anti-apoptotic effect on NB CSCs. However it did not cause significant anti-proliferative effect. We suggest that FB should be studied in combination with anti-proliferative agents in further studies.
Keywords: Neuroblastoma, Cancer Stem Cells, Flubendazole

Cell Proliferation, Apoptosis and DNA Damage Levels of FB treated NB CSCs

	Cell Proliferation (BrdU)	DNA Damage (H2AX)	Apoptosis (cleaved-PARP)
Control	26,9	7,2	4,1
300 mM FB	47,8	6,9	14,7
500 mM FB	29,1	3	42,5

Flow cytometric analysis of BrdU, cleaved-PARP and H2AX expression after 300 and 500 mM FB treatment on CD133+ NB stem cells.

P-004 - AUTOPHAGY INHIBITION ENHANCES PALLADIUM (II)-BARBITURATE COMPLEX - INDUCED APOPTOSIS IN PROSTATE CANCER

Erkisa Merve[1], Ari Ferda[1], Aydınlık Seyma[1], Cevatemre Buse[1], Aztopal Nazlıhan[1], Yılmaz Veysel T.[2], Ulukaya Engin[3]

[1]*Uludag University, Faculty of Arts and Sciences, Department of Biology, Bursa, Turkey*
[2]*Uludag University, Faculty of Arts and Sciences, Department of Chemistry, Bursa, Turkey*
[3]*Uludag University, Medical School, Department of Medical Biochemistry, Bursa, Turkey*

Introduction: Palladium (Pd) (II) complex were shown to have significant anti-tumor activities against different types of cancer cells. Chloroquine (CQ) as an inhibitor of autophagy is used prosurvival and resistance mechanism against cancer cells. Therefore, the combination of these two may be a realistic strategy for new treatment modality.
Materials and Methods: The androgen-sensitive human prostate adenocarcinoma cell line LNCaP and the human normal prostate cell line PNT1A were treated with different concentration of Pd (II) complex (1,56-100µM), CQ (0,6-40µM) alone or in combination with CQ. Viability was detected by MTT and ATP viability assays at 24 h and 48 h. Flow cytometry was used to determine the mode of cell death (apoptosis/necrosis/autophagy) responsible for the cytotoxicity in combination treatments. This results were confirmed with fluorescence images using triple staining method (Hoechst 33343, Annexin V, and Propidium iodide). Formation of acidic vesicular organelles (AVOs) was observed by fluorescence microscopy using acridine orange staining. Finally, protein expression levels associated to autophagy and cell death were determined by immunoblotting method and Luminex assay.
Results: The combination of CQ (5 µM) and Pd (II) complex (12.5 µM) at 48 h has enhanced cytotoxic activity resulted from the induction of apoptosis (indicated by the presence of pyknotic nuclei, increased the Annexin-V (+) cells and over expression of pro-apoptotic proteins). Importantly, the addition of CQ resulted in the suppression of autophagy that might have contributed to the enhanced cytotoxicity. In addition, PI3K/AKT/mTOR-related protein expression were altered after combination treatments. Treatment with Pd (II) complex alone resulted in increased AVOs formation, but the effect was potentiated by CQ-pretreatment.
Discussion: The combination of Pd (II) complex and CQ enhances apoptotic cell death, possibly via the inhibition of autophagy. This combination may be regarded as a novel and effective approach for the treatment of prostate cancer.
Keywords: Autophagy, Prostate Cancer, Apoptosis

P-005 - INHIBITION OF THE WNT SIGNALLING PATHWAY SENSITIZES BREAST CANCER STEM CELLS TO PD(II) COMPLEX-INDUCED APOPTOSIS

Aztopal Nazlihan[1], Karakas Didem[1], Cevatemre Buse[1], Ari Ferda[1], Yilmaz Veysel Turan[2], Ulukaya Engin[3]

[1]*Uludag University, Faculty of Arts and Sciences, Department of Biology, Bursa, Turkey*
[2]*Uludag University, Faculty of Arts and Science, Department of Chemistry, Bursa, Turkey*
[3]*Uludag University, Medical School, Department of Clinical Biochemistry, Bursa, Turkey*

Introduction: Cancer stem cells (CSC) are a rare cell population found in the tumor and responsible for drug resistance. Wnt signaling pathway is re-activated in CSCs and has an essential role in cell survival, self-renewal and proliferation. Therefore, elimination of these cells along with the other cancer cells is crucial for the successful treatment. In the present study, we evaluated the mechanism of cell death induced by palladium(II) complex, {[PdCl(terpy)](sac)·2H2O}, synthesized at the Uludag University and its combination with niclosamide which is an inhibitor of Wnt signaling pathway in breast cancer stem cells.
Material and Methods: Breast cancer stem cells (CD44+/CD24- cells) were propagated from parental MCF-7 cells and allowed to form mammosphere structures. After then, cells were pre-treated with Niclosamide (at the dose 1,5 μM) for 24h followed by exposure to Pd(II) complex (50μM) for 24 and 48h. Altered expression of proteins associated with stemness (Oct-4 and Bmi-1), Wnt signalling (LRP6, p-LRP6, Axin1, Naked2, Dvl3, Wnt5a/b, β-katenin) and cell death (Fas, PARP, Bax, kaspaz 8, p-c-Jun, p-SAPK/JNK, RIP1, Atg5, Beclin1, LC3II, p62/SQSTM1) were analyzed with western blotting.
Results: It was shown that the combinatorial treatment decreased the LRP6, p-LRP6, Dvl-3, Axin1, β-katenin levels and increased the Naked2 (Wnt antagonist) levels compared with the effect of complex or Niclosamide alone, implying the inhibition of Wnt signalling in a most effective manner. Stemness related proteins were also decreased. Apoptosis was induced via the activation of caspase 8 and cleavage of PARP as well as activation of stress-related proteins (JNK and c-Jun). Inhibition of necroptosis and autophagy was also observed.
Discussion: Inhibition of Wnt signalling resulted with the enhanced apoptotic activity possibly due to supression of autophagy and necroptosis. Therefore, this combination may be regarded as an effective approach to eliminate breast CSCs although in vivo experiments are required for the proof-of concept.
Keywords: Breast cancer stem cell, Wnt Signalling, Targeted therapy, Apoptosis

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P-006 - TARGETING EPIGENETIC REGULATION OF HISTONES WITH VALPROIC ACID LEADS TO APOPTOSIS IN BREAST CANCER STEM CELLS

Aztopal Nazlihan[1], Erkısa Merve[1], Ertürk Elif[2], Arı Ferda[1], Ulukaya Engin[3]

[1]*Uludag University, Faculty of Arts and Sciences, Department of Biology, Bursa, Turkey*
[2]*Uludag University, Vocational School of Health Services, Bursa, Turkey*
[3]*Uludag University, Medical School, Department of Medical Biochemistry, Bursa, Turkey*

Introduction: Cancer stem cells (CSCs) are a small subpopulation of cells within the tumor and lead to disease relapse because of an acquired resistance to apoptosis. In particular, epigenetic changes play a crucial role in the regulation of stemness and also have been implicated in the development of drug resistance. Therefore, in the present study, we focused on the cytotoxic and apoptotic activity of valproic acid (VPA) as an inhibitor of histone deacetylases (HDACs) against breast CSCs.
Material and Methods: Elevated expression of stemness markers were determined by western blotting in mammospheres (MCF-7s, cancer stem cell-enriched population) propagated from parental MCF-7 cells. Anti-growth activity of VPA was determined via ATP viability assay. The sphere formation assay (SFA) was performed to assess the inhibitory effect of VPA on the self-renewal capacity of MCF-7s cells. Acetylation of histon H3 was detected with ELISA assay. Cell death mechanism was determined via fluorescence microscopy (Hoechst dye 33342 and Propidium iodide staining), M30 (apoptosis) and M65 (primary or secondary necrosis) ELISAs and cytofluorimetric analysis (caspase 3/7 activity and annexin-V-FITC staining).
Results: VPA exhibited anti-growth effect against both MCF-7 and MCF-7s cells in a dose (0.6-20 mM) and time (24, 48, 72h) dependent manner. As expected, MCF-7s cells were found more resistant to VPA than MCF-7 cells. It was observed that VPA prevented mammosphere formation at lower doses (2.5 and 5 mM) in which acetylation of histon H3 was increased. VPA also increased the M30 and M65 levels at the same doses and secondary necrosis (late stage of apoptosis) was evidenced by nuclear pyknosis with propidium iodide staining positivity, annexin-V-FITC positivity and caspase 3/7 activation.
Discussion: Our results suggested that inhibition of HDACs sensitizes breast CSCs to apoptosis and targeting the epigenetic regulation of histones may hold significant promise for successful treatment of breast cancer.
Keywords: Breast cancer stem cell, Epigenetic regulation, Histone Acetylation, Apoptosis

P-007 - INVESTIGATION OF THE CYTOTOXIC AND APOPTOTIC EFFECTS OF COMBINATION OF THE HISTONE DEACETYLASE INHIBITOR AND WNT PATHWAY INHIBITOR ON LUNG CANCER CELL LINES

Akgun Oguzhan[1], Arı Ferda[1], Erkısa Merve[1], Ulukaya Engin[2]

[1]*Uludag University, Faculty of Arts and Sciences, Department of Biology, Bursa, Turkey*
[2]*Uludag University, Faculty of Medicine, Department of Medical Biochemistry, Bursa, Turkey*

Background and Aim: Epigenetic changes including histone modifications play an important role in the carcinogenesis. In this study, cytotoxic and apoptotic effects of histone deacetylase inhibitor valproic acid (VPA) in combination with Wnt pathway inhibitör niclosamide was investigated on lung cancer cell lines A549 and H1299.
Materials and Methods: Cytotoxic effects of VPA (500 µM) and niclosamide (0.08, 5 µM) combination was determined on A549 and H1299 cell lines after 72 h treatment by SRB and ATP cell viability assays. Nuclear morphology and plasma membrane integrity were visualized via Hoechst 33342, Propidium Iodide fluorescent staining for 72h. The change in mitochondrial transmembrane potential was evaluated using the cationic fluorescent indicator JC-1. Oxidative stress parameters were detected by H2DCFDA staining and flow cytometry.
Result: The combination of VPA with niclosamide dramatically decreased the viability, relative to either compound alone in A549 and H1299 cell lines. Also, the combination further increased mitochondrial membrane depolarization in cells. Moreover, combination treatment was increased oxidative stress dramatically in both cell lines, has been showed by flow cytometry and H2DFCA staining.
Conclusion: The combination of VPA and niclosamide enhances apoptotic cell death thorough disturbance of mitochondrial membrane potential and oxidative stress. Therefore, this novel combination deserves further attention for proof of concept in the treatment of lung cancer.
Keywords: Investigation, Cytotoxic, Apoptotic

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P-008 - GENOTOXIC ACTIVITY OF PALLADIUM(II) SACCHARINATE COMPLEX OF TERPYRIDINE ON BREAST CANCER STEM CELLS

Celikler Serap[1], Bozdemir Sezin[1], Karakas Didem[1], Ari Ferda[1], Aztopal Nazlihan[1], Yilmaz Veysel Turan[2], Ulukaya Engin[3]

[1]*Uludag University, Faculty of Arts and Sciences, Department of Biology, Bursa, Turkey*
[2]*Uludag University, Faculty of Arts and Science, Department of Chemistry, Bursa, Turkey*
[3]*Uludag University, Medical School, Department of Clinical Biochemistry, Bursa, Turkey*

Introduction: In the present study, we determined the potential DNA-damaging effect of palladium(II) comlex,{[PdCl(terpy)](sac)·2H2O}, synthesized at the Uludag University on MCF7 breast cancer cells and mammospheres (cancer stem cells-enriched population) derived from MCF7 cells.
Material and Methods: First, MCF7 cells and mammospheres were treated with Pd(II) complex (50 µM) for 12, 24 and 48 hours. The results of comet assay were analyzed by using Microsystem Comet Software Program (Argenit, Istanbul, Turkey) and comet lenght, tail lenght, percentage of DNA in the comet tail and head, Genetic Damage Index (GDI) and percentage of damaged cells (PDC) parameters were calculated.
Results: Based on the analyses, it has been found statistically significant increase in comet lenght, tail lenght and percentage of DNA in MCF7 cells in a time dependent manner compared to mammospheres ($p< 0.001$, $p< 0.001$ and $p< 0.005$, respectively). The percentage of DNA in head significantly decreased in MCF7 cells ($p<0.005$) and percentage of damaged cells and genetic damage index were also found more higher when compared to mammospheres ($p< 0.0001$ and $p< 0.0001$, respectively).
Discussion: Results indicated that MCF7 cells were more sensitive to complex than mammospheres. Hence, novel approaches are urgently needed to also target CSCs.
Keywords: Genotoxic, Activity, Palladium(II)

This study was supported by TUBITAK (The Scientific and Technological Research Council of Turkey) for the project that was numbered 212T147.

P-009 - MONOCYTIC AND GRANULOCYTIC MDSCS DISPLAY DISTINCT MOLECULAR PROPERTIES AND COORDINATE THE DYNAMIC SWITCHES BETWEEN EMT-MET IN BREAST CANCER MODEL

Lee Eunmi[1], Ouzounova Maria[1], Piranlioglu Raziye[1], Arbag Sena Nur[2], Korkaya Hasan[1]

[1]*Department of Biochemistry and Cancer Biology, Augusta University Georgia Cancer Center, Augusta, GA, USA*
[2]*Faculty of Medicine, Hacettepe University, Ankara, Turkey*

Background: It is widely accepted that the epithelial-mesenchymal plasticity of malignant cells is required during cancer metastatic cascade. The complex phenotypic changes highly depend on the collaboration of various molecular signaling and extracellular cues including other tumor cells, and stromal cells in the microenvironment. However, the specific mechanisms of how EMT plasticity spatiotemporally regulates metastasis are poorly defined. Emerging evidence suggests that immature myeloid cells promote angiogenesis and invasion in addition to their immunosuppressive functions.
Method: To study the roles of immune cells in breast cancer metastasis, we utilized murine breast cancer cells, non-metastatic EMT6 and metastatic 4T1. We isolated MDSCs from bone marrow, lung and primary tumor of tumor-bearing mice and co-cultured with non-metastatic EMT6 cells to examine whether MDSCs can enhance the metastatic ability in tumor cells.
Results: We showed that the metastatic 4T1 murine tumors induced early systemic expansion and mobilization of MDSCs in distant sites as well as primary tumor. Using co-culture experiments, we found that tumor infiltrating m-MDSCs from 4T1 tumor-bearing mice increased the expression of Vimentin and CK14 in EMT6 tumor cells as shown by qPCR and Immunofluorescence staining. Cell invasion assay showed that invasive ability of EMT6 cells were significantly increased when they were co-cultured with m-MDSCs. In contrast, g-MDSCs induced down-regulation of EMT markers while they increased cell proliferation as assessed by Ki67 staining. Furthermore, flow cytometry analysis showed the increased percentage of CD24, CD29 double positive cells, as a marker of murine cancer stem cell (CSC) phenotype, in EMT6 cells co-cultured with m-MDSCs. Tumor sphere assay confirmed that m-MDSCs enhanced sphere forming ability of tumor cells.
Conclusion: These data suggest that m-MDSCs derived from metastatic 4T1 tumor-bearing mice are able to confer EMT/CSC phenotype on tumor cells, while g-MDSCs are more potent in inducing epithelial phenotype and proliferation in tumor cells.
Keywords: Breast cancer, Metastasis, Myeloid-derived suppressor cells, EMT plasticity

P-010 - INFLAMMATORY CYTOKINE NETWORKS MODULATE HOST IMMUNE RESPONSES, GENERATING A PERMISSIVE MICROENVIRONMENT FOR METASTASIS

Arbağ Sena Nur[1], Lee Eunmi[2], Ouzounova Maria[2], Piranlioglu Raziye[2], Korkaya Hasan[2]

[1]*Faculty of Medicine, Hacettepe University, Ankara, Turkey*
[2]*Department of Biochemistry and Cancer Biology, Augusta University Georgia Cancer Center, Augusta, GA, USA*

Aim and Background: Establishment of pre-metastatic niche in distant organs is thought to be an important step during metastasis. Previous studies from our team and others established that tumors with activated inflammatory cytokines display aggressive epithelial mesenchymal (EMT) and cancer stem cell (CSC) phenotype.
Material and Method: In order to investigate the link between inflammation and breast cancer metastasis in syngeneic mouse model, we chose metastatic and non-metastatic murine tumors. 4T1 murine mammary tumor isolated from a BALB/c mouse has been characterized as metastatic while EMT6 as non-metastatic. To compare cytokine secretion between EMT6 and 4T1 cells, we collected conditioned media and performed cytokine antibody array. We also utilized flow cytometry analysis to check the infiltration of myeloid derived suppressor cells (MDSC)s in tumor sites.
Results: Our data showed that metastatic 4T1 tumor cells highly express inflammatory cytokines and growth factors, compared to non-metastatic EMT6. Mouse transcriptome analysis showed 781 genes that are differentially expressed between 4T1 and EMT6 tumors. We found a significant accumulation of both mMDSC and gMDSC in bone marrow, spleen, lung and tumor of 4T1 tumor-bearing mice. To examine whether 4T1 tumor secreted soluble factors can enhance metastasis in vivo, we intravenously injected EMT6 cells into mice with or without pre-treatment of conditioned medium(CM) from 4T1 tumor cells. It showed that injection of 4T1-CM significantly enhanced the metastatic ability of EMT6 tumors in lungs.
Conclusion and Discussion: These studies would provide an evidence that tumor secreted cytokines are capable of modulating early immune responses and establishing a pre-metastatic niche through recruitment of MDSCs and the reciprocal interactions of tumors and MDSCs facilitating metastatic steps such as dissemination and colonization in secondary organs.
Keywords: Breast cancer, Cytokines, Myeloid-derived suppressor cells, Pre-metastatic niche

P-011 - ANTI-METASTATIC EFFECT OF SINAPIC ACID IN PC-3 HUMAN PROSTATE CANCER CELL LINE

Eroğlu Canan, Kurar Ercan, Avcı Ebru, Vural Hasibe

Department of Medical Biology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

Introduction: Prostate cancer is one of important health problems diagnosed in men in many populations. Patients with prostate cancer has a good prognosis when it does not spread to other organs. However, the survival rate of patients sharply declines when it metastases. The treatment of the disease is often difficult due to metastasis. The aim of the study was to investigate the anti-metastatic effect of sinapic acid, is a phenolic compound and found in various vegetables and fruit species, in the PC-3 human prostate cancer cells.
Materials and Methods: Cytotoxic effect of sinapic acid was determined by using XTT assay. Total RNA isolation of control and dose groups (IC_{50} dose of sinapic acid) was conducted using TRIzol Reagent. Expressions of important genes in metastasis including *MMP-2, MMP-9, TIMP-1, TIMP-2, CDH1* and *CDH2* were investigated in control and dose groups by qPCR.
Results: IC_{50} dose of sinapic acid was detected as 1 mM for 72h in PC-3 cells. According to qPCR results, a significant increase in the expressions of *TIMP-1* and *CDH1* genes, and a significant decrease *MMP-9* gene were observed in the dose group, compared with the control group cells.
Discussion: It is thought that sinapic acid demonstrates anti-metastatic activity by regulating expression of important genes in metastasis on PC-3 cells. Furthermore, more detailed studies should be conduct to illuminate molecular mechanism of anti-metastatic activity of sinapic acid on prostate cancer.
Keywords: Metastasis, PC-3 cells, Prostate Cancer, Sinapic acid

P-012 - THE COMBINATION OF FERULIC ACID AND GEMCITABINE AFFECTS METASTASIS IN PC-3 HUMAN PROSTATE CANCER CELLS

Eroğlu Canan[1], Avcı Ebru[1], Seçme Mücahit[2], Dodurga Yavuz[2], Vural Hasibe[1], Kurar Ercan[1]

[1]*Department of Medical Biology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey*
[2]*Department of Medical Biology, Faculty of Medicine, Pamukkale University, Denizli, Turkey*

Introduction: Prostate cancer, the second causing of cancer-related death in men, can metastasize to the bone, lung and liver. Metastasis, associated with the aggressiveness of tumors and high mortality rate, is a multistep process. Ferulic acid (FA) is known as an abundant phenolic compound found in various fruit and vegetables. In this study, we aimed to determine the effects of FA and gemcitabine used either as a single agent or as a part of combination treatment in prostate cancer therapy, on metastasis in PC-3 human prostate cancer cell line.
Materials and Methods: The cytotoxic effects were determined by using XTT method after the treatment with FA, gemcitabine and combination of both of them. Total RNA was isolated with TRIzol Reagent. Expressions of genes are important in metastasis including *MMP-2*, *-9*, *TIMP1*, *2*, *CDH1*, *CDH2*, *COL4A3*, *VEGFA* and *HIF1A* were evaluated in four groups by qPCR.
Results: The IC_{50} doses of FA and gemcitabine were found to be 300 µM and 50 µM for 48h in PC-3 cells, respectively. For determination of combination effect, PC-3 cells were treated with $<IC_{50}$ doses (200 µM FA and 35 µM gemcitabine). When compared with the control group, qPCR results showed a significant decrease in the expressions of *MMP-2* and *VEGFA* genes; whereas, *TIMP-1* gene expression was decreased in the FA treatment group. After the treatment with gemcitabine, the expression of *TIMP-1*, *TIMP-2* and*CDH1* genes were significantly elevated, and the expression of MMP-2 and VEGFA genes were significantly downregulated. Furthermore, combination of FA and gemcitabine significantly increased expression of*TIMP-1*, *TIMP-2* and *CDH1* genes with higher fold change compared with other groups.
Discussion: In conclusion, it is thought that combination of FA and gemcitabine affected expression of metastasis genes with higher level compared with the single treatments in PC-3 cells.
Keywords: Ferulic acid, Gemcitabine, Metastasis, PC-3 cells, Prostate Cancer

P-013 - IDENTIFYING AND TARGETING NON-CODING RNAS TO INHIBIT LUNG METASTASIS IN TRIPLE NEGATIVE BREAST CANCER

Ersan Pelin Gülizar[1], Raza Umar[1], Eyüpoğlu Erol[1], Tokat Ünal Metin[1], Riazalhosseini Yasser[2], Alkan Can[3], Thieffry Denis[4], Gautheret Daniel[5], Şahin Özgür[1]

[1]*Bilkent University, Department of Molecular Biology and Genetics, 06800 Ankara, Turkey*
[2]*Centre for Molecular and Computational Genomics, McGill University, Montreal, Quebec, Canada*
[3]*Bilkent University, Department of Computer Engineering, 06800 Ankara, Turkey*
[4]*Ecole Normale Supérieure, Département de Biologie, IBENS - UMR ENS - CNRS 8197 - INSERM 1024 46 rue d'Ulm, 75230 Paris Cedex 05, France*
[5]*Institute for Integrative Biology of the Cell, CNRS, CEA, Université Paris-Sud - Bâtiment 400 91405 Orsay Cedex – France*

Introduction: Triple negative breast cancer (TNBC), the most aggressive breast cancer subtype, has high incidence rate of lung metastasis. Not only protein coding transcripts, but also noncoding transcriptome, such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), have active roles in cancer progression and metastasis. Additionally, lncRNAs can act as sponges for miRNAs. Here, we aimed i) to construct the first mRNA-miRNA-lncRNA network controlling metastasis in TNBC, and ii) to prevent lung metastasis by targeting identified central candidate genes.

Material and Method: We established primary tumor and human-in-mouse (HIM) and mouse-in-mouse (MIM) lung metastasis models using TNBC cell lines in nude and Balb/c mice, respectively. We visualized both primary and metastatic tumors using *in vivo* imaging system, harvested tumors and performed both RNA and small RNA sequencing. We obtained differentially expressed miRNAs, mRNAs and lncRNAs between primary and metastatic tumors. Using several bioinformatics tools, we did enrichment analyses, miRNA target predictions, and network construction.

Results: 45 and 91 miRNAs were differentially expressed between primary and metastatic tumors in HIM and MIM models, respectively. A miRNA family, whose role known in metastasis, as well as several other miRNAs were identified as highly differentially expressed in the same direction in both models. Moreover, 1127 and 3350 mRNAs, and 85 and 111 lncRNAs were differentially expressed in HIM and MIM models, respectively. DAVID bioinformatics tool showed significant enrichment of metastasis-related processes in metastatic tumors.

Discussion: Our study deciphered important candidates mediating lung metastasis in TNBC. Currently, we are integrating these 3 layers of data together with target predictions to construct the first mRNA-miRNA-lncRNA network controlling metastasis in TNBC. Identified central candidates will be tested in *in vitro* and *in vivo* metastasis assay models. Ultimately, our study will uncover lncRNAs that can be used as potential targets and/or biomarkers in breast-to-lung metastasis.

Keywords: Long non-coding RNA, Lung Metastasis, microRNA, Network Analysis, Triple negative breast cancer

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P-014 - INCREASED EXPRESSION OF TGF-β1 AND TSLP IN HIGHLY METASTATIC BREAST CANCER CELL LINES

Yitiz Nur[1], Yerlikaya Azmi[2], Erin Nuray[1]

[1]*1Akdeniz University, College of Medicine, Department of Medical Pharmacology, Antalya, Turkey*
[2]*Dumlupınar University, Faculty of Medicine, Department of Medical Biology, Kütahya, Turkey*

Aim: We performed proteomic analysis of exosomes derived from conditioned medium of breast cancer cells metastasized to vital organs such as brain, liver and heart. We found 1855 peptides in exosomes. Of these peptides, 225 of them, which corresponded to 83 proteins, were significantly altered in metastatic cell lines compared to non-metastatic cells. To support these results, we investigated the expression of two proteins which found higher in metastatic cell lines; Transforming growth factor beta (TGF-β1) and Thymic stromal lymphopoietin (TSLP). TGF-β1, a secreted protein, promotes cancer invasion and metastasis by inducing epithelial-to-mesenchymal transition. TSLP induces growth and metastasis of breast and pancreatic cancers through activating CD4+ T cells, inducing Th2-skewed immune responses and production of immunosuppressive factors.
Materials and Methods: We previously isolated liver, brain and heart metastatic cells of 4T1 murine breast carcinoma and named them as 4TLM, 4TBM and 4THM, respectively. In addition, we used non-metastatic 67NR breast cancer cells. In order to measure TGF-β1 levels, we treated the cell's supernatants with acid and base as recommended by the ELISA kit protocol; however, this protocol was unsuccessful. Afterwards, we used OASIS HLB 6cc (200 mg) Extraction Cartridges to concentrate and dissociate the proteins. TSLP levels measured directly from the conditioned mediums using ELISA.
Results: Depending on the subset of metastatic cells TGF-β1 levels were 6-13 fold higher compared to non-metastatic cells. Metastatic cells secreted markedly higher levels of TSLP (>15 fold).
Conclusions: Given the previous findings, our results suggests that increased levels of TGF-β1 and TSLP in exosomes of metastatic breast cancer cells mediate metastatic process and further studies targeting these molecules are required to determine possible therapeutic values of TSLP and TGF-b antagonists.
Keywords: breast cancer, metastasis, TGF-β1, TSLP

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P-015 - COMPARISON OF MIGRATION AND INVASIVE PROPERTIES OF SW-620 AND HT-29 COLON CANCER CELLS

Kandır Sinan[1], Karakurt Serdar[2]

[1]*Cukurova University, Ceyhan Faculty of Veterinary Medicine, Department of Physiology, Adana, Turkey*
[2]*Selcuk University, Faculty of Science, Department of Biochemistry, Konya, Turkey*

Introduction: Colon cancer is the third leading disease of death in the world wide especially industrial countries as well as developing ones. Current therapeutic approaches for colon cancer generally have variable efficiency, develop metastasis and drug-resistance, and have high toxicity to normal tissues. The aim of this study to clarify effects of phenolic compounds on metastatic and invasive properties of different colon cancer cell lines.
Material and Methods: SW-620 cells and HT-29 cells were grown in Leibovitz's L-15 Medium and McCoy's 5a medium, respectively and supplemented with 10% fetal bovine serum and 2 mM glutamine. Cytotoxicity of tannic acid on SW-620 cells and HT-29 cells were determined with Alamar blue and IC50 value was calculated. The effects of tannic acid on proliferation, invasive potential and metastasis of cancer cell lines were analyzed by wound healing, colony formation and matrigel chamber assays, respectively.
Results: In this study, we found that TA was found potential inhibitor against SW-620 than HT-29 and inhibited proliferation of both cell lines a concentration-dependent manner with an IC50 of 7.2 μM and 37.6 μM. TA treatment of cells led to a significant 90% and 85% decrease in motility and metastasis of PC-3, respectively. In addition, tannic acid treatment of SW-620 and HT-29 cells inhibits migration 82% and 73%, respectively as well as 73% and 55% inhibition were observed in invasive potential of cells.
Conclusions: These results show that tannic acid has more inhibitory action on the proliferation, invasive and migration potentials of colon cancer cell line SW-620 than HT-29 cells. SW-620 cells has the highest migration potential in all colon cancer cells. Hence it was shown that tannic acid have protective capacity upon the colon cancer.
Keywords: Colon cancer, SW-620, HT-29, Tannic acid, migration

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P-016 - ANALYSING OF SRP9 GENE EXPRESSION AND CORRELATION WITH CLINICOPATHOLOGIC PARAMETERS IN HUMAN BREAST CARSINOMA

Kaya Sare Burcu[1], Güven Mehmet[1], Tekin Bülent[1], Kelten Canan[2], Trabulus Didem[3]

[1]Department of Medical Biology, Cerrahpasa Faculty of Medicine, University of Istanbul, Istanbul, Turkey.
[2]Department of Pathology, Istanbul Education and Research Hospital, Ministry of Health, Istanbul, Turkey.
[3]Department of General Surgery, Istanbul Education and Research Hospital, Ministry of Health, Istanbul, Turkey.

Aim: Recently, post-transcriptional modification events, such as A-to-I RNA editing, catalysed by ADAR, has been revealed a new player in cancer, by changing the nucleotide sequence of target RNAs and introducing A-to-I/G "mutations''. Analysis, comparing genome and transcriptome sequencing revealed a generalized increase of A-to-I RNA editing events in metastatic breast cancer; particularly, the signal recognition particle 9 kDa (SRP9), involing in protein secretory pathways, showed a high frequency of adenosine-to-guanosine transition, which alters its protein sequence. Interestingly, when analysed by gene-expression array, the lobular breast cancer metastasis showed an upregulation of ADAR1 mRNA. From this point of view, the purpose of this study is to determine SRP9 gene expression levels in breast cancer tissue and to research its relation with clinical findings related to breast cancer by presenting the connection of these data with general increase in pre-mRNA modifications related to AID in breast cancer pathogenesis and their results. This study was supported by Istanbul University BAP unit; Project No: 34625.
Methods: The expression levels of SRP9 gene (mRNA) in tumor and corresponding adjacent normal tissue samples obtained from 50 breast cancer patients were analyzed by 'Quantitative Real Time-PCR' and 2-ΔΔCT method and statistically evaluated by 'Independent-t test' to investigate the possible role of SRP gene in breast cancer. The compariosion between the changes in SRP9 gene expression levels with clinical parameters were statistically analyzed by 'chi-square test' to investigate any possible relations.
Results: We found that there was a decrease of SRP9 gene expression levels in 18 (%36,0), increase in 23 (%46,0) and no change in 9 (%18) of the tumor tissue samples belonging to patients. There was an association between the increase of SRP9 gene and ER, PR expression (positive) respectively; (p=0,001*) and (p=0,009*).
Conclusion: According to our findings, SRP9 gene could have a potential role in increased expression of ER and PR in human breast carsinoma.
Keywords: ADAR1, Post-Transcriptional Modifications, SRP9 mRNA, Clinopathological Parameters, Breast Cancer Tissue

P-017 - EFFECTS OF TRPV1 AGONISTS AND ANTAGONISTS ON PROLIFERATION OF METASTATIC BREAST CARCINOMA CELLS

Akman Muhlis, Erin Nuray

Akdeniz University, School of Medicine, Department Of Medical Pharmacology, Antalya, Turkey.

Introduction: Transient Receptor Potential Cation Channel Subfamily V member 1 (TRPV1) or as known as capsaicin receptors mostly found on peripheral and central nervous system. There are few studies examining the expression and role of TRPV1 channels in cancer cells. The purpose of this study was to determine the effects of TRPV1 agonists and antagonists on survival and proliferation of metastatic breast carcinoma.
Materials and Methods: In this study, heart (4THM) and brain (4TBM) metastatic cells of 4T1 breast carcinoma cells which originally obtained from spontaneously formed breast cancer in a Balb-c mouse were used. First of all, expressions of TRPV1 on these cell lines were determined by western blot. A Balb-c mouse brain cortex was used as a positive control. Afterwards, 4THM and 4TBM cells were cultured on 96-well plate (500 and 1500 cell per well) and then treated with different concentrations (0.1 µM, 1 µM, 10 µM) of TRPV1 agonists (Resiniferotoxin, Capsaicin, MSK-195) and antagonists (Capsazepine, AMG-9810). Changes in cell proliferation were determined by using WST-1 after 72 hours incubation.
Results: Among TRPV1 agonists Capsaicin was the most effective and Resiniferatoxin was the least effective agent in inhibition of cell proliferation. Capsaicin dose-dependently inhibited cell proliferation in both 4THM and 4TBM cells. Surprisingly, TRPV1 antagonist AMG-9810 at 10 µM concentration markedly suppressed proliferation of metastatic cells. The effects of the other antagonist, Capsazepine was somewhat similar to AMG-9810 but the anti-proliferative effect was less marked.
Conclusion: Our findings show that TRPV1 channels are present on metastatic breast carcinoma cells and regulate cell proliferation. Further studies are required to determine the mechanisms of TRPV1-induced alterations on cell proliferation.
Keywords: AMG-9810, breast cancer, capsaicin, cell proliferation, TRPV1

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P-018 - THE EFFECTS OF PI3Kα AND PI3Kβ INHIBITORS ON MIP-2 AND KC SECRETION FROM METASTATIC BREAST CARCINOMA

Nizam Esra, Erin Nuray

Akdeniz University, School of Medicine, Dept. Of Medical Pharmacology, Antalya, Turkey.

Background: Phosphoinositide 3-kinases (PI3Ks) play key regulatory roles in many cellular processes including cell survival, proliferation, differentiation of cancer cells. Class 1 PI3Ks are composed of a p110 catalytic subunit, of which there are 4 isoforms (p110α, p110β, p110δ, and p100γ), and a p85 regulatory subunit. MIP-2 is a member of the CXC chemokine family, the mouse homologue of CXCL2, activates CXCR2 inducing angiogenesis and tumor progression. KC is a mouse homologue of CXCL1 and involved in the processes of angiogenesis, arteriogenesis, inflammation, wound healing, and tumorigenesis. Intracellular mechanisms involved in MIP2 and KC secretion from cancer cells are not entirely known. The goal of this study is to determine the role of PI3Kα and PI3Kβ on MIP-2 and KC secretion from breast carcinoma cells metastasized to brain (4TBM) and heart (4THM).
Materials and Methods: BYL719 is a selective PI3Kα inhibitor, and TGX-221, a potent, selective, and cell permeable inhibitor of PI3K p110β were used. 4THM and 4TBM cells were treated with 0,1-10µM doses of BYL719 and TGX221. Changes in MIP-2 and KC levels and cell proliferation were determined using Elisa and WST-1 respectively.
Results: BYL719 treatment dose dependently suppressed proliferation of 4TBM and 4THM cell when cells were seeded as 500 cells/96 well-plate. Secretion of MIP-2 and KC was markedly suppressed by 10µM BYL719. A partial inhibitory effect was also observed at doses of 1 µM. These effects were more pronounced in 4TBM cell line. TGX221 did not effect cell proliferation and MIP-2 secretion both cell lines. On the other hand TGX221 effectively suppressed KC secretion in 4TBM cells.
Conclusion: These results demonstrate that increased PI3-Kα activity might be involved in MIP-2 and KC secretion.
Keywords: Breast Cancer, MIP-2, BYL719, TGX221, PI3-K

P-019 - MUTATIONAL ANALYSIS OF AMINO TERMINAL DOMAIN OF INSULIN RECEPTOR SUBSTRATE-1 (IRS1) GENE IN GLIOBLASTOMA MULTIFORME PATIENTS

Gulacar Ismail Musab[1], Tuluce Yasin[1], Ozkol Halil[1], Gorgisen Gokhan[1], Yakut Sezin[2]

[1]*Medical Biology Department, Medicine Faculty, Yuzuncu Yıl University, Van, Turkey*
[2]*Medical Biology Department, Medicine Faculty, Akdeniz University, Antalya, Turkey*

Aim: In the recent studies about cancer-diabetes relations, Insulin Receptor Substrate (IRS) proteins that are mediator protein family and have pivotal roles in insulin signal pathway have become the focus of attention. Because IRS-1 is widely expressed in human tissues and has important role in insulin signaling, it is most studied member of this protein family. Due to becoming one of targeted protein and gene in both cancer and diabetes studies; genomic changes, expression/activation profiles and functional status of IRS-1 have been studied by a lot of researchers. We have detected some mutations in carboxy terminal domain of IRS-1 in our previous study. In this study, we want to also determine presence or absence of mutation in PH (pleckstrin homology) and PTB (phosphotyrosine binding) regions that are located in amino terminal domain of this protein.
Material and Methods: In this study, we have isolated and sequenced genomic DNAs from tumor samples of 28 Glioblastoma Multiforme tumors and 6 control tissues were obtained by autopsy and we looked for the presence or absence of mutations in the region PH and PTB domains.
Results: On the basis of our results, we detected p.A124S heterozygote changes in PH domain in 1 patient and p.G234G, c.702G>A heterozygote changes in PTB region of IRS1 in 1 of 28 patient samples compare to the controls.
Discussion and Conclusion: PH and PTB regions have a pivotal role in activation of IRS1 and diversity of signaling. Therefore, genomic changes in these regions may lead to tumorigenesis. Our results suggest that heterozygote changes in these regions of IRS1 may be involved in the modulation of IRS1 functions and could be relevant to Glioblastoma Multiforme.
Keywords: Insulin Substrate Proteins, IRS, Cancer, Glioblastoma Multiforme

P-020 - ASSESSMENT OF LOW DOSE EFFECT OF DOXORUBICIN ON APOPTOSIS AND MULTIDRUG RESISTANCE IN MCF-7 CELLS

Ercan Ayşe, Öncül Selin

Hacettepe University Faculty of Pharmacy Department of Biochemistry, Ankara

Introduction: As breast cancer is the most common cancer type amongst women worldwide, natural and synthetic drugs have been continuously developed and used as chemotherapeutic agents. It has been proved that many cancer types arise as a result of malfunctioning genes coding for anti-apoptotic proteins, apoptosis inhibitors or tumor suppressors. Doxorubicin (DOX) is an anthracycline based chemotherapeutic agent which inhibits topoisomerase II activity and halts replication by intercalating to the DNA helix. Alongside with various types of cancer, DOX is clinically used in the treatment of breast cancer. Multidrug resistance (MDR) is defined as simultaneous resistance towards different drugs which do or do not demonstrate structural resemblance and have different effects on their molecular targets. P-glycoprotein is a membrane protein coded by ABCB1 (MDR-1) gene which acts as an ATP-dependent pump and has a role on efflux of numerous drugs including DOX.
Methods: In this study MCF-7 breast cancer cells were treated with varying doses (50 nm-20 μM) of DOX and cell viability was determined using SRB assay. Subsequently, Tali® assay was performed to define the percentage of apoptosis and MDR assay was carried out to investigate the drug efflux level of DOX in MCF-7 cells.
Results: Cell viabiliy decreased significantly following application of DOX (IC_{50}=10 μM). The population of apoptotic cells were slightly elevated with treatment of 50, 200 and 800 nm of the drug and apoptotic cell ratios were measured to be 6.3, 10 and 17.7 %, respectively. Additionally, efflux of DOX was shown to be dramatically increased when MCF-7 cells were treated with same doses of the drug.
Conclusion: It was demonstrated that at low doses of DOX treatment, cell viability was decreased only partially through apoptosis and the dramatic increase detected in DOX efflux may contribute to the reduced apoptotic response in MCF-7 cells.
Keywords: Apoptosis, Breast Cancer, Doxorubicin, Multidrug Resistance

P-021 - GOLD NANOPARTICLE-SIRNA MEDIATED NF-κB SILENCING IN PROSTATE CANCER

Sünnetçi Akkoyunlu Deniz[1], Eren Keskin Seda[1], Çine Naci[1], İskenderoğlu Elmas Tuna[1], Yılmaz Elif Büşra[1], Devrim Nur[1], Kurtaş Ömer[2], Savlı Hakan[1]

[1]*Medical Genetics Department, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey*
[2]*Forensic Medicine Department, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey*

Background and Aim: Nuclear Factor kappa B (NF-κB) is a wellknown transcription factor which is activated in hematological malignancies and solid tumors. We found NF-κB overexpression using gene expression microarray in our previous studies about B-CLL after ionizing radiation exposure and prostate, papillary thyroid, breast cancers. Inhibition of NF-κB activation may allow selective killing of cancer cells. Based on this hypothesis, we aimed silencing NF- κB2 gene via gold nanoparticles functionalized with siRNA (small interfering RNA) in androgen-dependent prostate cancer cell line, NE-1-8.

Materials and Methods: Proliferation assay was performed using xCELLigence RTCA DP Real Time Cell Analyzer to determine the optimal cell concentration. The cells were transfected with gold nanoparticle-siRNA at a concentration of 2 nM, 4 nM and 8 nM at the 25th hour. Untransfected cells (cells only) were used as a control. The 24-hours post-transfection effect of gold nanoparticle-siRNA on NF- κB2 and apoptosis related genes (Bcl-2 and Bax) were determined by quantitative real-time PCR. IC50 value was calculated by cytotoxicity assays performed on the xCELLigence RTCA DP.

Results: We observed decreasing concentrations of cells transfected with gold nanoparticle-siRNA at a concentration of 8 nM, 4 nM and 2 nM from the 33rd, 39th and 45th hours respectively, whereas there was no change in cells only. NF-κB2 gene expression levels were decreased (1,352 folds in 2 nM, 3,063 folds in 4 nM) after transfection. Gene expression changes of Bcl-2 and Bax showed increased apoptosis with increasing molarity. Cytotoxicity assays determined IC50 value as 2.26974E-009 M.

Discussion and Conclusion: Our results suggest that gold nanoparticle-siRNA mediated NF- κB2 silencing may be an effective gene therapy for prostate cancer treatment. Further studies including modifications of gold nanoparticles for targeting tumor cells in tissue cultures and xenograft models for showing systemic effects of functionalized gold nanoparticles are needed.

Keywords: Cancer, Prostate Cancer, NF-κB, siRNA Delivery, Gold Nanoparticles

P-022 - SYNTHESIS OF PLGA COATED MAGNETIC NANOPARTICLES FOR CO-DELIVERY OF CANCER DRUG AND VITAMIN E TPGS

Metin Esra[1], Mutlu Pelin[2], Gündüz Ufuk[3]

[1]*Middle East Technical University, Department of Biotechnology, 06800, Ankara*
[2]*Middle East Technical University, Central Laboratory, Molecular Biology and Biotechnology R&D, 06800, Ankara*
[3]*Middle East Technical University, Department of Biological Sciences, 06800, Ankara*

Introduction: Magnetic nanoparticles (MNPs) which are used for targeted delivery of anticancer drugs under external magnetic field; maximize the efficacy of the drug, minimize its side effects and reduce systemic toxicity. Vitamin E TPGS, being a water-soluble derivative of natural Vitamin E has advantages in drug delivery such as extension of the half-life of the drug in plasma and enhancement of the cellular uptake of the drug. In the current study PLGA coated magnetic nanoparticles were designed for co-delivery of anti-cancer drug Doxorubicin and vitamin E TPGS.
Materials and Methods: Magnetic nanoparticles were synthesized by co-precipitation method. Doxorubicin and vitamin E TPGS loaded magnetic PLGA nanoparticles were produced by nanoprecipitation method. Synthesized nanoparticles were characterized by using FTIR, zeta potential, VSM, DLS, TGA, TEM analyses. Drug loading and release profiles were studied in vitro. Internalization of nanoparticles by MCF-7 cells was visualized under light microscope.
Results: According to FTIR and TGA analysis, Doxorubicin and vitamin E TPGS were loaded in PLGA coated MNPs successfully. DLS results confirmed that the size of the nanoparticles were in nano-scale. Moreover, the nanoparticles were superparamagnetic according to VSM results. Drug loading efficiency was high and loaded drug could be released in sustainable form. Finally, it was shown that the nanoparticles could be internalized into MCF-7 cells successfully.
Conclusion: PLGA coated magnetic nanoparticles could be successfully loaded by Doxorubicin and vitamin E TPGS. Internalization studies confirmed that these nanoparticles can be taken up by MCF-7 cells. These results showed that PLGA coated magnetic nanoparticles can be a suitable drug carrier for co-delivery of Doxorubicin and vitamin E TPGS.
Keywords: Magnetic Nanoparticles, Targeted Drug Delivery, Vitamin E TPGS

P-023 - THE CYTOTOXIC EFFECTS OF GREEN SYNTHESIS SILVER NANOPARTICLES ON MCF-7 CELL LINE

Şen Fatih[1], Şahin Birgütay[1], Kocacan Metin[2]

[1]*Faculty of Art and Science-Dumlupınar Universty/Kutahya*
[2]*Faculty of Medicine- Dumlupınar Universty/Kutahya*

In this study, silver nanoparticles (AgNPs) was obtained by using green synthesis method in the presence of Pomegranate (Punica granatum L). In this method, an aqueous extraction of Pomegranate (Punica granatum L) was mixed with AgNO3 for AgNPs synthesis. The resulting Ag NP' s cytotoxic effect have been investigated by the help of MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) test. After 24 hour incubation period at 20 mg/ml Ag NPs, IC50 of human breast cancer cell line (MCF7) were found to inhibit the proliferation. These results showed that Ag NPs supplemented with pomegranate have revealed the growth suppressive properties.
It was thought that Ag NPs supplemented with pomegranate may help the antiproliferative effect on MCF7 cell line.
Keywords: MCF-7, Pomegranate (Punica granatum L), MTT, Green Synthesis

P-024 - 6-(4-METYLPHENYL-8-(4-CHLOROPHENYL)IMIDAZO[1,2-A]PYRAZINE: A MOLECULE FOR TELOMERASE INHIBITION

Mutlu Ayşe Gül[1], Bilginer Abdülkerim[2], Kayağil İsmail[3], Yıldız Hülya[2], Demirayak Şeref[4]

[1]Mehmet Akif Ersoy University, Arts and Sciences Faculty, Department of Molecular Biology and Genetics, Burdur, Turkey.
[2]Mehmet Akif Ersoy University, Arts and Sciences Faculty, Department of Biology, Burdur, Turkey
[3]Mehmet Akif Ersoy University, Arts and Sciences Faculty, Department of Chemistry, Burdur, Turkey
[4]Medipol University, Faculty of Pharmacy, İstanbul, Turkey

Objective: Imidazopyrazine derivatives have been studied for their curative effects on some diseases like cancer and neurological problems; also, some of these molecules have been patented. Primary human cells exhibit limited replicative potential but the cancer cells divided indefinitely with passage in culture. This immortality is mainly a result of telomerase activity. We investigated the possible telomerase inhibitor effect and possible mtDNA damage action of two imidazopyrazine derivatives 6-(4-Metylphenyl-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine and 6-(4-Metylphenyl-8-(4-methoksiphenyl)imidazo[1,2-a]pyrazine.
Material and Methods: Telomerase activities were measured by the PCR-ELISA based TRAP method and mtDNA damage assays were achieved by quantitative PCR. We used zebrafish as a model organism for our research.
Results: In the application of 6-(4-Metylphenyl-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine (C19H14N3Cl), it was determined that they inhibit telomerase activities to a statistically significant degree. There were no significant differences among the groups in terms of mtDNA damage or copy number.
Conclusion: Cancer cells which can be divided limitlessly due to telomerase activation may lose this characteristics through inhibition of telomerase enzyme. According to these results, it was found out that this compound has the probability to be used as an anti-cancer agent as a result of detailed studies. All zebrafish applications were approved by the Ethical Committe of the Mehmet Akif Ersoy University (27.01.2014/ 57 and 24.02.2015/114).
Keywords: Anticancer drugs, mtDNA damage, Imidazopyrazine derivatives, Telomerase inhibition

P-025 - UKRAIN INHIBITS THE PROLIFERATION AND INDUCES APOPTOSIS OF BREAST CANCER CELLS COMPARED WITH TAMOXIFEN AND DOCETAXEL

Koçak Fatma Emel[1], Öztürk Bahadır[2], Koçak Cengiz[3], Vatansev Hüsamettin[2], Tekin Gülsüm[2]

[1]*Department of Medical Biochemistry, Faculty of Medicine, Dumlupinar University, Kutahya, Turkey*
[2]*Department of Medical Biochemistry, Faculty of Medicine, Selcuk University, Konya,Turkey*
[3]*Department of Medical Pathology, Faculty of Medicine, Dumlupinar University, Kutahya, Turkey*

Introduction: Ukrain is the trade-marked name of a semi-synthetic substance derived from the plant Chelidonium majus and promoted as a drug to treat of various cancer types. In this study, we investigated for the first time the effect of anticancer agent ukrain and compared with tamoxifen and docetaxel on MCF–7 (ER+, PR+, HER-2-) and MDA MB-231 (ER-, PR-, HER-2-) cell lines.
Material and Method: A real-time cell analyzer (xCELLigence, Roche Diagnostics GmbH, Penzbeerg, Germany) was used to evaluate the effects of different doses of ukrain (12,5-100µM), Tamoxifen (12,5-100µM) and Docetaxel (12,5-100nM) on the proliferation of both cell lines and determined IC50 for each drug. Cell blocks were prepared from cultured cells treated with drugs and formalin-fixed paraffin-embedded breast cancer cells were examined histopathologically using Haematoxylin&Eosin staining method. In addition, the expressions of Ki-67, Bcl-2, BAX, and cyclin-D1 were assessed immunohistochemically. Statistical analysis was performed GraphPad Prism version 6.05 (GraphPad Software, Inc, CA, USA).
Results: Ukrain inhibits the proliferation in both cell lines time and dose dependent manner. IC50 for Ukrain, Tamoxifen and Docetaxel in MDA-MB–231 and MCF–7 cells were 75 µM at 48h and 34 µM at 111h; 50 µM at 45h and 40 µM at 41h; 32 nM at 60h and 43nM at 40h, respectively. As a results of histopathologic and immunohistochemical analysis, Ukrain decreases Ki-67, cyclin-D1 and increases BAX/Bcl-2 ratio in both breast cancer cells.
Conclusions: The results of this study suggest that Ukrain decreases cell viability of breast cancer cells and induces apoptosis compared with Tamoxifen and Docataxel. It has been observed more effective for MDA MB–231 compared with MCF–7, thus supporting its use as a therapeutic agent for the treatment of reseptor negative breast cancer.
Keywords: Docetaxel, MCF-7, MDA MB-231, Tamoxifen, Ukrain

P-026 - EFFECT OF CDDO-ME, TAMOXIFEN AND DOCATAXEL ON MCF-7 AND MDA MB 231 CELL LINES

Öztürk Bahadır[1], Koçak Fatma Emel[2], Koçak Cengiz[3], Akyürek Fıkret[1], Tekin Gülsüm[1]

[1]*Department of Medical Biochemistry, Faculty of Medicine, Selcuk University, Konya,Turkey*
[2]*Department of Medical Biochemistry, Faculty of Medicine, Dumlupinar University, Kutahya, Turkey*
[3]*Department of Medical Pathology, Faculty of Medicine, Dumlupinar University, Kutahya, Turkey*

Introduction: Bardoxolone metyl (CDDO-me; (methyl-2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oate) is semi-synthetic triterpenoid. CDDO-me acts as an activator of the Nrf2 pathway and an inhibitor of the NF-κB pathway. It also has been used traditional medicine as anticancer and antiinflamatory agent. We have investigated the therapeutic efficacy of CDDO-Me, Tamoxifen and Docataxel in MCF–7 (ER+, PR+, HER-2-) and MDA MB-231 (ER-, PR-, HER-2-) cell lines.
Material and Method: A real-time cell analyzer (xCELLigence, Roche Diagnostics GmbH, Penzbeerg, Germany) was used to evaluate the effects of different doses of CDDO-me (10-200nM for MCF-7; 0,1-10 µM for MDA MB-231), Tamoxifen (12,5-100 µM) and Docetaxel (12,5-100nM) on the proliferation of both cell lines and determined IC50 for each drug. Cell blocks were prepared from cultured cells treated with drugs and formalin-fixed paraffin-embedded breast cancer cells were examined histopathologically using Haematoxylin&Eosin staining method. In addition, the expressions of Ki-67, Bcl-2, BAX, and cyclin-D1 were assessed immunohistochemically. Statistical analysis was performed GraphPad Prism version 6.05 (GraphPad Software, Inc, CA, USA).
Results: CDDO-me inhibits the proliferation in both cell lines time and dose dependent manner compared with Tamoxifen and Docataxel. IC50 for CDDO-me, Tamoxifen and Docetaxel in MDA-MB-231 and MCF-7 cells were 27nM at 65h and 82nM at 43h; 50 µM at 45h and 40 µM at 41h; 32 nM at 60h and 43nM at 40h, respectively. As a results of histopathologic and immunohistochemical analysis that CDDO-me decreases Ki-67, cyclin-D1 and increases BAX/Bcl-2 ratio in both breast cancer cells.
Conclusions: CDDO-me has been observed more effective for MDA MB-231 compared with MCF-7 and also has been more influential in both cell lines compared with docetaxel. CDDO-me may be helpful for the therapies of breast cancer according to our findings.
Keywords: CDDO-me, Docetaxel, MCF-7, MDA MB-231, Tamoxifen

P-027 - INVESTIGATION OF THE EFFECT OF POMEGRANATE EXTRACT AND PLATINUM NANOPARTICLE COMBINATION ON MCF-7 CELL LINE

Şen Fatih[1], Şahin Birgütay[1], Kocacan Metin[2]

[1]*Faculty of Art and Science-Dumlupınar Universty/Kutahya*
[2]*Faculty of Medicine-Dumlupınar Universty/Kutahya*

The present study aims to develop an easy and eco-friendly method for the synthesis of platinum nanoparticles using extracts from the medicinal plant, Punica granatum and evaluation of its anticancer properties. The various parts of P. granatum were screened and the root extract was found to have the highest potential for the synthesis of nanoparticles. The shell extracts were able to quickly reduce Pt+ to Pt0 and stabilized the nanoparticles. The synthesis of nanoparticles was confirmed by UV–Visible spectrophotometry and further characterized using Zeta sizer, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscope (TEM) and X-ray diffraction (XRD). The studies of phytochemical analysis of nanoparticles indicated that the adsorbed components on the surface of nanoparticles were mainly flavonoid in nature. Furthermore, nanoparticles were evaluated as cytotoxic against various cancer cell lines and 25 to 100 µg/mL nanoparticles showed good toxicity. The IC50 value of nanoparticles was found to be 25 and 50 µg/mL against MCF-7 cell lines, respectively. Additionally, the apoptotic effect of synthesized nanoparticles on normal and cancer cells was studied using trypan blue assay analysis. The results indicate the synthesized nanoparticle ability to kill cancer cells compared to normal cells.

Keywords: MCF-7, Pomegranate (Punica granatum L), Platinum nanoparticles, MTT, Green Synthesis

P-028 - INHIBITING CERAMIDASE ENZYME PROMOTES CELL DEATH IN HUMAN BREAST CANCER: AN IN VITRO STUDY

Kutlu Hatice Mehtap[1], Vejselova Djanan[1], Kuş Gökhan[2]

[1]*Anadolu University, Faculty of Science, Department of Biology, Eskişehir, Turkey*
[2]*Department of Health Programme, Open Faculty, Anadolu University*

Background: Acid ceramidases are enzymes with a vital role in metabolizing ceramide to sphingosine-1-phosphate that is an antiproliferative metabolite in ceramide pathway. Inhibition of exogenous ceramides with ceramidase inhibitors lead to augmented ceramide levels in cells in turn lead to cell cycle arrest and apoptosis.
Objective: Our study aimed at targeting ceramide metabolic pathway to induce apoptosis in human breast cancer cell line (MCF7) and we examined the antiproliferative and apoptotic activities of ceranib-2, an inhibitor of human ceramidase, on this cell line including with morphological changes caused by ceranib-2.
Material and Methods: Cytotoxic effects of ceranib-2 on MCF7 cells was detected via MTT test system. Dilutions prepared from the stock solution (in DMSO) of ceranib-2 was applied on MCF7 cells (1x104 cells/well) for 24 hours at 37 °C and 5% CO2 in air. The plates were read on ELISA reader (ELx808), at wavelength of 540 nm (n=3). For detecting the structural alterations IC50 concentration of ceranib-2 was applied on MCF7 cells for 24 hours. Treated cells were stained with Alexa fluor-488 phalloidine and acridine orange and observed under confocal microscope.
Results: Viability percentages and IC50 (13µM) value were determined. Morphological alterations detected on our confocal micrographs were damaged cytoskeleton as hole formation, shrinked cells and fragmented and condensed nuclei as apoptotic hallmarks.
Discussion: According to our results, ceranib-2 caused structural changes in MCF7 cells morphology.
Conclusion: We can conclude that ceranib-2 showed high cytotoxicity on MCF7 cancer cells in low concentrations and may be encouraging in designing of pharmaceutical products helpful in cancer treatment.
Keywords: Cytotoxicity, MCF7, Confocal microscopy

P-029 - APOPTOTIC EFFECT OF PRUNUS SPINOSA FRUIT EXTRACT ON HT-29 COLON CANCER CELL LINE

Qipa Egzona, Gökalp Fulya Dilek

Trakya University, Faculty of Science, Department of Biology, Edirne

Introduction: Colon cancer is the death-causing type of cancer, seen on both men and women. Natural compounds are mostly used lately and have shown prevention, inhibiton, and dilatory effects of different cancer types. In this study, it was aimed to investigate apoptotic effect of water extract of Prunus spinosa L. (Rosaceae) herb fruit traditionally known as Güvem or Çakal Eriği, on HT-29 colon cancer cell line.
Materials and Methods: Apoptotic effect on cell proliferation of different concentrations (44 g/ml, 88 mg/ml, and 4500 mg/ml) of water extracts of P. spinosa fruits on HT-29 cell line was investigated for 24 and 48 hours by using EB / AO (ethidium bromide /acridine orange) coloring method. The cells were morphologically evaluated and live, dead, necrotic and apoptotic cells were detected to differentiate the apoptotic effect on cell proliferation.
Results: It was shown that water extracts of P. spinosa fruits inhibited cell proliferation as the concentration and time increase and showed cytotoxic effect after 24 and 48 hour treatment. Cell death was significantly increased for both treatment period and for all tested concentrations compared to control ($p<0.01$; $p<0.001$). Probit analyze reports have shown IC50 values as 159.3 and 123.8 µg/ml for 24 and 48 hours treatment periods respectively.
Discussion and Results: The results showed that P. spinosa fruit extract inhibited cell proliferation depending on concentration and time increase and has a cytotoxic and apoptotic effect on HT-29 colon cell line. In the other investigations an anthocyanin compounds obtained from Prunus sp. also have cytotoxic effect on GLC, NCL-H460, A549 cancer cell lines. Further studies obtaining metabolites by using different extraction methods, and detecting activeness of metabolites might enhance economical value of Prunus sp and might have a potential to take part in anticancer pharmaceutical industry as a natural medicine.
Keywords: Prunus spinosa, Colon Cancer, EB/AO, Apoptosis, HT-29

P-030 - EFFECT OF 4-AMINOPYRIDINE ON PACLITAXEL ACTIVITY IN MCF-7 CELL LINE

Cüce Aydoğmuş Esra Münire, İnhan Garip Günseli Ayşe

Marmara University, School of Medicine, Biophysics Department, İstanbul, Turkey

Introduction and Purpose: Paclitaxel is a clinically proven antineoplastic agent that induces arrest at G2/ M phase. However its use is limited due to its side effects. There is a need for alternative methods to increase its effectiveness at low doses. It has been shown that pharmacological or genetic block of Kv channels reduces proliferation. 4-aminopyridine(4- AP) is a blocking agents in use.
This study aimed to determine changes that may occur in the activity of paclitaxel in MCF-7 breast cancer cell line preincubated with 4- aminopyridine.
Materials and Method: MCF-7 cells were grown in DMEM supplemented with 10% FBS, 2 mM L- glutamine and 100 U penicillin/streptomycin in 5% CO2 at 37 °C. Cells were seeded at 10000 cells/well into a 96-well plate and incubated overnight. Cells were incubated with 4- amino pyridine or paclitaxel or both for 24 hours and MTT assay was performed.
Results: MCF- 7 cells were incubated with low concentration (6 nM- 8 nM) of paclitaxel for 24 hours. A reduction by % 20 ±3 in cell viability was detected. Incubation with 4 mM and 5 mM concentrations of 4- AP for 24 hours caused % 20± 2 reduction in cell viability. After 24 h preincubation with specified concentration of 4- AP cells were incubated with same doses of paclitaxel for 24 h. Viability assay results showed no increase in paclitaxel activity. Conversely, an increase in cell proliferation of %15± 3 was observed.
Conclusion: There are two probabilities for this effect: 4- AP antagonized the cytotoxicity of paclitaxel by preventing MCF- 7 cells from entering the G2/ M phase, or Ca++ entry (by P2X7 receptors) upon blockage of Kv channels may have interfered with paclitaxel binding to tubuline.
Keywords: 4- Aminopyridine, MCF- 7 Breast Cancer Cell Line, Paclitaxel.

P-031 - INVESTIGATION OF ANTICANCER POTENTIAL OF ANTIHISTAMINE DRUG EBASTINE ON U266 MULTIPLE MYELOMA CELL LINE

Selin Gerekçi[1], Heval Ataş[2], Hepşen Hazal Hüsnügil[1], Aliye Ezgi Güleç[2], Can Özena,[2,3,4]

[1]*Graduate Program of Biochemistry,*
[2]*Graduate Program of Biotechnology,*
[3]*Central Laboratory, Molecular Biology and Biotechnology R&D Center,*
[4]*Center of Excellence in Biomaterials and Tissue Engineering, METU, Ankara*

Introduction: A new drug discovery and development process is very difficult, time consuming and costs approximately over $2 billion. However, reposition of drugs increases success rate of the treatment and can save time and money. Multiple myeloma (MM) is a hematological malignancy which is characterized by the uncontrolled growth of plasma cells in the bone marrow. This study shows that a second generation antihistamine drug Ebastine has a significant anticancer effect on MM U266 cell line.
Aim of Study: The aim of this study was to investigate anticancer potential of EBA on U266 MM cell line.
Materials and Methods: Dose and time response studies were performed with fluorescence-based CellTiter Blue Cell Viability Assay (Promega, USA). Caspase-3 levels were detected with PE Active Caspase-3 Apoptosis Kit (BD, USA) and performed on BD Accuri C6 flow cytometer.
Results: The anticancer effect of EBA was found to be time (12, 24, 48 hours) and dose (1-100 µM) dependent (IC50 15.97 ± 0.3 µM at 24 hour). Caspase-3 is one of the most important members of caspase family of cysteine proteases that plays a significant role in apoptosis. Active caspase-3 is used as a marker for cells undergoing apoptosis. To evaluate the apoptotic effect of EBA, cells were treated with 15 µM drug for 24 h. There was a significant increase in Caspase-3 activity of EBA treated cells.
Conclusion: Our results show that EBA has potent growth inhibitory and apoptotic effect on U266 cell line.
Keywords: Cancer, Multiple Myeloma, Anticancer Drug, Antihistamine, Drug repurposing

P-032 - IN VITRO ANTICANCER ACTIVIY OF CHLORPROMAZINE ON 266 MULTIPLE MYELOMA CELL LINE

Güleç Aliye Ezgi[1], Hüsnügil Hepşen Hazal[1], Ataş Heval[2], Gerekci Selin[1], Özen Can[3]

[1]METU, Biochemistry Department
[2]METU, Biotechnology Department
[3]METU, Central Laboratory Molecular Biology and Biotechnology R&D Center, Center of Excellence in Biomaterials and Tissue Engineering

Introduction: Multiple Myeloma (MM) is a cancer of plasma cells. MM accounts for 1% of all types of cancer and 13% of all hematological malignancies. Approximately three thousand people each year are diagnosed with MM in Turkey. MM has high morbidity and very low survival rates. Chlorpromazine (CPZ) is a phenothiazine and a marketed antipsychotic medication which is listed as an essential drug by the World Health Organization. It is primarily used to treat psychotic disorders such as schizophrenia, bipolar disorder, attention deficit hyperactivity disorder, nausea and vomiting, anxiety before surgery.
Aim of Study: The main goal of this study was to investigate the anticancer potential of CPZ on U266 MM cell line.
Materials and Methods: CellTiter-Blue Cell Viability Assay (Promega, USA) was used to analyze growth inhibitory effect of CPZ. Then, PE Active Caspase-3 Apoptosis Kit (BD, USA) was used to confirm the apoptotic effect of CPZ. Time- and dose-response studies were performed with nine doses of CPZ in the range of 1 to 100 µM and at 3 time points (12, 24, 48 h). For the apoptosis study, 20 µM CPZ was used.
Results: CPZ showed dose- and time-dependent inhibitory effect on cell viability. IC50 of CPZ was found as 22 ± 2.5 µM. Also, the increase in PE Active Caspase-3 flouresence signal after treatment indicates that CPZ exerts its cytotoxic effet on cells via inducing apoptosis.
Conclusion: Our results show that CPZ has potent growth inhibitory and apoptotic effect on U266 cell line.
Keywords: Cancer, Multiple Myeloma, Chlorpromazine, Drug Repurposing

P-033 - COMPUTATIONAL ANTI-CANCER AGENT REPURPOSING FOR PANCREATIC CANCER

Calimlioglu Beste[1], Gulfidan Gizem[2], Ozturk Emine Ravza[2], Nalbant Zeynep[2], Arga Kazim Yalcin[2]

[1]*Istanbul Medeniyet University, Bioengineering Department, 34700, Istanbul, Turkey*
Marmara University, Bioengineering Department, 34722, Istanbul, Turkey
[2]*Marmara University Bioengineering Department, 34722, Istanbul, Turkey*

Pancreas cancer is one of the known cancer types within late diagnosis and high mortality rates. Despite this fact, there is not sufficient literature information on prevention of disease, early diagnosis or efficient therapy strategies. On the other hand, investments including money, time and labor have gradually increased to fulfill demand of pharmaceutical industry. Elevated studies and improvements on omic technologies and their computational based analysis provide opportunities for further investigations such as drug repurposing.
This study aims to determine anti-cancer agent candidates for pancreatic cancer at transcriptome level.
4 transcriptome datasets (GSE19650, GSE16515, GSE22780, GSE32676) were acquired from Gene Expression Omnibus and chosen according to having healthy versus disease state. Each dataset was statistically analyzed independently to identify differential expressed genes (DEGs) with threshold p-value<0.01. All datasets were normalized and implemented in the affy and limma package of R/Bioconductor. Hub proteins were determined according to two metric (betweenness and degree) thru cytohubba plugin in CytoScape. Drugs were determined via DGIdb. Their significance was calculated via hyper geometric distribution. FDA approved anti-cancer agent list were arranged from National Cancer Institute lists for comparison.
Consequently, 16510 DEGs were determined as a combination of all datasets. We have found interactions between 509 genes & 2489 drugs which also covers the current pancreatic cancer drugs. 65 drugs were found as new candidate anti-cancer agents against pancreatic cancer although there are already used for other cancer types and some are also reported as candidates of pancreatic anti-cancer agents, recently. After statistical analysis, we have reduced the drug number based on threshold (p-value<0.001). Moreover, we have determined the hub protein-drug interactions. 9 drugs including Bosutinib, Dasatinib, Enzalutamide etc. were found in interaction with hub proteins.
Briefly, candidate anti-cancer agents were reported based on the computational analysis to investigate the effects on pancreatic cancer cases.
Keywords: Drug Repurposing, Pancreatic Cancer, System Biomedicine

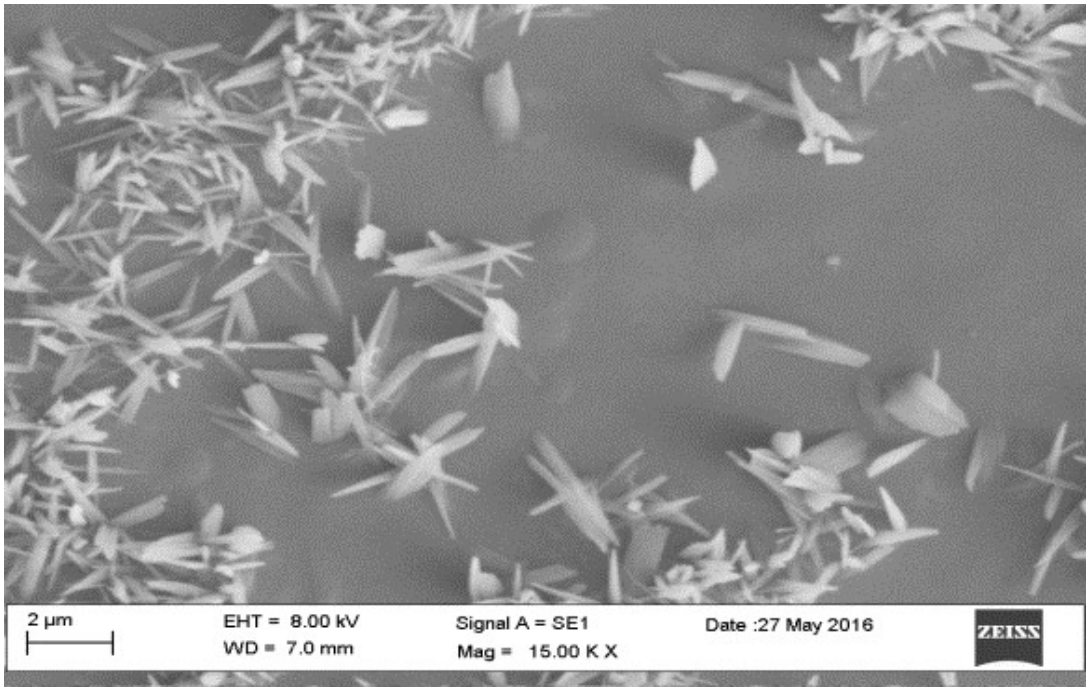
P-034 - PRODUCTION OF ORGANIC NANOPARTICLES BY USING NANOPOROUS MEMBRANES

Tuz Ali Ata, Şimşek Ahmet Kaan, Kazancı Murat

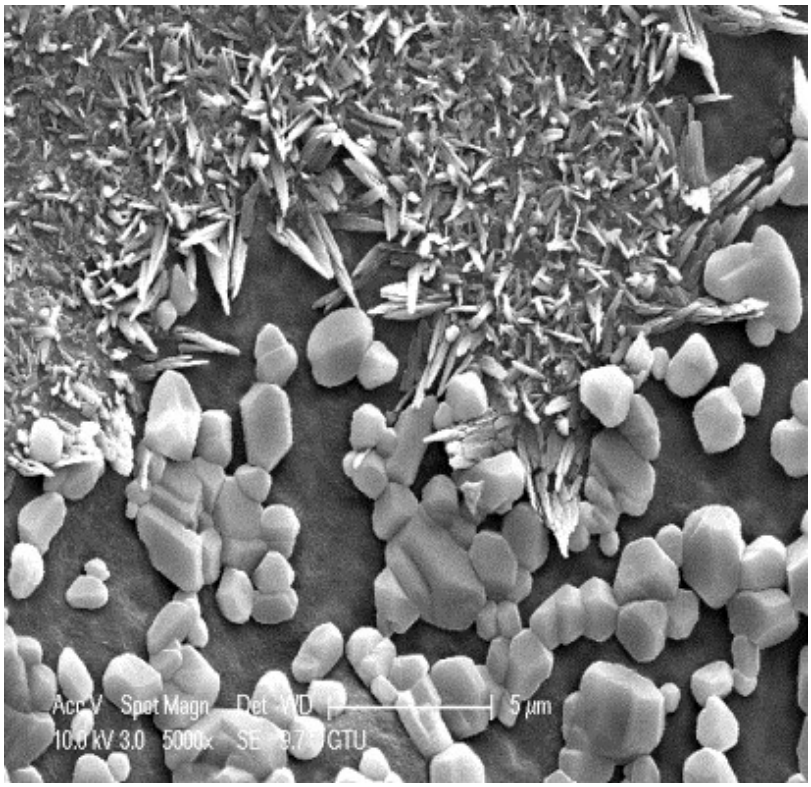
School of Medicine, Bahcesehir University, 34734 Kadikoy, Istanbul, Turkey

Background: The spatial and temporal control of the release of pharmaceuticals at the site of where they act is a key requirement for the therapeutic use of a drug.One method for realizing this objective is to create drug-loaded nanoparticles made out of biodegradable polymers.We present here an alternative strategy based on the use of a nanoporous membrane that separates the two liquids.In this work, we wanted to understand how to obtain nanoparticles under which conditions by using nanoporous membranes.We believe this knowledge can help to find most efficient way to produce the particles for different needs.
Methods: Two liquids, a feed solution and a receiver solution, are separated by a nanoporous polycarbonate track-etched(PCTE) membranes.The feed solution is pumped through the membrane into the receiver solution.The feed solution contains biopolymers such as chitosan,collagen and alginic acid brown algea.The receiver solution contains 1mM NaOH.According to these parameters, we aimed to find the ideal pore sizes and productionconditions.The particles are illustrated by SEM.
Results: Collagen nanoparticles with 0.4-2 µm diameter; algea nanoparticles with 2 µm diameter; chitosan needles with 0.2-3 µm diameter and 0.3-3 µm length are observed.
Conclusions: According to our findings, it is shown that different pore sizes affect the shape and length of the collagen particles.Particles that passed through big pores (10µm) were sphere-shaped, in contrast to particles which were rod-shaped at small pores (0.2µm).Moreover, small pores lead to particles with 2-3µm in length and 0,2-0,5µm in width.Big pore sizes lead to particles with 1µm radius.Different experimental conditions, such as pore sizes and velocity affected the features of the chitosan nanoparticles. It was shown that high velocity (100 µm/min) and low pore size (0,2 µm r) lead to not only fibrilles (0,5µm-3µminlength) but also sphere-shaped particles (0.1µm-1,5µm r). Chitosan is the only biomaterial that shows needles and sphere-shaped particles together.
Keywords: Nanoparticles, chitosan, collagen, alginic acid brown algae

SEM image of alginic acid brown algae needles



SEM image of chitosan nanospheres and needles



P-035 - IS IT USEFUL ADIPOSE DERIVED MESENCHYMAL STEM CELLS INDUCED BY THYMOQUINONE IN THE TREATMENT OF LUNG CANCER?

Konuk Hatice Büşra[1], Cemek Mustafa[2]

[1]*Gebze Technical University, Bioengineering Department, Gebze, Kocaeli; Yıldız Technical University, Bioengineering Department, Esenler, İstanbul*
[2]*Yıldız Technical University, Bioengineering Department, Esenler, İstanbul*

Introduction and Objective: Lung cancer is the most common cancer type and traditional therapies cannot provide effective treatment. Therefore, developments of targeted and effective cell therapies are very important. Human adipose tissue derived mesenchymal stem cells (hAD-MSCs) have the following properties: natural migration mechanism of cancerous tissue; easy to obtain, reproduce and genetic modifications; express to therapeutic molecules easily, and they don't generate an immune response when compared to other cellular therapy vehicles in cancer. However, hAD-MSCs can support cancer cells via paracrine factors; moreover they can increase migration, proliferation, angiogenesis, and metastasis of cancer cells. Thus, studies that eliminate the supporting features of MSCs to cancer cells are of great importance.
Thymoquinone inhibits expression of paracrine factors released from MSCs associated with cancer development.
In this study we aimed that co-culture of stimulated MSCs via thymoquinone and lung cancer cells *in vitro*to determine their interaction and to support the development of cancer treatments with stem cells.
Materials and Method: hAD-MSCs are stimulated by thymoquinone. After that, stimulated hAD-MSCs and lung adenocarcinoma A-549 cancer cells are co-cultured *in vitro*. Interaction between cells is determined by the change in the level of interleukin-8; vascular endothelial growth factor and MMP-2, MMP-9 metalloproteinase detected by ELISA assay. After interaction of cancer cells with stem cells; wound healing, colony formation and MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assays indicated migration, invasion and proliferation capacities of cancer cells are performed.
Results: Co-culture of lung cancer cells and stimulated hAD-MSCs will be demonstrated that expression level of above paracrine factors and proliferation, migration, invasion capacity of cancer cells compared to co-culture of lung cancer cells and without stimulation of hAD-MSCs.
Discussion and Conclusion: To identify the interaction between stimulated hAD-MSCs and cancer cells will be supported that produce new approaches about cellular therapy of lung cancer as well as other cancers.
Keywords: Lung cancer, adipose tissue derived mesenchymal stem cells, thymoquinone, paracrine factors, targeted cancer therapy

P-036 - CONCENTRATION DEPENDENT EFFECTS OF *ALOE VERA* LECTIN AND *A. VERA* LEAF AQUEOUS EXTRACT ON THE *IN VITRO* GROWTH OF DIFFERENT HUMAN CANCER CELL LINES

Çandöken Eda[1], Akev Nuriye[1], Özsoy Nurten[1], Süleymanoğlu Mediha[2], Gazioğlu Sema Bilgiç[3], Kuruca Serap Erdem[4]

[1]*Department of Biochemistry, Faculty of Pharmacy, Istanbul University, Istanbul*
[2]*Department of Molecular Medicine, Institute of Experimental Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul*
[3]*Department of Immunology, Institute of Experimental Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul*
[4]*Department of Physiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul*

Malignant transformation is known to be associated with various changes in cell glycosylation, therefore lectins, which have the ability to bind to specific carbohydrates on cell surfaces, can be useful in distinguishing cell differentiation and metastasis.
Aloe vera extracts have been reported to prevent or regress tumour growth. However, it is not well documented whether the lectins purified from *Aloe* species are responsible for the antitumour activity.
In the present study, we aimed to compare the concentration dependent effects of *A. vera* lectin (Aloctin), with *A. vera* leaf skin aqueous extract on the *in vitro* growth of some human cancer cell lines.
Aqueous extract was prepared from the leaf skin of the fresh plant leaves and Aloctin was isolated from this extract by ammonium sulphate precipitation and cyanogen bromide CNBr-Sepharose 4B-ovalbumin-affinity chromatography. Cytotoxicity experiments were done using human colorectal carcinoma HCT116, promyelocytic leukemia HL-60, chronic myeloid leukemia K562 cells using MTT assay. 5-fluorouracil (5-FU) and Imatinib (IM) were tested as positive controls. The cytotoxic effects were evaluated by comparing the cytotoxic concentration that provides 50% inhibition of cell growth (IC50). Induction of apoptosis and necrosis were monitored by flow cytometry using the Annexin V-FITC/PI kit.
Aloctin showed significantly strong cytotoxic effect on all cells tested, while *A. vera* extract have no effect on cells at the same concentrations. Apoptosis and necrosis were'nt detected as possible mechanisms of cytotoxicity. Positive controls, 5-FU and IM strongly inhibited the cell proliferation, as expected.
The anticancer potential of *A. vera* extracts is available in many publications. Since antitumour or cytotoxic effects of some lectins from other sources are also reported in literature, this research provides insight to the effects of the compounds responsible of the cytotoxic effects of *A. vera*.
Keywords: *Aloe Vera*, Cytotoxicity, Lectin

P-037 - CYTOTOXICITY AND STABILITY STUDIES ON INCLUSION COMPLEX OF QUERCETIN WITH METHYL-β-CYCLODEXTRIN

Güleç Kadri[1], İncesu Zerrin[2], Demirel Müzeyyen[3]

[1]Graduate School of Sciences, Department of Advanced Technologies, Program in Nanotechnology, Anadolu University, Eskişehir, Turkey
[2]Faculty of Pharmacy, Department of Biochemistry, Anadolu University, Eskişehir, Turkey
[3]Faculty of Pharmacy, Department of Pharmaceutical Technology, Anadolu University, Eskişehir, Turkey

Introduction and Aim: Quercetin (Qu) is one of the most effective plant originated antioxidant. Despite the potential usage in cancer treatment, stability problems and the scarcity of cellular bioavailability limit its applications. Cyclodextrins (CD) are the cyclic oligomers of glucose. They can form inclusion complexes with the drug molecules and change the physicochemical properties of the drugs. These changes may increase the therapeutic potential of the drugs. The aims of study were evaluation the impact of methyl-β-cyclodextrin (M-β-CD) on the cytotoxic effects of Qu and investigation of stability of Qu/M-β-CD complexes.
Materials and Methods: All Qu/M-β-CD (L1-L3-L4-SS) complexes were synthesized using varies methods and kept at 4±1°C, 25±1°C and 40±1°C during the 3 months. The stability was evaluated with particle size (PS), zeta potential (ZP) and Qu amount analysis. Cytotoxicity assay of the complexes were performed on HeLa and SKOV-3 cells. Hoechst staining and flow cytometry analysis were also performed.
Results: L3 (concentration range: 400µg/mL–195µg/mL) was found to be a potent cytotoxic complex on HeLa cells after incubation time. On the other hand, 235µg/mL of L4 treatment was decreased the cell viability of SKOV-3 cells after 24 h. The flow cytometry studies showed that the percentage of early apoptotic cells was found to be the highest value when L3 compare the other complexes by HeLa cells. The amounts of Qu in L3 were decreased 12-20 % after 3 months at different storage conditions.
Discussion and Conclusion: Cytotoxic effects of the complexes varies depending on the cell type. L3 complex has a potent cytotoxic effect on HeLa cells, while L4 complex is cytotoxic on SKOV-3 cells suggesting that the synthesis method used might be an effective role on the ability of cytotoxicity of complexes. L3 stored at 4±1°C in 3 months were the most ideal complex according to PS, ZP and Qu amount in stability test.
Keywords: Cytotoxicity, Inclusion Complex, Methyl-β-Cyclodextrin, Quercetin, Stability

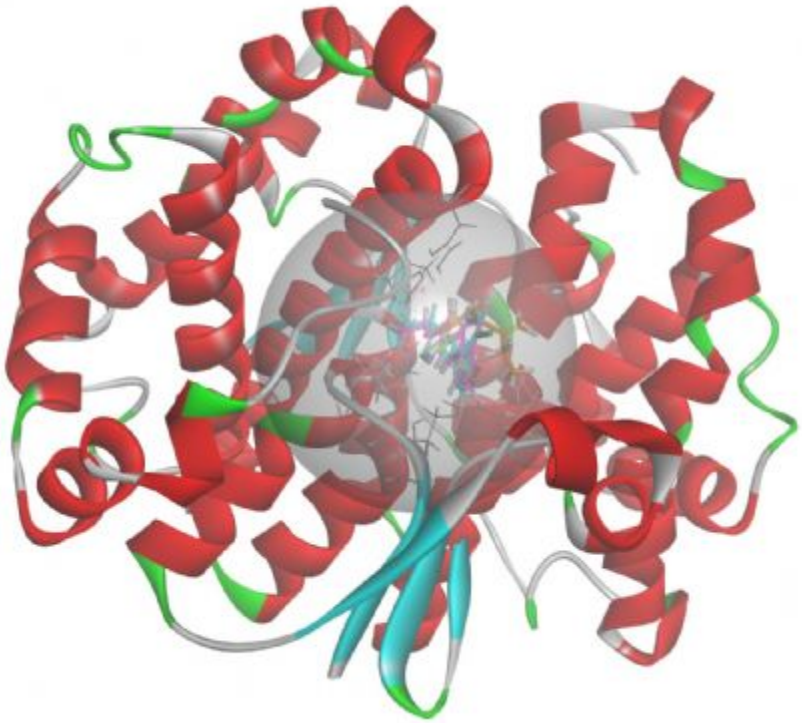
P-038 - OXADIAZOLE DERIVATIVES AS A POTENTIAL GLUTATHIONE S-TRANSFERASE INHIBITORS

Taşkın Tok Tuğba[1], Uzgören Baran Ayse[2]

[1]*Gaziantep University, Faculty of Sciences and Arts, Department of Chemistry, Gaziantep, Turkey*
[2]*Hacettepe University, Faculty of Science, Department of Chemistry, Ankara, Turkey*

Introduction and Aim: In the treatment of cancer, over production of Glutathione S-transferase is a one of the important problem. Because, this enzyme inactivate the chemotherapeutic agents and induce the drug resistance1. Nowadays, studies on inhibitors of these enzymes are progressing and for this, variety of chemical compounds are synthesized. For this reason, the structure-based relationships of these oxadiazoles in inhibiting the activity of hGST P1 (human pi-class glutathione S-transferase enzyme) based on inhibitors were studied in this study.
Materials and Methods: Oxadiazole-group containing compounds (1a, 1b, 1c, 1d and 1f) were chosen and elucidated based on the quantum chemical parameters2a,b. The various quantum mechanical parameters such as dipole moment, global hardness (η) and etc. were calculated at DFT/B3LYP/6-31G* basis set by using Gaussian09 program. Molecular docking2c was also carried out with these compounds in hGST P1 to obtain lead compound according to active site of the hGST P1.
Result: The proposed compounds were achieved using the above the human pi-class glutathione S-transferase. These compounds can be potential candidates for hGST P1 in cancer therapy. The enzyme/drugs activity relationship was investigated by theoretical calculations and molecular modeling methods.
Conclusion: The results indicated that the human pi-class glutathione S-transferase can easily be inhibited by our proposed compounds. Therefore, they represent potential drug leading compounds. Additionaly, molecular docking was performed to better visualize the interactions between the compounds and hGST P1. In the future, synthesis and characterization of the compounds and their effects on cancer cell lines will be studied.
Keywords: Glutathione S-transferase, Oxadiazole derivatives, Quantum chemical parameters, Molecular docking

Interaction of oxadiazole lead-ligand with hGST P1 from docking calculations.



Molecular docking was performed to better visualize the interactions between the compounds and hGST P1.

P-039 - INVESTIGATION OF THE EFFECT OF MELATONIN CELL VIABILITY AND APOPTOSIS IN HNSCC

Kalayci Yigin Aysel[1], Donmez Huseyin[2]

[1]*Department of medical genetics, Cerrahpasa medical faculty, University of İstanbul, İstanbul, Turkey.*
[2]*Department of medical genetics, Medical faculty, University of Selcuk, Konya, Turkey.*

Background and Aim: Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignancies which is characterized by aggressiveness, early recurrence and metastasis in humans. Despite significant advances in therapeutic approaches, little improvement has been achieved in overall survival rates for HNSCC. Melatonin has a wide range of biological effects due to an antioxidant, anti-inflammatory and anti-tumor activity. Melatonin has the ability of affecting cell survival, proliferation, and apoptosis associated with signal transduction pathways. The aim of this study is to determine the effect of melatonin on head and neck squamose cell carcinoma cell line (SCCl-MT1).
Materials ve Methods: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) analysis was used to investigate the cytotoxic effects of melatonin. Melatonin was applied interval of 0 and 200 µL/mL concentrations and incubated 24 h, 48 h and 72 h respectively in the parenteral SCCL-MT1 cell line. After MTT analysis, the number of apoptotic cells was measured by Annexin V/PI (propidium iodide) analysis. SCCL-MT1 was incubated at the concentration of 200 µL/mL for 24 h and treated with Annexin-V antibody and proprodium iodide. Consequently, apoptosis was evaluated by using flow cytometry.
Results: According to MTT results, dose of 200 µL/mL and 24 h treatment in SCCL-MT1 cell line was selected to apoptosis by flow cytometry. Treatment with 200 µL/mL melatonin for 24 h increased apoptosis in SCCL-MT1 (P=0.0061) cell line.
Conclusion: The results of the study, melatonin may decrease cell viability in head and neck squamose cell carcinoma cell line possibly through activation of apoptotic pathway.
Keywords: Head and Neck Cancer, Melatonin, Cell Viability, Apoptosis

P-040 - IN VITRO EFFECTS OF RESVERATROL ON EXPRESSION OF CYTOCHROME P450 ENZYMES AND PROLIFERATION OF PROSTATE CANCER CELLS

Gül Alev[1], Karakurt Serdar[1], Adalı Orhan[2]

[1]*Department of Biochemistry, Selcuk University, Konya, Turkey*
[2]*Department of Biological Sciences, Middle East Technical University, Ankara, Turkey*

Introduction: Trans-resveratrol, existing in many different plants especially grape, is the one of the important natural phytoalexins and their anticarcinogenic effects have been also demonstrated against various cancers. The main aim of this study is to investigate the in vitro effects of resveratrol on the xenobiotic metabolizing cytochrome P450 enzyme expression as well as proliferation, invasion and apoptosis of androgen non-dependent cancer cell line LnCAP.
Materials and Methods: LnCAP cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and 2 mM glutamine. Cytotoxicity of Resveratrol on LnCAP cell lines was determined with Alamar blue and IC50 value was calculated. The effects of Resveratrol on invasive potential of LnCAP cells were analyzed by matrigel chamber assays, respectively. Effects of Resveratrol on CYP3A4 and CYP2B6 protein and mRNA expression of LnCAP cells were determined using western blotting and qRT-PCR techniques.
Results: By investigating the effect of resveratrol on cell growth, it was found that IC50 value, half-maximal inhibitory concentration, is 76.2 for LnCAP cell line and 156.4 for PNT1A(healthy prostate cell line). Matrigel invasion assay showed that resveratrol significantly inhibit invasion of LnCAP cells as 43%. Western blot studies showed that both CYP3A4 and CYP2B6 protein expression were decreased 80% and 17%, respectively. On the other hand, qRT-PCR results demonstrated that mRNA expression of CYP3A4 interestingly increased as 2.2-fold while CYP2B6 mRNA expression was decreased 90 %.
Conclusion: As a result of this study, we demonstrated that resveratrol possesses anticarcinogenic effects on androgen independent LnCAP cells while there is less toxic effect on healthy prostate cell line PNT1-A in the same concentrations. Beside this, modulation of cytochrome P450 protein and gene expressions by resveratrol makes it one of the new drug candidate which may regules theuropathic drug in prostate cancer.
Keywords: Cytochrome P450, LnCAP, Proliferation, Prostate cancer, Resveratrol

This work was supported by TUBITAK, Project No: 113Z488.

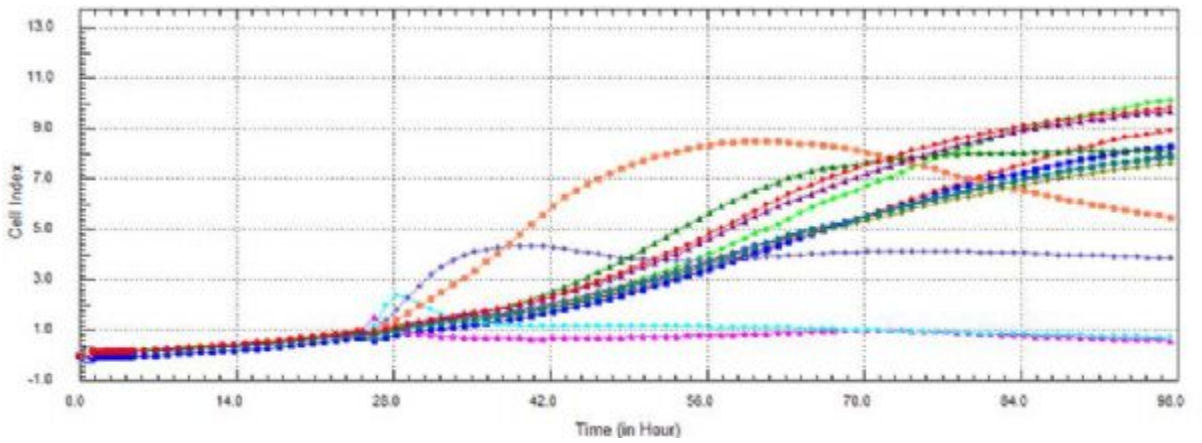
P-041 - REAL-TIME MONITORING OF THE EFFECTS OF TAMOXIFEN AND VITAMIN D ON PROLIFERATION OF BREAST CANCER CELL LINE

Yetkin Derya[1], Karagül Meryem İlkay[1], Kibar Kezban[1], Ballı Ebru[1], Yılmaz Şakir Necat[1], Türkegün Merve[2]

[1]*Medical Faculty, Histology and Embriyology, Mersin University, Mersin, Turkey*
[2]*Medical Faculty, Biostatistics, Mersin University, Mersin, Turkey*

Objectives: Breast cancer is the most common malign disorder seen in women. Tamoxifen is the most widely used endocrine agent in this disease. Tamoxifen, upon binding of ER changes receptor conformation and inhibits trancription. Vitamin D refers to a group of sterols which regulates cell proliferation and differantiation pathways. Vitamin D receptors (VDR) has been identified in many tumorigenic cell lines and it has an anti-proliferative effect on cancer. Current study was designed to investigate the anticarcinogenic effect of varying concentrations of tamoxifen and vitamin D on breast cancer cell line.
Methods: In our study we used xCELLigence RTCA system which measures cell proliferation and drug-mediated cytotoxicity in a time-dependent manner. 90 µL of the 3x104 cells/mL media mixture were added to the wells of E-plates. After 24 hrs, 10 µl of tamoxifen (60, 40, 20, 10, 1 µM) or vitamin D (125, 100, 75, 50, 10 nM) were added to each well. Cell proliferations were evaluated for 96 hrs.
Results: Tamoxifen treatment at 1 and 10 µM concentrations was found to be proliferative for 80 hrs and afterthat decrease in cell proliferation was observed. 20 µM of tamoxifen was found to be antiproliferative for approximately 50% of the population from 56th hrs. There was seen a significant decrease in the proliferation of almost all cells treated with 40 and 60 µM of tamoxifen. IC50 value of tamoxifen was calculated as 22 µM. For vitamin D, 10 and 50 nM concentrations were found to be proliferative whereas 75, 100 and 125 nM concentrations to be anti-proliferative.
Discussion: Our study showed that low concentrations of tamoxifen and vitamin D increased cancer cell proliferation. Vitamin D alone did not significantly decrease cell proliferation. Taken together, we propose to investigate the role of seperate or combined therapy of these agents on tumor growth.
Keywords: Breast cell, xCELLigence, Tamoxifen, Vitamin D

xCELLigence RTCA system which measures cell proliferation for TAM and Vitamin D



P-042 - ANTICARCINOGENIC AND ANTIOXIDANT PROPERTIES OF RESVERATROL ON PROSTATE CANCER PC-3 CELLS

Gül Alev[1], Karakurt Serdar[1], Adalı Orhan[2]

[1]*Department of Biochemistry, Selcuk University, Konya, TURKEY*
[2]*Department of Biological Sciences, Middle East Technical University, Ankara, TURKEY*

Introduction: Resveratrol that mainly found in berries and grapes has antioxidant, anticarcinogenic, anti-inflammatory effects and generally synthesized in response to pathogenic and abiotic stress. In this study, we aimed to investigate in vitro effects of resveratrol on antioxidant enzyme activities as well as protein and gene expressions in androgen independent prostate cancer cell line; PC-3. Beside these, the effects of resveratrol on proliferation, invasion and colony formation properties of PC-3 cells were also examined.
Materials and Methods: PC-3 cells were grown in Ham's F-12 and PNT1A cells RPMI 1640 medium supplemented with 10% fetal bovine serum and 2 mM glutamine. Cytotoxicity studies were performed using Alamar Blue test spectrophotometrically. GST and NQO1 enzyme activities were determined spectrophotometrically and protein and gene expression were determined by western blot analysis and qRT-PCR. Matrigel invasion, migration and colony formation assays were performed to see the effects of resveratrol on the characteristic properties of PC-3 cells.
Results: IC50 value of resveratrol on PC-3 cells were calculated as 76.2 µM while it is 156.4 µM for healthy prostate cells; PNT1A. Resveratrol significantly decreased migration, invasion and colony formation properties of PC-3 cells as 92.7%, 81% and 87.4%, respectively. GST and NQO1 enzyme activities were determined as 120.3±8 nmol/min/mg and 659.9±9 nmol/min/mg in resveratrol treated group while it was 286.6±4 nmol/min/mg and 1980±5 nmol/min/mg in control group. Moreover, resveratrol also significantly altered protein and gene expression of GST and NQO1.
Conclusion: Consequently, resveratrol was modulate expressions of antioxidant enzymes; GST and NQO1 which leads it one of the good candidates against aggressive prostate cancer.
Keywords: GST, NQO1, PC-3, prostate cancer, Resveratrol

This work was supported by a grant from TUBITAK, Project No: 113Z488

P-043 - THE CYTOTOXIC EFFECT OF ASTEMIZOLE AND İMIPRAMINE ON PROSTATE CANCER CELL LINE

Öztanrıverdi Onur[1], Yetkin Derya[2], Söğüt Fatma[3], Aşkın Ali[1], Çömelekoğlu Ülkü[4], Yılmaz Şakir Necat[2]

[1]*Faculty of Arts and Sciences, Biology, Mersin University, Mersin, Turkey*
[2]*Medical Faculty, Histology and Embriyology, Mersin University, Mersin, Turkey*
[3]*Vocational School of Medical Services, Perfusion Technology, Mersin University, Mersin, Turkey*
[4]*Medical Faculty, Biophysics Mersin University, Mersin, Turkey*

Introduction and Purpose: Cancer in the world and our country is a common disease and a high mortality rate. The prostate cancer is the common cancer in men. Studies have shown that the an antidepressant, imipramine and an antihistamine, astemizole affect some oncogenic proteins. Because of these features, the determination studies has gained importance whether these drugs are used in cancer therapy. In this study, it were aimed to investigate the cytotoxic effect of different doses of imipramine and astemizole on DU-145, prostate cancer cell lines.

Materials and Methods: İn this study, prostate cell line, DU-145 was used. This study was performed using the xCELLigence device to determine the effect of different doses of astemizole and imipramine on cell proliferation. n=4 were repeated for real time cell analysis in our study. RTCA (real time cell analysis) programme was useed. For blind, 100 µL media is added e-plate system. Later, to be 5x103 cells in 90 µL of medium was ultured in e-plate system. After 24 hours, the concentration of astemizole (1 nM, 10 nM, 100 nM,1 µM, 10 µM) and imipramine (10 nM, 100nM, 1 µM, 10 µM, 30 µM) in 10 µL and for 96 hour cell proliferation was evaluated.

Results: The conclusion of the statistical analysis in term of cell index were observed signifiant differences between ontrol and dose groups ($p<0.05$). İn the cell index values, all astemizole and imipramine group was signifiantly decreased compared to controls 96th hour.

Conclusion: The result of study, by signifiantly reducing proliferation of prostate cancer cells, clues were obtained about an antidepressant imipramine and an antihistamine astemizole can be used for prostate cancer therapy. But, the subject is need further studies using different doses.

Keywords: Prostate cancer, Astemizole, İmipramine

P-044 - GEN EXPRESSION PROFILING OF MTOR PATHWAY IN RECTUM CANCER

Yonar Kaplan Pelin[1], Mega Tiber Pinar[1], Ozden Ayse Sevgi[2], Orun Oya[1]

[1]*Marmara University, School Of Medicine, Biophysics Department, Maltepe, İstanbul*
[2]*Kartal Dr. Lutfi Kirdar Training And Research Hospital,Oncology Of Radiation Department,İstanbul*

Background: In this study, gene expression profile of mTOR pathway will be examined in advanced stage rectal cancer patients. mTOR is a member of serine/threonine protein kinase considering its role in the integration of extracellular and intracellular signals to regulate cell growth, proliferation and apoptosis. Many member as Deptor, REDD1 block the mTOR pathway is a substract of SCF3 ligase. The expression levels of the catalytic unit of SCF3 Ligase, SAG, and related genes NOXA, BNIP, p53 are compared in normal and tumor tissues of advanced stage rectal cancer patients.
Material and Methods: Tissue samples were collected from 32 patients who had histologically advanced cell carcinoma of rectum at Radiation Oncology Department of Kartal Education and Research Hospital. Total RNA was extracted from biopsies, total RNA was reverse transcribed using Transcriptor High Fidelity cDNA Synthesis kit and used as a template in real-time PCR reactions.
Results: The comparing expression levels of normal and cancer tissues, we observed SAG gene has 56% increasing, NOXA gene, 18%, BNIP gene 28% and p53 gene 47% increasing levels. SAG and NOXA has a reverse regulation in 68% patients. The catalytic component of the mTOR protein complex, SAG is an antiapoptotic proteins which are effective in cancer development and radiation response. NOXA is an apoptotic protein which is accumulated in the cells with a reduced level of SAG. BNIP is known as antiapoptotic protein and p53, tumor supressor genes.
Conclusion: This study showed that there is an inverse correlation between SAG and NOXA expression, also a high increase (upregulation) as expected in the p53 gene. After all the mTOR pathway protein expression determined, relationship with the prognosis of these levels will be determined.
Keywords: Apoptosis, mTOR Pathway, Noxa, Rectum Cancer, SAG

P-045 - THE CROSSTALK BETWEEN P38 AND AKT SIGNALING PATHWAYS ORCHESTRATES EMT IN NSCLC CELLS VIA REGULATION OF SATB2 EXPRESSION

Kucuksayan Hakan, Akca Hakan

Medical Biology Department, School of Medicine, Pamukkale University, Denizli, Turkey

Epithelial-mesenchymal transition (EMT) is a significant event for metastasis, and could be mediated by several pathways such as PI3K/Akt, MAP kinases and many epigenetic regulators. SATB2 is an epigenetic regulator involved in EMT and osteoblastic differentiation. Since preliminary results indicate that there is a crosstalk between p38 and Akt pathways in NSCLC cells, we aimed to determine whether this crosstalk has a regulatory effects on EMT and SATB2 expression in NSCLC cells. We used A549 and H1650 cells as a model to evaluate the effects of the crosstalk between p38 and Akt on EMT of NSCLC cells. Therefore, cell culture, inhibition of p38 activation via SB203580, transient expression assay for (CA-Akt), Western blot analysis, siRNA transfection for SATB2, wound healing and invasion assay were performed in this study. Firstly, the expression statues of E-cadherin, SATB2, p-p38, p38, p-Akt and Akt was examined in A549 and H1650 cells by Western blot analysis. We observed that Ecadherin and SATB2 are downregulated in A549 cells (highly active p38, lowly active Akt) compared to H1650 cells (lowly active p38, highly active Akt), suggesting that E-cadherin and SATB2 are associated with the crosstalk between p38 and Akt pathways. Our results demonstrated that p38 inhibition in A549 cells leads to decreased PTEN expression and subsequently increased Akt activation. Then, we found that p38 inhibition upregulated SATB2 expression, and reversed EMT in A549 cells. Furthermore, alone SATB2 knockdown is sufficient to induce EMT, and prevented the effects of p38 inhibition on EMT. All these results strongly indicate that the crosstalk between p38 and Akt pathways could regulate EMT of NSCLC cells by controlling SATB2 expression.
Keywords: EMT, NSCLC, p38, Akt, SATB2

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P-046 - EFFECT OF P53 ON DICLOFENAC AND IBUPROFEN-INDUCED ROS GENERATION-DEPENDENT APOPTOSIS IN PC3 PROSTATE CANCER CELLS

Akar Okan Remzi, Rencuzogullari Ozge, Arisan Elif Damla, Obakan Yerlikaya Pinar, Gurkan Coker Ajda, Palavan Unsal Narcin

Molecular Biology and Genetic, Istanbul Kultur Unıversity, Istanbul, Turkey

Prostate cancer is the most frequent type of cancer prevail among men. Collected evidence reveals that p53, in other words, “Guardian of genome” plays a critical role in cancer. Also, *in vivo* and *in vitro* studies suggest that regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce prostate cancer risk. In this study, we examined the effects of p53 on regulation of programmed cell death mechanisms that are triggered by ibuprofen and diclofenac in PC3 and PC3 $p53^{+/+}$ prostate cancer cells.

To investigate the role of p53, PC3 cells were transfected with p53 plasmid and correlated with immunoblotting. We observed that ibuprofen (1 mM) reduced cell viability by 25% in PC3 and 40% PC3 $p53^{+/+}$ cells. Diclofenac (250 µM) decreased cell viability by 60% in PC3 and 50% in PC3 $p53^{+/+}$ cells. Diclofenac induced apoptosis *via* caspase 8-dependent cleavage of RIP, which is a pro-apoptotic pathway regulator. Ibuprofen activated intrinsic pathway of apoptosis through upregulation of fas and cleavage of caspase 2, which are related with BID cleavage in PC3 cells. However, transfection of p53 downregulated SAPK/JNK signal axis which has an important role on activation of caspase cleavage and mitochondrial membrane potential loss. To better understand the effect of ibuprofen and diclofenac on ROS generation DCFDA staining was performed and flow cytometric analysis showed that diclofenac induced ROS generation.

In conclusion, the mechanism of transition between extrinsic and intrinsic pathways differ depending on presence of p53. Ibuprofen caused fas upregulation in PC3 cells, which was withdrawn by transfection of P53. Presence of p53 affects upregulation of Bax and Bak which are critical for mitochondrial apoptotis pathway. On the other hand, upon diclofenac treatment, activation of RIP was prevented through BID cleavage which is related with p53-dependent fas activation. Also diclofenac-caused ROS generation was observed, which is independent from p53.

Keywords: Apoptosis, NSAID, p53, Prostate Cancer, ROS

P-047 - CDK INHIBITORS ROSCOVITINE AND PURVALANOL INDUCED ER STRESS-MEDIATED APOPTOSIS IN HELA CERVICAL CANCER CELLS RELATED WITH INDUCTION OF OXIDATIVE STRESS

Sarikaya Bahar, Ozfiliz Kilbas Pelin, Arisan Elif Damla, Gurkan Coker Ajda, Obakan Yerlikaya Pinar, Palavan Unsal Narcin

Molecular Biology and Genetic, Istanbul Kultur Unıversity, Istanbul, Turkey

Novel cyclin-dependent kinase (CDK) inhibitors, roscovitine and purvalanol, induce apoptosis by triggering cell cycle arrest in cancer cells. Although the molecular targets of CDK inhibitors are well established, there are still unknown targets, which may affect their therapeutic potential. Endoplasmic reticulum (ER) stress is a mechanism that can be induced by elevated levels of oxidative stress, accumulation of unfolded proteins or starvation. Thereby prolonged ER stress in the cells activates unfolded protein response (UPR) and leads to apoptotic cell death. Our recent studies showed that purvalanol induced ER stress, which triggered apoptosis and autophagy in colon cancer cells [1]. In this study, our aim is to investigate the potential role of roscovitine and purvalanol on ER stress and apoptosis in HeLa cells.

Our findings indicated that both CDK inhibitors induced apoptosis as a late response, which was established by cleavage of PARP and activation of caspases. In contrary, drugs initiated ER stress as an early response via upregulating PERK, IRE-1α and ATF-6 levels in HeLa cells. In addition, pm-Cherry-tagged CHOP plasmid transfection studies showed the ER stress-related efficiency of drugs. The increased CHOP expression due to CDK inhibitors may underlie the potential effect of ER stress on apoptosis decision. This early response as ER stress induction may be related to the activation of oxidative stress. In order to evaluate the potential effect of CDK inhibitors, we showed ROS generation by DCFH-DA staining after time-dependent drug treatment. In further studies, the investigation of CHOP-related apoptotic decision is required to evaluate the potential molecular machinery of CDK inhibitors in HeLa cervical cancer cells.

Keywords: apoptosis, CDK inhibitors, ER stress, HeLa cells

P-048 - THE EFFECTS OF ELECTROMAGNETIC FIELD EXPOSURE AT 900 MHZ FREQUENCY EMITTED FROM MOBILE PHONES ON COCHLEAR CELLS

Aktaş Safiye[1], Altun Zekiye[1], Pamukoğlu Ayça[1], Demir Banu[1], Saygan Serkan[2], Çiçeksoy Burak[3], Erçetin Pınar[1], Serinan Efe[1], Olgun Yüksel[4], Olgun Nur[1]

[1]Institute of Oncology, Dokuz Eylul University, Izmir, Turkey
[2]Derinuzay Science and Techology Labs R&D Assc., Izmir, Turkey
[3]Deparment of Electric and Electronical Engineering, University of Economics, Izmir, Turkey
[4]Department of Otorhinolaringology, School of Medicine, Dokuz Eylul University, Izmir Turkey

Aim: Technological developments encountered radiofrequency field from mobile phones in our lives. Possible side effects of electromagnetic field (EMF) need to be investigated. The aim of this study is to evaluate cytotoxic, apoptotic and DNA damage effects of 900 MHz EMF emitted by mobile phones on House Ear Institute- Organ of Corti 1 (HEI-OC1 cell line) cochlear cells.
Methods: Cochlear cells were cultured in 6 well plates at 33oC, 10%CO2 in humidified conditions. They were exposed to 900 Mhz EMF in conditions of 5 minutes and 15 minutes, directly and 10 cm away from EMF. EMF was applied by a 3G cell phone and measured by Arduino EMF detector. Cell viability and apoptosis were evaluated after 24 and 48 hours by trypan blue and Annexin V methods. DNA damage related gene expressions was evaluated by real time PCR.
Results: Cell death was more prominent in cells 5 minutes of EMF at 48 h. The apoptosis ratio in cells situated 10 cm away from EMF were similar to cells that were directly exposed to 5 minutes of EMF. It was observed that DNA damage related gene expressions were increased in cells after EMF exposure in 48 hours. The expression levels are nearly same in cells that were 10 cm away from EMF. The genes that showed high expression than control are Bax, Gadd45a, Gadd45g, Mpg, Msh2, Rad51c and Xrcc3, which are related to apoptosis induction and DNA repair.
Conclusion: EMF at high dose for 5 minutes caused cell death via apoptosis in HEI-OC1 cell line in vitro. This result was supported by apoptosis detection and DNA damage related gene expressions. Apoptosis was prominent in 5 minutes and similar for both direct and close distance exposure. Further in vivo and in vitro studies with different doses and distances are needed.
Keywords: electromagnetic field, cochlear cells, mobile cell phones

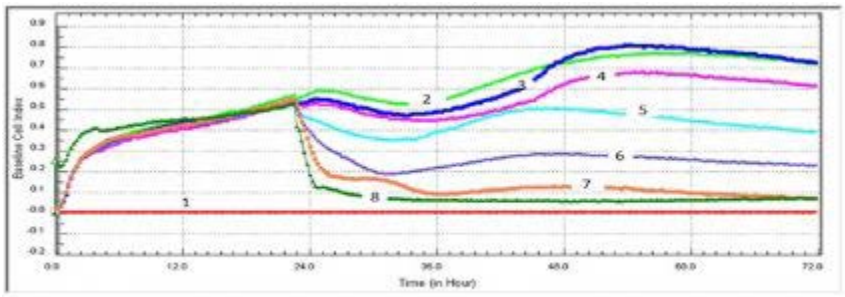
P-049 - THE EFFECT OF VITAMIN D ON MCF–7 BREAST CANCER CELL METABOLISM

Saraçlıgil Beyza, Öztürk Bahadır, Ünlü Ali, Abusoğlu Sedat, Tekin Gülsüm

Biochemistry Department, Selçuk University School of Medicine, Konya 42075, Turkey

Background and Aim: It has long been known that vitamin D is important for calcium absorption and bone health. However, recent studies have revealed that Vitamin D modulates breast cancer cell growth and epidemiologic studies have increasingly suggested that vitamin D may be associated with reduced breast cancer risk. The primary objective of this study was to highlight the effect of Vitamin D on MCF-7 breast cancer cell.
Materials and Methods: Changes in the number of cells and the particular cell culture in wells containing micro-electrodes, duration of the experiment were continuously monitored every 15 minutes. With the use of this data IC50 dose was calculated as 145nM. This pre-defined IC50 dose was subsequently applied to cells which were obtained. These samples were used to determine the apoptosis, levels analysis at cancer cells.
Results: We observed that the anti-proliferative effect of Vitamin D on MCF-7 breast cancer cell similar to the literature through real time cell electronic detection system. The apoptosis level which was 18% at the 24th hour vitamin D groups were respectively 28% at the 48th hour and 38.5% at the 72th hour.
Conclusion: Vitamin D was reduced cancer cell proliferation dose and time depending manner. This findings support the application of vitamin D in breast cancer prevention and treatment.
Keywords: Vitamin D, Breast cancer, Proliferasyon, Apoptosis

Figure 1



The dose dependent inhibitory effects of vitamin D on the growth of MCF-7 cell.1-DMEM,2-CONTROL, 3-ETANOL, 4-10 nM vit. D,5-100 nM vit., 250 nM vit. D, 500 nM vit. D, 1000 nM vit. D

Table 1

	7-ADD Negative, Annexin V Negative (%)	7-ADD Negative, Annexin V Positive (%)	7-ADD Positive, Annexin V Positive (%)
Control 24h	87.8	1.1	0.5
Vit.D 24h	74.1	18	3
Control 48h	90.6	1.1	0.8
Vit.D 48h	67.4	28.6	2.8
Control 72h	82.4	0.8	0.9
Vit. D 72h	52.3	38.5	6.5

The distribution of different phases of MCF-7 cell cycle after 1α,25(OH)2D3 treatment determined by flow cytometry with Annexin V-FITC and 7-AAD staining

P-050 - INVESTIGATION OF THE RELATIONSHIP BETWEEN TUMOR SIZE AND SOME AGNOR PARAMETERS ON BREAST CANCER

Köksal Mehmet, Doğan Serap, Öztürk Figen, Cücer Nurhan

Erciyes Üniversitesi Kayseri

Introduction: Tumor size is an important parameter used to determine the prognosis and stage of breast cancer. We investigated a probable correlation between AgNOR area and tumor size to examine wheter the AgNOR parameters can also be tools of detecting the stage and predicting of the prognosis.

Materials and Methods: The diameter of the tumor, AgNOR number and AgNOR area of 18 cases were obtained as a retrospectively from archive records. The mean AgNOR number and area were calculated via measurement of 50 cells from each cases. Patients were divided into groups according to the TNM classification system by tumor size. Thus, patients with a tumor diameter between 5-10 mm are included in T1b (n = 8), between 10-20 mm are included in T1c (n = 5), and between 20-50 mm are included in T2 (n = 5) groups. Obtained biopsy material was taken into methanol, spread on clean slides and air dried. After silver staining, 50 cells from each patient were evaluated by counting AgNOR spots and measuring AgNOR areas.

Results: Both mean AgNOR area and mean AgNOR count values had strong correlation with tumor diameters of patients (Respectively r=0.740,P<0.000, r=0.771;P< 0.000). When the statistical evaluations were done using the “Tumor Nod Metastasis class of the tumor instead of tumor diameter, correlation appeared milder (r=0,709;P<0,001, r=0,646;P<0,004 with same respect). Three groups of tumor diameter had significantly different for mean AgNOR area and mean AgNOR count values (Respectively;P=0,013;P=0,029).

Conclusion: Presence of positive correlations between tumor diameter and AgNOR parameters indicates that AgNOR parameters have potantial to be usefull tools for staging of breast cancer and predicting the prognosis of the condition. Because of the correlation with tumor diameter is more strong than TNM classification methods, we thought that the directly using of tumor diameter is more usuful.

Keywords: AgNOR, Prognosis, Tumor diameter

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P-051 - EVALUATION OF THE AGNOR PARAMETERS FOR THE EARLY DETECTION OF URINARY BLADDER CANCER VIA CELLS OBTAINED FROM URINE SAMPLES

Cucer Nurhan[1], Eroz Recep[2], Demirtas Abdullah[3], Akınsal Emre Can[3]

[1]*Erciyes University, Medical Faculty, Medical Biology Depart., Kayseri, Turkey*
[2]*Duzce University, Medical Faculty, Medical Genetics Depart., Duzce, Turkey*
[3]*Erciyes University, Medical Faculty, Urology Depart., Kayseri, Turkey*

Background and Aim: Urinary bladder carcinoma is one of the most generally diagnosed cancers after prostate, lung and colon cancers. The rates of bladder cancer is as the fourth most common cancer in men in the world. The detection, treatment, and staging of urinary bladder cancer have traditionally been based on an endoscopic examination - cystoscopy. Therefore additional methods for the early detection of the bladder cancer are important. Our goal was to determine the usage of cut-off values of some AgNOR parameters of exfoliated cells in urine samples from urinary bladder patients and healthy individuals for distinguishing malign and benign lesions by a non invasive method.
Materials and Methods: Exfoliative urinary bladder cells obtained from the urine samples of 11 healthy volunteers and 24 bladder cancer patients were spreaded onto the slides, fixed and air dried, and silver-stained. Images of the cells transferred into computer, and mean values of different AgNOR parameters were counted, measured and calculated.
Results: The mean age of patient group were 63.417±12.237 and of control group 55.546±15.572 and mean ages of these two groups were not statistically different (p=0.115).
The differences between control and patient groups were statistically significant for AgNOR numbers (respectively 1.950±0.535 and 3.06±0.863) and for TAA/NA (4.373±2.740 and 11.450±1.185 with the same respect) (p=0.000). According to the Baseian Statistic, the cutoff and AUC values were >2.73 ve 0.858 for AgNOR number($p<0.0001$), and >6.4 and 0.939 for TAA/NA values ($p<0.0001$). Cancer subgroups were significantly different for both AgNOR parameters ($p<0.05$).
Discussion and Conclusion: We showed the usefulness of AgNOR parameters in cells from urine, for detection of the presence of the tumor in suspected cases, with lesser time and cost and noninvasive methot.
Keywords: Urinary bladder cancer, AgNOR parameters, Early diagnosis

P-052 - GROWTH INHIBITION AND INDUCTION OF PROGRAMMED CELL DEATH IN HUMAN LUNG ADENOCARCINOMA CANCER CELLS BY SHORT TERM CARMOFUR TREATMENT

Kutlu Hatice Mehtap[1], Vejseloa Djanan[1], Çömlekçi Emre[2], Kuş Gökhan[3]

[1]Department of Biology, Faculty of Science, Anadolu University, Eskişehir, Turkey
[2]2Department of Molecular Biology and Genetics, Graduate School of Sciences, Bilecik Şeyh Edebali University, Bilecik, Turkey
[3]3Department of Health, Faculty of Open Education, Anadolu University, 26470, Eskişehir, Turkey

Background: Lung cancer is one of the type common cases in the World. In Turkey, the incidence of lung cancer in mans is abot %62 and %5 in womans. There is a fact that resistance to widely used chemotherapeutics occurs frequently. Thus novel agents for cancer treatment are required. Carmofur or HCFU (1-hexilcarbamoyl-5-florouracil) is a pirimidine analogue used as antineoplastic agent in treatment of breast and colorectal cancer types.
Objective: In the present study we evaluated the cell the cytotoxic and apoptotic effects of carmofur in human lung adenocarcinoma cells (A549).
Material and Methods: For cytotoxicity we used MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay. Stock solution of carmofur was prepared in dimethyl sulfoxide (DMSO) and further diluted in fresh culture medium. A549 cells were exposed to concentrations of carmofur ranging from 5 to 110 microM for 24 hours The percentages of viability and IC50 concentration of carmofur for 24 hours were determined. The morphological alterations on A549 cells caused by IC50 concentration of carmofur for 24 hours were investigated on confocal microscope. For confocal microscopy, A549 cells were double stained with acridine orange and phalloidine.
Results: Consequently carmofur inhibited the proliferation of A549 cells leading to morphological changes on the cells indicating apoptosis. On our micrographs we detected shrinked cells with damaged cytoskeleton, condensed and fragmented nuclei. In our results we demonstrated that carmofur was highly cytotoxic in low doses in A549 cells and caused damages in the cell structure.
Discussion: Furthermore, with the support of the results presented in this study, it may be concluded that carmofur holds potential to act as a beneficial agent in the treatment of cancer. In this research we showed the apoptotic effects of new agent as a carmofur may be using on human breast cancer cells.
Keywords: Carmofur, A549, Cytotoxicty, Confocal Microscopy

P-053 - SCREENING THE ANTI-PROLIFERATIVE EFFECTS OF SOME *HYPERICUM* SPECIES IN MCF-7 HUMAN BREAST CANCER CELLS BY USING REAL TIME CELLULAR IMPEDENCE TECHNOLOGY

Bender Onur[1], Ceylan Ramazan[2], Zengin Gokhan[2], Aktumsek Abdurrahman[2], Atalay Arzu[1]

[1]*Biotechnology Institute, Ankara University, Ankara, Turkey*
[2]*Department of Biology, Faculty of Science, Selcuk University, Konya, Turkey*

Introduction: Breast cancer is the most common type of cancer among women. Anti-neoplastic agents emerge as one of the major class of cancer therapeutics. Anti-cancer effects of *Hypericum* species, which is a natural agent, has been reported in different cancer types. In this study, we aimed to analyze the anti-proliferative effects of four previously unstudied *Hypericum* species (*H. salsugineum*, *H. olympicum*, *H. scabrum* and *H. pruinatum*) on MCF-7 cell line.
Materials and Methods: iCELLigence real time and label-free cell analysis system was used during the study. In order to investigate time and dose dependent effects; MCF-7 cells were seeded in E-plate L-8 and treated with various concentrations (2000, 1000, 500, 250, 125, 62.5 µg and DMSO-only control) of methanolic extracts of *Hypericum* species for 72h. Data analysis was performed by RTCA iCELLigence software. IC50 values were calculated based on the final readings taken at the 72 hour time point.
Results: After screening the anti-proliferative activity of four different *Hypericum* species, *H. salsugineum*was the most effective on cell proliferation which was followed by *H. olympicum*, *H. scabrum* and *H. pruinatum*, respectively. Our results demonstrated that *H. salsugineum* significantly decreased the proliferation of MCF-7 cells compared to control ones and other *Hypericum* species included in the study in all biological repeats.
Discussion: *H. salsugineum* displayed significant anti-proliferative effects on MCF-7 cells. These promising results will be the basis for further comprehensive functional assays on breast cancer by using *H. salsugineum*.
Keywords: Breast cancer, iCELLigence, *Hypericum*, Anti-proliferative, MCF-7

P-054 - INVESTIGATION OF SOME PLANT EXTRACTS AND CANCER DRUGS OF THE CYTOTOXIC EFFECT OF BCL 2- BAX GENE EXPRESSION AND EFFECT ON CELL CULTURE

Ghosigharehaghaji Atena[1], Toraman Emine[1], Sulukan Ekrem[2], Erdoğan Orhan[1]

[1]*Department of Molecular Biology and Genetics Faculty of Science Graduate School, Erzurum,Türkiye*
[2]*Aquacultural engineering Faculty of Science Graduate School, Erzurum,Türkiye*

Objective: Cancer is a term used to describe the uncontrolled growth and abnormal spread of cells. It is made for the treatment of cancer with chemotherapy and radiotherapy made by using cancer drugs that destroy cancer cells, to inhibit the growth and proliferation. In our study, it aimed to investigate of cytotoxic effects of different doses of various plant extracts and cancer drugs and effect on BAX and BCL2 gene-expression in cell culture.
Materials and Methods: Extraction was performed from plant tissue to be used in this study. Cancer drugs were selected to specific types of cancer to be studied. Plant extract (0.005 mg/ ml, 0.01 mg / ml, 0.015 mg / ml and 0.02 mg / ml) and cancer drug (0.5 µg / ml, 1.00 µg / ml, 2.00 µg / ml, 4.00 µg / ml) at different doses was applied to the cell cultures.Article of the MTT assay was performed at intervals of 24,48,72 hours. Cells were plated on a 6 well plate in place a certain amount. RNA isolation was performed after administration of plant extract and cancer drugs at certain dose. Real Time PCR was performed by performing cDNA synthesis.
Results: Showed increased of toxic effects on the cells both hours and increasing doses of cancer drugs at the of the study.The highest dose used in the experiment,72 h, that was found killed of medications and plant extracts more than 50% of cancer cells. Observed of increasing doses of the plant extract to decrease the BCL-2 gene expression, to increase the BAX gene expression according to Real Time PCR results.
Conclusion: As compared to the comparative plant extracts used cancer drug was found to lead to apoptosis in cancer cells.
Keywords: Bcl-2-BAX, plant extract, anticancer drugs, cell culture, cytotoxicity, gene expression, Real Time PCR

P-055 - INVESTIGATION OF CYTOTOXIC AND ANTI-METASTATIC EFFECTS OF *RHEUM RIBES* METHANOL EXTRACT ON MCF-7 BREAST CANCER CELLS

Çınar İlknur, Eroğlu Canan, Avcı Ebru, Çetinkaya Sümeyra, Dursun Hatice Gül, Kurar Ercan

Necmettin Erbakan University, Meram Medical Faculty, Medical Biology Department, Konya, Turkey

Rheum ribes species that one of the herbs commonly used in pharmacological researches, are grown in most Iran, Lebanon, and Eastern Turkey. This plant is determined to contain powerful active compounds. Studies about this plant are generally intended to assess the impact of antioxidant. Our aim in this study is to assess cytotoxic and metastatic changes by way of implementing methanol extract of the *R. ribes* (root) to the MCF-7 breast cancer cells.

Cytotoxic effect of *Rheum ribes* extract was evaluated by using the XTT (2,3-Bis(2-metoksi- 4-nitro-5-sulfofenil)-2H-tetrazolyum) test. In order to determine the dose of IC50, plant extracts were applied as time and dose dependent in the range of 10-500ug. In 72nd hour, the IC50 value is determined as 400ug. To examine the anti-metastatic effects of the extract, total RNAs were isolated from dose group and the control cells firstly, then cDNAs were synthesized. Expression profile of the target genes (MMP-2, MMP-9, TIMP-1, TIMP-2, CDH1, CDH2) are determined by qPCR.

According to the results, when the control group compared with the cells, it was determined that 1.6 and 2.07-fold respectively decrease in the gene expressions of MMP-2 and CDH2 of dose group cells. No significant difference was observed in the other genes examined.

Epithelial-mesenchymal transition (EMT) is a critical step for the initiation of cancer metastasis. N-cadherin and MMPs is known as significant mesenchymal marker. Our data suggested, the methanol extracts of *R. ribes* may not only have cytotoxic effect but also anti-metastatic effect on MCF-7 cells.

Keywords: Anti-metastatic, Cancer, Cytotoxic, *Rheum ribes*

P-056 - IMPACT OF NANOPARTICLES OF C60 FULLERENE ON GFAP AND NF-KB IN THE U373 GLIOMA CELL LINE

Ağca Can Ali[1], Nedzvetsky Victor[1], Tykhomyrov Artem[2]

[1]*Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Bingol University, Bingol, Turkey.*
[2]*Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (NASU), Kyiv, Ukraine.*

Backround: Gliomas are the most common type of primary tumor in brain and are characterized by high resistance to programmed cell death and invasion pattern. Water soluble nanoparticles C60 fullerene have been reported to be effective antioxidant and nontoxic antiproliferative agent. The malignant progression of astrocytic tumors is accompanied by a decrease in the proportion of cells expressing cytoskeleton protein glial fibrillary acidic protein (GFAP) as well as by a reduction in the GFAP content. The goal of this study was to elucidate antiproliferative effects of C60 and the role of cytoskeleton changes in astrocytic tumors on malignant glioma U373 cells.
Material and Methods: Glioma U373 cells were treated with fullerene C60, (1 μM) and H2O2 (500 mM) for 24h, and cells were treated with vehicle as control. Viability of cells were detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) analysis. GFAP and NF-kB proteins expression were checked by Western blot.
Results: MTT analysis showed that fullerene C60 inhibited cell proliferation in glioma U373 cells. There are observed an inhibition of proliferation in normal control on 16%. Contrary, in stressed cells C60 induced a rising of cell viability on 25%, that can be relate to power antioxidant ability of C60.Western blot revealed that GFAP and NF-kB expression decreased in U373 cell line with treated H2O2 on 48% and 64% respectively. The preincubation U373 with C60 fullerene 1 hour before H2O2 treatment ameliorates the reduction of glial intermediate filament and NF-kB expression almost to control level.
Conclusıon: These results illustrate that water soluble C60 fullerene provides protection against the system disturbance induced oxidative stress, especially ameliorates expression of cytoskeleton marker GFAP and key regulator for main pathways of cell response NF-kB. Along with antioxidant effect this nanoparticles C60 fullerene have an ability to inhibit the proliferation of malignant glioma U373 cells.
Keywords: C60 Fullerene, GFAP, NF-kB, Glioma

P-057 - EVALUATION OF CYTOTOXIC AND ANTI-ANGIOGENIC EFFECTS OF METHANOLIC EXTRACT OF *LAWSONIA INERMIS*

Barutca Banu[1], Altuğ Tasa Burcugül[1], İrdem Emine[2], Zurnacı Murat[2], Harzadın Münevver Arzu[3], Koparal Ayşe Tansu[1]

[1]*Department of Biology, Faculty of Science, Anadolu University, Eskişehir, Turkey*
[2]*Department of Biology, Faculty of Science and Letters, Eskişehir Osmangazi University, Eskişehir, Turkey*
[3]*Eskişehir Gelişim College, Eskişehir, Turkey*

Introduction and Aim: *Lawsonia inermis*, commonly named as henna, belongs to the *Lythraceae* family and its widely used for medicinal and cosmetic purposes. We aimed to determine the effects of methanolic extract of *L. inermis* on the human breast cancer cells (MCF-7), human colorectal adenocarcinoma cells (Caco-2), human neuroblastoma cells (SH-SY5Y) and human umbilical vein endothelial cells (HUVECs) and also whether use of the extract would be an alternative for current anti-cancer agents.
Material and Methods: The cytotoxic effects of the methanolic extract were determined by *in vitro* MTT and Neutral Red Uptake (NRU) assays. The anti-angiogenic effect of *L. inermis* extract on HUVECs differentiation was assessed by examining *in vitro* tube formation assay.
Results: The data of present study demostrated that methanolic extract of *L. inermis* induced cytotoxicity and significantly inhibited cell viability at concentrations above 25 µg/ml in time and dose dependent manner in all cells. According to MTT and NRU assays results, the most cytotoxic effect was observed on MCF-7 cells and IC50 values ranged 35.53 µg/ml to 40.87 µg/ml. The less cytotoxic effect was evaluated on Caco-2 cells and IC50 values ranged 96.95 µg/ml to 110.24 µg/ml. HUVECs and SH-SY5Y cells IC50 values of the extract of *L. inermis* ranged 69. 55 µg/ml to 80.75 µg/ml and 45.72 µg/ml to 48.63 µg/ml respectively. At low cytotoxic concentration on HUVECs, the methanolic extract was destroy the branching and the tube formation.
Conclusions: Our preliminary data indicates that the methanolic extract of *Lawsonia inermis* may be potential therapeutic agent for cancer therapy.
Keywords: *Lawsonia inermis*, Cytotoxicity, Tube Formation, Anti-Angiogenic

P-058 - THE DISTRIBUTION AND EFFECTS OF CDKN2 P16 540 C>G AND 580 C>T, AND MDM2 SNP309 T>G POLYMORPHISMS ON LARYNGEAL CANCER

Coşkun Cihan[1], Meshediyeva Roya[1], Verim Aysegül[2], Kucukhuseyin Ozlem[1], Hakan Mehmet Tolgahan[1],Yaylım Ilhan[1]

[1]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Molecular Medicine, Istanbul Turkey*
[2]*Otorhinolaryngology Clinic, Haydarpasa Training and Research Hospital, Istanbul, Turkey*

Background: Laryngeal squamous cell carcinoma (LSCC) is a multifaceted and genomically complex disease and cellular and preclinical studies have done wide ranging molecular mechanisms which underpin its development and progression. p16INK4, an important member of cell-cycle which inhibits cyclin D1–cyclin dependent kinase (CDK). It is known that changes in functions of p16 and MDM2 are related to tumor pathogenesis by enhancing cell proliferation and malign development. Recent reports have shown that p16 540C>G (rs11515), p16 580 C>T (rs3088440) and MDM2 SNP309 T>G polymorphisms were related to cancer development, prognosis and tumor aggressiveness.
Materials and Methods: Using PCR-RFLP technique, we determined SNPs in 79 patients with laryngeal tumors and 73 healthy volunteers without malignancy.
Results: We found no significant association for the distributions of CDKN2 p16 580 C>T and p16 580 C>T variants between cases and controls. Hovewer, the frequency of TT genotype for MDM2 SNP309 T>G was significantly 2.5 times higher ($p<0.001$) and possessing G allele had decreased risk ($p<0.001$) in laryngeal carcer than control group. We also found that the late tumor stage-laryngeal patients having TT genotype had higher 1.8 fold risk ($p=0.017$) than those with early tumor stage cancer.
Conclusion: These findings show that CDKN2 p16 540 C>G, CDKN2 p16 580 C>T and MDM2 SNP309 T>G variants may be risk factors for the development of laryngeal tumors.
Keywords: Laryngeal squamous cell carcinoma, p16, MDM2, Polymorphism

P-059 - SYNTHESIS AND CYTOTOXIC EVALUATION OF NEW 1,3,4-THIADIAZOLE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS

Altuğ Tasa Burcugül[1], Altıntop Mehlika Dilek[2], Sever Belgin[2], Özdemir Ahmet[2], Kaplancıklı Zafer Asım[2], Koparal Ayşe Tansu[1]

[1]*Department of Biology, Faculty of Science, Anadolu University, Eskişehir, Turkey*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey*

Introduction and Aim: The biological properties of 1,3,4-thiadiazole and its derivatives have been studied for more than one hundred years. They are widely known as compounds with various kinds of biological activities showing anticancer properties against human cancers. New 1,3,4 thiadiazole derivatives were synthesized via the reaction of 5-(4-fluoro/chlorophenylamino) 1,3,4-thiadiazole-2(3H)-thione with N-(thiazol/benzothiazol-2-yl)-2-chloroacetamide derivatives. We aimed to determined the effects of new 1,3,4-thiadiazole derivatives on human neuroblastoma cells (SH-SY5Y), human hepatocellular carcinoma cells (HEPG2), human umbilical vein endothelial cells (HUVECs).
Methods: MTT assay was carried out to determine the cytotoxic effects of the compounds on SH-SY5Y, HEPG2, HUVEC cell lines.
Results: Our results demostrated that new 1,3,4 thiadiazole derivatives significantly reduced cell viability all cell lines in time and dose dependent manner compared to untreated control cells. Cytotoxic effects of N-(Benzothiazol-2-yl)-2-((5-((4 chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)acetamide (I) on SH-SY5Y, HEPG2 and HUVEC cells IC50 values 400 µM. According to N-(6-Methylbenzothiazol-2-yl)-2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)acetamide (III) MTT assay results, the most cytotoxic effect was observed on SH-SY5Y cells and HEPG2 cells. The less cytotoxic effect was evaluated on HUVEC cells. Cytotoxic effects of N-(6-Methylbenzothiazol-2-yl)-2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio) acetamide (III) on SH-SY5Y and HEPG2 cells IC50 values 200 µM and 50 µM respectively and HUVEC cells IC50 value 400 µM.
Conclusions: The significant cytotoxic activity observed for I and III derivatives suggest that these derivatives may be potential anticancer agents.
Keywords: Cytotoxicity, Synthesis, Thiadiazole Derivatives, Anticancer

P-060 - BAG-1 INDUCES CELL SURVIVAL IN MDA-MB-231 BREAST CANCER CELL LINES

Kızılboğa Akgün Tuğba, Özden Can, Dinler Doğanay Gizem

Istanbul Technical University

BAG-1 (Bcl-2 associated athanogene) is a multifunctional protein that interacts with diverse array of cellular targets and modulates a wide range of cellular processes, including proliferation, cell survival, transcription, apoptosis, metastasis and motility. In human cells BAG-1 exists as three major isoforms (BAG-1S, BAG-1M and BAG-1L) derived by alternative translation initiation from a single mRNA, which allows interactions with various molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/proteasome machinery, Bcl-2, Raf-1 kinase, nuclear hormone receptors and DNA. Our work aims to investigate how altered Bag-1 expression levels affect cell survival in MDA-MB-231(ER,PR and HER2/Neu negative) breast cancer cell lines. We first cloned Bag-1L gene to a cloning vector, later we transfected MDA-MB-231 cells for overexpression of Bag-1. We also used Bag-1 siRNA to silence Bag-1 gene. Western blot analysis was applied to demonstrate relative expression levels of Bag-1, its interacting partners and certain proteins which are important for apoptosis pathway. We performed XTT cell viability assay for Bag-1 overexpressed cells to checkBag-1's impact on cell survival, and observed enhanced survival rates on cells compared to that of the untreated cells with Bag-1 overexpression. In addition, our study revealed that once BAG-1 forms a complex with C-Raf/B-Raf/Hsp70/Akt/Bcl-2, modulation of cell survival was observed. We believe that once the exact localization and involved molecular mechanisms of Bag-1 and its isoforms are found, the role of each Bag-1 isoform in cell survival can be understood better. This can further provide routes to study tumor development.

Keywords: Breast cancer, Bag-1, cell survival

P-061 - ANTI-PROLIFERATIVE AND APOPTOSIS INDUCING EFFECT OF FLUBENDAZOLE ON NEUROBLASTOMA CELLS

Ercetin Ozdemir Ayse Pınar[1], Altun Zekiye[1], Aydin Melek[1], Boskan Gizem[2], Gokdemir Ebru[2], Kavak Isa[3], Cecen Emre[4], Olgun Nur[5], Aktas Safiye[1]

[1]*Basic Oncology Department, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey*
[2]*Biochemistry Department, Faculty of Science, Ege University, Izmir, Turkey*
[3]*Genetic and Bioengineering Department, Faculty of Science, Kastamonu University, Kastamonu, Turkey*
[4]*Pediatric Oncology Department, Adnan Menderes University, Aydın, Turkey*
[5]*Pediatric Oncology Department, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey*

Aim: Neuroblastoma (NB) is the most common extracranial solid tumor of childhood. Flubendazole (Flu) inhibits microtubule activity that is responsible of proliferation and migration of eucaryotic cells as in metastasis and migration of tumor cells. Anti-proliferative effect of Flu has been shown in leukemia and multiple myeloma in the literature. According to this knowledge, the aim of the study was to evaluate the effect of Flu on the viability and characteristics of NB cells.
Method: After C1300 NB cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine, cells were incubated at various concentrations of Flu (100 nM, 200 nM, 400 nM, 500 nM, 1000 nM). Anti-proliferative effect was evaluated after 24, 48, and 72 hours of exposure by WST-1 test. Apoptotic cell levels were tested at 24 hours of exposure to the same concentrations by annexin V flow cytometric analysis. The migration of cells at LD50 dose for 72 hours was monitored and saved by wound healing and metastatic in vitro assays.
Results: Flu significantly inhibited cell proliferation in a concentration-dependent and time-dependent manner. LD50 doses of Flu were found to be 500 nM at 24-h treatment and 400 nM at 48-h. After 24-h treatment, WST-1 and the apoptosis test results were correlated. Apoptosis inducing effect of Flu was 2 to 6 times more than control. Flu decreased cell migration due to in vitro models.
Conclusion: According to this study, Flu is suggested as an anti-proliferative and apoptosis inducing agent on NB cells. At the 24-h treatment, WST-1 and apoptosis test results were correlated. In following studies the anti-tumoral effect and mechanisms of Flu is thought to be evaluated on the in-vivo animal models.
Keywords: Flubendazole, Neuroblastoma, Apoptosis, Anti-proliferation

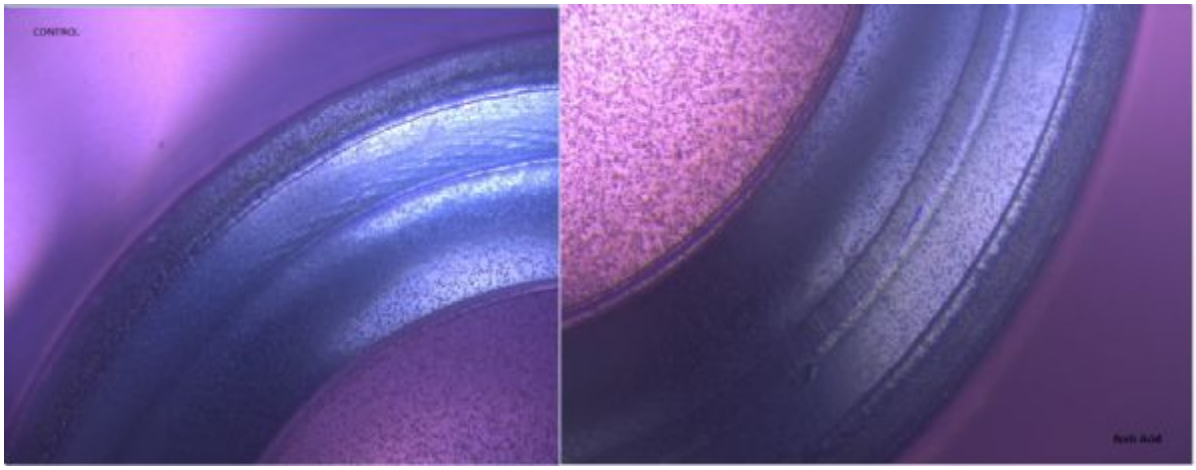
P-062 - IN VITRO EFFECT OF BORATE DERIVATIVES ON NEUROBLASTOMA CELLS

Erçetin Özdemir Ayşe Pınar[1], Aktas Safiye[1], Aydin Melek[1], Somen Meliha[2], Pasayigit Meryem[2], Olgun Nur[3], Altun Zekiye[1]

[1]*Basic Oncology Department, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey*
[2]*Biology Department, Faculty of Science, Ege University, Izmir, Turkey*
[3]*Pediatric Oncology Department, Institute of Oncology, Dokuz Eylul University, Izmir, Turkey*

Aim: Neuroblastoma (NB) which is the most common extracranial solid tumor of childhood, carries on to have a dismal prognosis for children diagnosed with advanced stage or relapsed disease. Boric acid (BA) which is an antiseptic, insecticide and precursor to other chemical compounds, has been shown to decrease cell growth in prostate cancer, osteosarcoma and malignant melanoma in previous studies but not in NB yet. Hence, we aimed to evaluate the effect of borat derivatives, BA and disodium pentaborate (DSP) of NB cell behaviour.
Method: Kelly (N-myc amplification positive) NB cells were cultured in RPMI 1640 supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine and incubated with BA (100 uM) and DSP (5 mM). Anti-proliferative effects were evaluated after 24 hours by WST-1 test. Apoptotic cell levels were detected by annexin V flow cytometric analysis. The migration of cells was monitored and saved by wound healing and metastatic in vitro assays in subgroups of control, BA and DSP treated cells.
Results: Both of the borate derivatives increased late apoptosis three times while total cell deaths were two times more than the control group. When evaluated seperately, BA was found to be inducing apoptosis 5 times more than DSP. According to in vitro migration models, cells migrated more in BA treated cells. Control cells and DSP treated cells showed similar disposition of migration.
Conclusion: The borate derivatives BA and DSP induced apoptosis on NB cells. However they did not show significant anti-metastatic effects. Particularly, the systemic effect of higher concentrations of BA is suggested to be evaluated on experimental NB animal models in future studies.
Keywords: Neuroblastoma, Boric Acid, Disodium Pentaborate

Boric Acid metastasis model



P-063 - INVESTIGATION OF EFFECTS OF OLIVE (OLEA EUROPAEA L.) LEAVES ON LIVER CELL LINES

Bektay M. Yunus[1], Güler Eray Metin[2], Uçkaya Fatih[3], Topçu Gülaçtı[4], Koçyiğit Abdurrahim[2]

[1]Bezmialem Vakif University, Faculty of Pharmacy, Department of Clinical Pharmacy 34093, Fatih, Istanbul, Turkey.
[2]Bezmialem Vakif University, Faculty of Medicine, Department of Medical Biochemistry, 34093, Fatih, Istanbul, Turkey.
[3]Bezmialem Vakif University, Faculty of Pharmacy, Department of Biochemistry 34093, Fatih, Istanbul, Turkey.
[4]Bezmialem Vakif University, Faculty of Pharmacy, Department of Pharmacognosy 34093, Fatih, Istanbul, Turkey.

Introduction: Olive (*Olea europaea* L.) is commonly consumed fruit. In addition to dietary consumption olive used for medical purposes for hundreds of years. Olive cultivated nearly everywhere around the world. However highest cultivation quantity concentrated around Mediterranean countries such as Spain, Italy, Greece Turkey, Syria. Main constituent of olive is fixed oil of the fruit and olive oil commonly used for dietary and medical purposes. Flavanoids, iridoids, secoiridoid glycosides, triterpenes, biophenols and many others are the secondary metabolites of olive. Oleuropein is the responsible compound for antioxidant, anti-inflammatory, anticancer, protection on Low Density Lipoproteins (LDL), antihypertensive effects of olive.
Material and Methods: In this study anti proliferative effects of Olea europaea L. leaves extract on liver cancer cell lines has been investigated. Olive leaves has collected around Iznik province of Turkey and these medical herbs extracted by maceration. Cytotoxic and genotoxic effects of extracts of *Olea europeae* L. Leaves extract have been performed with cell culture techniques. Hepatoma and liver normal cell lines were used due to stability and suitability for our work. Apoptosis induction of extracts has been performed with acridine orange assay. Used method for cytotoxicity determination was ATP assay. Intracellular Reactive Oxygen Species (ROS) has been detected according to Rajesh et al. (2010). Examination of genotoxicity made by comet assay.
Discussion: According to our results olive leave extract were more cytotoxic, genotoxic and apoptotic when compared with hepatoma cell lines.
Keywords: Olive, *Olea europeae* L., Oleuropein, Hepatoma, Anti cancer

P-064 - ISOLATION AND PURIFICATION OF ANTICANCER AGENTS CUCURBITACIN D AND I BY HPLC FROM *ECBALLIUM ELATERIUM* (L.) A. RICH

Tosun Emir, Baysar Ahmet

İnönü University, Faculty Of Engineering, Department Of Chemical Engineering, Malatya

Introduction and Objective: Ecballium elaterium (L.) A. Rich. (EE) fruit juice is used for the treatment of sinusitis and for several illnesses in Turkish folk medicine. EE also may be a great natural source for the development of new drugs and may provide a cost effective mean of treating cancers and other diseases in the developing world. It has been used against cancer in the last years. This effect arises from cucurbitacins contained in the plant. This study was designed to isolate and purify Cucurbitacin D and I from EE fruit juice by HPLC.

Material and Methods: EE fruits were collected from Adana, Turkey. The fruits were washed, pressed and the collected juice strained. The juice was extracted with chloroform. The aqueous phase in the fractions was removed and the process continued with the organic phase. The chloroform extract was reduced to 10 mL volume and then fractionated with flash chromatography. The residue obtained by removing the organic phase was dissolved in ethyl alcohol and then was fractionated by an analytical column HPLC. The optimised chromatographic conditions were acetonitrile-water [2:8, solvent A and 45:55, solvent B] with gradient elution analysis. Detection was achieved by a DAD detector at 229 nm wavelength. The Cucurbitacin D and I fractions were collected in the fraction collector. The fractions were lyophilized and Cucurbitacin D and I crystals were obtained.

Results: The isolated Cucurbitacin D and I were checked against standards (Extrasynthese, France). The concentrations of the isolated Cucurbitacin D and I were determined as 418 µM and 296 µM, respectively. The isolated Cucurbitacin D and I had the same purity as the standards.

Keywords: Ecballium elaterium (L.) A. Rich., Cancer, Cucurbitacin D and I, HPLC

P-065 - THE BLOOD NEUTROPHIL/LYMPHOCYTE RATIO IN BREAST CANCER

Koro Atakan[1], Aral Hale[1], Gürsu Rıza Umar[2], Trabulus Didem Can[3], Nazlı Mehmet Ali[4], Afşar Çiğdem Usul[2], Karaçetin Didem[5], Usta Murat[6]

[1]Istanbul Research and Training Hospital, Department of Medical Biochemistry, Istanbul, Turkey
[2]Istanbul Research and Training Hospital, Department of Internal Medicine, Istanbul, Turkey
[3]Istanbul Research and Training Hospital, Department of General Surgery, Istanbul, Turkey
[4]Istanbul Research and Training Hospital, Department of Radiology, Istanbul, Turkey
[5]Istanbul Research and Training Hospital, Department of Radiation Oncology, Istanbul, Turkey
[6]Giresun University, Faculty of Medicine, Department of Medical Biochemistry, Giresun, Turkey

Background: While the behavior of cancer involves numerous molecular cascades and processes, it is recognized that inflammation plays a major role in cancer biology. It is suggested that there is the stability of neutrophil/lymphocyte ratio (NLR) compared with the absolute leukocyte subtype counts that could be altered by various physiological, pathological, and physical factors. Moreover, NLR may represent the two opposing inflammatory and immune pathways that exist together in cancer patients. We aimed to investigate NLR in breast cancer, in our population.
Methods: Using data retrieved from the medical records, 66 women diagnosed primary breast cancer met our study inclusion criteria as they had a complete blood count with leukocyte differential performed before any anti-cancer therapy. And 44 women with benign mammary neoplasm/disease, followed up in the out-patient clinics of mammary disease and confirmed with sonographical/histopathological examination, made up our controls. Exclusion criteria included laboratory evidence of white blood cells count (WBC) > 10.5 × 10^9/L. Differential leukocyte counts were obtained by BC 6800 (Mindray Medical International Ltd., China), we examined WBC, neutrophil, lymphocyte, platelet counts, and hematocrite, NLR, mean platelet volume values.
Results: Although there is lack of evaluation of tumor-associated neutrophils and lymphocytes, higher NLR median values and lower lymphocyte mean counts (lymphopenia) were found in women with breast cancer ($p<0.0001$) as shown in Table I and Figure 1. There was a weak negative correlation in breast cancer between NLR values and platelet counts ($rs = -0.274$; $p = 0.026$).
Conclusion: Studying complete blood count and indices has advantages of short turn-around time, requiring no sample preparation and being cost-effective. The performance of NLR on assessing the risk of breast cancer should be investigated in pre and post-menopausal women in further studies at the molecular level including clinical outcomes and demographical/histopathological data.
Keywords: Blood, Breast, Cancer, Lymphocyte, Neutrophil

P-066 - INVESTIGATION OF THE EXPRESSION STATUS OF *TIMP3* IN LARYNX CANCER

Demokan Semra, Comert Sevde, Suoglu Yusufhan, Ulusan Murat, Dalay Nejat

Department of Basic Oncology, Oncology Institute, Istanbul University, Istanbul, Turkey,

Backround: Larynx cancer (LC) is the most common cancer type in the head and neck region. The incidence of LC is around 2.4% among all cancer types in the world. Despite of advances in medicine in recent years, there is not a significant reduce in the mortality ratio of the LC. Although environmental and genetic factors play a role in the development of LC the underlying mechanism is away from being clear.*TIMP3*, encoded by gene on chromosome 22, and also known as tissue inhibitor of metalloproteinases 3, is a member of the inhibitors of the matrix metalloproteinases which play a role in degradation of the extracellular matrix. Loss of *TIMP3* expression was recorded in various types of cancer such as pancreatic ductal and gastrict adenocarcinoma, non-small cell lung, endometrial and renal cell carcinoma. In our study, we investigated the association of differentially expressed level of *TIMP3* with LC.
Methods: The expression status of *TIMP3* was analyzed in tumor and matched-normal tissue samples of 44 patients with LC by the quantitative real-time polymerase chain reaction method (QRT-PCR).
Results: The *TIMP3* and the reference gene expression status were analyzed by calculating the threshold cycle numbers (Ct) as fold changes using the 2-ΔΔCt method, after performing QRT-PCR method. After evaluation of the expression levels obtained from tissue samples, we selected the ratio of >=2 as the threshold for differentially expressed *TIMP3*. The decreased expression ratio of *TIMP3* was observed as 65.9% (29/44) in LC patients. The results will be compared with the clinopathological data.
Conclusions: It is estimated that the differentially expressed levels of *TIMP3* was associated with the larynx carcinogenesis. Our study is still in progress to include a larger cohort of patients.
Keywords: TIMP3, Cancer, Investigation

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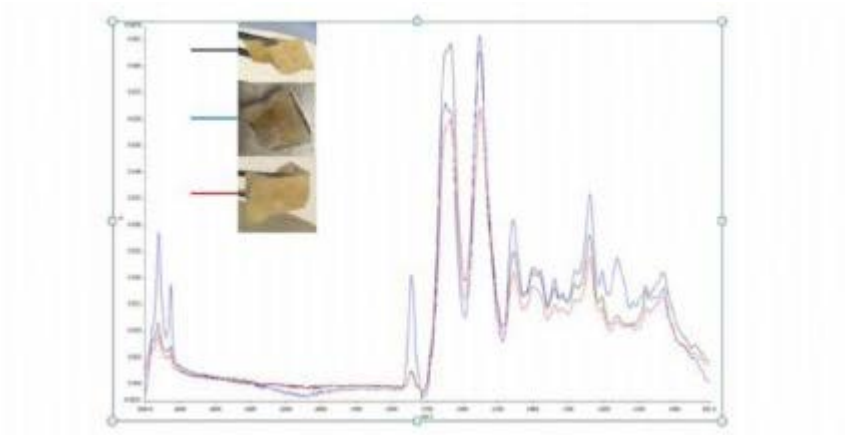
P-067 - CHEMOMETRIC DISCRIMINATION OF BREAST CANCER USING SPECTRAL HISTOPATHOLOGY

Kızıl Ramazan[1], Gedikli Bengisu Hidayet[1], İnce Ümit[2]

[1]*Istanbul Technical University*
[2]*Acıbadem Medical School*

Like cancer, most disease states are associated with chemical composition changes. Therefore, biochemical changes in a tissue can provide important molecular clues for diagnosis of cancer. These molecular clues can be considered as biological markers of disease state and have a complex chemical nature. Spectroscopic tools can be employed to sense molecular level changes between the healthy and tumor tissues, providing a means of spectral histopathology if they are validated by a standard clinical test.
This presentation covers determination of biochemical markers of breast cancer using Fourier transform Infrared spectroscopy at the attenuated total reflection mode at the molecular level and compare with the results of standard histopathological assessments to validate discrimination of tumor cells from healthy ones. The study involved seventeen breast cancer diagnosed cases from which twelve cases include samples from healthy and tumor cells. Spectral fingerprints suggest that breast cancer creates molecular changes in the amide I and II vibrational bands of protein along with DNA/RNA groups at 1240 and 1082 cm-1. Chemometric discrimination models were developed based on molecular information of amide, and DNA groups and tumor tissue was discriminated successfully from the healthy tissue. DNA modes were found to be the affected peaks so that class models based on DNA could provide the most accurate discrimination of tumor up to 90 percent. The poster will illustrate the methodology of the our spectral histopathology approach, spectral biological markers of breast cancer, use of a canonical variate analysis as a tool of tumor tissue discrimination. In addition to discrimination, spectral histopathology can also offer understanding of mechanistic cancer development by investigating lipid ratio in tumors and grading of cancer from spectral responses of DNA/RNA. The most striking advantage of spectral histopathology is based on rapid discrimination of tumor cells without the help of a priori information.
Keywords: Breast Cancer, Spectral Histopathology, Molecular markers of Cancer

Cancer Tissue and Spectra



The attached figure shows picture of three breast cancer tissues and their FTIR spectra. Spectra was used for histopathological assessment of breast cancer.

P-068 - THE EFFECTS OF SOME ARTIFICIAL SWEETENERS ON MTDNA

Mutlu Ayşe Gül[1], Kürklü Nesibe[2]

[1]*Department of Molecular Biology and Genetics, Faculty of Art and Science, Mehmet Akif Ersoy University, Burdur, Turkey, agmutlu@mehmetakif.edu.tr*
[2]*Department of Biology, Institute of Science, Mehmet Akif Ersoy University, Burdur, Turkey*

Objective: The aim of the current study, detection of the effects of some artificial sweeteners on mtDNA damage and copy number in Drosophila melanogaster. Artificial sweeteners are added to a wide variety of food, drinks, drugs and hygiene products. A cancer-inducing activity of one of these substances would mean a health risk to an entire population. DNA mutations generated by some artificial sweeteners have been investigated by some researchers but there is no information in the literature about the effects of these substances on mtDNA. mtDNA damage could potentially be more important than deletions in nDNA because the entire mitochondrial genome codes for genes are expressed. Also, somatic mtDNA mutations have been increasingly observed in primary human cancers.
Material and Methods: In this research, the QPCR method was used to measure mtDNA damage. The lesion present in the DNA blocked the progression of any thermostable polymerase on the template, so a decrease in DNA amplification was observed in damaged templates. We used Drosophila melanogaster as a model organism for our research.
Results: Aspartame created statistically significant mtDNA damage. There was no mtDNA damage in Saccharine+Cyclamate, Saccharine, Aceculfam K and Sucralose application groups.
Conclusion: Over half a century ago, Warburg initiated research on mitochondrial alterations in cancer. These alterations include changes in mtDNA content and mtDNA mutations. In recent years, many mtDNA mutations have been identified in various types of human cancer. Aspartame created mtDNA damage in Drosophila according to our research. These results indicate that the effects of aspartame in human should carefully detect.
Keywords: Artificial sweeteners, mtDNA damage, mtDNA copy number

P-069 - EFFECTS AND MECHANISMS OF VARIOUS CALORIE RESTRICTION METHODS ON EXPERIMENTAL CARCINOMA OF SPRAGUE DAWLEY RATS

Koç Yıldırım Ece, Balkaya Muharrem

Physiology Department, Veterinary Faculty, Adnan Menderes University, Aydın

Background: This study aimed to investigate the effects of different calorie restrictions, starting after tumor growth progressed to a certain degree, on tumor growth in induced breast cancer models.
Materials and Methods: A total of 34, 21-days old female Sprague Dawley rats were used. Breast cancer was induced via NMU (50 mg/kg/i.p.) on 29 rats. When tumors grow to 10-12 mm, rats were divided into 3 groups paired according to tumor size: animals in one group were given chow diet ad libitum and served as cancer controls (CC), while others undergo to 50% calorie restriction (CR-50%) or fed alternate days (ADF) for following 12 weeks. Other 5 animals were given physiological saline and served as ad libitum-fed healthy controls.
Body weights and, palpable, tumor diameters were recorded weekly. At the end of experiment tumors excised, weighed and their diameters measured. In sera prepared from blood samples, concentrations of the insulin, IGF-I, corticosterone, leptin and adiponectin were determined via ELISA.
Results: Rats undergoing CR-50% lost 26-38% of their initial live weights within 6 weeks, and experiment terminated for this group after 6 weeks. Mean body weight of controls was higher than that of CC, ADF and CR-50%. Tumor volumes at 6th week of CR-50% were less than that of CC. However, there was no difference in tumor volumes and weights between CC and ADF. Serum adiponectin concentration of controls and ADF and adiponectin/leptin ratio of ADF were higher than that of CC. Serum insulin, IGF-I, leptin and corticosteron concentrations did not differ between groups. All tumors from %50-CR group excised were benign. Highest incidence of malign and invasive tumors was encountered in CC.
Conclusions: ADF protocol did not delay tumor growth to rats undergoing this type of CR. CR remains a viable option as a dietary intervention in breast carcinoma.
Keywords: Alternate Day Fasting, Breast Cancer, Calorie Restriction, Sprague Dawley Rats, NMU

P-070 - SCREENING ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF *POLYPORUS SQUAMOSUS* AND *CANTHARELLUS CIBARIUS* EXTRACTS AND THEIR EFFECTS ON GLUTATHIONE-S-TRANSFERASE ACTIVITY

Shomali Naznoosh, Onar Okan, Akata Ilgaz, Yildirim Ozlem

Ankara University, Faculty of Science, Department of Biology, Ankara, Turkey

Medicinal mushrooms show an ideal food nature due to their low sugar and oil content, nutritional value and especially because of being good diet products. Nowadays there is a growing interest in new drugs against secondary metabolites derived from fungi and for the discovery of precursor compounds. These bioactive components are becoming popular sources of natural antioxidant, antitumor, antiviral, antimicrobial and immunomodulatory agents. In this study, the ethanol extracts of *Polyporus squamosus*and *Cantharellus cibarius* species were analyzed for the polyphenolic contents by using spectrophotometrically methods. The free radical scavenging and antimicrobial activities of extracts were evaluated by DPPH and disc diffusion assays. Besides, the mushroom extract effects were examined on the glutathione-S-transferase (GST) enzyme activity by kinetic assay. According to the results, ethanol extract of *P. squamosus* has been shown the highest total of phenolic and flavonoid contents with 25.65 ± 0.37 mg GAE/g and 12.36 ± 0.04 QE/g values, respectively. The highest DPPH radical scavenging were observed for ethanol extract of *P. squamosus* with 0.329641 ± 0.002275 mg/mL IC50 value. Also, the best activity profile for GST was observed with the crude ethanol extract of *P. squamosus*. In this study we additionally screened antimicrobial activity of mushroom extracts on the *Staphylococcus aureus*,*Escherichia coli* and *Pseudomonas aeruginosa* strains. However, ethanol extract of *P. squamosus* had shown moderate effects on the bacteria strains inhibition.

Keywords: *Polyporus squamosus*, *Cantharellus cibarius*, Antioxidant, Antimicrobial, Glutathione-S-transferase

P-071 - EFFECTS OF CHRONIC AND INTERMITTENT CALORIE RESTRICTION ON ADROPIN LEVELS IN MMTV-TGFα BREAST CANCER MOUSE MODEL

Guvenc Tuna Bilge[1], Atalay Pınar Buket[2], Altunbek Mine[3], Kalkan Batu Mert[4], Dogan Soner[4]

[1]*Department of Biophysics, Yeditepe University School of Medicine, Yeditepe University, Istanbul,Turkey*
[2]*Department of Medical Biology, Maltepe University School of Medicine, Maltepe University, Istanbul,Turkey*
[3]*Department of Genetics and Engineering, Faculty of Engineering and Architecture,, Yeditepe University, Istanbul,Turkey*
[4]*Department of Medical Biology, editepe University School of Medicine, Yeditepe University, Istanbul,Turkey*

Aim: Adropin, a recently identified peptide, has been implicated in insulin resistance and might be one of the potential growth regulator metabolic hormones. The aim of this study is to determine the effect of calorie restriction on circulating levels of adropin in MMTV-TGFα breast cancer mouse model and investigate the effects of adropin peptide on viability of MCF7 breast cancer cells in culture.
Methods: Mice were fed different dietary regimes ad libitum-fed (AL), chronic calorie restricted (CCR), and intermittent calorie restricted (ICR) from ten weeks of age up to 50 weeks. Serum adropin concentrations were evaluated using an enzyme-linked immunosorbent assay. In addition, cells were treated with 5,10,25,50ng/mL adropin for 24 hours. Cell viability was measured with WST-1 assay and cell cycle analysis was examined by flow cytometry.
Results: There was an inverse correlation between serum adropin levels and mouse age that was attenuated by calorie restriction. In the AL group, the level of adropin was significantly lower at week 50 (3.6±0.2ng/mL) compared to levels at week 10 (4.3±0.3 ng/mL). However, among the calorie restricted groups, serum levels of adropin remained high at week 50 (CCR 4.3±0.2ng/mL; ICR 4.7±0.2ng/mL). Incubation of MCF7 cells with 50ng/mL adropin for 24h resulted in a statistically significant decrease in cellular viability ($p<0.001$), while 24h treatment of 10 and 25ng/mL adropin did not show any significant effect. Flow cytometry analysis showed that MCF7 cells entered the early phase of apoptosis after treatment with 50ng/mL.
Conclusions: Our finding that calorie restriction can offset an age-dependent decrease in circulating adropin levels in a breast cancer mouse model suggests that adropin may be involved in the protective effects that calorie restriction has on breast cancer risk. Moreover the preliminary results of adropin on breast cancer cell viability provide a possible mechanism and indicate that additional studies are warranted
Keywords: Breast Cancer, Calorie Restriction, Adropin, MMTV-TGFα Mice

P-072 - THE INFLUENCE OF DIFFERENT CALORIE RESTRICTION PROTOCOLS ON SERUM PRO-INFLAMMATORY CYTOKINES, ADIPOKINES AND IGF-I LEVELS IN FEMALE C57BL/6 MICE: SHORT TERM AND LONG TERM DIET EFFECTS

Dogan Soner[1], Ray Amitabha[2], Cleary Margot P[2]

[1]*Department of Medical Biology, Yeditepe University School of Medicine, Istanbul*
[2]*Hormel Institute Medical Research Center, University of Minnesota, MN, USA*

Calorie restriction (CR) is an effective intervention to prevent chronic diseases in particular cancer. Although many factors including sex hormones, IGF-I and mTOR have been studied in response to CR, the molecular mechanisms of CR remain to be determined. Our objective was to determine the short and long-term effects of different CR protocols on pro-inflammatory cytokines. Our hypothesis was that Intermittent CR (ICR) will result in greater inhibition of pro-inflammatory serum cytokines compared to Chronic CR (CCR) as we previously found ICR to be more protective in prevention of mammary tumor development. From ten week old female C57BL6 mice were maintained on either ad libitum (AL) fed, ICR or CCR protocols for up to 74 weeks of age. Blood samples were collected for measurements of serum interleukin-6 (IL-6), tumor necrosis factor–alpha (TNF-alfa, adiponectin, leptin, IGF-I and insulin levels at specified ages and for ICR mice samples were collected by following restriction (ICR-R) and after refeeding (ICR-RF). In general, both modes of CR reduced serum IL-6, TNF-alfa, IGF-I and leptin levels significantly compared to AL feeding with IL-6 levels 24 and 3.5 fold and TNF-alfa levels about 11 and 1.5 folds lower in ICR and CCR groups, respectively at study termination. There was a trend for adiponectin and insulin to be highest in ICR-RF mice. Body weights were positively correlated with IL-6, TNF-alfa, insulin and leptin but negatively correlated with adiponectin-to-leptin ratio. Moreover, there was a positive correlation between IL-6 and TNF-alfa. Beneficial effects of ICR may function through pro-inflammatory cytokine pathways.

Keywords: Cytokines, calorie restriction, IGF-I, adipokines, inflammation

P-073 - CYTOTOXIC AND APOPTOTIC FUNCTIONS OF TOLFENOMIC ACID ON HUMAN PROSTATE CANCER CELLS

Kabadere Selda[1], Ertekin Rıfat[2], Kuş Gökhan[3]

[1]*Department of Physiology, Faculty of Medicine, Eskişehir Osmangazi University, Eskişehir-Turkey*
[2]*Institute of Health Sciences, Eskişehir Osmangazi University, Eskişehir-Turkey*
[3]*Department of Health, Faculty of Open, Anadolu University, Eskişehir-Turkey*

Prostate cancer is the common adenocarcinoma and second common cause of cancer death in men. The progression of prostate cancer is a multistep process. COX-2 is highly expressed in a number of human prostate cancers and has been identified as an important second messenger that play important roles in various aspects of cancer biology including inducing apoptosis and inhibiting proliferation of cells. We questioned whether tolfenamic acid (COX inhibitor) affects the survival and/or promotes apoptosis of prostate cancer cells (LnCaP) in vitro. After growing the cells in culture, we determined viability with MTT, apoptosis with flow cytometry and activity of COX-2 enzyme with real time PCR. Comparing to the control 1, 1, 5, 10, 25, 50 and 100 µM tolfenamic acid reduced the number of LnCaP cells to 91, 83, 82, 76, 61 and 49 % in 24 hr and 90, 83, 82, 78, 52 and 47 % in 48 hr, respectively. Early apoptotic rate of LnCaP cells were with were 2 and 46 % for 24 hr and 9 and 94 % for 48 hr, respectively. Tolfenomic acid (50 µM) increased the level of caspase-9 up to 4 fold comparing to the control. Moreover, 25, 50 and 100 µM tolfenamic acid reduced the level of COX-2 enyzme down to 1, 0.3 and 0.1 fold. Tolfenomic acid posseses a strong dose and time dependent antiproliferative effect on prostate cancer cell line, LnCaP by blocking COX-2 pathway of arachidonic acid metabolism and by activating caspase-9.

Keywords: Prostate cancer, tolfenomic acid, COX-2

P-074 - THE CYTOTOXIC ACTIVITIES OF NOVEL TEREPHTHALATO COMPLEXES

Öztopcu Vatan Pınar[1], Yildiz Ozge[2], Colak Alper Tolga[2]

[1]*Department of Biology, Faculty of Arts and Sciences, Eskisehir Osmangazi University, Eskisehir*
[2]*Department of Chemistry, Faculty of Arts and Sciences, Dumlupinar University, Kutahya*

Object: In this study, we examined cytotoxic activities of six new terephthalato complexes on two commonly used human prostate cancer cell lines (LNCaP and DU145). **Material and Method:** Cytotoxic activities of all complexes (1 to 500 µM) were performed prostate carcinoma cells and by MTT assay for 24 and 48 h. Cisplatin was used as a positive control. Enzyme-linked immunosorbent assay is used to identify apoptotic and necrotic cells. All statistical analyses were performed using one-way analysis of variance (ANOVA) and followed up by Tukey's multiple comparison tests.
Results: Complex of 6 was the most potent growth inhibitory effect against these cell lines. The IC50 values of complex 6 was determined as 43, 22 µM for LNCaP and 45, 18 µM for DU145 for 24 and 48 h, respectively. Prostate cancer cell lines were treated with various concentrations (10, 25, and 50 µM) of 6 complex for 24 h. This complex increased the apoptotic and necrotic cell death in both cell lines in a concentration dependent manner.
Conclusion: We evaluated growth inhibitory effect of six new terephthalato complexes two different human prostatic carcinoma cells. Among these compounds complex 6 had decreased significantly the cell viability time and dose dependently these cell lines. Especially after 24 h treatment, 6 complex increased the apoptotic and necrotic cell death in both cell lines in a concentration dependent manner.
Keywords: Cytotoxicity, MTT, Prostate Carcinoma, Terephthalate Complexes

P-075 - THE EFFECTS OF [CA+2]I MOBILISATION ON BINDING OF SKOV–3 CELLS TO FIBRONECTIN

Apaydın Elif[1], İncesu Zerrin[2]

[1]*Anadolu University,Health Sciences Institute, Department of Biochemistry, Eskişehir, Turkey*
[2]*Anadolu University, Faculty of Pharmacy, Department of Biochemistry, Eskişehir, Turkey*

Introduction and Aim: Intracellular calcium, [Ca+2]i increasing via endoplasmic reticulum (ER) stress is known to be involved in several cellular processes, such as adhesion, apoptosis and anoikis. Extracellular proteins such as fibronectin and vitronectin are essential compounds for microenvironment of high metastatic ovarian cancer cells. Particularly, fibronectin trigger the formation of spheroid structure, attachment and disaggregation of ovarian cancer cells.
Therefore, the aim of this study is an investigation of the effects of [Ca+2]i via stimulation of ER stress on binding capacity of SKOV–3 to fibronectin.
Materials and Methods: In the presence or absence of 12-24 µM of ER stress inducer, tunicamycin, the alteration of [Ca+2]i after binding of cells to 50 µg/ml fibronectin was investigated by calcium indicator dye, Fluo–3. The excitation wavelength used for Fluo–3 was 490 nm and the emission was detected at 510 nm. The rate of binding was measured by using RTCA after treatment of tunicamycin for 50 h. The rate of cell adhesion was calculated according to NCI formula.
Results: The amount of [Ca+2]i in SKOV–3 cells was increased after the treatment of 24 µM tunicamycin upon time–dependent manner. In the presence of 24 µM tunicamycin, the cell attachment to fibronectin was reduced to 0.09 NCI value as compared to control NCI value (1.03). This decline in binding was continued for 48 h. Light microscope images also supported these results.
Discussion and Conclusion: ER stress induced by tunicamycin affected on [Ca+2]i mobilisation in SKOV–3 cells, suggesting that increased cytoplasmic calcium might be replaced into mitochondria and therefore the apoptotic process could be triggered. On the other hand, ER stress also reduced the adhesion rate of SKOV–3 cells, suggesting anoikis could be induced as well. This issue still remains to be addressed.
This study is supported by Anadolu University Scientific Research Projects (Project No: 1308S303)
Keywords: Ovarium Cancer, ER Stress, Calcium, Adhesion

P-076 - RAB25 CONFERS RESISTANCE TO CHEMOTHERAPY-INDUCED CELL DEATH IN OVARIAN CANCER CELLS BY INHIBITING MITOCHONDRIAL APOPTOTIC PATHWAY

Giray Kurt Asli[1], Karakas Bahriye[2], Unver Gulsah[3], Temel Sehime Gulsun[4], Basaga Huveyda[2], Kozanoglu Ilknur[3], Kutuk Ozgur[1]

[1]Department of Medical Genetics, Baskent University School of Medicine, Adana Dr. Turgut Noyan Medical and Research Center, Adana, Turkey
[2]Molecular Biology, Genetics and Bioengineering Program, Sabanci University, Istanbul, Turkey
[3]Department of Physiology, Baskent University School of Medicine, Adana Dr. Turgut Noyan Medical and Research Center, Adana, Turkey
[4]Department of Histology and Embryology, Near East University School of Medicine, Nicosia, Northern Cyprus

Background: In spite of the increase in patient survival rates promoted by increased screening and prevention efforts, much faster tumor genome sequencing and developed smart targeted-therapies, de novo or acquired chemoresistance still remains to be a significant factor for treatment failure in cancer therapeutics. Conventional chemotherapy and radiotherapy constitute the main two approaches in addition to surgery in cancer treatment. BCL-2 protein family members regulate chemotherapy-induced mitochondrial cell death pathway and the release of cytochrome c into the cytosol, which is the point of no return for cell death. Rab GTPases play important roles in critical cellular processes such as intracellular vesicular trafficking and cellular viability and their contribution to carcinogenesis and cancer progression becomes increasingly clear. In particular, altered expression of Rab25 has been shown in ovarian, breast and hepatocellular cancers.
Objective: Here we explored how Rab25 regulates proliferation, cell death response and the expression of BCL-2 protein family members in ovarian cancer cells.
Methods: Confocal immunofluorescence microscopy was used to determine the intracellular localization of Rab25. The expression status of Rab25 and BCL-2 protein family members were detected by means of immunoblot analysis. CellTiter-Glo, colony formation assay and Annexin V/PI staining were used to evaluate cell death response. CyQUANT and MTT assays were used to assess cell proliferation.
Results: Rab25 was expressed only in two of eight ovarian cancer cells, in which immunofluorescence analysis revealed mainly cytoplasmic localization. Moreover, we demonstrated that Rab25 protected against carboplatin-, etoposide- and paclitaxel-induced cell death in ovarian cancer cells by blocking mitochondrial apoptotic pathway. Hence, siRNA-mediated knockdown of Rab25 in OVCAR3 and OVCAR4 cells resulted on decreased proliferation and increased sensitivity to chemotherapy.
Conclusion: Our results indicate a prominent prosurvival role for Rab25 in ovarian cancer cells, which is mediated through inhibition of mitochondrial apoptotic pathway.
Keywords: Ovarian cancer, Rab25, BCL-2, Apoptosis, Proliferation

P-077 - DETERMINATION OF APOPTOTIC EFFECT OF DEGUELIN ON GLIOMA CELL LINES

Oztopcu Vatan Pinar[1], Kabadere Selda[2]

[1]*Department of Biology, Faculty of Arts and Sciences, Eskisehir Osmangazi University*
[2]*Department of Physiology, Faculty of Medicine, Eskisehir Osmangazi University*

Aim: Glioblastoma multiforme is the most common and very aggressive type of brain tumors. Isolated from several plant species deguelin is a natural plant rotenoid. Several studies have shown that it is a promising cancer-preventive and therapeutic agent. Since the function of deguelin on glioma cells has not been defined yet, we decided to test its possible apoptotic effect on rat (C6) and human (T98G) glioma cell lines.
Methods: Alterations in the morphology and chromatin structure of glioma cells which underwent apoptosis, were examined by fluorescence dye 4',6-diamidino-2-phenylindole (DAPI) staining. Cells were seeded in a 24 well plate (3x104) and cultured for 24 h and were treated with or without 25 and 100 µM deguelin. At the end of 24 h, cells were collected and fixed in 0.5 % paraformaldehyde. Slides were stained with 1 µg/mL DAPI in the dark and were rinsed with PBS. At least 300 cells per condition were subjected to examination using a digital fluorescence microscope.
Results: Although untreated cells displayed a normal nuclear size, menadione treated and stained cells showed typical morphological features of apoptotic cells, with condensed and fragmented nuclei. Apoptotic changes have started at 25 µM dose and increased parallel with the dose increase to 100 µM both glioma cells.
Conclusions: In this study a new anticancer agent, deguelin, was examined for the first time in rat and human glioma cells and obtained some preliminary results about the apoptotic effects of this agent. The results indicated that deguelin induced apoptosis in a dose dependent manner on glioma cells.
Keywords: Apoptosis, Deguelin, DAPI, Glioma

P-078 - SKOV-3 CELLS BINDING TO FIBRONECTIN INDUCE MITOCHONDRIAL APOPTOTIC PATHWAY AFTER STIMULATION OF ENDOPLASMIC RETICULUM STRESS

Öğünç Yüksel[1], Ayrım Aysun[2], İncesu Zerrin[1]

[1]*Anadolu University, Faculty of Pharmacy, Department of Biochemistry, Eskişehir, Turkey*
[2]*Eskişehir Osmangazi University Life Sciences Institue, Department of Biosecurity and Biotechnology, Eskişehir, Turkey*

Introduction and Aim: [Ca2+]i increased by endoplasmic reticulum (ER) stress is transferred into mitochondria in order to reduce the [Ca2+]i to normal levels, This transition of [Ca2+]i is known to trigger the mitochondrial pathway of apoptosis. In vitro studies with ovarian cancer indicated that the mitochondrial membrane depolarization (MMP) can be occurred by [Ca2+]i mobilization and consequently, cytochrome c is released via Bax/Bak formation. The integrin activation by fibronectin is also induced the [Ca2+]i, however the role of SKOV-3 cells-fibronectin interaction in apoptosis has not investigated yet.
The aims of this study are the investigation of MMP and localisation of cytochrome c and Endo G releases from mitochondria in SKOV-3 cells attached to fibronectin after stimulation of ER stress.
Materials and Methods: MMP was investigated using flow cytometry after the cells were plated onto 50 µg/ml fibronectin coated wells. After 2 or 12 h, the cells were treated with either 18 µM tunicamycin or 10 µM dinitrofenol as a positive control. The localization of cytochrome c and Endo G was demonstrated using fluorescence labeling method.
Results: The adhesion of SKOV-3 cells to fibronectin for 2 or 12 h stimulated an increase in MMP (61,9±15,78 and 91,35±2,38, respectively) as compared to control (37,95±5,2). The fluorescence images showed that the accumulation of cytochrome c into the cytosol increased after adhesion of cells to fibronectin as compared to control at 120 min.
Discussion and Conclusion: SKOV-3 cell binding to fibronectin caused both mitochondrial membrane deposition and the releases of cytochrome c and Endo G from mitochondria after stimulation of ER stress. Induction of MMP, followed by cytochrome c and Endo G releases from mitochondria is a critical step of the apoptotic process. These results could be useful in clarifying the crosstalk between ER stress and apoptotic process in ovarian cancer.
Keywords: Ovarian cancer, ER stress, apoptosis, fibronectin

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P-079 - INHIBITION OF 5-HT1B RECEPTOR SIGNALING INDUCES CASPASE-INDEPENDENT APOPTOSIS IN PANCREATIC DUCTAL ADENOCARCINOMA CELLS

Gurbuz Nilgun[1,2], Ozpolat Bulent[1]

[1]*Department of Experimental Therapeutics, M.D. Anderson Cancer Center, The University of Texas, Houston, Texas, USA;*
[2]*Department of Medical Biology, School of Medicine, Suleyman Demirel University, Isparta, Turkey*

Pancreatic cancer (PaCa) is the fourth leading cause of cancer deaths with 100% mortality rate in Western countries. PaCa is intrinsically resistant to apoptosis and poorly responds to existing therapeutics. To be able to overcome drug resistance and improve PaCa therapy, novel molecularly targeted therapies have to be developed. Serotonin (5-hyroxytriptamin, 5-HT) was known to acts as mitogenic growth factor for several types tumor cells, including pancreatic carcinoid cells, breast and colorectal carcinomas. Among 5-HT receptors, we previously shown that 5-HT1B and 5-HT1D subtypes promotes pancreatic ductal adenocarcinoma (PDAC) cell proliferation and invasion (Gurbuz& Ozpolat, PlusONE 2012). Thus in this study, we aimed to investigate the effects of targeting 5-HT1B receptor using highly specific antagonist, SB216641, in PDAC cells. Inhibition of 5-HT1B receptor by SB216641 at 5 μM and 10 μM doses induced significant cell death of Panc-1 and MiaPaCa-2 cells by about 50% and 70%, respectively. The apoptotic protein expressions, inducing factor (AIF), the major mediator of caspase-independent apoptosis, and Cytochrome C (Cyt-C), the major mediator of mitochondrial apoptotic death, were evaluated both in mitochondrial and cytosolic fractions of PDAC cells treated SB216641. We found a marked induction and release of both of AIF and Cyt-C from mitochondria to cytosol compared to non-treated control cells. In conclusion, our data suggest that inhibition of 5-HT1B receptor-signaling induces mitochondrial dependent apoptosis. Considering the intrinsic apoptotic resistance, 5-HT1B receptor antagonists might be used to treat PDAC and further in vivo studies are warranted.

Keywords: Serotonin, 5-HT, AIF, Apoptosis, Pancreatic Cancer

P-080 - ANTI-TUMOR EFFECTS OF BEMIPARIN IN HEPG2 AND MIA PACA-2 CELLS

Alur İhsan[1], Dodurga Yavuz[2], Seçme Mücahit[2], Elmas Levent[2], Bağcı Gülseren[3], Gökşin İbrahim[1], Avcı Çığır Biray[4]

[1]*Department of Cardiovascular Surgery, Faculty of Medicine, Pamukkale University, Denizli, Turkey*
[2]*Department of Medical Biology, Faculty of Medicine, Pamukkale University, Denizli, Turkey*
[3]*Department of Medical Genetics, Faculty of Medicine, Pamukkale University, Denizli, Turkey*
[4]*Department of Medical Biology, Faculty of Medicine, Ege University, İzmir, Turkey*

Purpose: In this study, we examine the effects of bemiparin on apoptosis and cell cycle related gene expression, viability, colony formation and migration/invasion of the cultured MIA PaCa-2 pancreatic cancer cell and HepG2 hepatocellular carcinoma cells.
Material and Methods: Effects of bemiparin on cell viability and detecting of IC50 dose in MIA PaCa-2 and HepG2 cells were performed by using XTT assay. Total RNA was isolated from the cells exposed to IC_{50}doses of bemiparin using Trizol Reagent. Effects of the bemiparin on apoptosis and cell cycle related genes were determined via RT-PCR. Potential effects of the bemiparin on cell invasion, colony formation and cell migration were detected by matrigel-chamber, colony formation assay and wound-healing assay, respectively. The comparison of the control and dose groups has been analysed by "RT^2 Profiles PCR Array Data Analysis" through "Student t-test".
Results: IC_{50} dose of bemiparin was found to be 200 IU/mL in the 48th hour in the MIA PaCa-2 cell line and 50 IU/mL in the 48th hour in the HepG2 cell line. In HepG2 dose group, *CCND1* expression was reduced and *p53*, *caspase-3*, *p21*, *caspase-8* expressions were increased in the dose group cells when compared with the control group cells. In MIA PaCa-2 dose group, *CCND1*, *CDK4* and *CDK6* expressions were reduced and *p53* expression was increased. Cell invasion and migration was significantly inhibited and colony formation was significantly decreased through bemiparin treatment in both cell lines.
Conclusion: In present study, bemiparin inhibits cell proliferation by inducing cell cycle arrest, apoptosis and also decreases invasion, migration and colony formation in HepG2 and MIA PaCa-2 cells. Bemiparin may be a novel agent for treatment of hepatocellular and pancreatic cancers as a single agent or in combination with other agents.
Keywords: Apoptosis, Bemiparin, Cell cycle, HepG2, Mia Paca-2

P-081 - AN IN VITRO AND IN VIVO EVALUATION OF THE EFFECTS OF DOXORUBICIN AND CHLOROQUINE COMBINATION ON MICE EHRLICH ASCITES CARCINOMA (EAC)

Ikitimur Armutak Elif Ilkay[1], Aydinlik Seyma[2], Uvez Ayca[1], Ari Ferda[2], Sonmez Kiviicim[3], Esener Osman Behzat Burak[1], Ustun Alkan Fulya[4], Aktas Abit[1], Zeybek Sakir Umit[5], Ulukaya Engin[6], Dimas Konstantinos[7], Gurel Gurevin Ebru[8]

[1]Department of Histology and Embryology, Faculty of Veterinary Medicine, Istanbul University, 34320, Istanbul-Turkey
[2]Department of Molecular Biology, Faculty of Science, Uludag University, 16059, Bursa-Turkey
[3]Department of Pathology, Faculty of Veterinary Medicine, Istanbul University, 34320, Istanbul-Turkey
[4]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Istanbul University, 34320, Istanbul-Turkey
[5]Department of Molecular Medicine, The Institute of Experimental Medicine, Istanbul University, 34393, Istanbul-Turkey
[6]Department of Clinical Biochemistry, School of Medicine, Uludag University, 16059, Bursa-Turkey
[7]Department of Pharmacology, School of Medicine, Thessaly University, Larissa, Greece
[8]Department of Biology, Faculty of Science, Istanbul University, 34134, Istanbul-Turkey

Background and Aim: Cancer still remains the most horrifying disease due to treatment failure, cause development of resistance to chemotherapeutics.Autophagy is regarded as one of the key mechanism for drug resistance and chloroquine (CQ) is an inhibitor of autophagy.Therefore, the combination of Doxorubicin (DXR) and CQ may be a realistic strategy for a new treatment modality on Ehrlich Ascites Carcinoma (EAC) cells in vivo and in vitro.

Materials and Methods: EAC cells were treated with DXR (1µM) alone or in combination with CQ (8µM). ATP assay were perfomed to determine cytotoxicity after 48h treatment.Apoptosis and autophagy related proteins were also determined by Western blot analysis. Changes in survival pathway was detect by Luminex xMAP.For in vivo evaluation, 88 Balb-c mice with EAC were divided into control (n=8) and 8 experimental groups (n=10).DXR and CQ have been investigated following intraperitoneal administration of doses of 1.5 and 3 mg/kg X 3 and 25 and 50 mg/kg X 3 respectively.Tumor volume determined in vivo by caliper and relationship between apoptosis, proliferation and autophagy was evaluated by immunohistochemistry.

Results: According to ATP viability assay, combination of DXR and CQ caused a significant decrease in cell viability compared doxorubicin alone.Also 24h treatment of CQ and DXR combination showed increased autophagy protein levels.Cleavage of PARP1 and caspase-3, besides increase in expression of PTEN and FAS protein indicating the enhanced apoptosis.Moreover proteins related to survival pathway were found to decrease by using

Luminex xMAP.In vivo results showed the combination of CQ and DXR reduced tumor volume and increased apoptosis by suppressing the autophagy and proliferation.

Conclusion: The combination of DXR and CQ enhances apoptosis, possibly via the inhibition of autophagy and this might be a promising therapeutic strategy in EAC cells.

Keywords: Chloroquine, In vivo, Doxorubicin, Autophagy, Apoptosis

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P-082 - MIR376B IN BREAST CANCER

Akkoc Yunus[1], Tekirdag Ayse Kumsal[1], Dogan Ekici Asiye Isin[2], Gozuacik Devrim[1]

[1]*Department of Molecular Biology, Genetics and Bioengineering, Faculty of Engineering and Natural Science, Sabancı University, 34956 İstanbul, Turkey.*
[2]*Department of Pathology, Yeditepe University School of Medicine, Atasehir, 34755 Istanbul, Turkey*

Autophagy, is one of the most well known catabolic processes whose activation can degrade accumulated proteins as well as damaged organelles for maintaining cellular homeostasis. Beside this, autophagy was found to be associated with cancer. In addition, miRNAs have been implicated in several fundamental biological processes. Moreover, evidence also suggests that miRNAs play a role in cellular transformation and carcinogenesis. Thus, understanding the regulation of autophagic mechanisms through miRNAs might have tremendous importance in the field of cancer.
Overexpression of MIR376B in MCF-7 cells has been utilized and several mono clone cells picked and cultured under selection condition. For further analysis, mono clones were evaluated by their autophagic capacity via LC3 shift, p62 accumulation and MIR376B target protein status. After the characterization of clones, several growth analyses were performed either short or long term assays in vitro. On the other hand, Gamma-H2AX foci analysis and ROS measurement by DCFDA was carried out to identify the DNA damage and oxidative stress, respectively. We also evaluate the tumor growth capacity through in vivo nude mice xenograft model.
As a consequence of autophagy deregulation, accumulation of p62 was observed in MIR376B stable cells. Intriguingly, intracellular ROS level was also increased and accumulation of ROS localized around the mitochondria. In addition to susceptibility of oxidative stress, loss of autophagy makes cells more prone to DNA damage. Although in short term assays, growth attenuation of MIR376B stable cells was observed; in colony formation assay, those cells formed more and bigger colonies. In addition, we also figured out that MIR376B clones have a capacity to establish a bigger tumor in comparison to control clones in vivo.
We identified for the first time that MIR376B as a key miRNA which might has a role in tumorigenesis in breast cancer.
Keywords: MicroRNA, MIR376 Family, MIR376B, Cancer, Autophagy

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P-083 - MIR-548A-3P, MIR-548AS-3P AND MIR-8078 ARE RESPONSIBLE FOR TNF-α MEDIATED NF-KB INDUCTION OF NSCLC INVASION

Akgün Şakir, Küçüksayan Hakan, Tokgün Onur, Karagür Ege Rıza, Can Özge, Akça Hakan

Pamukkale University, School of Medicine, Medical Biology Depertmant, Denizli, Turkey

Introduction: Lung Cancer is the leading cause of cancer related deaths in the world and approximately 90% patients with lung cancer ultimately die from metastatic disease. Metastasis is the most dangerous step of cancer. In our recently published work showed that Akt/NF-kB pathway is continuously active and induces cellular invasion and PTEN suppresses cellular invasion via inhibition of Akt/NF-kB pathway. In this study we aimed to show NF-kB mediated induction of miRNA expression can responsible for inducing NSCLC invasion. Materials-Methods: We used Chromatin Immunoprecipitation (ChIP) Assay Kit for detection of TNF-α induced NF-kB mediated miRNAs. Therefore, H1299 and PC14 cells treated by TNF-α (30ng/ml) for ChIP assay. Chromatin regions, reading with ChIP-Seq, were analyzed using bioinformatics tools. We also performed additional bioinformatics search to find NF-kB related miRNAs which potentially take a role in NSCLC invasion. We investigated the effects of miRNA which determined at the bioinformatics analysis results on invasion using invasion chamber method.
Results: We found 16 miRNAs which potentially induced by NF-kB and related with NSCLC invasion. Our invasion results indicate that miR-548a-3p, miR-548as-3p, miR-8078, miR-1915, miR-6814-3p, miR-548q mimics can induce cellular invasion on H1299, miR- 548v, miR-548h-5p, miR-138-5p, miR-548a-3p, miR-548as-3p and miR-8078 mimics can induce cellular invasion on PC14. We also verified our results by qRT-PCR, because we want to sure that miRNAs which can induce invasion, can also transcriptionally regulated by NF-kB or not.
Discussion and Conclusion: We found that miR-548q, miR-548a-3p, miR-584as-3p, miR-1915 and miR-8078 in H1299, miR-138-5p, miR-548a-3p, miR-548as-3p and miR-8078 in PC14 can induce cellular invasion by NF-kB. As a conclusion, Our investigation indicate that NF-kB can induce NSCLC invasion via miR-548a-3p, miR-548as-3p and miR- 8078.
Keywords: NSCLC, microRNA, Invasion, NF-kb Pathway

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P-084 - EXPRESSION ANALYSIS OF MIR-181C, MIR-34A AND MIR-375 IN GASTRIC CANCER

Saadat Khandakar A. S. M.[1], Khailany Rozhgar A.[1], Khailany Rozhgar A.[2], Safdar Muhammad[1], Kanabe Belan O.[3], Junejo Yasmeen[4], Junejo Yasmeen[5]

[1]Department of Medical Biology and Genetics, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey
[2]Department of Biology, College of Science, University of Salahaddin, Erbil, Iraq
[3]Department of Biology, Gaziantep University, 27310 Sahinbey, Gaziantep, Turkey
[4]Department of Respiratory Medicine, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey
[5]National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan

Background: Gastric cancer (GC) has high incidence and casualty rate in different countries is still a standout amongst the most continuous and fatal diseases. MicroRNAs (miRNAs) initiate translational controlling and play a critical role in developmental timing. Presently, miRNAs have been determined to play an important roles in several pathological and physiological conditions, specially miRNA deregulation in difference types of cancer. In the current study, we aimed to exanimate the contribution of miR-181c, miR-34a and miR-375 expressions to unravel their role in gastric cancer tissue, the miRNAs to the risk of gastric cancer.
Methods: MiRNAs of 38 paired tumor and normal tissue samples that were grouped according to the types of gastric cancer and clinical characteristics of patients, including gender and average age were determined by quantitative real time polymerase chain reaction (qRT-PCR) technique.
Results: The expression level of miR-34a and miR-375 were significantly down-regulated while the expression level of miR-181c was significantly over-expressed in gastric cancer tissues according to gastric normal tissues.
Conclusion: Our results shows decreased expression in miR-34a and miR-375 might be a risk factor for gastric cancer. Further analysis is required to identify the responsible miRNAs rather than miR-181c, miR-34a and miR-375 in gastric cancer tissues.
Keywords: Gastric cancer, microRNA, Expression analysis, qRT-PCR

P-085 - EXPRESSION EVALUATION OF MIR-132-5P, MIR-184 AND MIR-34C-5P IN BREAST CANCER TISSUE

Khailany Rozhgar A.[1], Khailany Rozhgar A.[2], Safdar Muhammad[1], Saadat Khandakar A. S. M.[1], Kanabe Belan O.[3], Junejo Yasmeen[4], Junejo Yasmeen[5]

[1]Department of Medical Biology and Genetics, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey
[2]Department of Biology, College of Science, University of Salahaddin, Erbil, Iraq
[3]Department of Biology, Gaziantep University, 27310 Sahinbey, Gaziantep, Turkey
[4]Department of Respiratory Medicine, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey
[5]National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan

Background: Breast cancer is the most common malignancy among women in the worldwide, the incidence rates are highest in the Western world. non-coding RNAs that regulate the expression of target genes, microRNAs (miRNAs) belong non-coding RNA, play a significant role in the post-transcriptional regulation of gene expression by mRNA degradation or translational repression. The target of this study was to exanimate the expression level of miR-132-5p, miR-184 and miR-34c-5p in breast cancer tissues.
Methods: Expression determining of miR-132-5p, miR-184 and miR-34c-5p in 40 pairs of tumor and normal samples of breast cancer patients were performed by quantitative real time polymerase chain reaction (qRT- PCR).
Results: The expression level of miR-132-5p and miR-34c-5p in breast cancer tissue were significantly decreased (down- regulated) according to normal tissue samples. However, The expression level of miR-184 in breast cancer tissue was significantly increased according to normal tissue samples (over-expressed).
Conclusion: Decreased expression level of miR-132-5p and miR-34c-5p and increased expression level of miR-184 might be risk factors for breast cancer development, and suggesting these miRNAs expression alteration modify individual susceptibility to breast cancer. In order to understand molecular mechanism of breast cancer further analysis warranted.
Keywords: Breast cancer, microRNAs, Reverse transcription, qRT-PCR

P-086 - INVESTIGATION OF THE PROMOTER METHYLATION PROFILES OF THE MISMATCH REPAIR GENES IN LARYNX CANCER

Demokan Semra[1], Yalnız Zübeyde[1], Yılmazer Rasim[2], Çelik Mehmet[2], Süoğlu Yusufhan[2], Dalay Nejat[1]

[1]*Department of Basic Oncology, Oncology Institute, Istanbul University, Istanbul, Turkey*
[2]*Department of Otorhinolaryngology, Faculty of Medicine, Istanbul University, Istanbul, Turkey*

Background: The incidence of larynx cancer (LC) is around 2% among all cancer types. Although environmental and genetic factors play a role in the development of LC, the underlying mechanism is far from clear. Recent studies indicate that silencing of DNA repair genes by promoter hypermethylation may play a significant role in LC. The mismatch repair system (MMR) plays a crucial role in the maintenance of genomic stability. The primary function of the MMR genes is to remove mismatch base errors, insertions and deletions. The MGMT protein removes alkyl-adducts at the O6-position of guanine and prevents mutagenic effects. Cells with MMR deficiency may accumulate mutations and can progress to cancer. In this study we investigated methylation levels of six MMR genes (MLH1, MSH2, MSHS6, MSH3, MLH3, PMS2) and the MGMT gene in LC patients.
Material and Methods: The promoter methylation status of the MMR genes were analyzed by using Methylation-Specific Multiplex Ligation Dependent Probe Amplification (MS-MLPA) in the DNA obtained from tumor tissues from 73 LC patients. PCR products were analysed in a ABI 310 genetic analyzer. The peak areas of the signals were normalized by dividing to the areas of reference probes. A difference higher than 20% was considered positive.
Results: In 37 (50.6%) patients, more than one gene was methylated while only one metyhlated gene was observed in 24 (64.8%) patients. Methylation of two genes was shown in 6 (16.2%) patients. MGMT was the most frequently methylated gene and its methylation rate was 45.2% (33/73). There were statistically significant associations between stage (p=0,02), metastasis (p=0,0006) and the presence of methylation.
Conclusions: Our results indicate that methylation of the repair genes is a frequent event in LC and may play a role in the development of the diesase.
Keywords: Methylation, Epigenetics, Larynx Cancer, Mismatch Repair Genes, MGMT

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P-087 - A COMPARATIVE STUDY OF MCF-7S AND MDA-MB-435S FOR RELATIVE DATA NORMALIZATION TO ENDOGENOUS REFERENCE GENES

Bozkurt Şerife Buket, Öztürk Bahadır, Ünlü Ali

University of Selcuk, Konya

Purpose: microRNAs (miRNAs) play essential roles both physiological conditions and human malignancies. Additionally, obtain from miRNAs produced by cells shows their normal function state and these molecules have been examined as pathological biomarkers. A lot of methodologies have been adjusted to characterize quantitatively the expression patterns of miRNAs, one of them quantitative real-time PCR (qRT-PCR). In qRT-PCR evaluation of gene expression, normalization of data against RNA variant by using appropiate references gene is essential. The present study aimed to investigate the alterations in breast cancer cells MCF-7s and MDA-MB-435s of endogenous reference genes from 2 to 48 hours.
Methods: In order to determine the quantitative expression profiles of 84 miRNAs and 6 candidate housekeeping genes (SNORD68, SNORD72, SNORD95, SNORD96A, SNORD61, RNU6-2) in MCF and MDA-MB-435 cell lines were investigated by Fluidigm Microfluidic Dynamic Array. The software program NormFinder was used for selection of candidate housekeeping genes. Total RNAs including miRNAs were isolated from breast cancer cells at 2nd, 4th, 6th, 12th, 24th and 48th hours. Determinations of relative gene expression values were carried out by using the 2-ΔΔCt method (normalized threshold cycle (Ct) value of sample minus normalized Ct value of control).
Result: Our results demonstrated that statistically differences 6 housekeeping genes were detected in MCF-7 cells and MDA-MB-435 cells groups at different time period (4th, 6th, 12th, 24th, 48th hours) comparing with control groups (2nd hour). We found fluctuations in other housekeeping genes except for SNORD61. Thus we showed that, notably, SNORD61 is suitable and can be use for MCF-7 and MDA-MB-435 miRNA's normalization (foldchange<2, $p<0.05$).
Conclusion: This study provides for the time-dependent a comprehensive list of suitable housekeeping genes for experimental conditions of breast cancer cells. Thus, our result promote miRNA-based studies on MCF-7 and MDA-MB-435 cells.
Keywords: microRNA, Breast Cancer, Reference Gene, RT-PCR

P-088 - TIME-DEPENDENT EFFECT OF VITAMIN-D ON EXPRESSION OF MIR-548C-3P IN BREAST CANCER

Mercan Aslı[1], Bozkurt Şerife Buket[1], Öztürk Bahadır[1], Korucu Nedime[2], Ünlü Ali[1]

[1]*Selcuk Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya*
[2]*Necmettin Üniversitesi, Fen Fakültesi*

Purpose: Vitamin D is an important regulator via cellular differentiation and proliferation in a lot of cancer including breast cancer. Enormous evidence that microRNAs (miRNAs), which endogenous small RNAs, are known essential player in carcinogenesis. In this report, we overview detailed information on the vitamin D effect on level of miR-548c-3p from 2 to 6 hours in MCF-7 breast cancer cell.
Methods: Total RNA including miRNAs were isolated from 140 µM D vitamin treated (from 2 to 6hours) with MCF-7s and the expression of miR-548c-3p was examined by high-throughput real-time quantitative polymerase chain reaction (qPCR). All statistical analyses were performed using the Biogazelle's qbase PLUS 2.0 software.
Result: We observed significantly up-regulation (nearly sixty-fold regulation higher) of miR-548c-3p both 4th hour and 6th hour in 140 µM D vitamin treated MCF-7 cell compared with D vitamin non-treated MCF-7 cells (control group) ($p<0.05$). Besides miR-548c-3p target genes were related with pathways such as ErbB signaling pathway (hsa04012), Pathways in cancer (hsa05200), PI3 kinase/AKT (hsa04151).
Conclusion: miR-548c-3p may act as a tumor supressor-miRNA in breast cancer. Additionally, vitamin D increased expression of miR-548c-3p in MCF-7 cell. Thus, these data support that vitamin D may be an effective agent for breast cancer therapy.
Keywords: microRNA, vitamin D, MCF-7, Realtime-PCR

P-089 - MIR193B INHIBITS ERK ACTIVATION IN PANCREATIC DUCTAL ADENOCARCINOMA CELLS

Gurbuz Nilgun, Kosar Pınar Aslan

Department of Medical Biology, School of Medicine, Suleyman Demirel University, Isparta, Turkey

miRNAs have been known to play key roles in various cellular mechanisms, including cell proliferation, both in nontumorigenic and tumorigenic cells. Many miRNAs were shown to be involved in the progression of pancreatic ductal adenocarcinoma (PDAC) cells. Considering the strong regulatory effects, miRNAs might be used as a novel therapeutic approach for the prevention of PDAC, which has the highest mortality rate of all major cancers. For this purpose, we aimed to investigate the potential role of miR193b in cellular proliferation of PDAC cells, Panc-1 and MiaPaCa-2. To be able to evaluate the change in cell proliferation, ERK activation was detected both in cell lines transfected with miR193b or control miRNA. We clearly obtained that miR193b inhibits ERK activation compared to total ERK protein expression in Panc-1 and MiaPaCa-2 cells. When the inhibitory effect of miR193b on the ERK activation was compared with gemcitabine, which is currently used as a clinical drug for pancreatic cancer patients, we determined that miR193b was much more effective than gemcitabine. Having tumor suppressive properties, miR193b might be served as a novel gene therapeutic approach for pancreatic cancer patients and further in vivo studies are needed.

Keywords: miRNA, miR193b, Cell proliferation, MAPK, Pancreatic cancer

P-090 - DIFFERENTIAL EXPRESSION LEVELS OF DICER1 ANTISENSE RNA 1 (DICER1-AS1) AND DICER1 IN DIFFERENT CANCER CELLS

Bozgeyik Esra[1], Igci Mehri[1], Bozgeyik Ibrahim[1], Yumrutas Onder[2], Arslan Ahmet[1]

[1]*University of Gaziantep, Faculty of Medicine, Department of Medical Biology and Genetics, Gaziantep, Turkey*
[2]*Adiyaman University, Faculty of Medicine, Department of Medical Biology, Adiyaman, Turkey*

Introduction and Aim: As we all know, miRNAs are the most abundant member of the small RNAs that suppress gene expression at the post-transcriptional level. DICER1 is a type III ribonuclease involved microRNA biogenesis by producing active miRNAs. Additionally, long non-coding (Lnc) RNAs are a newer class of regulatory RNA molecules with a size longer than 200 nucleotides. A mass of indication suggest that Lnc RNAs are involved in the variety of cellular processes such as regulation of gene expression. Accordingly, the aim of the present study was to investigate interrelation between DICER1 and DICER1-AS1, a tail-to-tail natural antisense transcript.
Materials and Methods: HUVEC, and HEL299 normal cells and, MCF-7, DU-145, HeLa, and A549 cancer cells were included in the present study. For the analysis of gene expression levels RT-PCR method was used. Results were analyzed via Image J software.
Results: As a result, expression levels of DICER1 were found to be elevated in all cell lines as compared to DICER1-AS1 levels. In addition, DICER1 expression was found to be not much altered among cancer cell lines. Furthermore, DICER1-AS1 was shown to be differentially expressed in cancer cells. While its expression was up-regulated in MCF-7 and A549 cells, it was reduced in HeLa cervix cancer cells and not altered in DU-145 cells.
Discussion and Conclusions: DICER1-AS1 seems to have important regulatory function in cancers and further studies more functional studies are needed to understand its comprehensive regulatory role. Also, it is of great interest to determine the interrelation between DICER1 and DICER1-AS1.
Keywords: Cancer, DICER1, DICER1-AS1, NAT

P-091 - GENE EXPRESSION PATTERN OF MTUS1, A NEWLY IDENTIFIED TUMOR SUPPRESSOR, IN VARIOUS TYPES OF CANCERS

Bozgeyik Ibrahim[1], Igci Mehri[2], Yumrutas Onder[1]

[1]*Adiyaman University, Faculty of Medicine, Department of Medical Biology, Adiyaman, Turkey*
[2]*University of Gaziantep, Faculty of Medicine, Department of Medical Biology and Genetics, Gaziantep, Turkey*

Introduction and Aim: MTUS1 (Microtubule Associated Tumor Suppressor 1 gene or Mitochondrial Tumor Suppressor Gene 1) is a newly identified tumor suppressor gene located at chromosome 8p21.3-22. This tumor suppressor gene is known to encode five different protein isoforms (known as ATIPs; ATIP1, ATIP2, ATIP3a, ATIP3b and ATIP4) as a result of alternative exon usage. Furthermore, accumulating evidence suggest that MTUS1 is downregulated at various types of malignancies including prostate, bladder, liver, colon, head and neck, ovarian, and breast cancers. Thus, the aim of the present study was to evaluate differential expression of MTUS1 gene in various cancer and normal cell lines.
Materials and Methods: HUVEC, BEAS2B, and hfOB1.19 normal cells and U2OS, A549, DU-145, PC3, HGC27, BCPAP, and PANC1 cancer cells were included in the study. For the expression analysis of MTUS1 gene, Real-Time PCR method was used.
Results: As a result, expression level of MTUS1 gene were found to be highly reduced in BCPAP, PANC1, HGC27 and U2OS cancer cells and lost in DU-145 cancer cells. Surprisingly, MTUS1 expression was also found to be lost in HUVEC and hfOB1.19 normal cells. In contrast, expression levels of MTUS1 were found to be highly elevated in A549 and PC3 cancer cells. MTUS1 levels were also found to be reduced in BEAS2B bronchial epithelial cells compared to A549 cells.
Discussion and Conclusions: MTUS1 gene seems to be an important tumor suppressor regulating vital cellular process. In the present work, total MTUS1 expression levels were demonstrated. MTUS1 gene may have other cellular functions in different cancers. In the future studies, studying the role of MTUS1 gene variants can more informative in understanding its various functions.
Keywords: Cancer, MTUS1, Tumor Suppressor

P-092 - ACTIVATION OF IMMUNE RESPONSES TO COLORECTAL CANCER BY USING A COMBINATION OF RADIATION AND PROTEASOME INHIBITOR

Aydin Ali, Cacan Ercan

Department of Molecular Biology, Science and Art Faculty, Gaziosmanpasa University, Tokat, Turkey

Colorectal cancer (CRC) is the third most common cancer worldwide and the 5-year survival rate is lower than 60%. As CRC progress, tumor cells become less susceptible to the induction of apoptosis. Radiotherapy has been extensively used for cancer therapies including colorectal cancer. Sub-lethal doses of radiation can modulate gene expression, making tumor cells more susceptible to T-cell-mediated immune attack. However, radiation treatment alone isn't sufficient to generate strong immune responses. Inhibition of the ubiquitin-proteasome system causes inhibition of cell proliferation in all cells, including cancer cells. As inhibition of the ubiquitin-proteasome system is a promising strategy of cancer therapy, a combination treatment of proteasome inhibition and irradiation may further induce activation of tumor-specific immune responses. The goal of this study is to investigate the effects of the 26S proteasome inhibitor, bortezomib, alone or in combination with radiotherapy, on the expression of immunogenic genes in colorectal carcinoma cells. We examined two colorectal carcinoma cell lines (HCT116 and SW620) for changes in expression of multiple co-stimulatory molecules (OX40L and 41BBL) and death receptors (DR4, DR5 and CD95). Our results indicate a combination of 26S proteasome inhibition and sub-lethal radiation significantly increases the sensitivity of carcinoma cells to apoptosis. Combination treatment upregulates cell surface expression of death receptors and co-stimulatory molecules by increasing transcriptional activation of each gene. Thus, the combination treatment enhanced sensitivity to killing through FAS and TRAIL receptors by CD8+ T cells. Our studies show for the first time that combining radiotherapy and proteasome inhibition may simultaneously enhance tumor immunogenicity and the induction of antitumor immunity by enhancing tumor-specific T-cell survival and activation.

Keywords: Colorectal cancer, Bortezomib, HCT116, SW620

P-093 - ENHANCING SENSITIVITY OF BREAST CANCER CELLS TO ANTI-TUMOR IMMUNE RESPONSES BY RADIATION AND 26S PROTEASOME INHIBITION

Aydin Ali, Cacan Ercan

Department of Molecular Biology, Science and Art Faculty, Gaziosmanpasa University, Tokat, Turkey

Irradiation leads to DNA damage by inducing cellular stress responses, which result in activation of apoptotic pathways. Radiation treatment is an effective breast tumor therapy, however, its usage is limited by dose and toxicity. Sub-lethal doses of radiation can modulate tumor gene expression, making tumor cells more susceptible to immune responses, but radiotherapy alone is insufficient to generate strong tumor-specific immune responses. The proteasome presents a novel target for combination therapies in cancer: it plays a key role in cancer cell proliferation, inhibition of radiation-induced apoptosis and development of drug resistance. The objective of our study is to investigate the effects of the 26S proteasome inhibitor, Bortezomib, alone or in combination with radiotherapy, on the expression of immunogenic genes in breast cancer cells. Here, we examined MDA231 breast cancer cells for changes in expression of multiple immuno-stimulatory molecules and death receptors. Our preliminary data indicates a combination of bortezomib and low-dose radiation significantly increases the sensitivity of MDA231 cells to apoptosis. Furthermore, this novel combination treatment upregulates cell surface expression of multiple death receptors (TNFRSF10A, TNFRSF10B and Fas/APO-1) and co-stimulatory molecules (TNFSF4 and TNFRSF9) by increasing their transcriptional activation. These data will guide experiments to determine how this combination therapy can best enhance anti-tumor immune responses.

Keywords: Bortezomib, MDA231, 26S Proteasome

P-094 - COMPARISON OF HEMATOLOGICAL PARAMETERS IN PATIENTS WITH ENDOMETRIAL CANCER

Vatansev Hakan[1], Akyürek Fikret[2], Öztürk Bahadır[2], Vatansev Hüsamettin[2]

[1]*Necmettin Erbakan University Seydişehir Vocational School, Bakery Products and Technology, Konya, Turkey*
[2]*Selcuk University Faculty of Medicine, Department of Biochemistry, Konya, Turkey*

Introduction and Aim: Endometrial cancer is the most common gynecologic cancer after breast, lung and colon cancer especially in the elderly and postmenopausal women with the disease. The aim of our study was to compare levels of neutrophil, lymphocyte, mean platelet volume (MPV) and neutrophil/lymphocyte ratio (NLR) in patients with endometrial cancer and healthy control groups.
Materials and Method: Full blood samples were collected from 38 healthy control and 199 patients with endometrial cancer. The mean age for controls and patients were 63,15 ± 10,68 and 61,70 ± 8,56 respectively. Neutrophil, lymphocyte, MPV levels and NLR measured with Abbott Cell-Dyn 3700 Hematology Analyzer. Statistical analysis was performed with IBM SPSS 20 by using Mann-Whitney U and Wilcoxon T test.
Results: Neutrophil, lymphocyte, MPV levels and NLR parameters showed statistically significant difference between patients and healthy control groups ($p<0,01$).
Discussion and Conclusion: In our study, the healthy and endometrial cancer groups were compared with hematological parameters, it was observed that neutrophil levels and NLR as peripheral markers of systemic inflammatory response were decreased, lymphocyte and MPV levels were increased. As it has been shown in some studies that when NLRs decreasing MPV levels increase in advanced stage endometrial malignancies and can be used as a prognostic factor. Significant increase of MPV values which observed in our study support these studies.
Keywords: Endometrium, Mean Platelet Volume, Neutrophil/Lymphocyte Ratio, Cancer

P-095 - MPV VALUES IN PATIENTS WITH PROSTATE CARCINOMA

Akyürek Fikret[1], Vatansev Hakan[2], Yağcılar Hafize[1], Vatansev Hüsamettin[1], Öztürk Bahadır[1]

[1]*Selcuk University Faculty of Medicine, Department of Biochemistry, Konya, Turkey*
[2]*Necmettin Erbakan University Seydişehir Vocational School, Bakery Products and Technology, Konya, Turkey*

Introduction and Aim: In this study, our aim was to compare the level of Mean Platelet Volume (MPV) levels in Prostate Carcinoma patients and healthy control groups.
Materials and Method: Serum samples were collected from 36 healthy control and 23 patients with prostat carsinoma. The mean age for controls and patients were 70,47±7,60 and 69,43±9,26 respectively. MPV levels measured with Abbott Cell-Dyn 3700 Hematology Analyzer. Statistical analysis was performed with SPSS v21 by using independent sample t test.
Results: The MPV values in patients with prostate carcinoma (7,702±1,40) were higher from control group (6,97±0,83]) but this was not statically relevant (p=0,85).
Discussion and Conclusion: Although MPV is a inflammation marker, our analyses showed that MPV cannot be used for a inflammatory marker in patients with prostate carcinoma.
Keywords: Prostat, Mean Platelet Volume, Cancer

P-096 - PEPERS DNA PROTECTIVE ACTIVITY PROPERTIES

Karataş İbrahim

Gaziantep Üniversitesi Fen Edebiyat Fakültesi,Moleküler Biyoloji ve Genetik Ana Bilim Dalı,Gaziantep

Peppers, vegetables of the Solanaceae family, is a species in the genus Capsicum. Pepper and pepper products have strong antioxidant capacity. The origin of chili with antioxidants, especially to determine the mechanism of UV protection is extremely important. In this study, the Latin name Capsicum Annuum L. pepper is grown in Gaziantep. Type of color cayenne pepper green and red chili peppers and red sweet pepper varieties are dried in the shade. These three kinds of conditions suitable optimization pepper hexane, dichloromethane and methanol and water extracts were obtained. Different concentrations of each peppers were exposed to H2O2 and UV-C in four different solvents. Then the effect on DNA damage were investigated using plasmid pBR322 DNA. Compatible with the controls of the pepper extract DNA bands were displayed. When the results were analyzed, all examples of red sweet pepper, red chili pepper and green chillies have demonstrated a protective effect against DNA, Hydrogen peroxide and UV-C. While water extract of red pepper 10 mg concentration showed a protective effect, 40 mg concentration showed a protective effect in other extracts. But, all of the green pepper extract concentration of 10 mg was observed that the protectiveness effect. Compared with other types of green peppers DNA hydrogen peroxide and UV-C said to be more protective against the rich potential of the compound.

Keywords: Capsicum Annuum L., DNA, H2O2, pBR322, UV-C

P-097 - TRPV1 EXPRESSION DECREASES IN SPLEENS OF MICE BEARING METASTATIC BREAST CARCINOMA

Tanrıöver Gamze[1], Dilmaç Sayra[1], Erin Nuray[2]

[1]*Department of Histology and Embryology, School of Medicine, Akdeniz University, Antalya, Turkey*
[2]*Department of Medical Pharmacology, School of Medicine, Akdeniz University, Antalya, Turkey*

Introduction and Aim: Breast cancer is the leading cause of cancer-related deaths among women. Tumor induced inflammatory response is believed to be involved in metastasis of breast cancer. Transient Receptor Potential (TRP) channel 1 (TRPV1) is activated by warm temperatures and capsaicin and mainly expressed in sensory nerve endings. Recently it was also shown that TRPV1 is expressed in immune cells and might be involved in anticancer immunity.
Material and Method: We previously isolated liver (4TLM), heart (4THM) metastatic cells of 4T1 (metastatic breast tumor cell line) cells. 4TLM and 4THM cells (100.000 cell/mouse) were inoculated into the right upper mammary pad of 8-10 weeks old female Balb-c mice. Necropsies were performed 25-27 days after injection. TRPV1 immunoreactivities were examined in primary tumors and spleens by using immunohistochemistry. Tissues from control animals (no-tumor) were also used.
Results: We observed that TRPV1 expression was mostly confined to red-pulp and approximately 20% of monocytic cells were strongly positive and stained both cytoplasmic and membranous. Staining pattern was markedly altered in spleens of animals injected with 4TLM cells such that diffuse staining all though the red-pulp was observed. Strongly positive cells found in control spleens were lacking in spleens of 4TLM injected mice. TRPV1 positive cells were significantly decreased in spleens of 4THM injected mice. Occasional TRPV1+ immune cells were also observed in primary tumors.
Discussion: These results demonstrated that for the first time that TRPV1+ cells decreases in spleens of highly metastatic breast carcinoma cells. Further studies are required to understand significance of these findings in metastasis.
Keywords: TRPV1, metastatic breast cancer, immunohistochemistry

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P-098 - PROTEOMİC ANALYSİS OF NEURODEGENERATİVE FLY MODEL TBPH -/- AND ALS MODEL

Kalkan Hilal[1], Klima Raffaella[2], Feiguin Fabian[2]

[1]*Moleküler Biyoloji ve Genetik/İstanbul Üniversitesi, İstanbul/Turkey*
[2]*Neurobiology Laboratory İCGEB, Trieste/Italya*

Neurodegenerative diseases are group of pathologies characterized by the progressive loss of structure or functions of neurons. Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressive and fatal neurodegenerative disease affecting all ethnic groups. Both sporadic and familial cases of the disorder have been reported, where sporadic ALS (SALS) amounts to 90-95% of cases Familial ALS (FALS) accounts for 5-10% of the total ALS cases. The pathogenic mechanism of the ALS is not yet clear, this pathology could result from complex interaction between various cellular mechanisms, including genetic and environmental factors, alteration in RNA metabolism, oxidative stress, etc. Recent studies have been shown that several genes were mutated in AML. The first one reported was SOD1. Recently, TAR DNA-binding protein-43 (TDP-43) emerged as a key protein involved in the pathogenesis of ALS. TDP-43 is a highly conserved and ubiquitously expressed nuclear protein reported to be involved in pre-mRNA splicing, transcription, mRNA stability and mRNA transport. Recent studies documented role of TDP-43 as the main protein component of the intracellular inclusions observed in Amyotrophic Lateral Sclerosis (ALS) patient brain.

In the present study, we investigated the role of TDP-43 analogue in the model organism Drosophila melanogaster. We have used different transgenic flies expressing TDP-43 analogue TBPH and checked whether the expression of TBPH is causing the neurodegeneration as compared to the control groups. Expression of TPBH caused development of black spot (necrosis) in the eyes of the flies clearly suggested neurodegeneration in the eye. Moreover, 2D gel analysis on TBPH mutated flies showed reduced fat body proteins 1 (FBP1) levels in the eyes of mutated flies as compared to wild type, suggesting the role of TBPH in the protein aggregation. In conclusion our date showed TBPH is involved in the neurodegenerative process by enhancing the production of FBP1.

Keywords: 1.Neurodegenerative Disease, 2.Amyotrophic Lateral Sclerosis, 3.TDP-43

P-099 - SELENIUM ATTENUATES OXALIPLATIN-INDUCED OXIDATIVE STRESS, APOPTOSIS AND TRPV1 CHANNEL ACTIVITY IN MCF-7 BREAST CANCER CELLS

Çiğ Bilal[1], Nazıroğlu Mustafa[1,2]

[1]*Department of Biophysics, Medical Faculty, Suleyman Demirel University, Isparta, Turkey*
[2]*Neuroscience Research Center, Medical Faculty, Suleyman Demirel University, Isparta, Turkey*

Breast cancer is resulting in death is a serious global problem that especially a significant increase among women in recent years. Calcium ion accumulation into the cancer cells plays an important role in the survival and cell death. Accumulating evidences indicated that increase of intracellular free calcium concentration is the negative effect on the proliferation and metastasis of cancer cells. Transient receptor potential vanilloid1 (TRPV1) cation channels are non-selective calcium channels. In vivo studies in animals have shown that TRPV1 cation channel overstimulation of the sensory neurons selectively destroys the sensitivity of capsaicin. In a recent study, we observed modulator role of selenium (Se) on TRPV1 channel and oxidative stress in cancer cell and the similar effect of selenium may occur on oxaliplatin-induced oxidative stress and TRPV1 channel activity in MCF-7 breast cancer cells. In this study, aim of the study was investigate protective effect of selenium on TRPV1-mediated oxidative stress caused by an important chemotherapeutic oxaliplatin.
Keywords: Apoptosis, Oxaliplatin, Oxidative stress, TRPV1, Selenium

P-100 - EFFECT OF MELATHONIN ON PON LEVEL IN RATS GENERATED SEPSIS MODEL

Akbulut Hatice Feyza[1], Vatansev Hakan[2], Vatansev Hüsamettin[3], Sekmenli Tamer[4]

[1]*Süleyman Demirel University, Atabey Vocational High School, Isparta,Turkey*
[2]*Necmettin Erbakan University Seydişehir Vocational School, Bakery Products and Technology, Konya, Turkey*
[3]*Selcuk University, Faculty of Medicine, Department of Medical Biochemistry, Konya,Turkey*
[4]*Selcuk University, Faculty of Medicine, Department of Pediatric Surgery, Konya,Turkey*

Aim: There are two different methods that were used to create melatonin deprivation experimentally in rats. The exposure of rats to light for 24 h continuously, or the removal of the pineal gland operation.These methods cause to be done many researches related to biological rhythms as well as having the melatonin secretion destroying property of pineal gland.These methods also have to destroy property as well as the melatonin secretion of the pineal gland lot of research on biological rhythms are reasons to be made.The aim of this study was to determine the effect on the PON of change in the level of melatonin and to investigate therapeutic effect of melatonin.
Materials and Methods: In this study, 54 piece 4-5 month old adult male rats were used. First-third groups were hosted without food and water restriction under 12 h light-12 h dark at 21-22 °C. The rats in second group were hosted without food and water restriction under 24 h lights at 21-22 °C. All rats were hosted in this way for 10 days.Sepsis model was created by applying the cecum mount-drilling method(CLP). Rats in Group 2 to ensure the lack of melatonin were hosted under lights daily for 24 hours throughout hosted days. The level of PON in blood samples of rats was detected.
Findings: The levels of PON in bloods which were taken from two groups with and without sepsis after 24 hours were compared. The level of PON in group which was uncreated melatonin deprivation was found lower than that of created melatonin deprivation.
Results: The results obtained from this study suggest that low PON values in rats applied to pinealectomy can be due to the effect on PON values of melatonin. This case reveals that the level of melatonin may be the effect on the immune system.
Keywords: Pinealectomy, PON, Sepsis

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