

# Sequential Loss of Heterozygosity at Microsatellite Motifs in Preinvasive and Invasive Head and Neck Squamous Carcinoma<sup>1</sup>

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## ABSTRACT

Studies of sequential molecular alterations in noninvasive and invasive head and neck squamous carcinoma are few in number. Consequently, the genetic changes associated with the neoplastic transformation of these carcinomas have not been defined. To identify chromosomal alterations in preinvasive and invasive head and neck squamous carcinoma, we analyzed DNA from microdissected normal squamous epithelium, severe dysplasia, and invasive carcinoma samples from 20 patients for loss of heterozygosity (LOH) at microsatellite loci by multiplex PCR. Twenty-five microsatellite repeats on chromosomes 3p, 5q, 8p, 9p and 9q, 11q, 17p, 17q, and 18p and 18q regions were used. In informative cases, LOH in noninvasive lesions was observed in 9p (28%), 9q and 18q (10%), 11q and 17p (7%), and 3p and 18p (5%). A high incidence of LOH in invasive carcinoma was observed at 9p (72%), 8p (53%), 3p (47%), 9q (35%), and 11q (33%). LOH was also associated with DNA aneuploidy, high tumor stage, and poor histological differentiation. Our results indicate that: (a) the high incidence of LOH at loci on chromosomes 9p, 8p, 3p, 9q, and 11q implicate these regions in head and neck squamous carcinoma tumorigenesis; (b) 9p loci alterations are manifested in the early development of these tumors; (c) LOH is correlated with poor prognostic clinicopathological factors; and (d) LOH at 8p loci appears to be associated with the tumor's aggressive features.

## INTRODUCTION

Sequential molecular analysis of pathologically defined premalignant and malignant lesions should allow for determining the nature of the abnormality and the stage at which genomic alterations develop (1). Functional loss of tumor suppressor genes is one of the most common genetic alterations in human solid neoplasms. Defining chromosomal regions that harbor biologically important suppressor genes may, therefore, have broad practical implications on the diagnosis and management of these tumors (2, 3).

The superficial and limited nature of premalignant lesions and the difficulties in obtaining sufficient fresh samples have precluded extensive sequential molecular genetic analysis of these tumors (4, 5). However, the development of the widely distributed and highly polymorphic microsatellite repeat motifs and their suitability for PCR amplification now allow rapid and extensive genomic analysis of small DNA samples from such limited lesions (6-9).

Alterations of microsatellites have been reported in several solid neoplasms and have led to the identification of loci involved in early and late development of certain cancers (10-15). To better define the genomic changes associated with HNSC<sup>3</sup> tumorigenesis, fresh, microdissected normal squamous epithelium, and preinvasive and invasive squamous carcinoma specimens from 20 patients were analyzed using microsatellite markers of the commonly altered chromosomal regions reported in these tumors.

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<sup>3</sup> The abbreviations used are: HNSC, head and neck squamous carcinoma; LOH, loss of heterozygosity.

## MATERIALS AND METHODS

Normal-appearing squamous epithelium, severely dysplastic/carcinoma *in situ* mucosa, and invasive carcinoma from 20 patients with HNSC, prospectively accessioned in the Frozen Section Unit of the Department of Pathology, University of Texas M. D. Anderson Cancer Center from 1991 to 1993, formed the materials for this study. Peripheral blood lymphocytes from each patient were also collected. Normal squamous epithelium and epithelium with severe dysplasia/carcinoma *in situ*, exclusive of invasive carcinoma, were microdissected off the underlying submucosa after frozen section verification and localization. Histologically, normal squamous epithelium was obtained after frozen section verification from the farthest mucosal margin of the resected specimen. In most specimens at least 3 cm separated the edge of the carcinoma from this microdissected margin. Since precise grading of frozen dysplastic lesions is often difficult due to freezing artifacts, full-thickness disorganization, cellular atypia, and mitosis of the squamous epithelium were used as criteria for severe dysplasia/carcinoma *in situ*. Tissue samples from each specimen were immediately snap frozen and kept at -80°C until used.

**DNA Extraction.** For preparation of high molecular weight DNA, frozen tissues were ground in a lysis buffer containing 0.2 M Tris-Cl, 1% SDS, 0.25 M NaCl, and 25 mM EDTA (pH 8.5). Proteinase K was added to a final concentration of 200 µg/ml. DNA was then purified by extraction with phenol/chloroform and precipitated with ethanol.

**Microsatellite Analysis.** The microsatellites were selected to include those which have been involved in LOH of HNSC. DNA samples were amplified by PCR using primers (Research Genetics, Huntsville, AL) for the microsatellite motifs at chromosome 3p (D3S1307, GATA6F06, D3S1284), chromosome 5q (GATA3F03, GATA6E05, D5S408), chromosome 8p (LPL, D8S264), chromosome 9p (D9S168, D9S199), chromosome 9q (D9S301, D9S302, D9S158), chromosome 11q (D11S968), chromosome 17p (D17S799), chromosome 17q (D17S579, D17S795), chromosome 18p (D18S59, GATA11A06), and chromosome 18q (GATA2A12, D18S70).

We used multiplex PCR where two loci were amplified simultaneously in one reaction tube. One primer from each pair was end labeled using T4 polynucleotide kinase (USB, Cleveland, OH) and [ $\gamma$ -<sup>32</sup>P]ATP (10 mCi/ml; DuPont New England Nuclear, Boston, MA). PCR was performed in a final volume of 25 µl containing 200 ng genomic DNA, 0.0125 µM labeled primer, 0.5 µM of each unlabeled primer, 250 µM deoxynucleotide triphosphate, 6.25% DMSO, 0.25 mM spermidine (Sigma Chemical Co., St. Louis, MO), 10 mM Tris-HCl (pH 8.4), 40 mM NaCl, 1.5 mM MgCl<sub>2</sub>, and 0.5 units *Taq* DNA polymerase (Perkin Elmer/Cetus, Norwalk, CT). Twenty-five cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min were performed using an initial denaturation step and final elongation step of 94°C for 5 min and 72°C for 3 min, respectively. After addition of 10 µl loading buffer, the PCR products were heat denatured and electrophoresed on 7% urea-formamide-polyacrylamide gels at 80 W for 3 to 5 h, depending on the fragment size. The gels were exposed to Kodak X-OMAT-AR film (Eastman Kodak Co., Rochester, NY) at -80°C with intensifying screens.

**Acridine Orange Flow Cytometry.** Single-cell suspensions from solid tissues were prepared by mechanically mincing fresh tissue in RPMI 1640 media (Irvine Scientific, Santa Ana, CA).

Acridine orange staining was performed using a two-step method previously described (16).

## RESULTS

Table 1 presents the clinicopathological characteristics and the flow cytometric findings of our study. Ten females and 10 males, who ranged in age between 36 and 74 (mean, 70.0) years, were the study population. Tumor sites included mobile tongue (10 specimens),

Table 1 Clinicopathological factors, DNA flow cytometric data, and LOH in primary HNSC

New#	Age (yr)	Sex	Race	Site	Stage	Histology	DNA index	Proliferative index	LOH
1	68	F	W <sup>a</sup>	Larynx	II	MD	1.00	3	2
2	63	F	W	Larynx	II	MD	1.15	10	>2
3	68	F	W	Tongue	IV	PD	1.78	12	>2
4	58	F	H	BOT	IV	PD	1.30	16	>2
5	55	M	B	Larynx	III	MD	1.60	11	>2
6	48	M	B	Tongue	IV	MD	1.65	13	>2
7	39	F	B	Larynx	II	MD	1.00	13	0
8	68	M	B	Larynx	IV	PD	1.49	19	>2
9	36	F	W	Tongue	II	MD	1.00	12	>2
10	42	M	W	Tongue	II	MD	1.00	8	2
11	63	M	H	Tongue	III	PD	1.00	15	>2
12	70	F	W	Tongue	III	PD	1.42	15	>2
13	47	M	W	Tongue	IV	MD	1.00	9	>2
14	68	F	W	FOM	II	WD	1.00	10	0
15	69	F	W	Tongue	III	MD	1.00	10	>2
16	49	F	B	Tongue	II	MD	1.00	15	0
17	74	M	W	Tongue	III	PD	1.42	13	>2
18	59	F	B	Larynx	III	MD	1.50	21	>2
19	61	M	W	Larynx	II	MD	1.00	13	0
20	57	M	W	Larynx	IV	PD	1.00	23	>2

<sup>a</sup> W, white; H, Hispanic; B, black; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; BOT, base of tongue; FOM, floor of mouth.

larynx (8 specimens), floor of the mouth (one specimen), and mandible (one specimen). Histologically, there were 1 well-differentiated, 12 moderately differentiated, and 7 poorly differentiated carcinomas. Tumor stage was distributed as follows: eight, stage II; six, stage III; and six, stage IV tumors. Flow cytometric DNA content analysis was available on 17 tumors. Eleven tumors (62.5%) were diploid and six (37.5%) were DNA aneuploid. The proliferative fraction of these tumors ranged from 3 to 23%, with a mean of 14.9%. The proliferative fraction of 10 normal epithelium from surgical and fresh autopsy specimens ranged from 1 to 4%, with a mean of 2.2%.

Fig. 1 presents the chromosomal regions and the microsatellite loci chosen for the analysis. LOH was evaluated by visual comparison of the allelic density of the normal, dysplastic, and tumor samples. From complete loss and >50% reduction in allelic intensity were considered an LOH (Fig. 2).

No LOH at the informative loci of the different chromosomal regions was observed in any of the normal squamous epithelium samples when compared to autologous blood DNA. Of the dysplastic specimens, eight specimens (40%) showed LOH at different loci on chromosomes 3p, 8p, 9p, 9q, 17p, 18p, and 18q. The most frequent

abnormality (28%) was noted on 9p21 and 9p23. In all of these instances the corresponding carcinoma samples manifested the same abnormality. Of the carcinoma samples, 16 (80.0%) showed LOH in at least one of the microsatellites markers examined. The most frequent loss was observed at loci on 9p (72%), 8p (53%), and 3p (47%). LOH at the remaining chromosomal regions ranged from 17 to 35% (Fig. 3).

Table 2 presents the correlation between tumors with >2 and ≤2 LOH loci, and the clinicopathological and DNA content parameters. All tumors with aneuploid DNA content showed >2 loci with LOH; 6 of the 11 diploid tumors had 2 or less loci with LOH, and 5 showed >2 loci with LOH (P = 0.01). Similarly, all 12 stage III and IV tumors showed >2 loci with LOH, while 6 of the 8 stage II disease had ≤2 loci with LOH (P = >0.001). All poorly differentiated tumors (7 specimens) showed >2 loci with LOH, and 6 of 13 moderately differentiated tumors had ≤2 loci with LOH (P = 0.05). Although the mean proliferative index of tumors with >2 loci with LOH was higher than those with ≤2 loci with LOH, this was not statistically significant (P = 0.17). Of the regions with high LOH, only alterations at 8p loci were more frequent in high stage (8 in stages III and IV versus 2 in stage II) and had DNA aneuploidy (7 in stages III and IV versus 3 in stage II).

Chromosome	Location	Locus	SPECIMEN TYPE																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
3	p11	D3S1307	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p21-14	GATA6F06	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p13-14	D3S1284	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
5	q21-22	GATAGF03	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	q	GATA6E05	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	q35-ter	D5S408	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
8	p23-ter	D8S264	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p22	LPL	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
9	p22-23	D9S168	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p23	D9S199	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p21-22	D9S162	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p21	D9S126	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p21	D9S171	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p21	D9S161	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	q13-21	D9S301	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
11	q31-33	D9S302	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	q34-ter	D9S158	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
17	q11	D11S968	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p11	D17S799	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	q11-12	D17S579	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
18	q23-24	D17S795	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	p11	D18S59	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
	q22-23	GATA11A06	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	
q23-ter	GATA2A12	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗		
		D18S70	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	

Fig. 1. Allelotyping results of all dysplastic (D) and carcinoma specimens (T) at different chromosomal loci. ●, LOH; ⊗, retention of both alleles; ○, not informative.



Fig. 2. LOH in selected cases. N, normal squamous epithelium; D, severe dysplasia; T, invasive carcinoma.

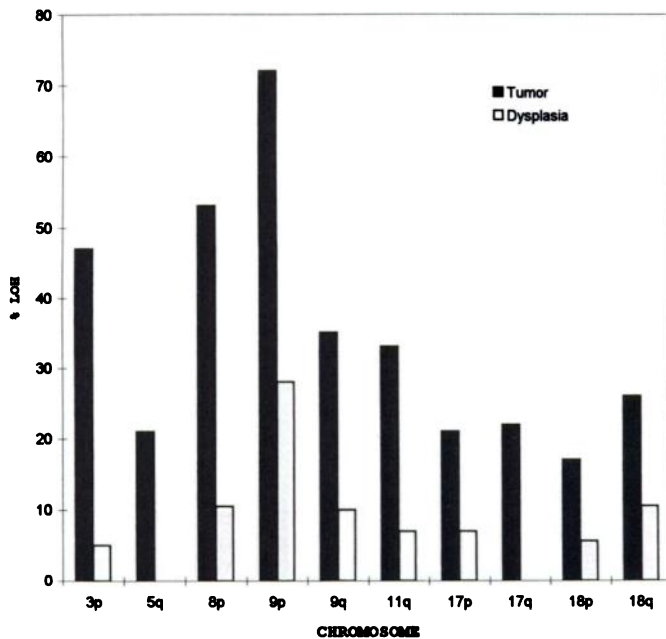


Fig. 3. Summary of the frequencies of LOH at different chromosomal arms in dysplasia and carcinoma samples.

This, however, did not reach a significant statistical correlation ( $P = 0.17$  and  $0.37$ , respectively). No apparent association between LOH at 3p markers and aggressive tumor characteristics was noted.

**DISCUSSION**

Previous cytogenetic and molecular genetic studies of HNSC have shown certain chromosomal regions with recurring alterations (17–26). The majority of these investigations have been performed on advanced, invasive neoplasms in which multiple genetic changes and

clonal heterogeneity confound the results (20, 26). Accordingly, characteristic temporal pattern and/or a stage at which alterations occur has not been defined. Since tumorigenesis is a sequential accumulation of genetic alterations (1, 27–29), analysis of early and late neoplastic lesions may define the genetic changes associated with the development and progression of HNSC.

In this study we microdissected freshly frozen autologous normal, severely dysplastic squamous epithelium, and invasive squamous carcinoma in patients with head and neck tumors. We found 9p loci to be most frequently reduced to homozygosity in invasive carcinoma (72%), in agreement with a previous study (25). Interestingly, 28% of noninvasive, dysplastic lesions also displayed LOH at 9p, indicating an early involvement of a gene(s) at this region in the genesis of these lesions. Of the 13 invasive tumors that showed 9p LOH, 38.4% of the autologous dysplastic lesions also displayed 9p alterations. Although, these findings corroborate the study of van der Veit *et al.* (25), the incidence of LOH at this region in our dysplastic lesions is lower than that reported in their series. The discrepancy between these results may be related to either interpretative differences and/or to the possible inclusion of a lesser grade dysplasia due to frozen section artifacts in some of our preinvasive lesions.

This same 9p region has been frequently altered in several other solid tumors (30–38) and implicated in the early development of others (5, 35). Among potential candidate genes in this region, the *MTS1/p16* and *p15* genes are a likely target for mutation (39–42). This, however, has more recently been contested by several recent studies indicating a lack of mutations in several primary neoplasms (41–44). In contrast to previous investigations, our study of autologous uninvolved squamous epithelium, noninvasive, and invasive samples are better able to define the progressive genomic alterations of HNSC, since it is done in a series of tissues from the same individual.

Overall, one third of noninvasive squamous lesions displayed LOH at microsatellite loci examined, with up to 15.7% showing 18q alterations. In invasive carcinoma, LOH was detected more frequently at 8p, 3p, and 9q than at 5q, 11q, 17p, 17q, 18p, and 18q loci. Contrary to previous reports (25), we observed LOH at 9q in 35% of the invasive cancers using the same microsatellite markers. Our results are in accordance with previous studies showing high frequency of LOH at 3p (45–49) and 9p (23, 25, 26) loci. We, however, observed a lower frequency of 5q LOH than Ah-see *et al.* did (26). This discrepancy may be related to differences in the patient population.

Our results showed no detectable LOH in histologically normal squamous epithelium. This is contrary to the findings of Lee *et al.* (4) relating to LOH for 13q loci. It is not clear, however, if the “nonmalignant mucosa adjacent to tumor” in their study means the absence of mild and/or moderate dysplasia. The lack of alterations in normal squamous epithelium in the field of excision in our study and those of others (10) suggest that such genetic abnormalities correspond to histological changes.

Table 2. Correlation between LOH at microsatellite markers and clinicopathological factors and DNA content data

Characteristic	LOH		P
	>2 Loci	≤2 Loci	
Stage			
II	2	6	>0.001
III and IV	12	0	
Differentiation			
Well and moderately	7	6	0.05
Poorly	7	0	
DNA ploidy			
Diploid	5	6	0.01
Aneuploid	9	0	
Proliferative index (mean)	13.8	8.4	0.17

Our data indicate that chromosomal aberrations at certain loci are present in preinvasive lesions and that additional abnormalities continue to develop during neoplastic transformation. The most likely region associated with early development is the 9p21 region and alterations at 8p loci may be associated with adverse biological features. In our study, tumors with marked LOH were preponderantly aneuploid, high stage, and poorly differentiated. These results corroborate similar studies in other tumors and indicate an association between the extent of LOH and tumor aggressiveness (14, 15, 49, 50). Additional studies to identify the timing at which this alteration occurs and to localize the putative suppressor genes at these locations are under way.

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## REFERENCES

- Vogelstein, B., and Kinzler, K. W. The multistep nature of cancer. *Trends Genet.*, **4**: 138–141, 1993.
- Nowell, P. C. Cancer, chromosomes, and genes. *Lab. Invest.*, **66**: 407–417, 1992.
- Lee, N. K. Tumor suppressor gene. *Head Neck*, **14**: 407–414, 1992.
- Lee, N. K., Ye, Y.-W., Li, X., Schweitzer, C., and Nisen, P. D. Allelic loss on chromosome 13 can proceed histological changes in head and neck cancer. *Int. J. Oncol.*, **5**: 205–210, 1994.
- Nees, M., Homann, N., Discher, H., Andl, T., Enders, C., Herold-Mende, C., Schumann, A., and Bosch, F. X. Expression of mutated p53 occurs in tumor-distant epithelia of head and neck cancer patients: a possible molecular basis for the development of multiple tumors. *Cancer Res.*, **53**: 4189–4196, 1993.
- Sutherland, G. R., and Richards, R. I. DNA repeats—a treasury of human variation. *N. Engl. J. Med.*, **331**: 191–193, 1994.
- Louis, D. N., Deimling, A. V., and Seizinger, B. R. A(CA)<sub>n</sub> dinucleotide repeat assay for evaluating loss of allelic heterozygosity in small and archival human brain tumor specimens. *Am. J. Pathol.*, **141**: 777–782, 1992.
- Litt, M., Hange, X., and Sharma, V. Shadow bands seen when typing polymorphic dinucleotide repeats: some causes and cures. *Biotechniques*, **15**: 280–284, 1993.
- Gruis, N. A., Abelin, E. C. A., Bardeol, A. F. J., Devilee, P., Frants, R. R., and Cornelisse, C. J. PCR-based microsatellite polymorphisms in the detection of loss of heterozygosity in fresh and archival tumor tissue. *Br. J. Cancer*, **68**: 308–313, 1993.
- Sidransky, D., Frost, P., von Eschenbach, A., Oyasu, R., Preisinger, A. C., and Vogelstein, B. Clonal origin of bladder cancer. *N. Engl. J. Med.*, **326**: 737–740, 1992.
- Uchida, T., Wada, C., Ishida, H., Egawa, S., Yokoyama, E., Kameya, T., Ohtani, H., and Koshiba, K. Alterations of microsatellite repeats in bladder cancer. *Oncol. Rep.*, **1**: 967–970, 1994.
- Osborne, R. J., and Leech, V. Polymerase chain reaction allelotyping of human ovarian cancer. *Br. J. Cancer*, **69**: 429–438, 1994.
- Fishel, R., Lescoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. The human mutator gene homology MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell*, **75**: 1027–1038, 1993.
- Emi, M., Fujiwara, Y., Ohata, H., Tsuda, H., Hirohashi, S., Koike, M., Miyaki, M., Monden, M., and Nakamura, Y. Allelic loss at chromosome band 8p 21.3-p22 is associated with progression of hepatocellular carcinoma. *Genes Chromosomes Cancer*, **7**: 152–157, 1993.
- Jen, J., Kim, H., Piantadosi, S., Liu, Z.-F., Levitt, R. C., Sistonen, P., Kinzler, K. W., Vogelstein, B., and Hamilton, S. R. Allelic loss of chromosome 18q and prognosis in colorectal cancer. *N. Engl. J. Med.*, **331**: 213–221, 1994.
- Traganos, F., Darzynkiewicz, Z., Sharpless, T., and Melamed, M. R. Simultaneous staining of ribonucleic and deoxyribonucleic acids in unfixed cells using acridine orange in a flow cytofluorometric system. *J. Histochem.*, **25**: 46–56, 1977.
- van Dyke, D. L., Worsham, M. J., Benninger, M. S., Krause, C. J., Baker, S. R., Wolf, G. T., Drumheller, T., Tilley, B. C., and Carey, T. E. Recurrent cytogenetic abnormalities in squamous cell carcinomas of the head and neck region. *Genes Chromosomes Cancer*, **9**: 192–206, 1994.
- Cowan, J. M., Beckett, M. A., Ahmed-Swan, S., and Weichselbaum, R. R. Cytogenetic evidence of the multistep origin of head and neck squamous cell carcinomas. *J. Natl. Cancer Inst.*, **84**: 793–797, 1992.
- Osella, P., Carlson, A., Wyandt, H., and Milunsky, A. Cytogenetic studies of eight squamous cell carcinomas of the head and neck. *Cancer Genet. Cytogenet.*, **59**: 73–78, 1992.
- Sreekantaiah, C., Rao, P. H., Xu, L., Sacks, P. G., Shantz, S. P., and Chaganti, R. S. K. Consistent chromosomal losses in head and neck squamous cell carcinoma cell lines. *Genes Chromosomes Cancer*, **11**: 29–39, 1994.
- Rodriguez, E., Sreekantaiah, C., and Chaganti, R. S. K. Genetic changes in epithelial solid neoplasia. *Cancer Res.*, **54**: 3398–3406, 1994.
- Kiaris, H., Spandidos, D. A., Jones, A. S., and Field, J. K. Loss of heterozygosity and microsatellite instability of the *H-ras* gene in cancer of the head and neck. *Int. J. Oncol.*, **5**: 579–582, 1994.
- Nawroz, H., van der Reit, P., Hruban, R. H., Koch, W., Ruppert, J. M., and Sidransky, D. Allelotype of head and neck squamous cell carcinoma. *Cancer Res.*, **54**: 1152–1155, 1994.
- Huang, D. P., Lo, K.-W., van Hasselt, C. A., Woo, J. K. S., Choi, P. H. K., Leung, S.-F., Cheung, S. T., Cairns, P., Sidransky, D., and Lee, J. C. K. A region of homozygous deletion on chromosome 9p21–22 in primary nasopharyngeal carcinoma. *Cancer Res.*, **54**: 4003–4006, 1994.
- van der Reit, P., Nawroz, H., Hruban, R. H., Corio, R., Tokino, K., Koch, W., and Sidransky, D. Frequent loss of chromosome 9p21–22 early in head and neck cancer progression. *Cancer Res.*, **54**: 1156–1158, 1994.
- Ah-See, K. W., Cooke, T. G., Pickjord, I. R., Soutar, D., and Balmain, A. An allelotype of squamous carcinoma of the head and neck using microsatellite markers. *Cancer Res.*, **54**: 1617–1621, 1994.
- Schantz, S. P. Experimental head and neck oncology. *Curr. Opin. Oncol.*, **3**: 500–506, 1991.
- Ankathil, R., and Nair, M. K. Molecular genetic mechanisms underlying oral oncogenesis. *Oncol. Rep.*, **1**: 1011–1015, 1994.
- Jaffe, D. R., Montero-Puerner, Y., Beckett, M. A., Cowan, J. M., Weichselbaum, R. R., and Diamond, A. M. Differential retention of tumor and differentiations suppressor functions in cells derived from a human squamous cell carcinoma. *Mol. Carcinog.*, **5**: 278–285, 1992.
- Devlin, J., Keen, A. J., and Knowles, M. A. Homozygous deletion mapping at 9p21 in bladder carcinoma defines a critical region with 2 cM of IFNA. *Oncogene*, **9**: 2757–2760, 1994.
- Keen, A. J., and Knowles, M. A. Definition of two regions of deletion on chromosome 9 in carcinoma of the bladder. *Oncogene*, **9**: 2083–2088, 1994.
- Cairns, P., Shaw, M. E., and Knowles, A. Preliminary mapping of the deleted region of chromosome 9 in bladder cancer. *Cancer Res.*, **53**: 1230–1232, 1993.
- Orlow, I., Lianes, P., Lacombe, L., Dalbagni, G., Reuter, V. E., and Cordon-Cardo, C. Chromosome 9 allelic losses and microsatellite alterations in human bladder tumors. *Cancer Res.*, **54**: 2848–2851, 1994.
- Holland, E. A., Beaton, S. C., Edwards, B. G., Kefford, R. F., and Mann, G. J. Loss of heterozygosity and homozygous deletions on 9p21–22 in melanoma. *Oncogene*, **9**: 1361–1365, 1994.
- Isshiki, K., Seng, B. A., Elder, D. E., Guerry, D. P., and Linnenbach, A. J. Chromosome 9 deletion in sporadic and familial melanomas *in vivo*. *Oncogene*, **9**: 1649–1653, 1994.
- Coleman, A., Fountain, J. W., Nobori, T., Olopade, O. I., Robertson, G., Houseman, D. E., and Lugo, T. G. Distinct deletions of chromosome 9p associated with melanoma versus glioma lung cancer and leukemia. *Cancer Res.*, **54**: 344–348, 1994.
- Mead, L. J., Gillespie, M. T., Irving, L. B., and Campbell, L. J. Homozygous and hemizygous deletions of 9p centromeric to the interferon genes in lung cancer. *Cancer Res.*, **54**: 2307–2309, 1994.
- Merlo, A., Gabrielson, E., Mabry, M., Vollmer, R., Baylin, S. B., and Sidransky, D. Homozygous deletion on chromosome 9p and loss of heterozygosity on 9p, 6p, and 6q in primary human small cell lung cancer. *Cancer Res.*, **54**: 2322–2326, 1994.
- Mori, T., Miura, K., Aoki, T., Nishihira, T., Mori, S., and Nakamura, Y. Frequent somatic mutation of the *MTS1/CDK31* (multiple tumor suppressor/cyclin-dependent kinase 4 inhibitor) gene in esophageal squamous cell carcinoma. *Cancer Res.*, **54**: 3396–3397, 1994.
- Kamb, A., Gruis, N. A., Waver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, E., Day, R. S., III, Johnson, B. E., and Skolnick, M. H. A cell cycle regulator potentially involved in genesis of many tumor types. *Science (Washington DC)*, **264**: 436–440, 1994.
- Okamoto, A., Hussain, S. P., Hagiwara, K., Spillare E. A., Rusin, M. R., Demetrick, D. J., Serrano, M., Hannon, G. J., Shiseki, M., Zariwala, M., Xiong, Y., Beach, D. H., Yokota, J., and Harris, C. C. Mutations in the p16<sup>INK4</sup>/MTS1/CDK2n, p15<sup>INK4B</sup>/MTS2, and p18 in primary lung cancer. *Cancer Res.*, **55**: 1448–1451, 1995.
- Otsuki, T., Clark, H. M., Wellmann, A., Jaffe, E. S., and Raffeld, M. Involvement of Cdkn2 (p16<sup>INK4A</sup>/MTS1) and p15<sup>INK4B</sup>/MTS2 in human leukemias and lymphomas. *Cancer Res.*, **55**: 1436–1440, 1995.
- Liu, Q., Neuhausen, S., McClure, M., Frye, C., Weaver-Feldhaus, J., Gruis, N. A., Eddington, K., Allalunis-Turner, M. J., Skolnick, M. H., Fujimura, F. K., and Kamb, A. Cdkn2 (MTS1) tumor suppressor gene mutations in human tumor cell lines. *Oncogene*, **10**: 1061–1067, 1995.
- Sun, Y., Hidesheim, A., Lanier, A. E. P., Cao, Y., Yao, K.-T., Raab-Traub, N., and Yang, C.-S. No point mutation but decreased expression of the P16/MTS1 tumor suppressor gene in nasopharyngeal carcinoma. *Oncogene*, **10**: 785–788, 1995.
- Partridge, M., Kiguwa, S., and Langdon, J. D. Frequent deletion of chromosome 3p in oral squamous cell carcinoma. *Oral. Oncol. Eur. J. Cancer*, **30**: 248–251, 1994.
- Maestro, R., Gasparotto, D., Vukosavljevic, T., Barzan, L., Sulfaro, S., and Boiocchi, M. Three discrete regions of deletion at 3p in head and neck cancers. *Cancer Res.*, **53**: 5775–5779, 1993.
- Latif, F., Fivash, M., Glenn, G., Troy, K., Orcutt, M. L., Hampsch, K., Delisio, J., Lerman, M., Cowan, J., Beckett, M., and Weichselbaum, R. Chromosome 3p deletions in head and neck carcinomas: statistical ascertainment of allelic loss. *Cancer Res.*, **52**: 1451–1456, 1992.
- El-Naggar, A. K., Lee, M. S., Wang, G., Luna, M. A., Goepfert, H., and Batsakis, J. G. Polymerase chain reaction-based restriction fragment length polymorphism analysis of the short arm of chromosome 3 in primary head and neck squamous carcinoma. *Cancer (Phila.)*, **72**: 881–886, 1993.
- Field, J. K., Tsiroyiotis, C., Howard, P., Spandidos, D. A., and Jones, A. S. Allele loss on chromosome 3 in squamous cell carcinoma of the head and neck correlate with poor clinical prognostic indicators. *Int. J. Oncol.*, **4**: 543–549, 1994.
- Li, X., Lee, K. N., Ye, Y.-W., Waber, P. G., Schweitzer, C., Cheng, Q.-C., and Nisen, P. D. Allelic loss at chromosomes 3p, 8p, 13q and 17p associated with poor prognosis in head and neck cancer. *J. Natl. Cancer Inst.*, **86**: 1524–1529, 1994.

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