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Cytogenetic abnormalities in laryngeal carcinoma: a case report

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Cytogenetic analysis of metastastic squamous cell carcinoma of the larynx was undertaken in a 62 year old male smoker. All the metaphases obtained were in the hypotriploid range with a modal number of approximately 64. Despite definitive radiotherapy and radical surgery recurrence and metastasis was seen. [Turk J Cancer 1994;24(3):163-7]

Key words: Squamous cell carcinoma, larynx, cytogenetics

Squamous cell carcinoma is the most common malignancy of the larynx (1) and is usually seen among cigarette smokers over the age of 40. Men are affected more than women, and environmental irritants such as exposure to radiation, are probably important in its development. However, there also appears to be an operative genetic factor.

Cytogenetic studies of laryngeal carcinomas are very limited (2-7). We present here the cytogenetic abnormalities found in a metastatic squamous cell carcinoma of the larynx.

Case Report

A 62 year old male with a history of smoking, 80 pack-years was admitted to the otorhinolaryngology unit with the complaint of hoarseness for the last 4 months. Direct laryngoscopy and biopsy was performed and a well-differentiated squamous cell carcinoma of the right vocal cord, staged as T1NOMO was diagnosed. He was treated with definitive radiotherapy (68 Gy). Eight months later local recurrence, which was staged as T3N1M0, was noted, total laryngectomy and right radical neck dissection was performed. Pathological examination revealed a well-differentiated squamous cell carci-

noma and cervical lymph node metastasis. Seven months following the operation, he developed a fixed mass of 3x3 cm in the right supraclavicular region and right upper mediastinal dissection was performed.

Cytogenetic study of the tumor sample obtained from the excision material was transported to the laboratory immediately. The specimen was trimmed of fat, necrotic material, stroma and was minced in HBSS (Hanks' balanced salt solution, Biochrome) with 200 µg/ml streptomycin, 200 U/ml penicilin, then transferred into a centrifuge tube and washed twice in prewarmed RPMI 1640 medium without serum and resuspended in RPMI 1640 medium supplemented with 100 U/ml penicilin, 100 µg/ml streptomycin, 1% L-glutamine and 17% FBS (Fetal Bovine Serum, Flow) (8). Three or four tissue fragments were placed into the 25 cm^2 tissue culture flasks with the aforementioned medium and incubated in the CO_2 incubator at 37°C. The cultures were fed with fresh medium twice a week. After 10 days, when the cultures had sufficient number of mitosis, harvest was performed by conventional methods (8). Harvesting procedure included colcemid treatment (0.05 µg/ml) for 2 hours, hypotonic treatment with 0.075M KCl and methanol/acetic acid (3:1) fixation steps (8,9).

Prior to G-banding, slides were incubated overnight at 60° C and then banded with Leishman's stain and karyotypes were interpreted according to the International System for Cytogenetic Nomenclature (10). Clonality was defined as the same structural abnormality or extra chromosome in at least two metaphases or the same missing chromosome in at least three metaphases (8,10).

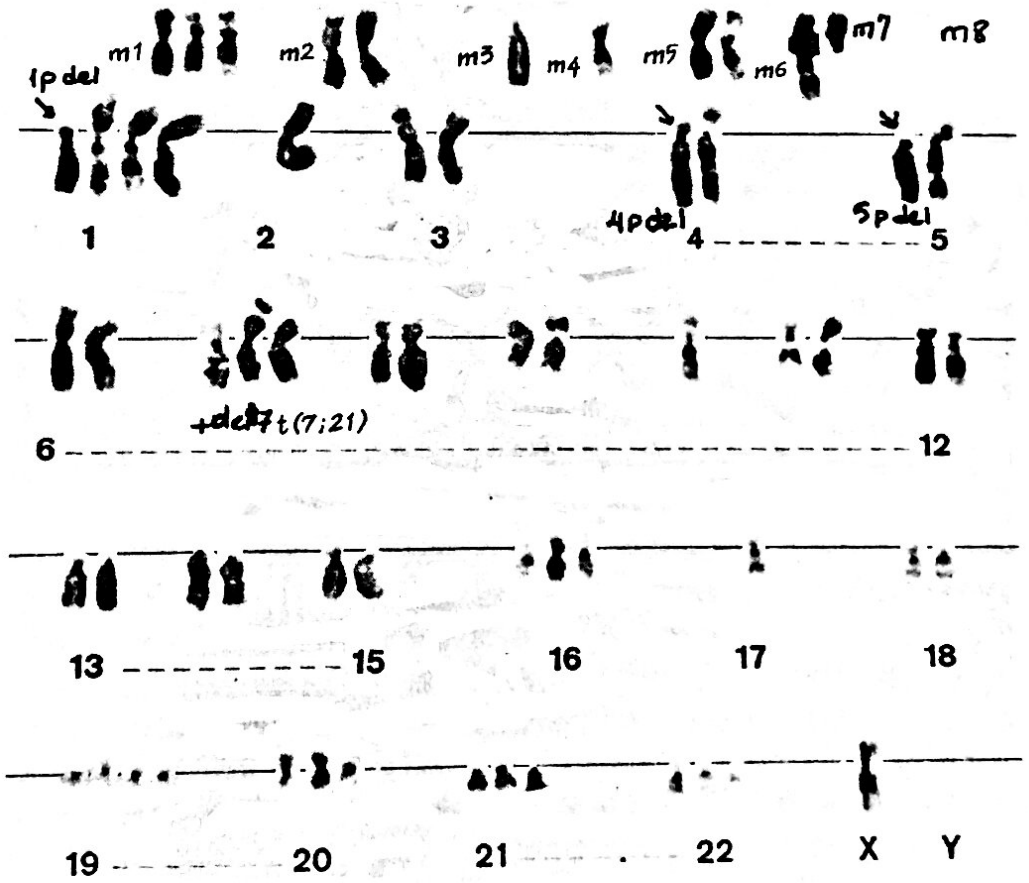

Fig 1. Representative karyotype of metaphase I from squamous cell carcinoma of the larynx: 63 X,-Y,+1,-2,-10,+16,-17,+19,+19,+20,+21,+22, +del (1) (p22), del (4) (p15), del (5) (p13), +der7t (7;21) (p22;p11), +mar1, +mar1, +mar1, +mar2, +mar2, +mar3, +mar4, +mar5, +mar5, +mar6, +mar7, +mar8

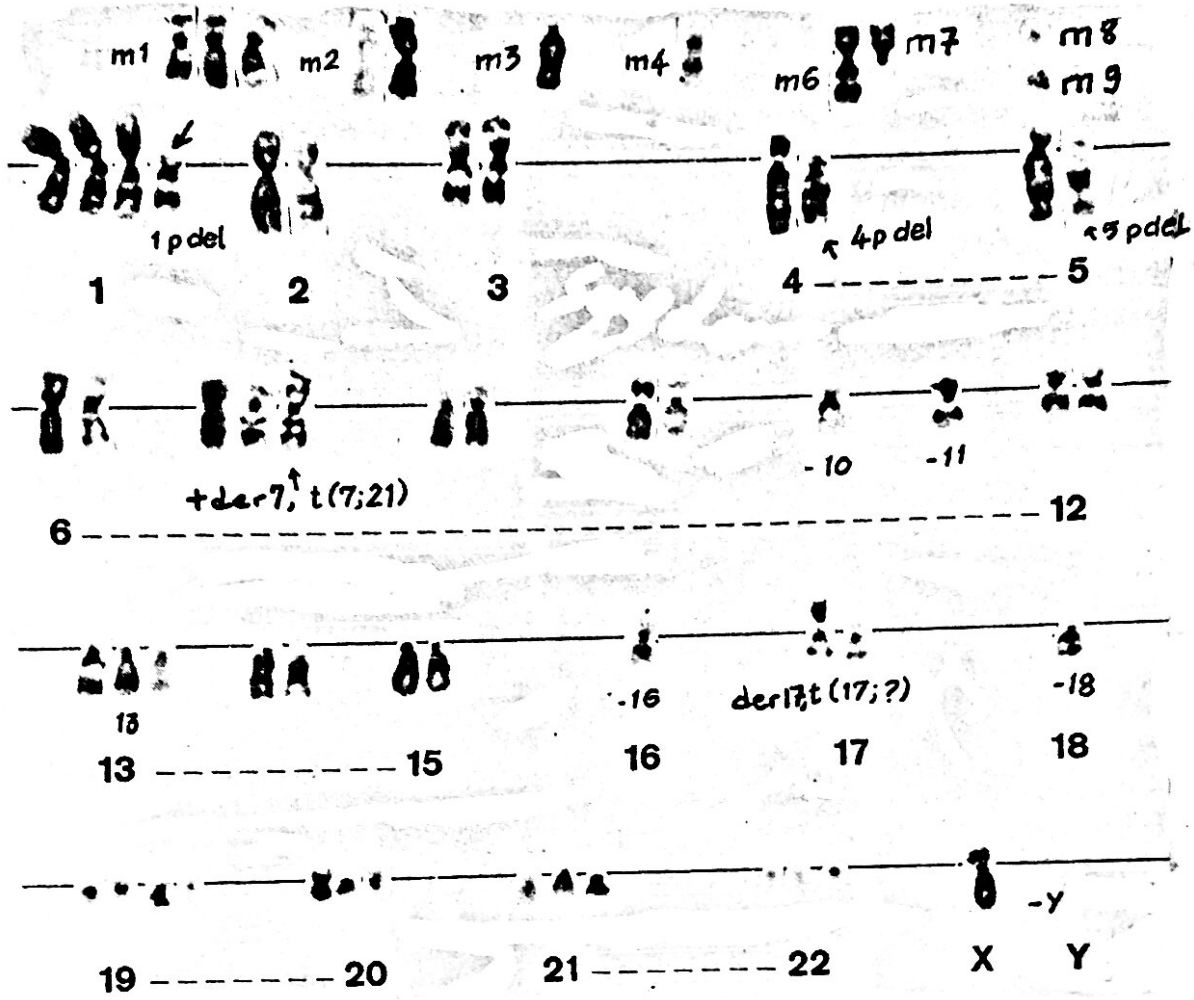

Fig 2. Representative karyotype of metaphase II from the squamous cell carcinoma of the larynx: 61 X,-Y,-10,-11,+13,-16,-18,+19,+19,+20,+21,+22,+del (1) (p22), del (4) (p15), del (5) (p13), +der7t (7;21) (p22;p11), der17t (17;?) (p?,p?) +mar1, +mar1, +mar1, +mar2, +mar2, +mar3, +mar4, +mar6, +mar7, +mar8, +mar9

Cytogenetic findings are presented in table 1 with modal chromosome number, and numerical and/or structural abnormalities in 13 metaphases. All metaphases were in hypotriploid range (modal number 64). Karyotypes of two metaphases are given in figures 1 and 2.

Discussion

With the use cytogenetic studies in cancer, we can determine clonal chromosomal aberrations for diagnostic and prognostic purposes as well as determining the locations of oncogenes. Studies performed on solid tumors are limited in literature and the principal aim of these studies is to detect primary or clonal abnormalities in spesific malignancies.

In our study, karyotype analysis was performed in the short-term (10 day) cultures of metastatic carcinoma cells of a patient, clinically and pathologically diagnosed as laryngeal carcinoma. Several subclones with various abnormalities were found in the karyotype analysis which may point to tumor progression and, despite definitive radiotherapy and radical surgery recurrence was seen.

Although diploid chromosome numbers had been reported in the studies concerning the same cancer type (2-7), modal chromosome number was observed to be 64 in our case. A diploid clone was not detected and this seems compatible with reports showing increased DNA content in cell populations in laryngeal cancer (11,12). Increased DNA content and hyperdiploidy are indicators of genetic instability such as mitotic abnormalities. Increased susceptibility to chromosome breakage, nondysjunction, ploidy changes, and other genetic alterations would increase the probability of a more malignant cell population selection (11-13). The absence of the Y chromosome, which was a constant numerical abnormality in all metaphases in our case, has been previously described in some laryngeal squamous cell carcinomas (4,7).

Table 1
Numerical and structural abnormalities detected (13 metaphases investigated)

Chromosomal abnormality	No of metaphases
-Y t(7;21) (p22;p11) +del (1) (p22)	13 (Clonal)
del (4) (p15) del (5) (p13) der (17) +1 +7 +19, +19 +20 +21 +22 +mar1 +mar2 +mar3 +mar4 +mar6 +mar7 +mar8	> 2 (Clonal)
-10	> 3 (Clonal)
+13 +16 +mar5 +mar9	< 2 (Non clonal)
- 2 -11 -16 -17 -18	> 3 (Non clonal)

Deleted chromosome 1 from p22, which was reported as a non-clonal abnormality previously is another clonal abnormality in our case (7). The constant abnormality in all the metaphases analysed, t(7;21) (p22;p11) seems to be unique to our case. We determined loss of chromosome 11 in one of the metaphases and it was not clonal. Monosomy 11 and deletion of the short arm of chromosome 3 in the region p21-23 were also found in high grade laryngeal carcinomas and are thought to be associated with tumor progression (7).

Because of the limited information on solid tumors, each result about clonal abnormalities should be taken seriously and considered as a causative primary abnormality. However, one should be aware that the results obtained in vitro may not represent the conditions in vivo.

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