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Expression levels and clinical significances of hsa-miR-29 family and their target genes in the bone marrow of patients with multiple myeloma

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The microRNA (miR)-29 family has been deregulated in several types of hematologic malignancies. However, role of this family and their target genes DNMT3A (DNA methyltransferase 3A) and TET2 (Ten-Eleven Translocation 2) remains unclear. Here, we have made an attempt to determine the relative expression levels of three miRNAs and target genes in patients with newly diagnosed Multiple myeloma (MM) using quantitative real-time PCR. Moreover, the expression levels of selected miRNAs and genes and their correlations with clinical parameters were compared and analyzed. The ROC curve was used to analyze their diagnostic efficacy for MM. The expression level of hsa-miR-29b-3p was significantly higher in patients with newly diagnosed MM compared with the control group. ROC analysis showed that hsa-miR-29b-3p demonstrated a moderate diagnostic power in MM. The relative expression level of hsa-miR-29b-3p in patients with high LDH levels was markedly reduced compared to that in patients with normal and low LDH levels. *DNMT3A* expression level was significantly increased in patients with high LDH levels and patients with lambda light chain. Our results indicate that hsa-miR-29b-3p may be used as a potential biomarker in the diagnosis of MM.

Keywords: Blood cancer, DNA methylation, Hematological cancer, Kahler's disease

Cancer is a serious global public health problem and is the second largest cause of mortality in the United States[1]. Multiple myeloma (MM), also known as Kahler's disease, is an incurable hematopoietic (blood) cancer characterized by uncontrolled proliferation of neoplastic plasma cells[2]. It is associated with high mortality among hematological cancers[3]. Despite significant improvements in the treatment of people with multiple myeloma (MM), the majority of patients eventually relapse and cannot be cured[4,5]**.** Therefore, it is urgent to discover new prognostic biomarkers and therapeutic strategies for MM patients.

The miRNAs, small noncoding RNAs with approximately 19-25 nucleotides length, are responsible for the regulation of multiple genes by inhibiting the expression of target genes[6]. Recent studies have gradually revealed the role of miRNAs and their target genes in MM pathogenesis, offering possible new approaches for treatment interventions for MM[7-9]. Few miRNAs have high diagnostic and prognostic accuracy and can be considered as promising biomarkers in MM[10,11]. The hsa-miR-29 family, consisting of hsa-miR-29a, miR-29b and miR-29c, regulates DNA methylation and controls a number of cellular functions such as differentiation, apoptosis, and metastatic properties[12.13]. This family has been reported as a tumor suppressor, controlling numerous oncogenic pathways in various cancers including haematological malignancies[14].

DNMT3A is involved in the transfer of a methyl group to the fifth carbon of a cytosine residue and is localized to chromosome 2p23[15]. Suppression of the *DNMT3A* gene leads to increased self-renewal of hematopoietic stem cells. It is required for the differentiation of hematopoietic cells and plasma cells[16]. The TET2 gene, a component of the TET enzyme family, is located on chromosome 4q24. Its protein product, TET2, regulates DNA hydroxymethylation by converting 5-methylcytosine (5 mC) to 5-hydroxymethylcytosine (5 hmC) to induce DNA demethylation[17]. Decreased TET2 expression leads to an increase in the number of hematopoietic stem cells (HSCs) and enhances their self-renewal ability[18]. *DNMT3A* has been experimentally validated as a direct miR-29s target in many cell types but the molecular regulation of TET2 is poorly understood[19]. The miR-29s-TET2 pathway plays an important role in various cancers such as prostate and colorectal cancer but its role in MM is

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still unclear[20,21]. Hence, in this study, we sought to identify the clinical significance of has-miR-29s and two target genes in MM and to analyze whether TET2 is also a putative target of miR-29s and contributes to the molecular pathogenesis of MM alone or together with *DNMT3A*.

Materials and Methods

A total of 48 bone marrow samples were collected from newly diagnosed MM patients without any treatment in the Department of Haematology-Oncology, Mersin University Faculty of Medicine Hospital between the period from 2019 to 2020. Inclusion criteria were: (i) the diagnosis of primary MM patients was performed according to the 2016 International Myeloma Working Group guidelines[22]; (ii) patients' age ≥ 18 years; (iii) patients without any treatment before bone marrow sample collection; and (iv) patients who signed informed consents. Exclusion criteria: (i) the age of patients <18; (ii) patients treated before bone marrow collection; and (iii) patients suffered from other hematologic diseases. A total of 11 healthy individuals with no family history of hematologic disease served as the control group and bone marrow samples from this group were collected from the sternum during cardiac surgery by the Cardiology Department, Mersin University Faculty of Medicine Hospital. Mersin University Health Sciences Ethics Committee approved the present study with the decision numbered 2022/607, dated 31/08/2022. Clinical parameters of the MM patients and healthy controls are described in Table 1.

RNA extraction

Up to 5 mL BM sample was extracted from each participant. The total RNA from all bone marrow samples was extracted using TRIzol reagent (Invitrogen). The room temperature for RNA isolation was 22°C. Following RNA extraction, the purity of all RNA samples was measured using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). All samples showed high purity and integrity (OD 260/280 nm ratio >1.8).

Reverse transcriptase PCR reactions (RT-PCR)

RT-PCR conditions for hsa-miR-29a, miR-29b and miR-29c with a final reaction volume of 15 µL included 5 µL of extracted total RNA, 50 nM stem–loop RT primer, 1× RT buffer, 0.25 mM each of dNTPs, 50 units of modified M-MuLV Reverse Transcriptase (Thermo Scientific, Vilnius, Lithuania), 25 units of RiboLock RNase inhibitor (Thermo Scientific, Vilnius, Lithuania) and nuclease-free water. The reaction was carried out on an automated Thermal Cycler (Thermo Scientific, Vilnius, Lithuania) RT PCR conditions included 30 min at 16°C, 30 min at 42°C, 5 min at 85°C and then held at 4°C. RT-PCR conditions for DNMT3A and TET2 genes contained 2 µg/µL of extracted total RNA, 200 U/µL Revertaid Reverse Transcriptase (Thermo Scientific, Vilnius, Lithuania), 5 × RT buffer, poly-T

Table 1 — Basic characteristics of the study subjects

	Patients (n)	Healthy individuals (n)
Total	48	11
Male	29	7
Female	19	4
At age diagnosis (years)		
≤65	25	ND
>65	23	ND
ISS-R Stage		
I	6	ND
II	35	ND
III	7	ND
Durie-Salmon stage		
I	17	ND
II	20	ND
III	11	ND
β2-MG, mg/L		
<3.5	18	ND
3.5-5.5	20	ND
>5.5	10	ND
ALB, g/dL		
Normal	24	ND
Low	24	ND
LDH, U/L		
Normal	19	ND
High	18	ND
Low	11	ND
CRP, mg/L		
≤5	22	ND
>5	26	ND
Calcium, mg/L		
Normal	36	ND
High	3	ND
Low	9	ND
Creatinine, mg/L		
≤0.9	21	ND
>0.9	27	ND
Hemoglobin, g/dL		
Normal	24	ND
Low	24	ND
Heavy-chain type		
IgG	38	ND
IgA	10	ND
Light-chain type		
kappa	33	ND
lambda	15	ND

[LDH, lactate dehydrogenase; ISS-R, Revised international staging system; Ig, immunoglobulin; β2-MG, Beta2 microglobulin; CRP, C-reactive protein; ALB, albumin; ND, not determined]

primer, 2 mM each of dNTPs, 40 U/μL of RiboLock RNase inhibitor (Thermo Scientific, Vilnius, Lithuania) and nuclease-free water to a final reaction volume of 50 μL. RT-PCR conditions included 60 min at 37 °C, 5 min at 95°C and then held at 4°C. Obtained cDNAs were stored at −20°C.

Quantitative Real-time PCR (qPCR) Analysis

To determine the expression levels of DNMT3A, TET2, and miRNAs, qPCR analysis was performed with an ABI Prism 7500 Real-Time PCR (Applied Biosystems) using the TaqMan probe system. For miRNAs, the reaction tube contained 5 μL cDNA template, 12.5 μL 2 X Master Mix (Solis Biodyne, Tartu, Estonia), 1.5 μL universal primer, 1.5 μL forward primer, 0.6 μL of probe, and 5 μL nuclease-free water. For DNMT3A and TET2 genes, the reaction mixture contained 12.5 μL TaqMan Gene Expression Master Mix (Applied Biosystems), 5 μL cDNA template, primers (2.5 μL each), probes (0.6 μL each), and 5 μL nuclease-free water. The final volume of each tube was 25 μL for both genes and miRNAs. Reaction conditions were reached after preincubation at 50°C for 2 min and denaturation at 95°C for 10 min, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. The relative expression levels of miRNAs were normalized by using miR-26b-5p as an endogenous control, and β-actin was used as an endogenous control to validate DNMT3A and TET2 gene expressions. Responses for the genes under study and endogenous genes were read in triplicate. Primer and probe sequences specific for miRNAs and their target genes (Metabion International AG, Martinsried, Germany), shown in Tables 2 and 3, were designed using Primer Express 3.0 software (Applied Biosystems, Waltham, MA, USA). In the probe design for miRNAs, the cytosine analog 5-(1-propynyl)-dC (pdC) was added instead of the cytosine nucleotide to maintain target specificity to increase the melting temperature by 2.8°C per substitution. In addition, Zip Nucleic Acids (ZNA) probes, the effective alternative to Minor Groove Binder (MGB) and Locked Nucleic Acid (LNA)-containing oligonucleotides, were used to obtain target specificity[23]. The $2^{-\Delta\Delta Ct}$ method was used to estimate the relative changes in expression levels of selected miRNAs and their target genes.

Statistical analysis

The Shapiro-Wilk test was performed to test the suitability of the normal distribution of the numerical data. Descriptive analyses were presented using median (25th percentile-75th percentile) or mean±standard deviation (sd) based on distribution normally or not. Comparison of independent two groups were made using the Independent samples t-test or Mann-Whitney U test for continuous endpoints and the Chi-Square test for categorical endpoints. For comparison of three groups, ANOVA or Kruskal Wallis (KW) tests were used according to the distribution assumption. Bonferroni (for ANOVA)

Table 3 — Primer-probe sequences of taget genes used in RT-PCR

Genes	Gene ID	Primer-probe sequences
DNMT3A	1788	F-5′-GCCGCTGAGCTCGTTTTG-3′ R-5′-GTAGATGGCTTTGCGGTACATG-3′ PR-FAM-GCGTTCCACCAGGCCACGTACA-BHQ-1-3′
TET2	54790	F-5′-GAGCCCACTTACCTGCGTTTC-3′ R-5′-CGAGTGAAGGCATATGGAGATG-3′ PR- FAM- 5′-CTCTTG CCGAAAGGACCATGTC CGT-BHQ-1-3′
ACTB (β-actin)	60	F 5′-GGCACCCAGCACAATGAAG-3′ R 5′-GCCGATCCACACGGAGTACT-3′ PR 5′-Yakima Yellow-TCAAGATCATTGCTCCT CCTGAGCGC-BHQ-1-3′

[BHQ, Black Hole Quencher; F, forward; PR, probe; R, reverse]

Table 2 — Primer-probe sequences of miRNAs used in Real-time PCR

miRNAs	miRNA ID	Primer-probe sequences
hsa-miR-26b-5p	407017	RT- 5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACACCTAT-3′ F-5′-GCCGCTTCAAGTAATTCAGG-3′ PR-FAM-5′-TG(pdC)ATA(pdC)GA(pdC)A(pdC)CTATCC-ZNA4-BHQ-1-3′
hsa-miR-29a-3p	407021	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACTAACCG-3′ F-5′- GCCGCTAGCACCATCTGAAAT-3′ PR-FAM-5′-TG(pdC)ATA(pdC)GA(pdC) TAACCG-ZNA4-BHQ-1-3′
hsa-miR-29b-3p	407024	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACAACACT-3′ F-5′- GCCGCTAGCACCATTTGAAATC-3′ PR-FAM-5′- TG(pdC)ATA(pdC)GA(pdC) AACACT-ZNA4-BHQ-1-3′
hsa-miR-29c-3p	407026	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACTGCATACGACTAACCG-3′ F-5′- GCCGCTAGCACCATTTGAAAT-3′
Universal primer		PR-FAM-5′- TG(pdC)ATA(pdC)GA(pdC) TAACCG-ZNA4-BHQ-1-3′ R-5′GTGCAGGGTCCGAGGTAT-3′

[BHQ, Black Hole Quencher; F, forward; miRNA, microRNA; PR, probe; R, reverse]

and Dunn (for KW) tests were used as post hoc tests. Roc curve analysis was performed to determine the diagnostic power of expression levels. Spearman rank correlation coefficient was used for correlation analysis. Box plot and Scatter plot were drawn for visualization. The analyses were performed in STATISTICA version 13.3.1 (TIBCO Software Inc., Palo Alto, CA, USA) and MedCalc (MedCalc Software Ltd, Ostend, Belgium). A *P* value of less than 0.05 was considered statistically significant.

Results

A total of 48 MM patients and 11 healthy subjects were included in the present study. The control group included 4 females and 7 males with an average age of 62 years and an age range of 22-80 years. The patient group included 21 females and 27 males with an average age of 70 years and an age range of 47-91 years. Six of the 48 patients were diagnosed with revised-ISS (R-ISS) stage 1, 35 with stage 2, and 7 with stage III.

hsa-miR-29b-3p expression level was upregulated in the bone marrow samples of MM patients

To determine whether the hsa-miR-29 family and the target genes DNMT3A and TET2 are normally or abnormally expressed in MM, we performed qRT-PCR to measure their levels in bone marrow cells taken from patients and control subjects. Thus, the expression levels of these three miRNAs and the target genes were evaluated between MM patients and the controls. qPCR results showed no significant association was detected between the expression levels of hsa-miR-29a-3p, hsa-miR-29c-3p, DNMT3A and TET2 genes in MM compared with the control, except hsa-miR-29b-3p (p=0.422, p=0.387, p= 0.602, p=0.654, respectively). We observed different expression patterns of hsa-miR-29b-3p between control and patient groups. This miRNA was upregulated in the patient group (p=0.03, Fig. 1)

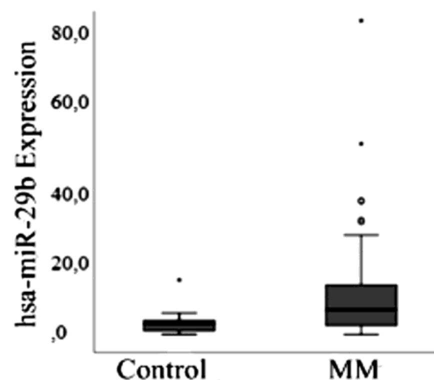

Fig. 1 — Boxplot graphic of hsa-miR-29b-3p expression between MM and control. Stars represent high variance of expressions between individuals

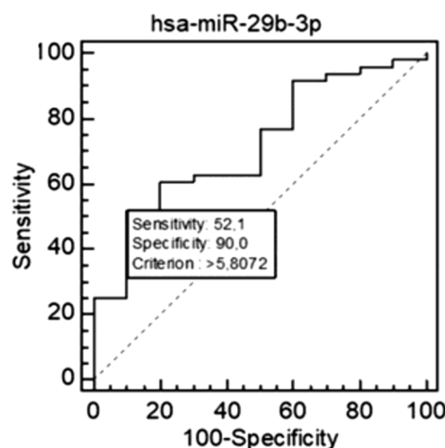

Fig. 2 — ROC curve analysis of hsa-miR-29b-3p in bone marrow. The diagnostic value of hsa-miR-29b-3p in MM patients was determined by constructing a ROC curve

Diagnostic value of bone marrow hsa-miR-26b-3p in MM

The ROC curve was performed to clarify the diagnostic value of bone marrow hsa-miR-26b-3p in MM patients. As shown in Fig. 2, hsa-miR-26b-3p could distinguish MM patients from healthy controls with an AUC of 0.719 [95% confidence interval (CI), 0.585-0.829; p = 0.006], the sensitivity of 52.1%, and the specificity of 90%. These results suggest moderate value of hsa-miR-26b-3p in the diagnosis of MM.

Correlation of expression of hsa-miR-29s and the target genes

Correlation analysis of the relative expression levels of hsa-miR-29a/b/c and the target genes in the total 59 research subjects from MM and the control groups was performed using Spearman's rank correlation analysis. A moderate positive significant correlation was found between the expression levels hsa-miR-29a-3p and hsa-miR-29b-3p (rs = 0.553, *P* <0.001), hsa-miR-29b-3p and hsa-miR-29c-3p (rs= 0.560, *P* <0.001). There was a strong significant correlation between hsa-miR-29a-3p and hsa-miR-29c-3p (rs = 0.844, *P* <0.001, Fig. 3). The expression levels between the hsa-miR-29b-3p and TET2 gene showed a weak positive correlation (rs = 0.306, p = 0.020). However, no significant correlation was found between expression levels hsa-miR-29a-3p/ hsa-miR-29c-3p and the two target genes or between DMT3A and TET2.

Differences between the relative expression levels of hsa-miR-29a-3p, hsa-miR-29b-3p and hsa-miR-29c-3p in terms of the clinical characteristics of MM patients

We then analyzed the differences between the expression levels of hsa-miR-29a-3p, hsa-miR-29b-

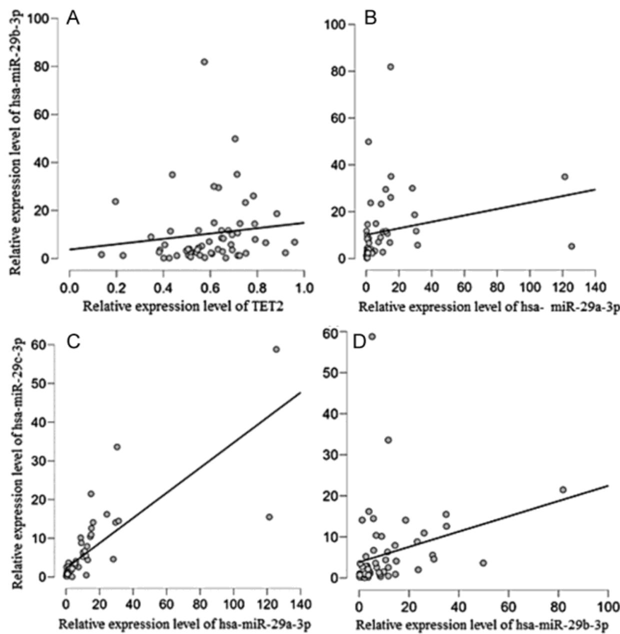

Fig. 3 — The Correlation analysis between hsa-miR-29a/29b/29c and target genes as well as between hsa-miR-29 members (A) a weak positive correlation between hsa-miR-29b-3p and TET2; (B) a moderate positive correlation between hsa-miR-29b-3p and hsa-miR-29a-3p; (C) a strong positive correlation between hsa-miR-29c-3p and hsa-miR-29a-3p; and (D) a moderate positive correlation between hsa-miR-29c-3p and hsa-miR-29b-3p

3p, and hsa-miR-29c-3p in relation to the clinical characteristics of MM patients. As summarized in Table 4, the expression level of hsa-miR-29b-3p was significantly decreased (p= 0.015) in patients with elevated lactate dehydrogenase (LDH) levels, while other miRNAs showed no obvious difference in patients with elevated LDH levels compared to patients with normal LDH levels. Moreover, no association was found between the relative expression levels of these three miRNAs and MM patients with different age, gender, Durie-Salmon stages, R-ISS stages, creatinine levels, light chain types, heavy chain types, hemoglobin levels, albumin levels, β2-microglobulin levels (p all >0.05). These results showed that the bone marrow level of hsa-miR-29b-3p was significantly decreased in MM patients with high LDH levels and was independent of age, sex, R-ISS stage, Durie-Salmon stage, light chain type, heavy chain type, and albumin, creatinine, hemoglobin, and β2-microglobulin levels (Table 4).

Differences between the relative expression levels of DNMT3A and TET2 in terms of the clinical characteristics of MM patients

Differences between these two genes in relation to the clinical characteristics of MM patients showed that DNMT3A expression level was significantly increased in patients with high LDH levels (p=0.035) and patients with lambda light chain (p=0.045). These results indicated that DNMT3A might be related to MM. However, as shown in Table 4, no association was found between these two genes and other clinical parameters.

Discussion

Over the past years, the molecular mechanism behind MM tumorigenesis has been gradually revealed with discovery of novel therapies[24]. Growing evidence points to the pivotal roles of miRNAs in the regulation of human malignancies by acting as inhibitory factors of downstream target genes, including MM[25]. For example, Liu *et al.*[26] demonstrated that the tumor-suppressor role of miR-215-5p inhibits the tumor formation of MM cells via negatively regulating RUNX1 gene. Recent investigations highlighted that miRNAs were promising options for the treatment of MM patients[27,28].

Recent reports have highlighted the potential of hsa-miR-29 family members as diagnostic biomarkers in various cancers such as prostate cancer, glioblastoma, colon cancer, colorectal cancer, as well as MM[29-31]. The miR-29 family members have been considered as potential tumor suppressors in MM. For instance, Amodio *et al.*[32] showed that overexpression of hsa-miR-29b was involved in the suppression of SOCS-1 gene and negatively regulated the growth and migration of MM cells. In another report by Wang *et al.*[25] miR-29b inhibited proliferation but induced cell cycle arrest and apoptosis of MM cells via down-regulating FOXP1. In the current report, hsa-miR-29b was significantly upregulated in MM patients compared with the control, which was independent from age, gender, stage, serum levels of β2-MG, albumin, calcium, creatinine, myeloma protein and haemoglobin. In agreement with our results, Zhang *et al.*[33] demonstrated that hsa-miR-29b-3p expression was increased in MM patients compared with healthy controls. Dysregulated miR-29b was only correlated with high LDH levels of MM patients. In addition, ROC curve analysis revealed a moderate value of the hsa-miR-29b as a bone marrow marker for MM diagnosis.

In order to better understand the characteristics of expression levels and regulation of hsa-miR-29s in MM, we searched the miRDB database. DNMT3A

Table 4 — Comparison of the relative expression level of hsa-miR-29s and the target genes in patients with different clinical parameters of multiple myeloma

Characteristics	Cases	hsa-miR-29a-3p*	hsa-miR-29b-3p*	hsa-miR-29c-3p*	DNMT3A†	TET2†
Age						
≤65	25	1.438 (0.587-12.516)	6.801 (2.364-11.702)	1.511 (0.785-4.908)	1.33±0.46	0.56±0.13
>65	23	3.501 (0.624-11.904)	5.190 (2.464- 23.729)	1.991 (0.580-8.810)	1.30±0.35	0.62±0.17
		p=0.672	p=0.845	p=0.942	p=0.820	p=0.228
Sex						
Male	29	1.76 (0.602-10.120)	8.605 (2.343-14.550)	1.065 (0.478-6.311)	1.24±0.29	0.59±0.17
Female	19	3.501 (0.830-14.693)	4.431 (2.679-20.316)	2.896 (0.834-7.796)	1.41±0.50	0.59±0.14
		p=0.400	p=0.747	p=0.324	p=0.164	p=0.920
R-ISS Stages						
I	6	1.113 (0.443-16.781)	10.128 (3.085-34.595)	2.263 (0.267-7.766)	1.26±0.46	0.49±0.23
II	35	3.501 (0.602-12.948	7.015 (2.464-11.707)	2.536 (0.871-10.118)	1.25±0.37	0.61±0.15
III	7	1.089 (0.624-5.334)	2.883 (2.343-8.324)	0.797 (0.759-2.915)	1.47±0.40	0.59±0.58
		p=0.667	p=0.475	p=0.311	p=0.056	p=0.290
Durie-Salmon stage						
I	17	2.725 (0.465-14.719)	6.801 (1.694-24.901)	2.536 (0.912-10.656)	1.37±0.31	0.59±0.16
II	20	3.364 (0.810-12.241)	6.791 (2.516-11.195)	2.141 (0.897-5.883)	1.25±0.40	0.61±0.14
II	31	1.438 (0.521-9.130)	5.190 (2.639-14.550)	0.871 (0.344-3.634)	1.34±0.53	0.55±0.18
		p=0.714	p=0.998	p=0.404	p=0.646	p=0.615
β2-MG, mg/L						
<3.5	18	1.505 (0.443-11.949)	10.151 (2.059-18.532)	1.038 (0.294-4.452)	1.32±0.36	0.59±0.15
3.5-5.5	20	8.120 (0.849-14.801)	6.137 (2.508-10.790)	4.409 (0.987-10.796)	1.21±0.42	0,60±0.17
5.5	10	1.124 (0.611-7.238)	5.778 (2.591-13.900)	1.191 (0.797-3.283)	1.51±0.40	0.57±0.14
		p=0.714	p=0.998	p=0.404	p=0.166	p=0.871
ALB, g/dL						
Normal	24	5.618 (0.836-12.732)	9.433 (2.760-14.081)	2.725 (0.919-9.791)	1.37±0.35	0.57±0.14
Low	24	1.505 (0.566-10.800)	6.137 (2.260-13.064)	0.952 (0.366-4.360)	1.25±0.44	0.61±0.17
		p=0.187	p=0.550	p=0.080	p=0.326	p=0.357
LDH, U/L						
Normal	19	4.494 (1.287-12.732)	10.243 (6.680-14.081)	3.078 (1374-8.185)	1.14±0.32	0.60±0.14
High	18	1.366 (0.506-12.547)	3.395 (1.590-11.416)	0.941 (0.294-6.680)	1.45±0.45	0.58±0.19
Low	11	0.959 (0.589-11.328)	2.552 (1.343-17.431)	0.979 (0.714-6.644)	1.41±0.35	0.58±0.13
		p=0.352	**p=0.015**	p=0.210	**p=0.045**	p=0.941
CRP, mg/L						
≤5	22	3.364 (0.567-10.611)	5.448 (2.622-11.697)	1.861 (0.466-5.488)	1.24±0.38	0.55±0.13
>5	26	1.505 (0.618-14.585)	7.377 (1.923-26.933)	2.203 (0.791-10.183)	1.37±0.42	0.63±0.17
		p=0.722	p=0.605	p=0.591	p=0.273	p=0.082
Calcium, mg/L						
Normal	36	1.507 (0.558-9.871)	4.811 (2.055-11.697)	1.419 (0.504-4.548)	1.34±0.38	0.59±0.16
High	3	1.084 (0.624-9.902)	8.324 (2.343-11.250)	0.870 (0.770-3.305)	1.58±0.36	0.56±0.11
Low	9	10.120 (2.330-22.691)	11.386 (4.813-22.365)	5.534 (1.370-12.507)	1.12±0.46	0.62±0.15
		p=0.120	p=0.216	p=0.192	p=0.273	p=0.082
Creatinine, mg/L						
≤0.9	21	3.087 (0.572-13.497)	7.015 (3.139-13.129)	1.730 (0.881-8.900)	1.31±0.44	0.56±0.11
>0.9	27	1.742 (0.624-11.904)	6.568 (2.192-18.655)	1.991 (0.759-6.311)	1.32±0.37	0.61±0.18
		p=0.811	p=0.596	p=0.670	p=0.960	p=0.259
Hemoglobin, g/dL						
Normal	24	5.618 (0.618-14.801)	9.433 (2.425-22.461)	2.725 (0.762-10.796)	1.34±0.33	0.60±0.16
Low	24	1.505 (0.607-8.720)	5.448 (2.508-10.119)	1.170 (0.778-4.200)	1.28±0.47	0.58±0.15
		p=0.293	p=0.303	p=0.303	p=0.608	p=0.597
Heavy-chain type						
IgG	38	2.906 (0.744-12.300)	6.254 (2.595-12.418)	1.529 (0.767-6.935)	1.31±0.42	0.58±0.16
IgA	10	1.179 (0.239-8.153)	8.215 (1.351-19.878)	2.725 (0.724-6.940)	1.34±0.34	0.63±0.11
		p=0.274	p=0.871	p=0.774	p=0.608	p=0.597
Light-chain type						
kappa	33	1.160 (0.624-30642)	2.674 (2.010-14.872)	0.874 (0.759-30604)	1.49±0.35	0.59±0.15
lambda	15	5.334 (0.587-14.693)	7.954 (3.395-13.129)	2.551 (0.771-10.248)	1.23±0.40	0.59±0.16
		p=0.178	p=0.151	p=0.151	**p=0.035**	p=1.000

[LDH: lactate dehydrogenase, ISS-R: Revised international staging system, Ig: immunoglobulin, β2-MG: Beta2 microglobulin, CRP: C-reactive protein, ALB: albumin. *Variables were summarized as mean±standard deviation. † Variables were summarized as median (25P-75P). Bold types show the statistical significance]

and TET2 genes were two targets of for all members of this family[34]. Dysregulation of DNMT3A, one of the epigenetic modifiers of DNA methylation machinery, may contribute to tumorigenesis[35,36]. For example, Bollati *et al*.[37] reported a decrease DNMT3A expression in MM, supporting its tumour-suppressive role. In the current study, no statistically significant differences in the bone marrow DNMT3A expression profiles of MM patients were observed. This result was consistent with another study regarding MM. Luzna *et al*.[19] reported that DNMT3A expression in bone marrow of MM patients did not differ between the healthy and control group (possibly due to low patient counts). Serum lambda light chain correlates with the tumour-bearing status of MM patients. LDH, a commonly used prognostic indicator for melanoma patients, can indicate tumour-proliferating activity, making it valuable for evaluating the therapeutic efficacy and prognosis[38]. We found that relative DNMT3A expression was significantly higher in patients with high LDH levels compared to patients with normal and low LDH levels and patients with lambda light chain, suggesting that DNMT3A expression level may be involved in disease progression. Further studies should pay attention to larger patient counts so that we can conduct a more comprehensive analysis for the expression pattern of DNMT3A in MM.

As another epigenetic player, TET2 is expressed in brain, kidney and hematopoietic system. Loss of TET2 function has been associated with the development of myeloid malignancies[39]. Although the impacts of TET2 expression have been studied in various hematologic malignancies including AML[40] and CML[41], to the best of our knowledge, no studies have been evaluated to assess TET2 expression profiles in patients with MM. Thus, this is the first report demonstrating the importance of TET2 expression in MM pathogenesis. However, the expression level of TET2 in MM was similar to the level found in the controls. In addition, no significant associations between TET2 expression and clinical parameters were found.

However, in contrast to several studies showing a correlation between DNMT3A and hsa-miR-29 family[42], we did not find a correlation between them. For the first time, we investigated the relationship between hsa-miR-29s-TET2 regulation. There was no correlation between relative expression levels of hsa-miR-29 family and TET2 gene in MM. It should be noted that MM is defined as a genetically and clinically highly complex and heterogeneous disease and hsa-miR-29s target many genes.

Conclusion

In summary, we characterized the expression feature of hsa-miR-29a-3p/hsa-miR-29b-3p/hsa-miR-29c-3p and their target genes DNMT3A and TET2 in Turkish Multiple myeloma (MM) patients. We demonstrated that hsa-miR-29b-3p expression level was abnormally increased in the bone marrow of multiple myeloma (MM) patients and demonstrated a moderate diagnostic power in the diagnosis of MM. These results suggest that it may serve as an auxiliary diagnostic tool in MM diagnosis. Inhibiting hsa-miR-29b-3p could be a promising therapeutic strategy for MM therapy.

Conflict of Interest

Authors declare no competing interests.

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