Chromosome Abnormalities in Eighty-three Head and Neck Squamous Cell Carcinomas: Influence of Culture Conditions on Karyotypic Pattern

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ABSTRACT

Short-term cultures from 115 squamous cell carcinomas (SCC) of the head and neck were cytogenetically investigated. Thirty-six of the tumors have been reported previously, whereas 79 are new cases. The material was divided into two series based on the medium used. The 80 tumors of series I were cultured in RPMI 1640 supplemented with fetal calf serum, glutamine, antibiotics, insulin, cholera toxin, and epidermal growth factor. The 35 tumors of series II were cultured in a chemically defined, serum-free medium with a low calcium concentration, MCDB 153, which stimulates epithelial growth while inhibiting fibroblasts. A total of 83 tumors with clonal karyotypic abnormalities were detected in the two series. Series II had a higher proportion of tumors with complex karyotypic changes than series I (43% versus 15%), a lower proportion of tumors with pseudo- or neardiploid clones characterized by simple rearrangements (3% versus 34%), and a lower frequency of unrelated clones (3% versus 24%), indicating that the different culture conditions favored growth of different cell populations. Except for rearrangements of Ip22, which were mainly found in series I, the distribution of breakpoints in structural aberrations was similar in the two series and clustered to seven chromosome arms: 3p, 7q, 8p, 11q, 13p, 14p, and 15p. Unbalanced structural aberrations were more common in series II, frequently leading to loss of segments from chromosome arms 3p, 7q, 8p, 11q, 13p, 14p, and 15p, whereas gain of genetic material often involved chromosome arms 1q, 3q, 8q, and 15q.

INTRODUCTION

Head and neck SCC3 represent 2–3% of all malignant tumors in humans but approximately 95% of all malignancies of the head and neck region. Because of the technical difficulties encountered in solid tumor cytogenetics in general, and in the analysis of SCC in particular, published data on head and neck SCC with clonal structural abnormalities are limited to only 49 cases (1–11).

Cytogenetic analyses of short-term cultures of head and neck tumors have with few exceptions revealed small, pseudodiploid clones characterized by simple balanced rearrangements, multiple unrelated clones, and a high frequency of cells displaying non-clonal structural aberrations. The findings are at odds with those unrelated clones, and a high frequency of cells displaying non-clonal structural aberrations. The findings are at odds with those of other clones characterized by complex karyotypic changes (12–15). The findings in short-term cultured SCC also differ from what has been the general pattern in most other cytogenetically analyzed neoplasms, in which histological subtypes are found to be associated with specific chromosome changes, the cytogenetic findings indicate a monocellular tumor origin, and the complexity of the aberrations (in particular the level of aneuploidy) is correlated with malignancy grade (16, 17).

The above considerations, the discrepancy between the short-term culture chromosome findings and data obtained from flow cytometric DNA content studies (18–22), and the occasional identification of tumors with massively rearranged karyotypes all give rise to the following question. "Do the pseudodiploid clones really belong to the tumor parenchyma?" A recent examination of short-term cultures from nonneoplastic skin and pharyngeal mucosa revealed both clonal and nonclonal aberrations in metaphase cells that probably originated from subepithelial fibroblasts, casting further doubt on the importance of the small, pseudodiploid clones (23).

In the present study, we utilized a new culture technique including a chemically defined, serum-free medium with low calcium concentration, MCDB 153, to culture 35 SCC of the head and neck. We compare the results with the findings obtained from 80 tumors examined after short-term culturing in a conventional, serum-containing medium.

MATERIALS AND METHODS

The material we present includes all 115 head and neck SCC that we have analyzed during 5 years (1987–1991). Samples obtained at surgery or from diagnostic biopsies were divided in two parts: one for histopathological examination; the other for cytogenetic analysis. Tumor location and histopathological differentiation of the 50 new cases with clonal chromosome aberrations (see below) are summarized in Tables 1 and 2. There were 47 primaries and 3 recurrences (cases 30, 36 and 41). The two latter were the only patients who had received genotoxic treatment prior to cytogenetic analysis. All tumors were classified according to International Union Against Cancer Criteria (24).

Eighty tumors (series I) were cultured according to previously described procedures (2), 36 of these (33 with karyotypic aberrations) have already been published (2–8). In brief, the fresh samples were minced, disaggregated overnight in collagenase, and plated on collagen-coated chamber slides in RPMI 1640 with 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid buffer and supplemented with fetal calf serum, glutamine, antibiotics, insulin, cholera toxin, and epidermal growth factor. The in situ preparations were harvested after 5–10 days. Before banding with Wright's stain, the slides were aged overnight at 60°C followed by submersion in 2× standard saline-citrate at 60°C for 3–4 h.

The remaining 35 tumor specimens (series II) were cultured in a chemically defined medium (25). Techniques for tumor disaggregation, in situ harvesting, and staining were the same as described above. The modifications lay in the culture medium and its supplementation, which were as follows.

1. The basic growth medium was MCDB 153. Dissolve powder (17.7 g) in 900 ml tissue culture grade water on a magnetic stirrer.
2. Add 1.176 g sodium bicarbonate and, when all is dissolved, adjust pH to 6.4–6.6 with 1 M HCl or 1 M NaOH. Add water to 1000 ml and sterilize by filtering.
3. Supplement with (final concentrations given): 10 ng/ml epidermal growth factor; 6 μg/ml insulin; 0.4 μg/ml hydrocortisone; 0.1 μg/ml ethanolamine; 0.1 μg/ml phosphoethanolamine; 0.23 mg/ml l-glutamine; 100 IU/ml penicillin; 0.2 mg/ml streptomycin; 2.5 μg/ml amphotericin B.

The clonality criteria and the description of karyotypes were according to the International System for Human Cytogenetic Nomenclature (1991) (26).
RESULTS

Cytogenetic Findings in the 79 New Cases of Head and Neck SCC. A total of 50 tumors revealed clonal chromosome abnormalities (Tables 1 and 2). Clonal structural abnormalities were found in 25 tumors (9 in series I, 16 in series II). Simple numerical aberrations as the only clonal change were seen in 25 tumors (18 in series I, 7 in series II). The remaining 29 tumors (17 in series I, 12 in series II) had a normal chromosome complement.

Among the 25 SCC with simple numerical aberrations only, loss of the Y chromosome was the most common change, found in 20 of the 21 male patients. It was the sole anomaly in 14 cases and was found together with other simple numerical changes in another 6. In addition, –Y was also present in 11 tumors with clonal structural aberrations. In four tumors, loss of the Y chromosome was seen in clones with complex karyotypes. The second most common simple numerical aberrations were gain of the Y chromosome and trisomy 7, each found as the only change in a clone in six tumors. In addition, +Y and +7 were present as part of a complex karyotype in another one and four cases, respectively. The remaining simple numerical changes were gain of an X chromosome in three tumors and trisomy for chromosomes 3 and 18 in one tumor each.

Of the 25 SCC with clonal structural chromosome abnormalities, 9 (eight of which in series I) had simple (less than 4 aberrations per clone) balanced aberrations. Cytogenetically unrelated clones were seen in five of these cases. The remaining 16 tumors (1 in series I, 15 in series II) displayed complex karyotypes (at least 4 aberrations per clone) and were often highly aneuploid. The modal number in these tumors was hypodiploid in one, diploid in four, hyperdiploid in one, neartriploid in five, hypertriploid in two, and hypotetraploid in three tumors. Representative karyotypes from two such tumors (cases 35 and 38) are shown in Fig. 1. The chromosomal bands most frequently involved in aberrations were all centromeric or near-centromeric: 1p11, 3p11, 8q10, 14q10, and 15q10. Except for one case with rearrangement of 3p11 in series I, all tumors with these breakpoints were found in series II. An isochromosome i(8q) was detected in four tumors. Cytogenetic evidence of gene amplification, hsr, was found in three tumors. A hsr was located in 11q13 in two tumors and in 1q11 and 2p11 in one tumor each (in case 45, one hsr was located in 2p11 and another in 11q13).

Influence on Karyotypic Pattern of Differences in Tissue Culture Technique. In order to estimate fully the effect of the use of different culture media, we now expand the data base by adding to series I the cytogenetic findings of the 36 tumors (33 of them karyotypically abnormal) that we have published previously (2–8). The major karyotypic characteristics of the two series are presented in Table 3. Three major differences are apparent: the frequency of clones with simple structural aberrations was higher in series I (34%) than in series II (3%); the frequency of complex, often markedly aneuploid karyotypes was higher in series II (43%) than in series I (15%); and the frequency of unrelated clones was higher in series II (24%) than in series I (3%).

DISCUSSION

Simple numerical aberrations, mostly –Y, +Y, and +7, were found as the sole anomalies in both series in a total of 23 tumors. Numerical sex chromosome aberrations and +7 have recently been described in short-term cultures of nonneoplastic brain, lung, and kidney tissue (27–30). It is therefore possible that the clones with –Y, +Y, and +7 as the sole anomalies are not representative of the tumor parenchyma. We favor the interpretation that they represent nonneoplastic cells, be they epithelial, stromal fibroblasts, or inflammatory cells (31).

Unbalanced structural rearrangements and numerical changes coexisting with structural aberrations were more frequently detected in series II than in series I, and loss of chromosome material occurred more often than gain. Chromosomes 9, 12, 13, 8, and Y were the ones most frequently lost, whereas deletions of chromosome segments...
often involved chromosome arms 3p, 7q, 8p, 11q, 13p, 14p, and 15p (more than six cases). Gain of genetic material was most often seen in chromosome arms 1q, 3q, 8q, and 15q (in at least four tumors).

The distribution of chromosomal breakpoints corresponding to the structural aberrations of our combined series (23 new and 30 previously published cases) is depicted in Fig. 2. With the exception of band 1p22, which was almost exclusively rearranged in series I, the distribution of breakpoints was similar in the two series. The chromosomal bands most frequently involved were 11q13 (12 tumors), 1p22 (11 tumors), 1p11-1p12 and 3p11-q11 (10 tumors each), 5q13 (9 tumors), 1q25 and 15q10 (8 tumors each), and 8q10 (7 tumors).

Aberrations involving 11q13 were seen in all 7 tumors with complex karyotypes in series I (in two of them as a hsr) and in 2 of the 12 tumors carrying complex karyotypes in series II (in both cases as a hsr). Structural abnormalities affecting band 11q13 have been reported in a variety of other human tumors (32) and have also recurrently been found in head and neck SCC cell lines (14, 15, 33). The new data corroborate our previous conclusion (8) that band 11q13 is nonrandomly involved in head and neck SCC and contains loci of importance in the pathogenesis of these tumors. At least nine genes have been mapped to band 11q13 (32). Among them, oncogenes BCL1, INT2, and HST1 have recently been shown to be amplified in subsets of head and neck SCC (34-38). Whether this really is the pathogenetically essential correlate to the cytogenetic evidence of amplification we observe, or if some other gene is of the essence, is still unknown.

Chromosome 1 was involved in structural rearrangements in 32 tumors, with a clustering of breakpoints to bands 1p22, 1p11-1p12, and 1q25. Involvement of chromosome 1 in structural rearrangements is common in a number of different tumors (39) and is generally considered to be acquired during tumor progression. It may be worthy of note that the breakpoints of chromosome 1 involved in unbalanced aberrations seemed to cluster to the pericentromeric region (bands Ip22, Ip11-1p12, and Iq25). Involvement of chromosome 1 in structural rearrangements occurs also in the other histological subgroups of lung cancer (41-43).

Unbalanced aberrations affecting chromosome region 3p11-q11 were detected in 10 tumors, leading to loss of 3p material in 7 tumors. Deletions of 3p, with breakpoints clustering to 3p11, 3p12, and 3p21, have also been detected in a high frequency of head and neck SCC cell lines (13, 15). Loss of material from 3p was originally described as a specific change in small-cell lung carcinomas (40) but it is now known to occur also in the other histological subgroups of lung cancer (41-43).

More recently, deletions of 3p have been reported in a number of other malignancies, including renal cell carcinoma (44), mesothelioma (45), ovarian cancer (46), and breast cancer (47, 48). It is evident that
loss of 3p material constitutes a common tumorigenic event in the
development of many malignancies.

An identical anomaly, i(8), was shared by six tumors (four in series
II, two in series I) with a complex karyotype. The same abnormality
has repeatedly been reported in adenocarcinomas of the lung and other
solid tumors (39). The formation of such an isochromosome leads to
gain of 8q and loss of 8p, and there are no good data to indicate which
of the two is pathogenetically more important.

Robertsonian translocations were frequent in tumors with markedly
aneuploid karyotypes. They involved chromosome 15 in eight tumors,

\[\begin{array}{cccccc}
1 & 2 & 3 & 4 & 5 & 6 \\
7 & 8 & 9 & 10 & 11 & 12 \\
13 & 14 & 15 & 16 & 17 & 18 \\
19 & 20 & 21 & 22 & X & Y \\
\end{array} \]

Fig. 1. A, karyotype of case 35 in series II showing a hypodiploid clone with complex changes. B, karyotype of case 38 in series II showing a hypotetraploid clone with massive
rearrangements. Arrowheads, breakpoints.

| Table 3 Comparison of main karyotypic features of head and neck squamous cell
<p>| canceromas cultured in serum-containing (series I) and chemically defined (series II) growth medium |</p>
<table>
<thead>
<tr>
<th>Karyotype pattern</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series I ((n = 80))</td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>20 (25)*</td>
</tr>
<tr>
<td>Simple numerical changes only</td>
<td>21 (26)</td>
</tr>
<tr>
<td>Structural aberrations</td>
<td>39 (49)</td>
</tr>
<tr>
<td>I. Complex clones</td>
<td>12 (15)</td>
</tr>
<tr>
<td>II. Simple clones</td>
<td>27 (34)</td>
</tr>
<tr>
<td>Cytogenetically unrelated clones</td>
<td>19 (24)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of cases.
in four as der(15;21). An identical der(15;21) has been described in seven tumors of six different histologies (39).

The most striking cytogenetic differences between series I and series II were the higher frequency of complex, markedly aneuploid clones in series II and the higher frequency of pseudo- or near-diploid stemlines in series I (Table 3). This shows that different culture conditions favor proliferation of different cell populations. The chemically defined, serum-free MCDB 153 medium used in series II favors...
growth of markedly aneuploid cell populations, which must correspond to tumor parenchyma cells. On the other hand, serum-supplemented RPMI 1640 seems to favor the proliferation of pseudo- or near-diploid cells. A recurring feature in these tumors was the occurrence of cytogenetically unrelated clones, present in 24% of the tumors of series I compared with 3% in series II. Such multiclonality has also been a recurrent finding in short-term cultures of skin tumors (49–53). If the clonal aberrations are present in neoplastic cells, then either the primary genetic alterations are submicroscopic whereas the visible aberrations are secondary or the findings indicate multiclonal carcinogenesis within a cancer-prone epithelial field (54, 55). However, another explanation would be that the clonal aberrations, most of them simple balanced translocations, are not representative of the tumor parenchyma at all but are instead present in stromal fibroblasts or other subepithelial cells.

We have previously pointed out (7) that the short-term cultures examined in series I consisted of a mixture of what appeared to be both epithelial and mesenchymal cells (Fig. 3). Use of the chemically defined medium yielded better growth of epithelial-like cells with simultaneous inhibition of the fibroblast-like cells (Fig. 4). Cultures dominated by fibroblast-like cells usually had a normal karyotype or pseudodiploid clones. In all four cases that had pseudodiploid clones with structural changes in series II, the cultured cells looked like fibroblasts. Two of these tumors were recurrences (cases 36 and 41) and the patients had received genotoxic therapy for the primary tumor, lending further support to the interpretation that the pseudodiploid clones reflect genomic damage suffered by cells other than those belonging to the tumor parenchyma.

The serum-supplemented RPMI 1640 that we used in series I allows stromal fibroblasts to overgrow the tumor parenchyma cells. Not only does a high serum content stimulate fibroblasts but also some of the major growth factors in the serum, such as platelet-derived growth factor, may promote differentiation and inhibit proliferation of epithelial cells. The MCDB 153 medium, on the other hand, favors the growth of keratinocytes while inhibiting fibroblast growth. The latter effect is accounted for by the absence of serum and by the low concentration of calcium (30 times lower than in ordinary media). That the level of extracellular calcium can have dramatic effects on the growth and differentiation of epithelial cells has been demonstrated repeatedly (56–59). In the cytogenetic context, the major disadvantage of the serum-free medium is that sometimes the technical quality of the metaphase chromosomes is lower. Supplementation with 5% fetal bovine serum 2–3 days before harvesting may increase the mitotic activity of the tumor cells and improve chromosome quality. Another disadvantage of this serum-free medium is that normal epithelial cells may grow better in vitro than the tumor cells. It is therefore important to harvest the cultures as early (3–5 days) as possible, preferably without passing.

REFERENCES


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