Genetic Progression Model for Head and Neck Cancer: Implications for Field Cancerization

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Abstract

A genetic progression model of head and neck squamous cell carcinoma has not yet been elucidated, and the genetic basis for “field cancerization” of the aerodigestive tract has also remained obscure. Eighty-seven lesions of the head and neck, including preinvasive lesions and benign lesions associated with carcinogen exposure, were tested using microsatellite analysis for allelic loss at 10 major chromosomal loci which have been defined previously. The spectrum of chromosomal loss progressively increased at each histopathological step from benign hyperplasia to dysplasia to carcinoma in situ to invasive cancer. Adjacent areas of tissue with different histopathological appearance shared common genetic changes, but the more histopathologically advanced areas exhibited additional genetic alterations. Abnormal mucosal cells surrounding preinvasive and microinvasive lesions shared common genetic alterations with those lesions and thus appear to arise from a single progenitor clone. Based on these findings, the local clinical phenomenon of field cancerization seems to involve the expansion and migration of clonally related preneoplastic cells.

Introduction

Annually, there are approximately 500,000 new cases of head and neck squamous cell carcinoma worldwide and over 50,000 cases in the United States (1). Patients with early stage cancer frequently manifest minimal physical findings and symptoms, resulting in delayed diagnosis and poor survival in many patients. Head and neck cancer is often associated with widespread epithelial histopathological alterations, and despite successful treatment of a primary head and neck cancer, there is a high likelihood of occurrence of a second primary tumor of the upper aerodigestive tract and lung (2—5). These observations led Slaughter et al. (6) to propose the concept of “field cancerization”; the hypothesis that there are carcinogen-induced changes throughout the mucosa of the upper aerodigestive tract of head and neck cancer patients.

It is now generally accepted that most sporadic solid tumors result from a multistep process of accumulated genetic alterations (7). In the past several years, a model for the initiation and progression of colorectal cancer has become a paradigm for other human solid tumors, including brain and bladder (8—12). Like colorectal cancer, HNSCC is thought to progress through a series of well-defined clinical and histopathological stages.

Areas of frequent chromosomal loss have recently been identified in head and neck cancer, but little is known about the timing of these genetic changes in malignant transformation (13). A variety of genetic changes frequently found in primary head and neck cancer have recently been described, including specific alterations of proto-oncogenes and tumor suppressor genes as well as chromosomal losses thought to indicate inactivation of other critical tumor suppressor genes (14—16). Ten tumor suppressor loci were chosen for this analysis based on the following criteria: (a) they have displayed a substantial proportion of loss (>40%) in invasive lesions by allelotype analysis and/or (b) they identify a minimal area of loss at a putative tumor suppressor locus (see “Materials and Methods,” “Selection of Loci”).

PCR-based microsatellite marker analysis enables analysis of microscopic lesions at different histopathological stages to establish a general order of progression for specific genetic changes. This study correlated genetic changes and histopathological progression within individual premalignant and malignant head and neck lesions, as well as histopathologically benign lesions frequently observed in patients at risk for head and neck cancer. Histopathologically distinct areas within single lesions were also analyzed and were particularly helpful in defining molecular progression. Based on this information, we have constructed a preliminary genetic progression model for HNSCC. Moreover, we have examined the concept of field cancerization based on the order of genetic changes established in this model.

Materials and Methods

Selection of Loci. The 9p21 locus corresponds to an area of genetic loss common to many solid tumors containing p16, a cyclin-dependent kinase inhibitor involved in cell cycle regulation (17, 18). At this time, this area demonstrates the locus with the most frequent LOH in HNSCC (16). 11q13 includes the bcl-1/int-2 locus, an amplicon carrying the proto-oncogene cyclin D1 (19), one of the few proto-oncogenes implicated in HNSCC. Apparent LOH at this locus actually represents amplification of cyclin D1, as confirmed by studies using fluorescence in situ hybridization (20). The p53 gene locus is commonly mutated in HNSCC (16). The p53 gene locus is located at 17p13, which also corresponds to an area of frequent LOH in HNSCC (21). We analyzed both LOH at this locus and p53 sequence in those lesions that demonstrated LOH to describe the time course of p53 inactivation and 17p13 loss in HNSCC progression. Chromosome 3p has been shown to contain at least three putative HNSCC tumor suppressor loci (22, 23). We analyzed markers from each of these three loci for those specimens with histopathologically distinct areas and in lesions biopsied over time. For the sake of clarity, however, only data for the 3p21 locus is included the calculations for LOH in Table 2. 13q21 contains an area with frequent LOH near the retinoblastoma locus that is now thought to identify a second, novel tumor suppressor gene locus (24). Chromosomes 4q26—28 and 14q31—32.1 contain loci mapped by microsatellite analysis in our laboratory that are also lost at high rates in HNSCC. Chromosomes 8 and 6p contain loci thought to contain putative tumor suppressor genes that have not been precisely mapped and are as yet
unidentified, but these regions are included since they are lost at a high rate (−40%) in invasive tumors (13). Microsatellite markers included were D3S1067, D3S1284, D3S1038, D3S1007 (chromosome 3p), D4S1613, FABP2 (chromosome 4), D6S265, TCEA1, D6S105 (chromosome 6p), D8S261, D8S262, D8S273, D8S257, D8S167 (chromosome 8), IFN-α, D9S736, D9S171 (chromosome 9p21), D11S873, INT-2, PYGM (chromosome 11q13), D13S170, D13S133 (chromosome 13q21), D14S81, D14S51 (chromosome 14q), TP53, and CHRNBP1 (chromosome 17p13).

**Tissue and DNA Extraction.** Eighty-seven specimens from 83 patients with head and neck lesions of the upper aerodigestive tract were analyzed. Four patients were biopsied twice at an interval of 1−12 months. Thirty-four lesions were diagnosed as benign squamous hyperplasia with no evidence of neoplastic transformation, 31 lesions were diagnosed as dysplasia, 21 lesions were diagnosed as squamous cell CIS, and 1 lesion was diagnosed as a small focus of squamous carcinoma with microscopic invasion arising in a background of benign squamous hyperplasia. Five of these lesions had sharp borders demarcating histopathologically distinct areas within a single biopsy. Tissue was obtained from archival, paraffin-embedded blocks from the Johns Hopkins Department of Surgical Pathology or from fresh-frozen tissue obtained with consent from Johns Hopkins Hospital patients and from the M. D. Anderson Cancer Center Head and Neck Cancer Tumor Bank. Tissue was chosen solely on the basis of the initial histopathological diagnosis from the pathology departments of both institutions. Representative sections from tissue used for DNA extraction were stained with H&E, and diagnosis was confirmed for each lesion by two pathologists (R. C. and W. W.). Fresh-frozen tissue was meticulously dissected on a cryostat to ensure that the specimen contained at least 75% epithelial cells. Approximately thirty-five 12-μm sections were then collected and placed in 1% SDS/proteinase K (0.5 mg/ml) at 58°C for 24 h. Paraffin-embedded tissue was sectioned into twenty-five 14-μm sections. Each individual section was placed on a glass slide and individually microdissected using a dissecting microscope to obtain >75% epithelial cells. The samples were placed in xylene overnight to remove the paraffin, pelleted in 70% ethanol, and then incubated in SDS/proteinase K at 58°C for 72 h. Digested tissue from both sources was then subjected to phenol-chloroform extraction and ethanol precipitation as described previously. Normal control DNA was obtained by either (a) venipuncture and isolation of lymphocyte DNA as described previously (25); (b) microdissection of purely nonneoplastic normal tissue in the previously mentioned archival, paraffin-embedded biopsy specimens; or, if necessary, (c) isolation of DNA from distant (normal), nonneoplastic, paraffin-embedded tissue from archival paraffin blocks other than the biopsy specimen in the manner described above.

**Analysis for Allelic Loss.** Microsatellite markers suitable for PCR analysis were obtained from Research Genetics. Prior to amplification, 50 ng of one primer from each pair was end labeled with [γ-32P]ATP (20 mCi; Amersham) and T4 kinase (New England Biolabs) in a total volume of 50 μl. PCR amplifications of each primer set were obtained from Research Genetics. Prior to amplification, 50 ng of one primer from each pair was end labeled with [γ-32P]ATP (20 mCi; Amersham) and T4 kinase (New England Biolabs) in a total volume of 50 μl. PCR reactions were carried out in a total volume of 12.5 μl containing 10 ng genomic DNA, 0.2 ng labeled primer, and 15 ng of each unlabeled primer. The PCR buffer included 16.6 mM ammonium sulfate, 67 mM Tris (pH 8.8), 6.7 mM magnesium chloride, 10 mM β-mercaptoethanol, and 1% DMSO, to which were added 1.5 μM deoxyribonucleotide triphosphates and 1.0 units Taq DNA polymerase (Boehringer Mannheim). PCR amplifications of each primer set were performed for 30 to 35 cycles consisting of denaturation at 95°C for 30 s, annealing at 50−60°C for 60 s, and extension at 70°C for 60 s as described. One third of the PCR product was separated on 8% urea-formamide-polyacrylamide gels and exposed to film from 4 to 48 h as described. For informative cases, allelic loss (or possible allelic imbalance in the case of the p53 gene using Taq-CS polymerase cycle sequencing (27, 28). Samples displaying mutations were reamplified and resequenced to confirm mutations.

**Statistical Analysis.** The primary statistical outcome for the analysis of these data is the proportion of chromosomal loci showing LOH at different histopathological points in the progression to cancer. To make comparisons between the different loci, it is convenient to model the proportion with LOH as a quantity which increases from benign squamous hyperplasia through dysplasia, CIS, and reaching a maximum with invasive cancer. To accomplish this, we used a simple model with the following characteristics: (a) the diagnostic categories from benign to invasive cancer were represented on an ordinal scale; (b) the proportion with LOH increases in a linear fashion across diagnoses; and (c) the slopes and intercepts of these linearly increasing proportions can differ from locus to locus. This degree of structure permits us to quantify and test differences between loci using a familiar Poisson regression approach. It is true that the assumption of an ordinal scale across diagnostic categories is arbitrary, as is the linear component of the Poisson model. However, the purpose of the analysis is to quantify and reveal structure in the data rather than to model them rigorously.

The results of this analysis demonstrate that the increase in LOH across the diagnostic categories (modelled as a linear trend) is significantly greater than 0 (P < 0.0001). Pairwise tests comparing trend in different loci do not show statistically significant differences.

**Results**

**Timing of Genetic Loss.** We examined 87 preinvasive lesions and paired normal samples using microsatellite analysis to establish the presence of allelic loss at 10 critical loci frequently lost in HNSCC (see "Materials and Methods"). The incidence of LOH at all loci (mean allelic loss) corresponding to each stage of histopathological diagnosis is shown in Table 1. Nearly all samples of dysplasia and CIS and less than one third of benign epithelial hyperplastic lesions displayed LOH in at least one of these specific loci. Calculation of the mean number of loss events (maximum of 10) shows that LOH at an increasing number of loci correlates with histopathological progression. As seen in Table 2, the highest frequency of LOH occurred in benign squamous hyperplastic lesions at 9p21 (20%), followed by 3p21 (16%) and 17p13 (11%). This finding indicates that LOH at these loci is an early event in HNSCC tumor progression. Dysplasia, considered to be an intermediate step in histopathological progression, showed additional loci with LOH at increased incidence at 11q13 (29%), 13q21 (32%), and 14q31 (23%). As expected, there was an increase in frequency for losses at 9p21 and 3p21 in the step from benign hyperplasia to dysplasia, and then an additional increase in CIS to a plateau level that was not greater in the invasive cancers. This is consistent with the role of 9p21 and 3p21 as early loss events. Later events in progression are indicated by a significant rise in the frequency of LOH (at least 2-fold) from the CIS to the invasive stage. These loci included 6p (19% and 38%, respectively), 8p (21% and 40%), 8q (20% and 38%), and 4q26−28 (21% and 47%). Statistical analysis shows that both the initial proportion of LOH and the rates of progression differ significantly among the loci (P < 0.0001, see "Materials and Methods").

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Benign squamous hyperplasia</th>
<th>Dysplasia</th>
<th>CIS</th>
<th>Invasive</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of lesions</td>
<td>35</td>
<td>21</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Mean loci lost ± SD</td>
<td>0.7 ± 1.3</td>
<td>2.7 ± 1.5</td>
<td>3.3 ± 2.0</td>
<td>3.6 ± 2.2</td>
</tr>
<tr>
<td>LOH at least one locus (%)</td>
<td>11 (31)</td>
<td>30 (97)</td>
<td>21 (100)</td>
<td>29 (97)</td>
</tr>
</tbody>
</table>

* A total of 10 chromosomal loci were tested, minimal losses were initially defined in 30 invasive tumors (for 3p21, 6p, 8, and 11q13). Subsequently, the minimal region was further mapped in at least 70 primary tumors at 9p21, 17p13, 13q21, 4q26−28, and 14q31−32.1.
LOH of 17p13 is thought to result in the loss of a second p53 allele after the first allele has been inactivated by a mutational event. However, our previous work indicated that p53 mutation was uncommon in preinvasive lesions, occurring in only 25% of CIS lesions and rarely in dysplastic lesions (16). In invasive tumors, LOH of 17p occurs in 80% of lesions with a p53 mutation. To test specifically for p53 inactivation, every lesion with 17p13 loss was sequenced. We found that none of the benign squamous hyperplastic lesions, only three of nine dysplastic lesions, and three of nine CIS lesions with 17p13 loss had detectable p53 mutations. This observation is consistent with other reports indicating the presence of a second tumor suppressor gene or amplification indicating a putative proto-oncogene at 17p (21, 29, 30). The high frequency of 17p13 LOH in early lesions unaccompanied by p53 mutation also implies that 17p loss may herald a separate, earlier event in head and neck cancer progression.

**Evidence from Intralesional Progression.** To further determine the general order of progression of early genetic events, areas of intralesional histopathological progression were evaluated. Five lesions with histopathologically distinct areas were analyzed by microdissecting each area separately and comparing patterns of genomic microsatellite loss. Allelograms (diagrams showing the pattern of LOH at multiple tumor suppressor gene loci) for two of these lesions are shown in Fig. 1, along with representative autoradiographs in Fig. 2. In these cases, the histopathologically advanced area displayed the same pattern of LOH (i.e., the same genetic events) as the less advanced area (Fig. 1b). However, in four of the five cases (including Fig. 1a), the histopathologically advanced area demonstrated additional LOH at one or more loci. We were also able to map the location of identical boundaries between an area of loss and retention at one or more loci in four of five of these pairs of lesions, including those shown in Fig. 1. If two samples from one patient share concordant losses of identical alleles with identical boundaries between loss and retention, this provides strong evidence that the cells in the two areas are clonally related. This finding is consistent with the hypothesis that more benign-appearing, histopathologically distinct areas adjacent to more malignant regions in aerodigestive mucosa arise from a common clonal progenitor. Progression, therefore, is thought to involve clonal outgrowth of a subpopulation of cells with yet additional genetic alterations. Although the number of lesions examined in this fashion was not sufficient for a statistical analysis, we noted frequent LOH at either 9p21 or 3p21 shared by both histopathologically early and advanced regions, supporting the role of these loci as important early events in tumor progression.

**Discussion**

In the past several years, tumor progression models have been constructed for a few tumor types by correlating specific genetic changes with histopathological progression. The identification of allelic losses in tumors within the context of a tumor progression model has led to the identification of critical putative tumor suppressor genes. The initial description of molecular progression proposed by Fearon and Vogelstein (8) indicated that (a) tumors progress via the activation of oncogenes and the inactivation of tumor suppressor genes, each producing a growth advantage for a clonal population of cells; (b) specific genetic events generally occur in a distinct order of progression; but (c) the order of progression is not necessarily the same for each individual tumor, and thus it is the accumulation of genetic events that determines tumor progression.

In the HNSCC progression model derived in this study, allelic loss has been used as a molecular marker for inactivation of putative tumor suppressor genes. However, allelic imbalance may also occur from oncogene amplification (e.g., cyclin D1 on 11q13). In the case of HNSCC, chromosomal loss and amplification at these 10 loci have been confirmed by other complementary methods (29). A preliminary molecular progression model developed from this work is presented in Fig. 3. This model supports the initial observations of the colorectal molecular progression model, in that (a) both oncogenes and tumor suppressor genes are involved in tumor progression; (b) specific events in head and neck cancer generally occur in a distinct order of progression, with loss at 9p21 or 3p among the earliest detectable events; and (c) it is the accumulation and not necessarily the order of

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**Table 2 LOH for histopathological diagnosis and chromosomal locus**

<table>
<thead>
<tr>
<th>Chromosomal locus</th>
<th>Benign squamous hyperplasia</th>
<th>Dysplasia</th>
<th>CIS</th>
<th>Invasive</th>
</tr>
</thead>
<tbody>
<tr>
<td>9p21</td>
<td>8/35 (20)</td>
<td>17/30 (57)</td>
<td>17/21 (80)</td>
<td>54/74 (73)</td>
</tr>
<tr>
<td>3p21</td>
<td>5/31 (16)</td>
<td>15/29 (52)</td>
<td>12/20 (60)</td>
<td>18/27 (67)</td>
</tr>
<tr>
<td>17p13</td>
<td>4/35 (11)</td>
<td>10/30 (33)</td>
<td>9/17 (47)</td>
<td>34/46 (55)</td>
</tr>
<tr>
<td>11q13</td>
<td>2/24 (6)</td>
<td>9/21 (39)</td>
<td>8/20 (40)</td>
<td>14/23 (61)</td>
</tr>
<tr>
<td>13q21</td>
<td>1/32 (3)</td>
<td>9/28 (32)</td>
<td>7/20 (35)</td>
<td>31/60 (52)</td>
</tr>
<tr>
<td>14q31–32.1</td>
<td>1/31 (3)</td>
<td>7/20 (33)</td>
<td>6/19 (33)</td>
<td>32/73 (44)</td>
</tr>
<tr>
<td>6p</td>
<td>23/34 (18)</td>
<td>6/20 (30)</td>
<td>4/21 (19)</td>
<td>9/24 (38)</td>
</tr>
<tr>
<td>5p</td>
<td>3/34 (8)</td>
<td>22/30 (73)</td>
<td>4/21 (19)</td>
<td>9/24 (38)</td>
</tr>
<tr>
<td>8q</td>
<td>4/28 (14)</td>
<td>3/24 (13)</td>
<td>4/19 (21)</td>
<td>8/20 (40)</td>
</tr>
<tr>
<td>4q26–28</td>
<td>1/26 (4)</td>
<td>2/23 (9)</td>
<td>4/19 (21)</td>
<td>30/64 (47)</td>
</tr>
</tbody>
</table>

* Values given in loss/informative cases (% loss).

* From previously published and unpublished data in 30–70 tumors for each locus.

**Fig. 1. Genetic analysis of lesions undergoing histopathological progression. a, microdissected lesion shows genetic progression with loss at 6p and 11q13 accompanying histopathological progression from dysplasia to CIS within the biopsy sample. Note identical boundaries between LOH (●) and retention (□) at 3p, 8q, and 9p21. Similar genetic progression was noted in three other biopsy samples with histopathological progression. b, microdissected lesion showing identical genetic changes despite histopathological progression from benign squamous hyperplasia (BSH) to dysplasia. Again, note identical boundaries of LOH (●) and retention (□) at 3p and 11q13. NI, noninformative.
The finding that 30% of histopathologically benign squamous hyperplastic lesions already consist of a clonal population of cells with selective loss of the bottom allele in the area of CIS for marker D11S873 (middle), demonstrating genetic progression from dysplasia to CIS at this locus.

Our observations provide insight into a possible mechanism for field cancerization. This term was originally coined by Slaughter et al. (6) in a study of oral cancer. They examined resection specimens of invasive squamous cell carcinoma of the oral cavity and found histopathological abnormalities in the epithelium surrounding the invasive cancer. These abnormalities included additional areas of invasive cancer, CIS, dysplasia, hyperkeratosis, and epithelial hyperplasia with frequent multiple foci of the more aggressive lesions. Both Slaughter et al. (6) and, more recently, other investigators have reported an increased incidence of second primary head and neck, pulmonary, and esophageal cancers in patients with head and neck cancer (33). Based on these observations, field cancerization was used to describe the phenomenon by which an entire field of tissue developed malignant or premalignant change in response to a carcinogen.

Our analysis of lesions with areas of apparently benign mucosa adjacent to premalignant lesions demonstrate that early genetic events may be shared by cells in a local anatomical area, which are apparently derived from a common clone. Other studies of areas of histopathological abnormality surrounding lung carcinomas show allelic-specific loss in both chromosome 3p and 9p loci, consistent with a common clonal origin of these cells (34, 35). The original observations of Slaughter et al. (6) may be interpreted in the context of these data, suggesting that areas of histopathological abnormality surrounding malignant and premalignant lesions are all generally derived from a common single progenitor clone. In fact, Slaughter et al. (6) noted that “forty-three of these eighty-eight patients [with] two separate tumors occurred in the same anatomical area of the oral cavity.” Subsequent genetic events in various subclones produce different phenotypic alterations, resulting in a variety of histopathologically diverse regions in a local anatomical area and possible outgrowth of genetic “pathway” to malignancy. However, the natural clinical history of benign squamous hyperplasia is not usually associated with progression to carcinoma (although Fig. 1b is consistent with a benign hyperplasia undergoing progression to dysplasia). This is consistent with statistical models based on age-specific incidence of head and neck cancer that this malignancy requires multiple (perhaps 10 or more) genetic alterations to develop an invasive phenotype (10). Therefore, it might be expected that lesions with only a few genetic events would display a rather benign morphological phenotype. It is also a reasonable, although an untested hypothesis, that other subclinical lesions or histopathologically normal mucosa may contain similar genetic alterations and may be placed early on the progression pathway.

Existence of an alternate gene in this region has been implicated in the genetic progression of brain and breast cancer (30, 31). Therefore, it might be expected that lesions with only a few genetic events that determines progression. This model is certainly not complete, and the temporal placement of two closely occurring events in progression may in fact be arbitrary. Our data support the notion that events spaced at greater intervals in the model are likely to occur in the indicated order in most tumors. Progression by accumulation of genetic events is also supported by the fact that generally “late” genetic events occasionally occur as the only detectable change in early lesions (e.g., one benign hyperplastic lesion showed loss solely at the 8p locus). In many tumor types, including HNSCC, p53 inactivation apparently occurs in the transition from the preinvasive to the invasive state (16). A second 17p13 locus may be altered earlier in progression. Existence of an alternate gene in this region has been implied in the genetic progression of brain and breast cancer (30, 31).

The finding that 30% of histopathologically benign squamous hyperplastic lesions already consist of a clonal population of cells with shared genetic alterations characteristic of those seen in head and neck cancer suggests that a substantial proportion of these lesions are on a genetic "pathway" to malignancy. However, the natural clinical history of benign squamous hyperplasia is not usually associated with progression to carcinoma (although Fig. 1b is consistent with a benign hyperplasia undergoing progression to dysplasia). This is consistent with statistical models based on age-specific incidence of head and neck cancer that this malignancy requires multiple (perhaps 10 or more) genetic alterations to develop an invasive phenotype (10). Therefore, it might be expected that lesions with only a few genetic events would display a rather benign morphological phenotype. It is also a reasonable, although an untested hypothesis, that other subclinical lesions or histopathologically normal mucosa may contain similar genetic alterations and may be placed early on the progression pathway.

Genetic changes in areas of differing histopathology contained within individual lesions have also been examined, similar to recent investigations of genetic progression in dysplastic colonic crypts (32). These lesions quite possibly provide snapshots of tumor progression, demonstrating common early genetic events, the common (clonal) origin of these histopathologically distinct areas, and the association of additional genetic loss with progression to a more malignant phenotype.

Our analyses of lesions with areas of apparently benign mucosa adjacent to premalignant lesions demonstrate that early genetic events may be shared by cells in a local anatomical area, which are apparently derived from a common clone. Other studies of areas of histopathological abnormality surrounding lung carcinomas show allelic-specific loss in both chromosome 3p and 9p loci, consistent with a common clonal origin of these cells (34, 35). The original observations of Slaughter et al. (6) may be interpreted in the context of these data, suggesting that areas of histopathological abnormality surrounding malignant and premalignant lesions are all generally derived from a common single progenitor clone. In fact, Slaughter et al. (6) noted that “forty-three of these eighty-eight patients [with] two separate tumors occurred in the same anatomical area of the oral cavity.” Subsequent genetic events in various subclones produce different phenotypic alterations, resulting in a variety of histopathologically diverse regions in a local anatomical area and possible outgrowth of genetic "pathway" to malignancy. However, the natural clinical history of benign squamous hyperplasia is not usually associated with progression to carcinoma (although Fig. 1b is consistent with a benign hyperplasia undergoing progression to dysplasia). This is consistent with statistical models based on age-specific incidence of head and neck cancer that this malignancy requires multiple (perhaps 10 or more) genetic alterations to develop an invasive phenotype (10). Therefore, it might be expected that lesions with only a few genetic events would display a rather benign morphological phenotype. It is also a reasonable, although an untested hypothesis, that other subclinical lesions or histopathologically normal mucosa may contain similar genetic alterations and may be placed early on the progression pathway.
a subclone that has acquired a particular selective growth advantage. Findings in our laboratory related to the origin of these multiple primary tumors are discussed by Bedi et al. (36).

A genetic progression model for head and neck cancer has several important implications. From a scientific viewpoint, identification of targeted tumor suppressor genes and proto-oncogenes at these loci may be critical for understanding the biological initiation, behavior, and progression of head and neck cancer. Determination of the genetic status of a primary tumor or of the tissues surrounding invasive cancer may have prognostic significance for tumor recurrence and/or progression of remaining clonal epithelial populations toward a more malignant phenotype. For example, the presence of transforming clonal events in surrounding normal epithelium at the time of cancer resection may predict late local recurrence in some patients. Identification of early genetic events would also provide the best targets for screening of saliva or sputum to identify premalignant transformation. Knowledge of early events could also be used to identify residual clonal populations by molecular margin analysis to more accurately assess the success of surgical resections (37). A recent molecular analysis of urine sediment identified microsatellite alteration or LOH in 95% of cancer patients in our laboratory, similar strategies could be adopted for screening of saliva specimens (38). Moreover, prevalent and early events such as 9p21 loss offer attractive targets for therapeutic strategies based on pharmacological or genetic modification in lesions of all stages.

References

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