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Chromosome Abnormalities in Oral Squamous Cell Carcinomas

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Strong evidence in favour of the somatic mutation theory of cancer, which states that genomic rearrangements are early and essential events in tumour development, has during the past two decades been obtained from both cytogenetic and molecular genetic studies of neoplastic cells. More than 14 000 neoplasms with acquired clonal chromosome aberrations have been reported; the majority have, however, been haematological malignancies, whereas still little is known about the karyology of the quantitatively more important carcinomas. For oral squamous cell carcinomas (SCC), which constitute a substantial subset of human malignancies, only 63 short-term cultured tumours with karyotypic aberrations have been described. Simple numerical changes, mostly −Y, +Y, or +7, have been detected as the sole anomalies in 19 tumours, but these aberrations are probably not causally related to the neoplastic process. The remaining 44 SCC have had structural changes of varying complexity, often together with numerical aberrations. An assessment of the karyotypic imbalances resulting from these aberrations reveals that chromosomes 9, 13, 18 and Y are recurrently lost, and that deletions frequently involve chromosome arms 3p, 7q, 8p, 11q, 17p and the short arms of all acrocentric chromosomes. The chromosomal breakpoints in structural rearrangements frequently involve the centromeric regions of chromosomes 1, 3, 8, 14 and 15 as well as bands 1p22, 11q13 and 19p13. At least one of these bands has been rearranged in 70% of SCC with structural aberrations and they probably contain loci of importance in oral squamous cell carcinogenesis. A comparison of data obtained from oral and other types of SCC—laryngeal, oesophageal, lung, cervical, and anal canal—indicates that some of the events in the multistep process of SCC development involve the same genetic pathways irrespective of site of origin.
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INTRODUCTION

THE IDEA that neoplasia at the cellular level is a genetic disease goes back to the beginning of this century, when Theodor Boveri presented his book Zur Frage der Entstehung Maligner Tumoren [1]. Based on his own experiments and results reached by others, in particular by David von Hansemann who had described abnormal mitoses in carcinomas [2], he outlined the hypothesis that a neoplasm begins in one cell, i.e. it is monoclonal, and that transformation from normal to neoplastic is due to an acquired change in the cell's genetic make up, that can be seen as a chromosomal imbalance. With time, a vast amount of evidence has accumulated indicating that Boveri's hypothesis, now known as the somatic mutation theory of cancer, was essentially correct. Perhaps the most solid corroboration stems from cytogenetic analyses of neoplastic cells.

Attempts to test Boveri's ideas were for a long time hampered by technical difficulties, with mammalian tumour cytogenetics mainly being restricted to studies of direct preparations from highly malignant ascites tumours. With the use of colchicine to arrest dividing cells in metaphase and hypotonic solutions to improve spreading of metaphase chromosomes, and with the advent of improved tissue culture techniques during the 1950s, both normal and neoplastic human tissues became available for cytogenetic analyses. After the correct chromosome number of man was established in 1956, several investigators focused on congenital syndromes that long had been suspected to be caused by chromosomal abnormalities, and within a few years a large number of constitutional chromosome aberrations had been identified [3]. Although Nowell and Hungerford had already in 1960 described a characteristic chromosome aberration, the Philadelphia chromosome, in the neoplastic cells of patients with chronic myelogeneous leukaemia (CML) [4], a similar break-through in cancer cytogenetics did not occur until the early 1970s, when chromosome banding techniques were developed.

Chromosome aberrations in cancer

To date, over 14 000 neoplasms with clonal chromosome aberrations have been reported [5], and the knowledge derived from these data has been of great importance for our current understanding of neoplasia. It seems biologically justified to trichotomise cancer chromosome anomalies into primary aberrations, secondary aberrations, and cytogenetic noise [6, 7]. Primary abnormalities are non-random, are sometimes seen as the only deviation from the constitutional chromosome complement, and are considered early and essential events in carcinogenesis. It has been speculated that one consequence of the acquisition of a primary cancer chromosome rearrangement is increased genetic instability

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[8]. This in turn predisposes to new abnormalities, some of which will provide additional proliferative advantage. Such secondary aberrations are also non-random; their distribution depends on both the primary abnormality and the tumour type in which they occur, and possibly also on environmental factors such as what cytotoxic treatment the patient may have received. The acquired instability will also result in cytogenetic noise, random rearrangements with little or no selective value.

Primary rearrangements are non-randomly distributed throughout the genome. Mitelman and Heim compiled data from almost 10 000 cytogenetically aberrant neoplasms and found that the primary structural abnormalities clustered to only 71 out of the 329 chromosome bands [9]. The pinpointing of these breakpoints has been valuable for studies utilising molecular genetic techniques. One of the important observations is the connection between cellular oncogenes and cancer chromosome breakpoints. Not only do they cluster to the same chromosome bands [9], but it has been shown that some rearrangements may affect either the expression (quantitative activation) or the primary structure (qualitative activation) of oncogenes that are located at the breakpoints [10–12]. Since the detection of the Philadelphia chromosome in CML it has also become increasingly evident that different tumour types are associated with different chromosome rearrangements, some of which even appear to be pathognomonic [6, 7]. This could imply that the set of genes active in different cell types limits the number of rearrangements that are allowed to occur or to be expressed [10]. It has been hypothesised that in a structural rearrangement involving two chromosome bands, one band harbours a proliferation-determining gene, and the other, a gene associated with the differentiation of that particular cell type [13].

Due to the technical difficulties encountered in solid tumour cytogenetics in general, and in the analysis of epithelial tumours in particular, these tumour types account for less than 25% of the cytogenetically aberrant neoplasms so far studied with chromosome banding techniques [5]. The remaining karyologic data stem from haematological malignancies, which comprise a minority of human neoplasms. The recent improvements of short-term culture methods [14–16] have, however, allowed rapid progress also in solid tumour cytogenetics, and the conclusions drawn from haematological neoplasms seem to also hold here [17, 18]. Cytogenetic characterisation has been the first step in the recent identification of genes that seem to be pathogenetically involved in myxoid liposarcomas, thyroid carcinomas and Ewing's sarcomas [19–21].

CYTOGENETIC NOMENCLATURE

Chromosomes are classified according to their size, the location of the centromere, and the banding pattern along each arm [22, 23]. The autosomes are numbered from 1 to 22 in descending order of length; the sex chromosomes are referred to as X and Y. Each chromosome consists of a continuous series of alternating light and dark transverse bands. Each band can be individually designated by first listing the chromosome number, then the chromosome arm (the centromere divides the chromosome into a short "p" and a long "q" arm), the region (a region is an area delimited by specific landmarks), and the band number within the region. Thus 9q34 indicates chromosome 9, long arm, region 3, band 4.

In a karyotype the chromosome number is given first, followed by the sex chromosome complement and a description of the numerical and structural aberrations. Gains or losses of whole chromosomes are identified by a + or − sign before the chromosome number; increase or decrease in the length of chromosome arms is indicated by + or − after the chromosome arm symbol. The breakpoints are specified by their band position. The following abbreviations are used for the most common structural rearrangements:

add (addition) = additional material of unknown origin,
del (deletion) = loss of chromosome material,
der (derivative chromosome) = structurally altered chromosome,
dup (duplication) = doubling of a chromosome segment,
i (isochromosome) = mirror image chromosome consisting of two identical arms,
ins (insertion) = material from one chromosome is wedged into another chromosome or to another place within the same chromosome,
inv (inversion) = rotation of a segment 180°,
t (translocation) = exchange of material between two or more chromosomes.

CYTOGENETIC FINDINGS IN ORAL SQUAMOUS CELL CARCINOMAS

Because of the problems with initiating short-term cultures of epithelial neoplasms, existing data on oral squamous cell carcinoma (SCC) karyology is very limited. To date, cytogenetic analyses of short-term cultures from a total of 88 oral SCC have been reported. Twenty-five of these have displayed only normal karyotypes, probably representing normal epithelial cells or stromal fibroblasts which frequently overgrow neoplastic cells *in vitro*, whereas 63 have had clonal karyotypic aberrations [24–32].

Simple numerical changes

Simple numerical changes, mostly −Y, +Y, or +7, have been found as the sole anomalies in 19 tumours. Loss of the Y chromosome is frequently detected also in non-neoplastic tissues of elderly men, and is not likely to be related to the neoplastic process *per se*. Also, trisomy 7 has been found in short-term cultures of non-neoplastic brain, lung, and kidney tissue as well as in several types of pseudo-neoplastic disorders [33–37]. Most probably, the cells with +7 or +Y as the sole change do not belong to the tumour parenchyma and the pathogenetic role, if any, is unclear [37].

Structural rearrangements

Forty-four tumours have shown structural rearrangements of varying complexity: 28 of these have had pseudo- or near-diploid clones carrying relatively simple aberrations, whereas the remaining 16 tumours have had complex karyotypes with massive numerical and structural changes (a representative example is presented in Fig. 1). 26 of the 44 cases have had unbalanced structural rearrangements (with or without numerical changes) leading to loss or, less frequently, gain of chromosome segments (Fig. 2). Loss of whole chromosomes has mostly involved chromosomes 9, 13, 18, and Y, whereas partial deletions frequently have involved chromosome arms 3p, 7q, 8p, 11q, 17p, and the short arms of the acrocentric chromosomes, i.e. chromosomes 13–15 and 21–22. Gain of genetic material has been particularly common for chromosome arms 1q, 3q, 8q, and 15q.

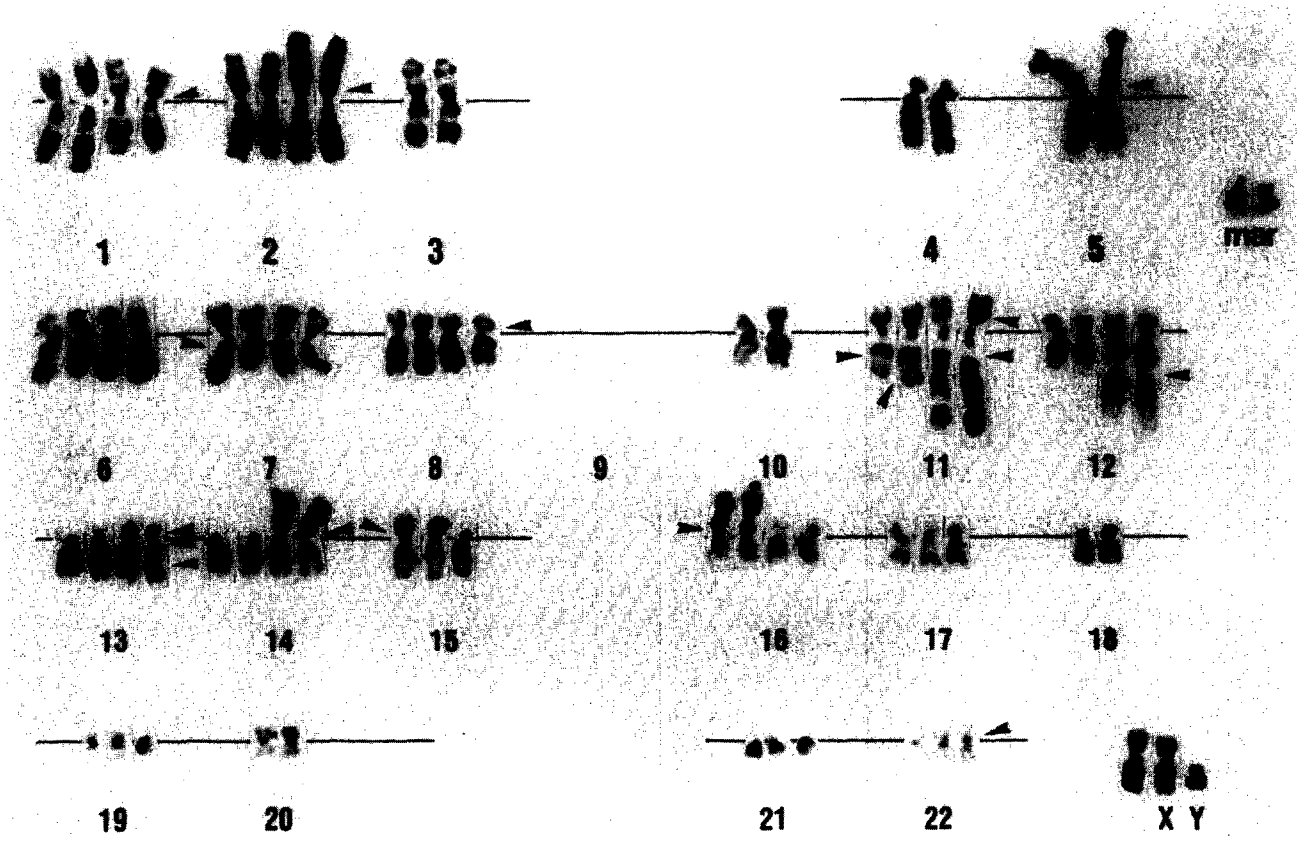

Fig. 1. Karyotype of a squamous cell carcinoma of the oropharynx showing multiple numerical and structural aberrations.

A map of the distribution of chromosome breakpoints in the 44 tumours with structural abnormalities (Fig. 3) shows that all chromosomes have been involved, but that several breakpoint cluster regions—here defined as bands or segments affected in at least six tumours—can be identified: 1p11–q11 (10 tumours), 1p22, 1q25 (six tumours each), 3p11–q11 (10 tumours), 8p11–q11 (nine tumours), 11q13 (nine tumours), 14p11–q11 (11 tumours), 15p10–p11 (10 tumours), and 19p13 (six tumours).

Clonal heterogeneity

Cytogenetically unrelated clones with completely different structural aberrations have been a recurrent feature of oral SCC, seen in 17 tumours [24, 26–28, 31, 32]. The unrelated clones have often had a pseudo- or near-diploid chromosome number (45–47 chromosomes) and have displayed simple balanced translocations.

IMPLICATIONS OF CYTOGENETIC FINDINGS

Loss of chromosome material

Chromosome deletions or losses are thought to be associated with functional loss of tumour suppressor genes [38]. Several such loci have been implicated in human tumours, including the *RB1* gene (13q14) in retinoblastoma, osteosarcoma and carcinomas of the breast, bladder and lung, *WT1* (11p13) in Wilms' tumour, *FAP* (5q21) in familial adenomatous polyposis and carcinoma of the colon, *TP53* (17p12–13.1) in various neoplasms, *DCC* (18q21) in carcinoma of the colon, *NF1* (17q11.2) in neurofibromatosis type 1 and *MEN1* (11q13) in tumours of the parathyroid glands, pancreas, pituitary and adrenal cortex [38,39]. It was initially thought that different loci were involved in different tumours, but it is now becoming clear that loss of these segments occurs in a variety of tumour types and may be of general importance in carcinogenesis. Loss of chromosomes 13 and 18 or material from 11q and 17p in oral SCC might thus be pathogenetically important through loss or inactivation of the tumour suppressor genes *RB1*, *DCC*, *MEN1* or *TP53*. The *TP53* gene is the only tumour suppressor gene that has been studied in oral SCC by molecular techniques and it seems that a high proportion of the tumours (14 out of 15) contains a mutated *TP53* gene [40].

Frequently rearranged bands

Rearrangements of chromosome 1 have been the most prevalent changes in oral SCC, observed in 27 tumours. Although the aberrations have been diverse and affected both the short and long arm, a clustering can be seen at bands 1p22, 1q25 and the centromeric region. The 1p22 aberrations, mostly found in cells with a relatively simple, pseudo- or near-diploid karyotype, could possibly lead to rearrangement of the oncogene *NRAS*, which maps to this band [41] and that has been shown to be amplified in a subset of head and neck SCC [42]. Breakpoints in unbalanced aberrations, mostly as part of a complex karyotype, cluster to bands 1p12–q11.

Structural aberrations involving chromosome 3, notably bands 3p11–q11, have been detected in 20 tumours, leading to loss of 3p material in 10. Deletions of 3p, with breakpoints clustering to bands 3p11–p12 and 3p21, are also frequent in head and neck SCC cell lines [43, 44]. Recent molecular studies have shown that loss of heterozygosity at two chromosomal loci—D3S3 (3p14) and RAF1 (3p25)—are common in head and neck SCC and nasopharyngeal carcinomas [45, 46].

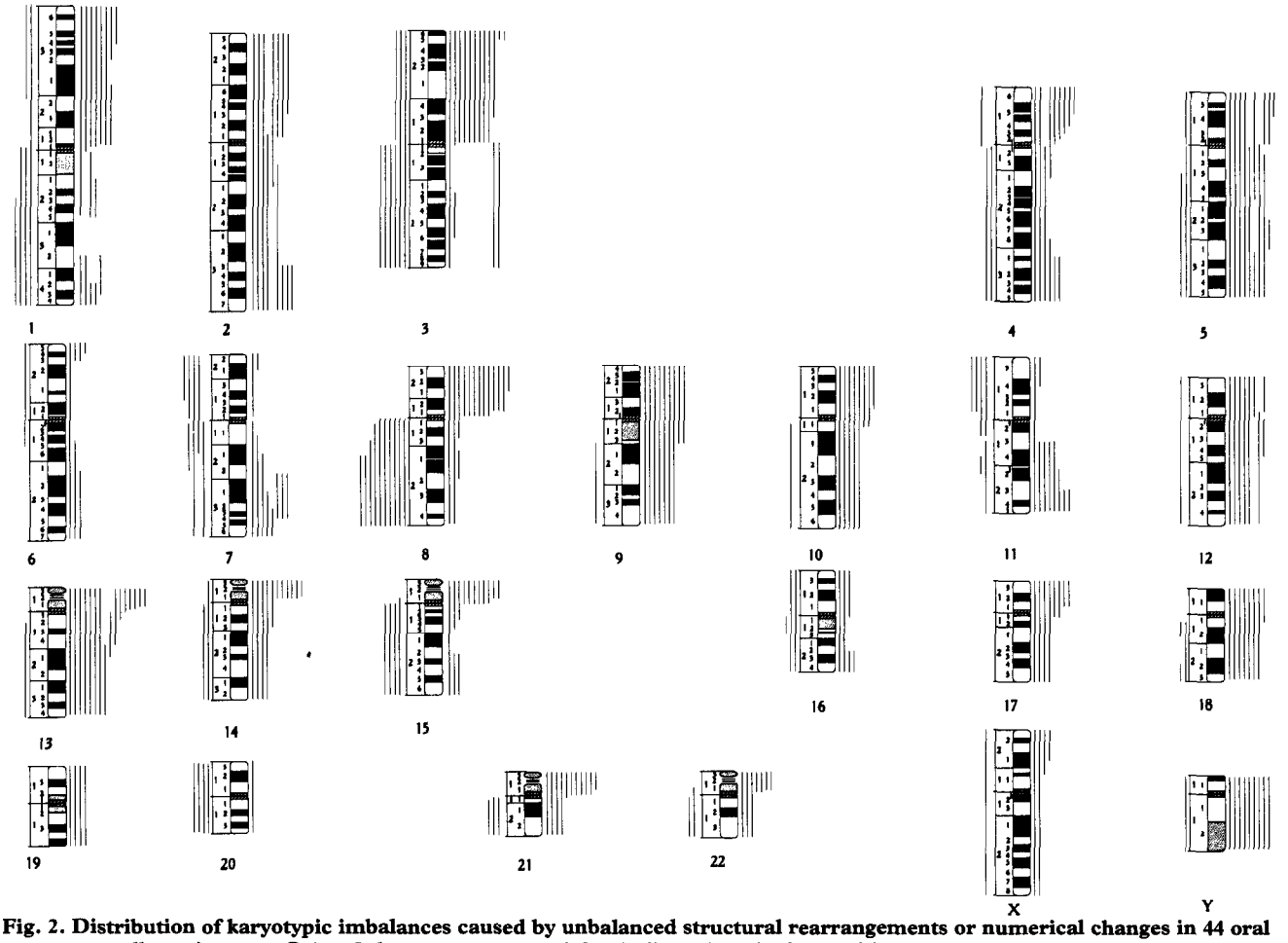

Fig. 2. Distribution of karyotypic imbalances caused by unbalanced structural rearrangements or numerical changes in 44 oral squamous cell carcinomas. Gain of chromosome material is indicated to the left and losses to the right of the chromosomes. Each bar represents one aberration.

Loss of material from 3p was first associated with small-cell lung carcinoma [47], but is now known to occur also in other histological subgroups of lung cancer [48–50]. More recently, deletions of 3p have also been demonstrated in a number of other malignancies, including renal cell carcinoma [51], ovarian cancer [52], and breast cancer [53, 54]. Both molecular genetic and cytogenetic data thus suggest that 3p deletions—possibly through loss of function of a tumour suppressor gene—may be a common tumorigenic event in the development of many epithelial malignancies.

An isochromosome i(8q) has been found in six oral SCC with complex karyotypes. The same abnormality is recurrent also in adenocarcinomas of the lung [5], but since it has never been detected as the sole aberration, it probably represents a secondary aberration. The formation of an i(8q) leads to gain of 8q and loss of 8p, and there are at present no good data to indicate which of the two is pathogenetically more important.

Chromosomal band 11q13 has been rearranged in nine of the 17 tumours with aberrations of chromosome 11. Structural rearrangement of 11q13 is associated with at least two other neoplasms; as t(10;11)(p14;q13–q14) in acute non-lymphocytic leukaemia and as t(11;14)(q13;q32) in lymphocytic malignancies [17]. In the latter setting, the rearrangement of 11q13 involves the putative oncogene *BCL1* [55]. A homogeneously staining region (hsr), a cytogenetic sign of gene amplification, has been found in this band in three oral SCC. In line with these observations is the recent molecular evidence that the oncogenes *BCL1*, *INT2*, and *HST1*, all of which map to band 11q13, are amplified in a subset of head and neck SCC [56–61].

Rearrangements involving band 19p13 have been found in six tumours. In three of them, they were part of a complex karyotype and described as add(19)(p13), i.e. the material added to 19p13 could not be identified. Other solid tumours known to have a 19p+ marker as a consistent anomaly are malignant fibrous histiocytoma [62] and ovarian cancer [52]. The aberration in these tumour types occurred together with numerous other chromosome changes, suggesting that it is a secondary change. The 19p+ marker in malignant fibrous histocytoma was found to be associated with an increased relapse tendency [63].

Cytogenetically unrelated clones

The finding of cytogenetic heterogeneity, shown as the presence of two or more unrelated clones, in a substantial subset of oral SCC could be interpreted in two different ways. Provided that the clonal aberrations are present in tumour parenchyma cells, either an invisible primary genetic alteration preceded the chromosomal aberrations implying that visible aberrations are secondary changes, or the unrelated clones reflect multicellular carcinogenesis within a cancer-prone epithelial field. Support for the latter interpretation would be the frequent observation of multicentric, synchronic or metachronic tumour development in the oral mucosa [64–69]. The

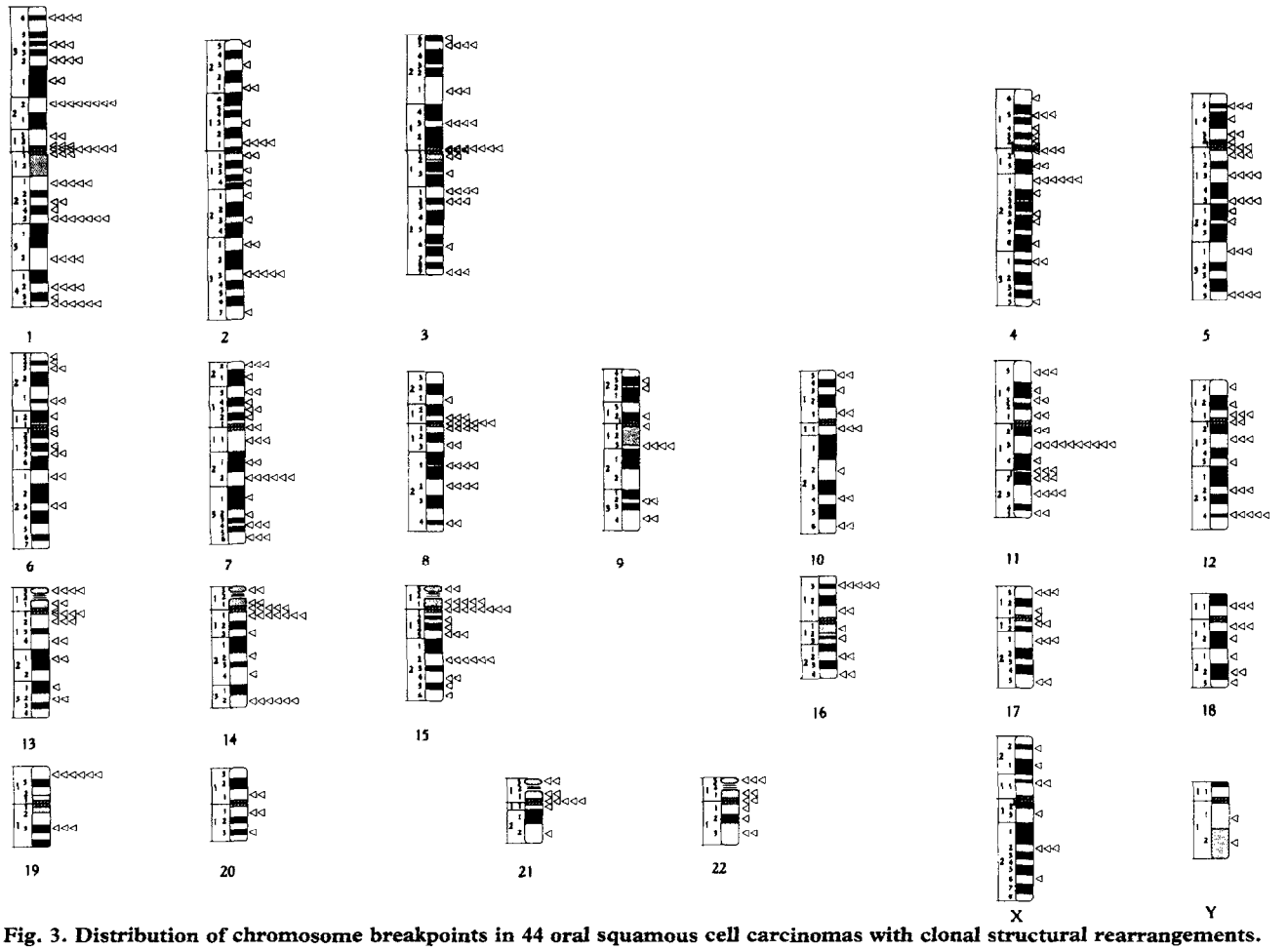

Fig. 3. Distribution of chromosome breakpoints in 44 oral squamous cell carcinomas with clonal structural rearrangements.

occurrence of multiple primary tumours may be regarded as a manifestation of a fundamental, general mucosal disturbance, "field cancerisation", in which a large epithelial field is predisposed to malignant transformation [64]. Experimental evidence in support of this hypothesis comes from findings in 7,12-dimethylbenz (α) anthracene (DMBA) and v-*ras* gene induced skin papillomas, a subset of which are polyclonal from the earliest morphologically recognisable stages [70,71]. Of importance in the pathogenesis of head and neck cancers is probably the massive exposure of the mucosa to a wide variety of ingested and inhaled exogenous carcinogens. Such exposure increases the probability that several cells will undergo mutational changes simultaneously.

A note of caution should, however, be added to this interpretation. The short-term cultures that are analysed mostly consist of a mixture of what appear to be epithelial and fibroblast-like cells. As a consequence, it is not possible to know if any given metaphase belongs to the stroma or to the tumour parenchyma. The possibility exists, therefore, that the chromosome changes may occur in subepithelial fibroblasts and not in the neoplastic cells. A recent examination of short-term cultures from non-neoplastic skin and pharyngeal mucosa revealed both small pseudo-diploid clones and non-clonal aberrations in metaphase cells that probably originated from subepithelial fibroblasts lending support to the argument that similar changes in oral SCC may not belong to the tumour parenchyma [72]. Further support for this interpretation may be provided by our recent finding that different culture media favour proliferation of different cell populations [28]. By using a chemically defined serum-free medium, which favours the growth of epithelial cells, the frequency of aneuploid clones with complex aberrations has increased, whereas the frequency of pseudo- or near-diploid clones and unrelated clones has decreased compared with the results obtained using a serum-containing RPMI 1640 medium, which stimulates the growth of fibroblasts. If the small pseudo- or near-diploid clones are not neoplastic, then it remains to be determined whether the rearrangements are caused by the tumour or whether they are the consequences in stromal cells of the carcinogenic exposure that in the neighbouring mucosal epithelial cells caused a malignant tumour.

CYTOGENETIC SIMILARITIES BETWEEN ORAL AND OTHER SCC TYPES

Laryngeal SCC is another group of head and neck SCC that has become accessible to cytogenetic analysis through the recent advances in cell culture technique. Clonal chromosome aberrations have so far been described in a total of 26 tumours, 23 of which have had clonal structural changes [27, 28, 30, 32, 73–76]. Six of them have displayed markedly complex karyotypes, with hsr in band 11q13 in one of them [27]; the rest have had pseudo- or near-diploid, often multiple unrelated, clones. Chromosome bands 1p22, 2q13, 5q13 and 14p11 have been recurrently involved in structural rearrangements. Of these breakpoints only 1p22 and the centromeric region of chromosome 14 are shared with oral SCC.

Karyotypic information on oesophageal SCC has so far been restricted to only five primary tumours and 14 cell lines

[77, 78]. All karyotypes have been complex and have shown great heterogeneity, but some genomic sites nevertheless seem to be non-randomly involved, namely 1p11–q11, 1q31, 3p11–12, 3p14, 11q11–q12, 15p11–q11 and 21q11. The 11q aberrations in oesophageal SCC have often been mapped to 11q11–q12, in contrast to 11q13 in oral SCC. It is possible, therefore, that different genes in 11q11–q13 are affected in the two types of SCC. However, cytogenetic evidence of gene amplification, i.e. hsr located to bands 11q12–q13 has been detected in both tumour types, and also in oesophageal SCC molecular evidence indicates that the oncogenes *INT2* and *HST1* are repeatedly amplified [58,61]. The cytogenetic similarities between oral and oesophageal SCC are further accentuated by the high frequency of aberrations leading to loss of 3p material through deletions or unbalanced translocations at bands 3p11–p12, rearrangements of the centromeric region of chromosome 1, and the frequent centric fusions that are seen between the acrocentric chromosomes.

A review of the cytogenetic data on lung, cervical and anal canal SCC indicates that also these SCC are cytogenetically similar to oral SCC. Structural aberrations involving chromosomal bands or regions 1p12–q11, 1p22, 3p11–p14, 11p13–p15, 11q11–q14 and 15p13–q11 are common [5]. Involvement of these genomic regions is not specific for SCC, however. They have also been observed in other types of epithelial carcinomas, e.g. adenocarcinoma of the breast, transitional cell cancer of the bladder, and non-SCC lung cancer, indicating that some of the events during multistep epithelial tumorigenesis are the same, regardless of the site of origin or the type of carcinoma.

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