Chromosome abnormalities in squamous cell carcinoma of the human oral cavity

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Received April 26, 1989

Cytogenetic studies carried out in tissues of 75 patients with squamous cell carcinoma of the oral cavity gave satisfactory results in 12 cases. Remarkable variation characterized the modal chromosome numbers of these tumors, ranging from marked hypodiploidy to tetraploidy. Chromosomes which were lost belonged to group A whereas chromosomes which were gained belonged to groups C, D, E, F and G. Marker chromosomes were present in three cases. There was no correlation between the chromosome abnormalities observed and the clinical stages of the disease. The pattern of chromosome abnormalities ranging from marked hypodiploidy to tetraploidy observed in oral cancer tissues suggests an association of DNA oncogenic virus possibly Herpes simplex virus Type I (HSV-1) with oral cancer.

Key words: Oral cancer, chromosomes, karyotype, oncogenic virus.

Carcinoma of the oral cavity is one of the ten most common cancers in the world, and in many countries it represents about one-third of all cancers. Oral cancer constitutes 18.14% of all cancers seen at the Regional Cancer Centre, Trivandrum, South India, and is the commonest cancer among men and the third commonest cancer among women treated at this Centre [23]. The incidence of premalignant lesions/conditions of the oral cavity like oral leukoplakia, oral submucous fibrosis, etc., are also reported to be high in this region as compared to other parts of the world [12].

A relatively high frequency of tumors of varying histology is seen in oral cavity. The most predominant type is the squamous cell carcinoma constituting about 90%, and in India the most common site is the buccal mucosa [23]. However, in spite of this high incidence, chromosome reports on squamous cell carcinoma of the oral cavity have been scarce in the literature.

The present study was undertaken to investigate the abnormalities of the chromosome constitution in the tissues of patients with the above type of cancer.

Materials and methods

Seventhy-five patients with squamous cell carcinoma of the oral cavity, attending the clinics of the Regional Cancer Centre, Trivandrum, South India, were entered in this study. All were histopathologically proved cases and they were in different

grades of histologic differentiation. The patients were selected before the start of any kind of therapy. The age distribution and sex of the subjects are shown in Table 1. Cytogenetic studies were attempted in fresh tumor tissue of these patients by making direct preparations, following the method of BERGER [5] with slight modifications. In short, fresh tumor tissue was scraped with a surgical blade in 60 mm Petri dish to single tumor cells and pipetted several times to obtain a cell suspension. The tumor cell suspension was then transferred to culture flask. The culture medium used was a 1 : 1 mixture of RPMI 1640 and Ham's F12 medium supplemented with 17% fetal bovine serum (GIBCO) and 0.03% L-glutamine, 100 μg/ml streptomycin, 100 μg/ml penicillin and 80 U/ml gentamycin.

Table 1. Age distribution and sex of the subjects

Subjects	Number	Age in years (mean ± SD)	No. of subjects with satisfactory metaphases
Males	45	59.4 ± 11.5	7
Females	30	61.5 ± 13.4	5
Total	75	60.8 ± 12.3	12

For direct chromosome preparations colcemid (GIBCO) was added immediately to the cultures at a final concentration of 0.2 μg/ml for 3—4 h, or overnight at a final colcemid concentration of 0.02 μg/ml. Serial harvests (24, 48, 72 and 96 h) were also made in each tumor when possible. Short-term culture of 10—14 days were sometimes done. After exposure to colcemid, the cells were treated with prewarmed Ohnuki's hypotonic solution (equimolar solution of 0.55 mol KCl, $NaNO_3$ and CH_3COONa at a ratio of 10:5:2) for 90—100 min. Fixation was done with freshly prepared fixative (methanol: acetic acid at a 3 : 1 ratio). Slides were prepared by the air drying method and stained with Giemsa's stain. Satisfactory metaphases considered suitable for karyotyping were photographed through a 100× objective. Karyotypes were made according to the International System for Human Cytogenetic Nomenclature [11]. Even though banding of these chromosomes was attempted, technical difficulties interfered in obtaining satisfactory results.

Malignant cells from oral cancer tissues showed extensive numerical changes. Deviations of the karyotypes were recorded in terms of gains and losses of whole chromosomes as compared to the normal male or female karyotype. Those abnormal chromosomes which differed in length and/or centromere position and with a peculiar morphology not seen in the normal karyotype were recognized as markers. The predominant number in cells displaying a wide range of chromosome numbers was designated as the modal chromosome number.

Results

Out of the 75 patients studied, analyzable metaphases were obtained in 12 cases. In these 12 patients 4 to 11 metaphases per specimen were obtained and chromosome

counts were made in a total of 83 metaphases. Chromosome counts revealed extensive numerical changes, i.e. gain or loss of whole chromosomes. The details of the chromosome numbers which ranged from 38—92 are given in Table 2. Each tumor showed great variability in the number of chromosome per cell. Remarkable variation characterized the modal chromosome numbers of these tumors ranging from marked hypodiploidy to tetraploidy. Modal chromosome numbers in triploid region were more predominant, while at the lower end of the scale modal chromosome numbers of 38 and 44 were also observed. Among these 12 cases, the modal chromosome number was in the near-triploid region in 6 cases, in one case it was in near-tetraploid region and five cases showed near-diploidy as modal chromosome range (Table 3, Fig. 1). Karyotype analysis was carried out in good metaphases of the 12 patients. Some of the tumor cells with 46 chromosomes were proved to be pseudodiploid on karyotype analysis. Out of the 4 pseudodiploid metaphases analyzed, in three one chromosome from group C was missing and was replaced by an extra chromosome in group D. In the fourth pseudodiploid metaphase, a chromosome in group E was missing and compensated by an additional marker chromosome.

Deviations in the chromosome numbers from the normal 46 were due to losses and/or gains of chromosomes. The chromosomes which were lost frequently belonged to group A, whereas the chromosomes which were gained belonged to groups C, D, E, F and G. The karyotypic patterns in the 12 cases are given in Table 4.

Table 2. Chromosome counts in 12 patients with squamous cell carcinoma of the oral cavity

Case No.	Sex	Age	Histologic grade	No. of counted metaphases	Range of chromosome	Modal chromosome No.
1	male	66	I	4	45—46	46
2	female	65	I	8	44—65	65
3	male	66	I	7	38—47	38
4	male	68	II	7	45—49	46
5	male	62	II	6	51—70	68
6	female	63	II	8	52—69	69
7	male	58	II	9	53—62	59
8	female	69	II	7	46—72	46
9	male	67	III	6	48—76	76
10	female	70	III	11	72—92	92
11	female	67	III	5	42—50	44
12	male	64	III	5	41—77	72

Table 3. Distribution of modal chromosome numbers

Category	Chromosome range	Patients	
		No.	percentage
Near-diploid	35—57	5	41.7
Near-triploid	58—80	6	50
Near-tetraploid	81—103	1	8.3

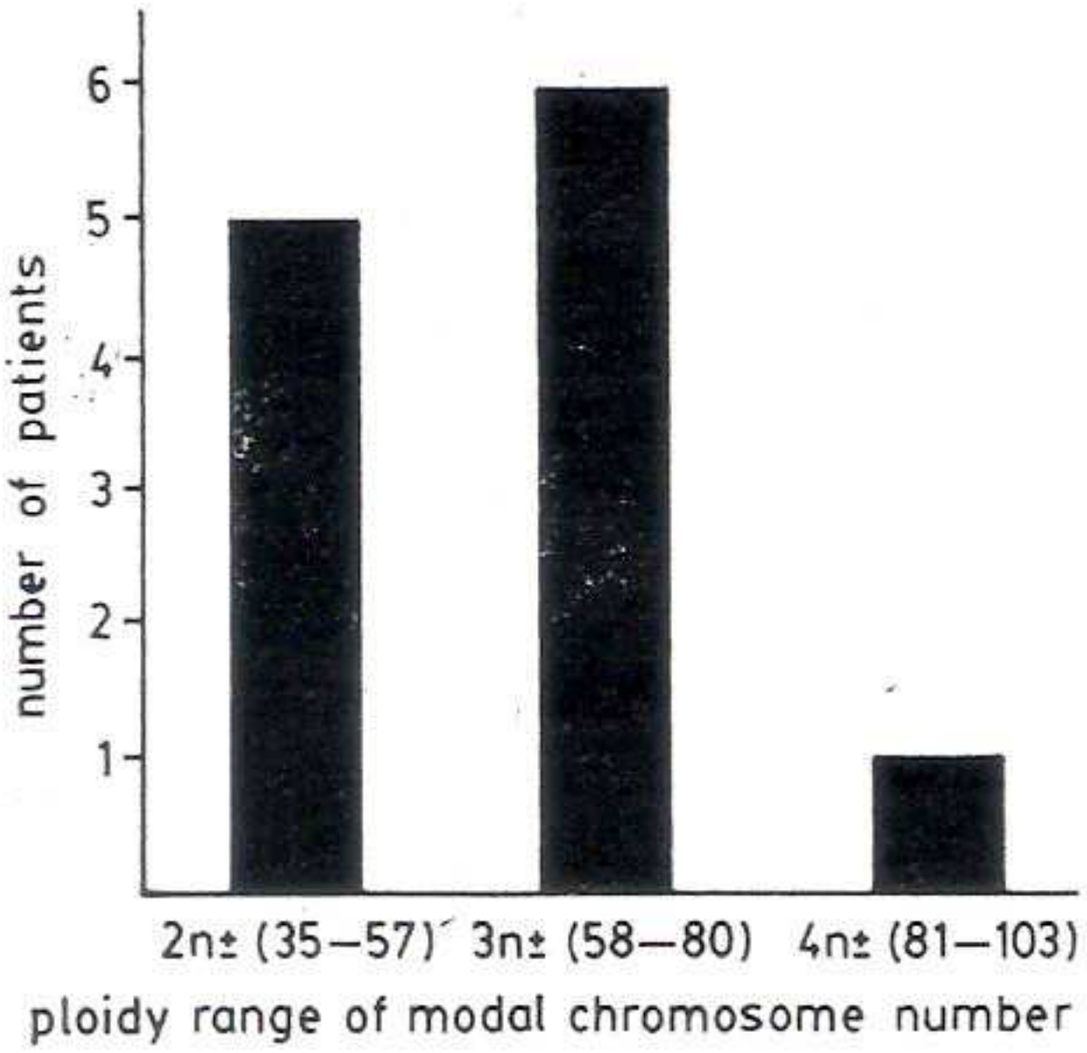

Fig. 1. Ploidy range of modal chromosome number in 12 cases of oral cancer.

Marker chromosomes were present in three patients. The morphology and number of markers differed in the three cases. One marker was acrocentric, of the size of group C chromosomes. Another marker was submetacentric of the size of group D chromosomes. The third type was very small metacentric marker, smaller than group G chromosomes.

Table 4. Karyotypic pattern in 12 cases of oral cancer indicating the total number of extra/missing chromosomes in each group

Patient S. No.	Sex	Modal karyotype	Total number of extra/missing chromosomes in groups									
			A	B	C	D	E	F	G	X	Y	Marker
1	male	46 XY										
2	female	65 XX			+9	+6	+3					+1
3	male	38 XY	—3	—1	—2			—2				
4	male	46 XY					—1					+1
5	male	68 XY			+9	+6	+3	+2	+2			
6	female	69 XX			+11	+7	+3	+2				
7	male	59 XY			+7	+3	+3					
8	female	46 XX			—1	+1						
9	male	76 XY			+13	+6	+6	+2	+2			+1
10	female	92 XXXX	+6	+4	+14	+6	+6	+4	+4	+2		
11	female	44 XX	—2									
12	male	72 XY			+11	+6	+5	+2	+2			

Discussion

Most tumors by the time they reach macroscopic size and demonstrate malignant characteristics display cytogenetic alterations, and these changes generally indicate a clonal growth pattern. Both in solid neoplasms and in leukemias, cytogenetic chan-

ges suggesting a clonal pattern of growth can be observed at all stages of the neoplastic process [3, 13, 20, 21, 25]. There are characteristic chromosome changes associated with some neoplasias, among them the Ph chromosome in chronic myeloid leukemia [24], and the chromosome No. 14 with an extra segment on the long arm in Burkitt's lymphoma [19].

Even though cytogenetic studies were carried out in 75 patients, we observed satisfactory chromosome spreads only in 12 cases. A significant percentage of oral cancer tissues had a very low mitotic index, and so it was very difficult to obtain adequate numbers of dividing cells for cytogenetic investigations. This may be the reason why reports on chromosome constitution of human oral cancer tissues are scarce in literature.

The tissues of the 12 cases of oral cancer showed extensive numerical chromosome changes, i.e. loss and/or gain of whole chromosomes. As a result, gross chromosome deviations from the diploid to tetraploid levels were encountered. The chromosomes which were lost frequently belonged to group A. Gains of chromosome numbers were observed in the autosomes belonging to the group C to G. However, sex chromosome were not at all involved in any of the cases. LEVAN and MITELMAN [16] in a survey of chromosome data on 15 types of tumors had reported that chromosome aberrations are non-random, but tend to cluster in 12 autosomal chromosomes of the 24 chromosome types of the human karyotype. In each of the tumors, only a few chromosomes were preferentially involved and 8 chromosomes were involved each with at least 3 disorders. It has been suggested [22] that the selectively involved chromosomes carry genetic material of importance to the normal development and/or proliferation of cells.

In our study, the distribution of chromosome numbers was generally broad, with aneuploid metaphases seen in 66% of the evaluable mitoses. Both hypodiploid and hyperdiploid populations were seen in the same specimen, suggesting a high incidence of aneuploidy and heterogenous DNA populations. Hypodiploid metaphases as a result of chromosome loss have been observed in 2 cases with modal chromosome numbers of 38 and 44, respectively. Loss of chromosomes can be explained by non-disjunction or chromosome lag at anaphase. The chromosomes which were lost mainly belonged to group A. Tumors with DNA values suggesting hypodiploidy to octoploidy and chromosome counts from 44 up to 80 per cell have been described [3, 8, 9, 18, 28]. Using microspectrophotometry, squamous cell carcinoma of the tongue and buccal mucosa were found to have considerable variations of DNA suggesting aneuploidy [8].

Most tumors exhibit a modal chromosome number which, however, may fall anywhere within a wide range, either around the diploid level or around the triploid--tetraploid range [10]. Out of the 12 cases in the present study, 6 cases showed near--triploidy as the modal chromosome range, 5 cases showed a near-diploid modal chromosome range and in one case it was near-tetraploid. These commonly observed near-triploid/triploid numbers could be achieved by a series of non-disjunctions or by a combination of chromosomal loss through non-disjunction and a complete doubling [1]. A very high chromosome number in the tetraploid range may be achieved by a doubling of the complete set. ATKIN [2] reported remarkable variation in the modal chromosome numbers in primary and metastatic cancers, ranging from marked hypodiploidy to more than 100 chromosomes.

The presence of one or more marker chromosomes is a noticeable feature of tumor metaphases [2]. We encountered marker chromosomes in three cases. The morphology

and number of the markers differed in the three cases. One marker was an acrocentric, of the size of group C chromosomes. Another marker was submetacentric of the size of group D chromosomes. The third type was a very small metacentric marker, smaller than the group G chromosomes. The presence of marker chromosomes in the three cases is an indication that structural changes also had actually occurred. The morphology of the chromosomes in oral cancer tissues was often fuzzy and less than optimal for a detailed analysis, so banding studies could not be carried out, and this prevented the identification of the origin of marker chromosomes. When the habits of these three patients who exhibited marker chromosomes were examined, no contributing facts emerged. These three patients had the same chewing habits like the other oral cancer patients included in the study who chewed natively processed tobacco leaves or tobacco stem along with betel leaves (*Piper betel*), arecanut (*Areca catechu*), and shell lime.

We observed no correlation between the chromosome abnormalities observed and the clinical stages of the disease. Chromosome abnormalities were encountered in all the stages of the disease.

Studies on both human and experimental animals suggest that viruses may play a role in the etiology of human cancers [15]. Infection of human diploid cells by oncogenic viruses (both DNA and RNA viruses) brings about chromosomal abnormalities [7]. With DNA viruses, chromosome changes progress toward hypodiploidy and hypotetraploidy, while RNA viruses cause an evolution toward trisomies and double trisomies [6]. In this study we have encountered chromosome numbers in the range of hypodiploidy to hypotetraploidy. This tempts us to postulate an association with DNA oncogenic virus, possibly Herpes simplex virus type-I (HSV-I) with oral cancer causing these wide range of chromosome abnormalities. Herpes simplex virus type I had been reported to produce lesions of the oral cavity [26]. KUMARI et al. [14. 15] reported an association between HSV-1 and oral cancer.

Many specific chromosomal lesions implicated in neoplasia can be correlated directly with known locations of specific oncogenes in the human genome. A majority of the DNA tumor viruses have genes that encode protein products that actively participate in the creation of a cancer cell and the transformed phenotype [27]. DNA tumor virus oncogenes act through or interact with the cellular protooncogenes to affect a transformation event [17]. Further studies on detection of oncogenes in the DNA of human oral cancer tissues and their association with chromosome abnormalities may help to unravel the mechanism of oncogenesis of oral cancer.

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