ORAL CANCER BACKGROUND PAPERS

Chapter II: Histopathology, Biology and Markers

Working Draft

A. State of the Science

The development of oral cancer seems to begin in many cases with exposure of the mucosal surfaces of the upper aerodigestive tract to topical carcinogens, predominantly alcohol and tobacco.[1-4] In some persons exposed to these carcinogens or co-carcinogens, premalignant and malignant lesions develop in a multi-step process within the mucosa.[5,6] However, oral cancers occur in some patients with no history of tobacco or alcohol usage and no other apparent risk factors. Additionally, it is not clear that all of the tumors have an apparent "precancerous" state.

There is an emerging body of evidence that persons who develop head or neck cancer may have undergone alterations in p53 or other tumor suppressor genes; however, this has not been proven.[7-13] In addition, there is evidence that altered p53 genes may cooperate with other oncogenes, such as ras, to generate cells with a growth advantage for tumor progression, a multifunctional process associated with cutaneous carcinogenesis as well.[14] Increasing immunosuppression from HIV infection also appears to be a factor in predisposing oral mucosa to malignant changes (see Chapter III).

Neoplasms of diverse cellular origin arise in the oral regions, including nasopharyngeal carcinoma, lymphoma, mucosal melanoma, sarcomas, and salivary gland tumors. This chapter will focus on squamous cell carcinomas and their variants, as these cancers constitute over 90% of oral malignancies.

The earliest detectable morphologic changes are the appearance of the "premalignant" lesions of leukoplakia and erythroplakia. Genetic alterations in these premalignant lesions have also been demonstrated (see Chapter IV).[17-19] *Leukoplakia* is a white plaque that cannot be removed by gentle scraping and for which no other etiology can be identified. Microscopically, leukoplakias exhibit hyperplasia of keratinocytes, as represented by hyperorthokeratosis, hyperparakeratosis, and/or acanthosis.

The term *dysplasia* is reserved for lesions showing combinations and degrees of cytologic atypia (e.g., hyperchromatism, increased nuclear size, pleomorphism, dyskeratosis, and increased or abnormal mitotic figures).[1,18] Atypia confined to basilar and parabasilar keratinocytes constitutes *mild dysplasia*, whereas atypia extending into the midspinous layer is termed *moderate dysplasia*. When cellular atypia extends to the surface layer, the terms *severe dysplasia* and *carcinoma in situ* (complete top-to-bottom cytologic atypia) are applied. Architectural changes are also a feature of dysplasia, the most significant being a bulbous or teardrop shape of rete ridges. For oral mucosa in general, up to 20% of clinically defined leukoplakias that are biopsied may exhibit dysplasia; lesions located in the floor of the mouth approach a 40% prevalence of dysplastic change.[19] Dysplastic leukoplakias have a high propensity to progress to invasive squamous cell carcinoma. However, leukoplakias without present evidence of dysplastic changes may progress to dysplasia and subsequently to carcinoma; still, many leukoplakias fail to undergo malignant transformation.[20,21]

Erythroplakia is a velvety red patch of oral mucosa that does not conform to other defined oral disease processes that appear red clinically. There is a high prevalence of dysplastic change among these lesions, approaching 80-90%, and progression to invasive carcinoma is high.[1,2,22] Although dysplastic, the epithelium is usually atrophic, and submucosal vasodilation with inflammatory cell infiltration is a consistent finding. When erythroplakias coexist with white foci, they are termed speckled leukoplakias or erythroleukoplakias; such lesions will exhibit hyperkeratosis in the white areas.

Malignancies arising from the mucosa of the oral cavity are epithelial in origin and are, therefore, classified as squamous cell carcinomas more than 90% of the time.[23,24] According to the degree of differentiation, three subtypes are defined: (1) well-differentiated squamous cell carcinoma showing more than 75% keratinization; (2) moderately differentiated squamous cell carcinoma with 25-75% keratinization; and (3) poorly differentiated squamous cell carcinoma with less than 25% keratinization.[1,2] The majority of cases are of moderate differentiation. A clear relationship between histologic differentiation and clinical prognosis has not been established, although a lack of differentiation has been associated with more rapid growth and spread. The morphologic classification of squamous cell carcinoma by degree of differentiation is used in the description of the histopathologic specimen.

There are histopathologic variants of squamous cell carcinoma, all of which are rare, that affect prognosis and the selection of therapeutic modalities. Spindle cell or sarcomatoid squamous cancers, occasionally found in the oral cavity, are most frequently encountered on the lip and in the larynx. Radiation therapy to a pre-existing conventional squamous cell carcinoma is a common antecedent event; however, spindle cell carcinomas may arise de novo.[25] Other rare variants of oral, head, and neck carcinoma include pseudoglandular, basaloid, and small cell neuroendocrine carcinomas, the latter two being radiosensitive. Because these tumors share histopathologic features with other neoplasms (i.e., melanomas, neuroblastomas, lymphomas), the use of specific immunohisto-chemical markers is warranted.

Verrucous histopathologic patterns characterize a subset of oral epithelial tumors. Because there is evidence that these carcinomas evolve from leukoplakias that also exhibit a verrucoid architecture, they are termed proliferative verrucous leukoplakia (PVL).[17] Two specific forms of squamous cancers may arise from PVL lesions. The first, verrucous carcinoma, is characterized by marked hyperparakeratosis, acanthosis, parakeratin crypts, and large "pushing" bulbous rete ridges, said to resemble "elephant's feet."[26] It should be noted that verrucous carcinomas do not have dysplastic cytologic features; they also do not metastasize.

The second variant, also often preceded by PVL, is the papillary form of squamous cell carcinoma. Histologically, these lesions exhibit either an exophytic papillary pattern of growth or a verrucous inverting architecture.[27,28] Both patterns harbor dysplastic cytologic changes; a small number (less than 10%) have been shown to metastasize to regional nodes.

Oncogenes and proto-oncogenes are DNA sequences that encode factors that drive the cell cycle and include growth factors, their ligands, internal signaling pathway protein kinases, cyclins, cyclin-associated kinases, and DNA transcription factors, many of which can be demonstrated in tumor tissues. Conversely, anti-oncogene or tumor suppressor gene protein products retard or inhibit the cell cycle or activate pathways that lead to programmed cell death (apoptosis). Loss of suppressor gene product or inactivation by mutation of both alleles will favor cell proliferation and malignancy. These proteins can also be detected in tissues by immunohistochemical and molecular analytic methods.

Oncogenes and tumor suppressor genes are currently being studied in both defining the multistep carcinogenetic process and as prognostic factors for disease-free and overall survival.[1,6] For example, amplification of the epidermal growth factor receptor (EGFR) gene has been demonstrated in human specimens.[29,30] In one study, EGFR levels were shown to be higher in poorly differentiated tumors than in well-differentiated or moderately differentiated tumors.[31] In addition, increased EGFR levels have been shown to correlate with larger primary tumor lesions.[32] On the other hand, studies of the erb-B-2 oncoprotein have indicated that the expression of erb-B-2 is common in human head and neck cancer but does not seem to be of prognostic significance.[33]

Normal p53 protein serves as a suppressor of cell growth; a possible correlation between p53 mutation and prognosis is being investigated. Generally, mutations of p53 are demonstrated in about 50% of head and neck cancers and have also been seen in premalignant lesions.[34] The incidence and specific type of p53 mutation may depend on the risk factor exposure pattern.[35-38] A retrospective study by Brachman et al.[7] suggested that tumors with p53 mutation had a shorter time to treatment failure than tumors lacking a p53 mutation. Shin et al. reported on tumor samples from 118 patients[8] (tumor sites were the oral cavity, oropharynx, larynx, and hypopharynx); median survival was significantly shorter in patients with a p53 mutation in their primary tumor specimen than in those with no such mutation. However, there was no difference in recurrence rates of the primary tumor according to p53 status, although patients with p53 mutation had a higher likelihood of developing a second primary malignancy.

Studies of the chromosome 9p21-22 indicated mutations in this region in over 70% of examined head and neck tumors;[9,10] a similar incidence of allelic loss was found in preinvasive lesions. These findings suggest that loss of genetic information on chromosome 9p is an early event in head and neck squamous cell carcinogenesis. It also appears that information on the myc oncogene may be useful for prognosis, but this remains controversial.[6] Decreased expression of the nuclear retinoid receptor (RAR-beta) has also been associated with head and neck carcinogenesis.[39]

B. Emerging Trends

The most interesting emerging trend in this area is using molecular biology to define more carefully

a tumor's biological behavior ("biological staging"). Another interesting trend currently being investigated is the use of the polymerase chain reaction (PCR) to determine if surgical margins obtained at the time of surgery that are histopathologically free of tumor contain a small amount of histologically undetectable tumor cells. Specifically, the use of PCR to detect specific p53 mutations identified in the primary tumor in the histopathologically negative surgical margin could be very useful, as these mutations would indicate the presence of residual (histopathologically undetected) tumor cells. It will be very important to establish whether the presence of submicroscopic tumor cells contributes to prognosis and clinical outcome.

The recent development of in situ PCR will allow amplification of both DNA and RNA directly in tissue section; this technique should be extremely helpful in the future to localize tumor cells containing altered oncogenes or tumor suppressor genes. An improved understanding of the molecular biology of head and neck cancer may also contribute to future therapeutic improvements. Finally, molecular probes may be used to facilitate the early detection of second malignancies.

Head and neck cancer increasingly appears to be a complex disease entity that requires highly specialized input from pathologists, surgeons, radiologists, and medical and oral oncologists. The need for laboratory scientists; specialists in social services, speech, and swallowing disorders; restorative dentists; and maxillofacial prosthodontists clearly identifies this malignancy as one that should be studied and treated at large academic centers with ongoing clinical and laboratory research programs. Such an environment will promote the use of increasingly refined laboratory techniques for improved diagnosis and therapy.

A good deal of current laboratory and clinical research is focusing on identifying the relative contributions of certain oncogenes and tumor suppressor genes on carcinogenesis, tumor stage, and clinical outcome. Although abnormalities of some oncogenes and tumor suppressor genes have been identified, their relative contribution and optimal use in diagnosis, prognosis, or treatment remain unknown. The same might be said for the role of Epstein-Barr, hepatitis, and herpes simplex viruses; further clinicolaboratory studies will be needed to define which are clinically relevant and when they should be investigated. Figure 1 shows biomarkers that may prove useful in assessing cycling cells in precancerous lesions and at surgical tumor margins, and in predicting aggressive behavior, invasion front, and metastatic potential.[40] Genetic analysis at a molecular/chromosomal level is emerging as a science that may aid in identifying risk and possibly prevention as well.

Finally, a reliable and predictable histopathology grading system should be developed to include, in addition to differentiation of tumor cells, such factors as basement membrane protein expression and invasion patterns, perineural invasion, and immunologic responses.[41]

Figure 1: Biomarker Predictors in Oral Precancerous & Cancerous Lesions

Marker	Detection Method	Target
Proliferation		
PCNA, Ki67, BrU	IHC	Cycling cells
Histone	mRNA ISH	
AgNORs	Silver stain	
Genetic		
Ploidy	FC	Aneuploid cells
Oncogenes		
C-myc	IHC	Cycling cells
Tumor Suppressor		
p53 mutations	IHC, PCR	Cycling cells
Cytokeratin 8/19	IHC	Anaplasia
Blood Group Antigens	IHC	Anaplasia
Integrins/ECM Ligands	IHC	Invasion and metastatic potential

Abbreviations:

IHC	immunohistochemistry
FC	flow cytometry
PCR	polymerase chain reaction
ISH	in-situ hybridization

C. Opportunities and Barriers to Progress

The emergence of molecular biology with its new prognostic and, ultimately, therapeutic tools represents an enormous opportunity. The use of biologic markers to screen patients who are at increased risk may help to predict the probability of disease progression, aid in the diagnosis made by routine histopathologic studies, assess the prognosis of the individual cancer patient, develop treatment protocols, and evaluate the response to therapeutic agents. A major barrier to progress is a health care climate in which a large proportion of patients receive either uncoordinated "multispecialty" or traditional surgical care without proper usage of laboratory, clinical, and therapeutic investigational tools. Thus, research is slowed at single institutional and national levels (e.g., Cooperative Groups). Proper recognition that survival rates are too often poor with "standard therapy" in patients with advanced disease should lead to a greater appreciation for research.

Another barrier to progress is the cost of biologic markers combined with the failure of third parties

to cover them. Laboratory standardization of biologic marker techniques and variability in interpretation of tissue results compromise the diagnostic significance of these markers.

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