An Update on Molecular Diagnostics of Squamous and Salivary Gland Tumors of the Head and Neck

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**Context.**—Molecular testing in anatomic pathology is becoming standardized and can contribute valuable diagnostic, therapeutic, and prognostic information for the clinical management of patients. In head and neck pathology, recent advances in molecular testing have provided important targets in several different diagnostic areas, with particular emerging clinical applications in squamous and salivary gland pathology. In squamous mucosal-derived lesions, human papilloma virus has emerged as an important pathogenic etiology in a subset of oropharyngeal squamous cell carcinomas. Within the category of salivary gland tumors, 3 tumors have recently been recognized that contain oncogenic translocations.

**Objective.**—To describe the current state of information about the molecular alterations in squamous lesions and in salivary gland tumors of the head and neck.

**Data Sources.**—Published literature on squamous and salivary gland tumors of the head and neck.

**Conclusions.**—The different approaches to identification of viral-associated tumors include assays using polymerase chain reaction, in situ hybridization, and immunohistochemistry. Most mucoepidermoid carcinomas harbor MECT1-MAML2 gene rearrangement. The MYB-NFIB translocations have recently been identified in adenoid cystic carcinomas. Finally, a newly described tumor of salivary gland, mammary analogue secretory carcinoma, harbors the ETV6-NTRK3 translocation. Although these translocations are just emerging as diagnostic targets, future roles may evolve as potential therapeutic targets. (Arch Pathol Lab Med. 2011;135:602–609)

**Head and neck pathology** is a diverse subspecialty because of the highly variable tissue types and the many different organs that are found within this anatomic region. Two unique areas of study within head and neck pathology are squamous mucosal lesions and salivary gland tumors. Tumors of the mucosal and salivary gland sites include both interesting morphologic entities and newly described, controversial, and diagnostically challenging entities. Although morphology, histology, and immunohistochemistry remain the mainstay of diagnosis in these locations, recent advances in molecular anatomic pathology are providing interesting new potential targets for pathologists to consider adding for the diagnostic approach. This review will discuss several unique and interesting tumors of the head and neck mucosal and salivary gland regions that have consistent molecular alterations.

**SQUAMOUS MUCOSAL LESIONS**

Most malignant mucosal tumors of the head and neck are derived from the squamous epithelium and are squamous cell carcinomas (SCC). There is a well-established and accepted progression pathway for SCC. The precursor lesions are diagnosed along the spectrum of squamous dysplasia, generally graded as mild, moderate, or severe dysplasia/carcinoma in situ, based on histologic architectural alterations and cytologic atypia. Tumors with invasion through the basement membrane are classified as invasive squamous cell carcinoma.

Although most squamous malignancies are conventional keratinizing squamous cell carcinoma, there are a number of well-described variant morphologies that occur throughout the upper aerodigestive tract. Both conventional SCC and the variants have some unique molecular characteristics that can provide important diagnostic, prognostic, and potentially, therapeutic benefit, beyond what can be diagnosed purely at the morphologic level.

**Squamous Dysplasia**

The histologic features of squamous dysplasia include both architectural alterations and cytologic atypia. At the architectural level, a dysplastic epithelium demonstrates lack of maturation and disorganization. As the healthy epithelium matures from the base to the surface, the cells change in nuclear orientation (organization) and in the nuclear to cytoplasmic ratio (maturation). At the base, the cells have large nuclei and scant cytoplasm and the long axis of the nucleus is often polarized perpendicular to the basement membrane. At the surface, they have small, pyknotic nuclei and abundant cytoplasm and are flattened with their long axis parallel to the surface. Customary organization and maturation are lost in dysplasia. Cytologically, the nuclei of dysplasia show atypia, including features such as hyperchromasia, enlargement,
and nuclear membrane irregularity. The degree of dysplasia is generally graded according to the thickness of epithelium involved. When only the lower or basilar third is altered, the designation of mild dysplasia is typically used. Atypia in the middle third qualifies as moderate dysplasia, and full-thickness alteration is diagnosed as severe dysplasia/carcinoma in situ.

Keratinizing dysplasia is a unique form of dysplasia in the head and neck that is particularly difficult to diagnose and grade. Keratinizing dysplasia can be associated with invasive SCC, even in the absence of full-thickness atypia (so-called drop-off carcinoma). For this reason, grading these lesions can be misleading because the grade may not be reflective of the malignant potential. Instead, some authors argue for simply categorizing this condition as *keratinizing dysplasia.*

The molecular alterations in squamous dysplasia are often associated with tobacco-related DNA damage because most dysplasia and head and neck SCC are related to smoking. DNA damage to tumor suppressor genes is quite common in the dysplasia-SCC pathway, and studying these alterations has been a common technique for examining the so-called field effect of tobacco-related products. The *field effect* implies that all mucosal surfaces exposed to tobacco products will be at risk for DNA-damage that may precede both clinical and histologic changes. Molecular studies of apparently uninvolved mucosa in patients with SCC have supported the field effect, showing multiple molecular changes in tumor suppressor genes even in histologically normal areas.

The most common tumor suppressor gene or genomic loci with loss of heterozygosity in squamous dysplasias are 3p, 9p, and 17p. Some studies have even proposed that these markers can be used diagnostically to predict prognosis and/or progression of dysplasia. Although the mutation burden does appear to correlate with prognosis and outcome, practically speaking, these assays have not become common tools in the diagnostic laboratory.

Some specific tumor suppressor genes have been studied at the protein expression level, as well. These include p53 (associated with 17p) and CDKN2A (associated with 9p). Studies have been inconclusive about the significance of expression of p53 and p16 in dysplasia, and part of this controversy is likely due to the confounding variable of human papillomavirus (HPV, see discussion below). Several studies have shown, however, that p53 and p16 expression is seen in dysplasias in oral cavity locations that are not typical of HPV-associated SCC and that they may, therefore, be markers of neoplastic transformation.

**Conventional SCC**

Conventional SCC typically displays some degree of keratinization. Tumors with abundant keratin production are classified as well differentiated, and those with little or no keratinization are characterized as poorly differentiated. A spectrum of possibilities between these 2 extremes exists; because many conventional SCCs end up in the moderately differentiated category, grading is inexact and does not have strong clinical significance.

The molecular findings in conventional SCC are an extension of those identified in the precursor lesions. Again, the most common findings are related to tumor suppressor gene alterations, which notably includes 3p, 9p, and 17p, which is the p53 locus. Importantly, p53 alterations are very common in tobacco-associated SCC.

These alterations can be detected by loss of heterozygosity analysis on DNA extracted from tumor cells, or they can be identified at the protein immunohistochemistry level. The one caveat is that overexpression of p53 at the protein level does not have a strong correlation to the presence of biallelic inactivation of the tumor suppressor genes. In other words, the protein expression can be altered in the absence of either detectable, somatic, inactivating point mutations or loss mutations. Despite this potential pitfall, p53 expression has been associated with poor prognosis and progression in SCC of the head and neck. p53 alterations have also been associated with chemotherapy and radiation therapy sensitivity.

Perhaps the most heavily studied molecular area within head and neck SCC in the past decade is the association with epidermal growth factor receptor (EGFR) alterations. The strong interest in EGFR originated from the availability of molecular-targeted therapeutics, in the form of small molecular tyrosine kinase inhibitors and monoclonal antibodies against EGFR. The most notable EGFR alteration in head and neck SCC is altered expression at the immunohistochemistry level in tumor samples. The protein appears to be overexpressed in most SCCs. At the DNA level of the tumor, most studies have shown relatively low rates of EGFR somatic mutations in exons 18 to 21, which have been described in some types of lung carcinoma. A mutant variant, EGFRvIII, which was first described in brain tumors, has been identified in some head and neck SCCs. Potential treatment of squamous carcinomas with EGFR-inhibitor therapies does not depend on any molecular or immunohistochemical testing for EGFR because no correlation with response to therapy has been found.

In head and neck SCC, surgical resection with clear margins is one of the mainstays of therapy, and positive surgical margins are associated with local recurrence and poor prognosis. One of the most interesting applications of molecular assays in SCC has been the exploratory investigation of margin status. Several studies have examined whether molecularly positive and histologically negative margins have an association with tumor recurrence. These studies of margins using molecular diagnostics have mostly assessed p53 mutations. Again, in support of the cancerization field effects, molecular mutations are found in histologically uninvolved margins and do appear to be associated with tumor recurrence and poor prognosis.

**HPV-Related SCC**

In the past, SCC in nonsmokers and nondrinkers was uncommon because most squamous carcinomas were associated with tobacco use. In the past decade, however, an increase in the incidence of SCC has been identified worldwide, with a particular increase in the incidence of oropharyngeal tumors. Recent molecular advances have shown that many of these tumors have a strong association with HPV. This association is presumably secondary to sexual practices. There is hope that the use of the HPV vaccine will eventually lead to a decrease in incidence of these tumors. These tumors have recently been described as a separate variant category identified as HPV-assoicated SCC (HPV-SCC).

The most frequent location for HPV-SCC is the oropharynx, including the tongue base and tonsils. In these anatomic locations, in North American studies, up to 65% to 70% of the tumors are associated with HPV. Tumors of the oral tongue, in contrast, have a low
incidence of HPV. The HPV-associated SCCs characteristically have a different histologic appearance than most tumors of the oral cavity. The HPV-SCCs are more likely to be nonkeratinizing or basaloid in their morphology, with limited or absent keratinization (Figure 1, A through C). Although most HPV-SCC will be nonkeratinizing, occasional keratinizing tumors in the oropharynx can be HPV-related. Recent literature has also illustrated that there is a subset of tonsil/tongue base tumors that resemble undifferentiated nasopharyngeal carcinoma, with lymphoepithelial carcinoma morphology, that are HPV positive (Figure 1, D).

The HPV in tumors has interesting effects on the expression patterns of other tumor suppressor gene-related proteins, including p53, Rb, and p16. These alterations are secondary to expression of the viral proteins E6 and E7. At the diagnostic level, this means that HPV-SCC is almost always associated with wild-type p53 (nonmutated and not overexpressed) and is almost always associated with overexpression of the p16 protein. Not surprisingly, HPV-SCCs also demonstrate differences in other molecular parameters, including the promoter methylation patterns and the micro-RNA patterns, as compared with tumors that are HPV negative.

Various HPV detection methods have been described in the literature with no standardized approach. These have included polymerase chain reaction (PCR)-based assays, real-time or quantitative PCR, and in situ hybridization. The PCR-based assays are presumably more sensitive than the in situ hybridization assays (some have argued they may be overly sensitive). The in situ hybridization assay has the advantage of being adequately sensitive, with the added value of allowing for visual identification of the cellular localization and integration of virus in tumor cells. Probes are available to detect either a single subtype of HPV or a cocktail of high-risk (and low-risk) HPV subtypes. HPV-16 is the most common subtype of HPV in SCC, although HPV-18 and the other high-risk subtypes can also be found in up to 5% to 10% of SCCs. Recent literature has demonstrated a very strong correlation between HPV
There are strong clinical prognostic and therapeutic reasons for the pathologist to identify HPV-SCC in tissue specimens, and occasional cases will benefit from diagnostic testing. An example of a diagnostic application is the patient who presents with a metastatic cystic squamous cell carcinoma in neck lymph nodes and an unknown primary carcinoma. In these patients, detecting HPV in the metastasis strongly suggests an oropharyngeal origin. For prognostic and therapeutic purposes, HPV testing also has an important emerging role. Patients with HPV-SCC have a better long-term prognosis and a lower rate of second primary tumors than patients with smoking-related carcinomas. Patients with HPV-SCC may have better overall responses to therapy, although large randomized controlled trials are needed to confirm these findings. The pathologist, therefore, should consider testing all oropharyngeal tumors for an association with HPV through p16 immunohistochemical staining and/or HPV molecular testing using in situ hybridization or PCR-based assays.

SALIVARY GLAND LESIONS

Three salivary gland tumors have newly described, interesting molecular profiles that warrant discussion: mucoepidermoid carcinoma, adenoid cystic carcinoma, and a recently described tumor, mammary analogue secretory carcinoma.

Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma (MEC) is a common salivary gland tumor that occurs across a broad age range and can appear in both minor and major salivary gland locations. Even in the first descriptions of the tumor from Stewart, Foote, and Becker in 1945, the 3 cell types of the tumor were noted: epidermoid, intermediate, and mucinous cells. In that original article, the tumor was described as having a highly variable clinical prognosis, with some tumors behaving exceptionally well and others having a poor prognosis. Early grading schemes relied entirely on the percentage of the tumor that was composed of cystic spaces (>80% had a good prognosis). More recent schemes have proposed point systems to more objectively grade MEC.

Little had been known about the molecular pathogenesis of mucoepidermoid carcinoma until the past 5 to 10 years. The prior literature has sporadic references to occasional alterations in tumor suppressor genes and oncogenes, such as a relatively low reported rate of HRAS oncogene mutations (<20%). One of the most important discoveries in the study of MEC, however, has been the identification of a translocation between the CRTC1 gene and the MAML2 gene. Interestingly, the translocation initially appeared to be much more prevalent in low- and intermediate-grade MEC than in high-grade MEC. A recent study that carefully excluded mucosally based adenosquamous carcinoma (which is a mimic of high-grade MEC) showed higher rates of translocation positivity in high-grade MEC.

The MECT1-MAML2 translocation can be identified using fluorescent in situ hybridization or with assays based on reverse transcription–polymerase chain reaction (RT-PCR). Virtually no other malignant tumors of the salivary gland have been shown to have this translocation.

Figure 2. p16 and human papillomavirus (HPV) in human papillomas virus–squamous cell carcinomas (HPV-SCCs) of the head and neck. A, This image is an example of p16 staining, which is diffusely and strongly positive, in an HPV-related SCC of the head and neck. B, This is an example of HPV-16 in situ hybridization that is demonstrating the dotlike staining pattern consistent with integrated virus. C, This is an example of HPV-16 in situ hybridization with an episcopal pattern, which shows diffuse blue staining in cells (p16 stain, original magnifications ×200 [A and B] and ×400 [C]).

detected by molecular techniques and p16 expression by immunohistochemistry (Figure 2, A). It may be possible to use p16 immunohistochemical staining alone as a surrogate marker for the presence of virus.
tumor. Some studies have demonstrated its presence in Warthin tumor, whereas others have not found this association. Because there can be mucinous metaplasia in Warthin tumor, some authors have argued that diagnostic discrepancies may also be confounding this argument.

**Adenoid Cystic Carcinoma**

Adenoid cystic carcinoma (ACC) is a tumor that can occur in both minor and major salivary gland locations throughout the head and neck. The tumor often has a relentless clinical course that includes late recurrences and distant metastatic disease. Histologically, ACC fits into the category of biphasic salivary gland tumors, meaning that it contains both epithelial and myoepithelial cell components. The growth patterns that are seen fall into 3 distinctive groups: tubular, cribriform, and solid types. Although these components are often mixed, the presence of a significant percentage of solid growth portends a worse prognosis and should be noted in diagnostic reports. These components have variable percentages in tumors, with epithelial-rich tumors often being associated with a poorer prognosis and more aggressive disease (solid growth pattern or high-grade transformation).

Interest in adenoid cystic carcinoma at the molecular level began from the identification of CKIT (CD117) overexpression at the protein immunohistochemical level. There was initial hope that the overexpression would provide a therapeutic target, similar to gastrointestinal stromal tumors. However, multiple studies have found no evidence of CKIT mutations in ACC; one surprising recent article described a high percentage of cases with CKIT mutation, which was in sharp contrast to all previous reports. A few clinical trials and case reports of ACC have reported mixed results using imatinib, which targets CKIT, although overall, it appears to have low efficacy. Importantly, overexpression of CKIT is not seen only in ACC and cannot be used as a diagnostic marker. Polymorphous low-grade adenocarcinoma and epithelial-myoepithelial carcinoma can have overexpression of CKIT.

Several recent articles describe a translocation between chromosomes 6 and 9 in ACC of both the head and neck and the breast. This translocation fuses the MYB gene with the NFIB gene, which leads to a chimeric transcript. Very interestingly, part of the deleted area of the MYB gene includes the 3′-untranslated region, which also contains several sites that have been shown to bind micro-RNA molecules. These micro-RNAs have, in turn, been found to customarily prevent expression of MYB, and when this regulatory area is through the fusion, MYB is overexpressed.

Some research has also investigated ACCs for allelic losses and gains through the use of comparative genomic array technology and loss of heterozygosity studies. These clinically indolent tumors are generally fairly stable genetically and do not show widespread loss and gain mutations that are common in more aggressive tumors. However, isolated genomic losses have been detected at individual genomic loci, including loss of heterozygosity of 12q, 1p, and 9p. The tumor suppressor gene p53 is probably involved in high-grade transformation of conventional ACC; these aggressive tumors are often associated with overexpression and mutations of the p53 gene.

**Mammary Analogue Secretory Carcinoma of the Salivary Gland**

A very recent article has described a new salivary gland tumor that histologically resembles secretory carcinoma of the breast, but occurs in salivary gland locations. Most of these tumors have likely been diag-
nosed as acinic cell carcinomas in the past because of some histologic overlap between these 2 tumors. The tumor grows in an infiltrative pattern, with microwystic and tubular patterns. Bubbly secretory material within the luminal spaces is PAS positive and diastase resistant. The low-grade nuclei are relatively bland and monomorphic, show vesicular-type chromatin, and have prominent, centrally placed nucleoli. Immunohistochemically, the tumors are strongly positive for CK7, vimentin, and S100 (Figure 3, A through C).

Similar to the namesake tumor in the breast, the salivary gland mammary analogue secretory carcinoma harbors a translocation between the ETV6 and the NTRK3 genes.111,112 In the recent report, which is the first to describe these tumors, 13 of 14 cases (93%) were positive for the translocation by RT-PCR.113 This translocation can be detected using an RT-PCR assay, or a fluorescent in situ hybridization-based approach.

CONCLUSIONS

With our expanding understanding of the pathogenesis and molecular alterations in different tumor types, new targets will continue to be proposed for diagnosis, prognosis, and therapeutic applications in all of pathology. The pathologist needs to be familiar with the molecular alterations that have strong potential for successful integration into diagnostic algorithms with good clinical applications.

References


