Distinct Patterns of Chromosomal Alterations in High- and Low-Grade Head and Neck Squamous Cell Carcinomas

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Abstract

Comparative genomic hybridization was performed on 30 primary head and neck squamous cell carcinomas. Fractional or entire DNA loss of chromosome 3p was a basic finding that occurred in 29 cases (97%). Additional DNA underrepresentations were observed in more than 50% of the cases on chromosomes 1p, 4, 5q, 6q, 8p, 9p, 11q, 13q, 18q, and 21q. Deletions on chromosomes 3p, 13q, and 17p were confirmed by loss of heterozygosity analysis. Entire or partial DNA copy number increases were identified for chromosome 3q in 26 cases (87%) with high-level amplifications at 3q24 and 3q27-pter. Overrepresentations were found in decreasing order of frequency at 11q13 (70%), 8q (57%), 19q (50%), 19p (47%), and 17q (47%). The use of comparative genomic hybridization superkaryograms of the group of well-differentiated carcinomas (G1) indicated that the deletions on chromosomes 3p and 9p along with the overrepresentation of 3q are associated with early tumor development. Accordingly, the undifferentiated tumors (G2) were characterized by additional deletions on chromosomes 4q, 8p, 11q, 13q, 18q, and 21q and overrepresentations on 1pter, 11q13, 19, and 22q, suggesting that these changes are preferentially associated with tumor progression.

Introduction

Tumors of the head and neck represent 6% of all cancers, of which 90–95% are squamous cell carcinomas. The overall 5-year survival rate for patients with HNSCCs is among the lowest of the major cancer types and has not improved dramatically during the last decade. The lack of progress in head and neck oncology emphasizes the importance of molecular genetic studies to define alterations that may correlate with tumor behavior. Although a considerable amount of cytogenetic and molecular genetic data on HNSCC have been accumulated during the last years, the picture how the genetic alterations interfere with the different steps of tumor progression is still incomplete. In a recently proposed model of tumor progression of HNSCC, deletions on chromosome 3p that were located at 3p23 (D3S647), 3p24 (THRB), and 3p26 (D3S2358) were identified for chromosome 3q in 26 cases (87%) with high-level amplifications at 3q24 and 3q27-pter. Overrepresentations were found in decreasing order of frequency at 11q13 (70%), 8q (57%), 19q (50%), 19p (47%), and 17q (47%). The use of comparative genomic hybridization superkaryograms of the group of well-differentiated carcinomas (G1) indicated that the deletions on chromosomes 3p and 9p along with the overrepresentation of 3q are associated with early tumor development. Accordingly, the undifferentiated tumors (G2) were characterized by additional deletions on chromosomes 4q, 8p, 11q, 13q, 18q, and 21q and overrepresentations on 1pter, 11q13, 19, and 22q, suggesting that these changes are preferentially associated with tumor progression.

Materials and Methods

Tumor Samples. Tumor specimens were obtained from surgical resections of 30 primary HNSCCs treated at the Department of Otorhinolaryngology of the Humboldt-University Berlin. The tumors comprised 13 laryngeal carcinomas, 3 carcinomas of the oral cavity, and 7 carcinomas each of the oropharynx and hypopharynx. Grading was performed according to the criteria defined by Ammeroth et al. (3). The majority of the tumors showed a moderate differentiation (G3). Three tumors were highly differentiated (G1), whereas five were undifferentiated (G2). The specimens were fresh-frozen in liquid nitrogen within 1 h after operation. DNA was extracted from several 30-µm cryostat sections by proteinase K and phenol-chloroform extraction, which was verified to consist of a minimum of 70% tumor cells in each case.

CGH and Digital Image Analysis. CGH, image acquisition, and digital image analysis were performed as described previously (4). Briefly, the tumor DNA was labeled by nick translation with biotin-dUTP and the normal DNA with digoxigenin-dUTP (Boehringer Mannheim, Mannheim, Germany). In combination with human Cot1 DNA, both genomes were hybridized in equimolar amounts to normal metaphase chromosomes prepared from peripheral blood lymphocytes. After 3 days of hybridization at 37°C, the tumor and the normal DNA were specifically detected by fluorescein-avidin (Vector Laboratories, Burlingame, CA) and antidigoxigenin-rhodamine (Boehringer Mannheim, respectively), whereas the chromosomes were counterstained with DAPI. All hybridizations were performed sex neutral, i.e., tumor DNA, reference DNA, and metaphase chromosomes were either derived from male or female donors. The quality of the metaphase spreads was controlled as described by Kallioniemi et al. (5).

Three fluorescence images per metaphase were acquired on an Axioskop epifluorescence microscope (Zeiss, Oberkochen, Germany) using selective filter sets for DAPI, FITC, and TRITC. They were stored as 8-bit gray level images under the TIFF format. Digital image analysis was performed using a CGH program that was developed in our laboratory (6). Briefly, the three fluorescence images of each metaphase were superposed and a ratio (FITC/TRITC) image was calculated. The chromosomes were arranged to a CGH karyogram. By averaging at least 10 metaphases/karyograms, a CGH sum karyogram with mean ratio chromosomes and mean ratio profiles including the 95% confidence interval was produced per case. If the mean ratio profile exceeded the monosomy or trisomy thresholds, i.e., fluorescence ratios of 0.75 and 1.25 corresponding to the theoretical value of a monosomy/trisomy in 50% of the tumor cells of an otherwise diploid tumor, it was considered as a deletion or overrepresentation, respectively (7). An overrepresentation was defined as a high-copy amplification if the fluorescence ratio exceeded at least the value of 1.5. In an extension of the concept, tumor subgroups were defined by averaging the CGH sum karyograms to superkaryograms.

Detailed information about the CGH preparation and the digital image analysis are available on our web server (http://ambo.rz.charite.hu-berlin.de/cgh).

LOH Analysis. Paired samples of tumor and normal DNA were assessed for allelic loss by microsatellite polymorphism analysis. Three different markers on chromosome 3p that were located at 3p23 (D3S5647), 3p24 (THRB), and...
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3p25 (D3S1110), two markers at the Rb locus at 13q14 (D13S788, D13S887), and two markers near the p53 locus at 17p13 (TP53, D17S513) were investigated. The primer sequences and the PCR conditions were extracted from the Genome Data Base via Internet (http://gdbwww.gdb.org/). The microsatellite polymorphisms were assessed by nonradioactive detection (8).

Results

The summary of all alterations is shown in Fig. 1. Vertical lines on the left side of each chromosome idiogram demonstrate loss of genetic material in the tumors, whereas those on the right side correspond to overrepresentations. Amplification sites are represented as solid bars.

DNA Losses. Chromosome 3 was most frequently affected and showed deletions in 29 cases. DNA underrepresentations of the entire short arm occurred in 15 cases. Fourteen tumors exhibited interstitial deletions with minimal nonoverlapping regions at 3p11-12, 3p14, 3p21-22, and 3p24-pter.

Deletions were frequently observed on chromosome 9p (22/30 cases), 9q (21/30 cases), 13q (20/30 cases), and 18q (19/30 cases). For chromosome 9p, the deleted region included in 18 cases the chromosome band 9p21, which harbors the p16 TSG. For 13q, CGH analysis indicated deletions at the Rb locus 13q14 in 12 cases.

Chromosomes 4, 6q, and 21q were deleted in 60% (18/30). Further deletions with an incidence above 25% were observed in decreasing order of frequency at the following chromosomal locations: 8p (16 cases), 11q (16 cases), 1p (15 cases), 11p (14 cases), 2q (13 cases), 14q (10 cases), 12q (9 cases), 17p (9 cases), and 10q (9 cases).

DNA Gains. Overrepresentations of the 3q arm were found in 26 tumors. Seven high-copy amplifications were mapped to two nonoverlapping regions at 3q24 and 3q27-pter. In eight cases, an overrepresentation of the entire chromosomal arm 3q was accompanied by the loss of the entire chromosome 3p, suggesting the formation of a 3q isochromosome.

We observed overrepresentations at 11q13 in 20 cases. DNA gains were seen less frequently on chromosomes 8q in 17 cases, 19q in 15 cases, and 19p and 17q in 14 cases each. Other copy number increases with an incidence above 25% were found at 22q (13 cases), 9q (12 cases), 16q (12 cases), 1q (11 cases), 5p (11 cases), 20q (11 cases), 7 (10 cases), 12q (9 cases), and 12p (8 cases).

High-copy amplifications were mapped to chromosomes 1p32, 1p35-36, 1q21-23, 2p16-21, 2q31-33, 5p15, 7q22, 8q21-23, 8q24, 9q34, 10p11-13, 10q21, 10q22, 10q25-26, 12p12-13, 12q13-14, 14q32, 15q26, 17q11-21, and 17q25.

LOH. The result of a LOH analysis is shown in Fig. 2. The three markers at 3p23, 3p24, and 3p25 showed allelic losses in 57% of the informative cases. By CGH, 22 tumors (73%) showed telomeric deletions covering the region 3p23-26. In 88% of the informative cases, the LOH and CGH data were consistent. The two markers at 13q14 indicated 38.5% of LOH, which was confirmed by CGH in all cases.

Fig. 1. Summary of all genetic alterations in the 30 HNSCCs. Lines on the left of the chromosome ideogram represent a DNA loss; lines on the right, DNA gains. Solid bars, high-copy amplification.
cases. The analysis for the polymorphic markers on chromosome 17p demonstrated 33% of allelic loss. The overall correlation between CGH and LOH for the informative cases varied between 70 and 88% for the three chromosomal arms tested.

Patterns of Chromosomal Imbalances. The superposition of all well-differentiated carcinomas to a CGH superkaryogram (Fig. 3A) revealed a characteristic pattern of alterations consisting of DNA loss on chromosomes 9p and 3p associated with a DNA overrepresentation of 3q. The superkaryogram of all undifferentiated carcinomas (Fig. 3B) showed additional changes, i.e., deletions on chromosomes 4q, 8p, 11q, 13q, 18q, and 21q and overrepresentations on 1p, 3q, 11q13, 19, and 22q. In 16 tumors we observed a pattern of changes that involved deletions at 9p and 13q combined with an overrepresentation at 11q13.

Discussion

Only a few years after its initial description (9), CGH has been established as a powerful screening method in tumor genetics. Its main advantage is the detection of multiple alterations within a single experiment, which results in a genetic pattern of DNA gains and losses at specific chromosomal sites. CGH has been previously applied to a limited number of HNSCCs (10, 11). However, there is still little known about the prevalence of specific changes in the different stages of tumor development as well as in different tumor subgroups. In the present study, we put particular emphasis on the characterization of the deletions and the extension of the genetic analysis to define tumor subgroups. In general, the high incidence of genomic imbalances as detected by CGH indicates that chromosome aberrations represent a more important mechanism of genetic instability in HNSCC than microsatellite instability (12).

Genetic Events in Tumor Progression. In our series, we observed deletions in more than 50% of the cases on chromosomes 1p, 3p, 4, 5q, 6q, 8p, 9p, 11q, 13q, 18q, and 21q. The use of CGH superkaryograms indicated that deletions on chromosomes 3p and 9p were preferentially associated with high tumor differentiation. This is in accordance with a recently proposed model of HNSCC tumor progression in which the authors observed allelic loss on 9p and 3p in precursor lesions of HNSCC (2). In addition to the 3p loss, the superkaryogram indicates that gain of chromosomal material on chromosome 3q occurs early in tumor development consistent with a recent study indicating that the 3q gain is detectable in severe dysplastic squamous epithelium of the cervix (13).

The CGH superkaryogram of the undifferentiated carcinomas suggested that deletions on chromosomes 4q, 8p, 11q, 13q, 18q, and 21q and overrepresentations on 1p, 11q13, 19, and 22q are associated with tumor progression. DNA loss on chromosomes 4q, 11q, and 13q have been described as late events in the progression model of HNSCC (2). We did not find a high incidence of deletions on 6p, 14q, and 17p, which is in contrast to allostotype studies (14, 15). It might indicate that deletions at these sites are more frequently of small size and thus beyond the resolution capacity of CGH. The correlation between LOH and CGH of our study is within the range reported in the literature (5).

The observations of van Dyke et al. (16), who reported that loss of 18q is associated with poor prognosis, and Soder et al. (17), who demonstrated that chromosome 18 deletions occur particularly in metastasizing HNSCC, are consistent with our data. Deletions on chromosome 21q have been described in HNSCC at an incidence of 5–26% (14, 15).

Amplifications on chromosome 11q13 have been correlated with poor prognosis in HNSCC (18). In our analysis, 11q13 amplification occurred simultaneously with distal 11q loss in poorly differentiated tumors, supporting the notion that deletions of 11qter are as important as the amplifications for clinical outcome.

Putative Mechanisms for Chromosomal Imbalances. The CGH analysis indicated that deletions are frequently associated with DNA gains on the same chromosome. Simultaneous imbalances were particularly observed for chromosomes 3, 5, 8, 9, and 11. This corresponds to the fact that squamous cell carcinomas are prone to the formation of 3q, 5p, and 8q isochromosomes (19).

Chromosome 3 was often affected by small DNA gains on 3q as well as DNA losses on 3p. This pattern was almost as frequent as the previously described formation of the putative 3q isochromosome (10, 11). Intrachromosomal rearrangements might play a role in this diversity of changes, since chromosomal breakage is a main mechanism that leads to amplifications and deletions (20). Hypothetically, the initial event is triggered by extrinsic factors like carcinogen exposure or a viral infection which is enhanced by the alteration of an oncogene (21) or TSG (22). In this regard, it is interesting that the FHIT gene at chromosome 3p14.2 contains the FRA3B fragile site that coincides with a spontaneous HPV16 integration site (23). Altered transcripts of the FHIT gene were reported in about 40% of squamous cell carcinomas of the lung (24), which makes it a candidate TSG for HNSCC. However, the fact that chromosome 3 displays a variety of lesions since it is frequently inactivated as recently shown (25). In combination with the loss of the RB locus, which is corroborated by LOH studies (27), supporting the notion that additional TSGs are located on this chromosome arm.

The DPC4 gene on chromosome 18q21.1 is infrequently harbors
Fig. 3. A, CGH superkaryogram of the three well-differentiated HNSCCs. Deletions are depicted in red, amplifications in green, and equilibrium between the tumor and normal DNA in blue. Characteristic changes of the G1 tumors are deletions on chromosome 3p and 9p as well as an overrepresentation of 3q. B, CGH superkaryogram of the undifferentiated (G2) carcinomas suggests that deletions on chromosomes 4q, 8p, 11q, 13q, 18q, and 21q and overrepresentations on chromosomes 1p34-pter, 11q13, 19, and 22q are associated with tumor progression.
mutations in HNSCC (28). To our knowledge, no candidate TSG has been identified on chromosomes 4q, 8p, and 21q.

Our study indicated that high- and low-grade HNSCC are characterized by distinct patterns of chromosomal imbalances. Deletions were generally more prevalent than amplifications and seem to be particularly important for clinical outcome (4, 29, 30). The follow-up of our patients will show whether a subgroup of specific alterations or the bare number of changes can predict an individual prognosis for HNSCC patients.

References

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