

Mucoepidermoid carcinoma

The presenting signs and symptoms of laryngeal mucoepidermoid carcinomas mimic those of squamous cell carcinoma of the larynx. Hoarseness is common and some patients have hemoptysis, foreign body sensation, dysphagia, or a neck mass. Reported lesions have varied in size from about 0.5-5.0 cm. Nothing in their gross appearance distinguishes them from squamous cell carcinomas. Microscopically, low-grade mucoepidermoid carcinomas of the larynx resemble the same type of tumor found in other sites, and recognition is usually not too difficult. High-grade mucoepidermoid carcinomas may resemble poorly differentiated squamous cell carcinomas. The behavior of these laryngeal tumors has been referred to as unpredictable. This may partly result from analyses that contain different numbers of high-grade adenosquamous carcinomas, which often are difficult to separate from mucoepidermoid carcinomas. Although histological grading influences treatment, the most important factor in therapy is the clinical stage. Total laryngectomy has been the most frequently employed treatment, but appropriately small or limited lesions have been treated with vertical hemilaryngectomy or supraglottic laryngectomy. In the presence of clinically enlarged neck lymph nodes, neck dissection should generally be performed. On the basis of our own experience and that of the Armed Forces Institute of Pathology, it is safe to presume that except for low-grade mucoepidermoid carcinomas, other supposed grades of that carcinoma in the larynx are much more likely to be adenosquamous carcinomas.

Adenosquamous carcinoma

This highly malignant neoplasm may arise from overlying surface mucous or from the ducts of minor salivary glands. Microscopically, adenocarcinomatous and squamous carcinomatous components should be present in a single neoplasm with intercellular bridges or keratin demonstrable in the squamous component. Approximately 40 cases have been reported in the larynx. However, the diagnostic criteria are not universally accepted, and some authors do not distinguish between adenosquamous and high-grade mucoepidermoid carcinoma. Furthermore, whether primary adenosquamous carcinoma of the minor salivary glands exists is controversial, most authors consider this carcinoma of surface origin.

Adenocarcinomas

There is also a frustrating lack of clarity in what constitutes an "adenocarcinoma" of the larynx after exclusion of adenoid cystic carcinoma. From the literature one gets the distinct impression that the so-called adenocarcinomas are poorly differentiated, large, bulky, and preponderantly supraglottic neoplasms that are subsurface in origin. When photomicrographic illustrations are available, many, if not most, have a neuroendocrine appearance.

Miscellaneous carcinomas

A few cases of laryngeal acinic cell carcinoma have been reported in the literature. Epithelia-myoeplithelial carcinoma, myoeplithelial carcinoma, carcinoma expleomorphic adenoma and salivary duct carcinoma have also been reported to occur in the larynx.

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Gene alterations in precancerous and cancerous lesions of the larynx

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Stratified squamous epithelium is composed of several layers with distinct biological functions. Cells in the basal layer, which make contact with basal membrane, are stem cells with the ability to proliferate and provide new elements for the upper layers. However, the main proliferative activity is detected by Ki67 expression in cells located immediately above them. Going upwards, the prickle cell layer is the most populated, as well as the most morphologically characteristic. These cells are still metabolically active, their main function being the production of keratin. Cells that enter the prickle cell layer express p21WAF1 instead of Ki67, but this expression is transitory because it is seen only in the lower layers (1).

Appropriate mechanisms regulating cell growth and differentiation maintain the normal turnover that controls epithelial thickness, perhaps through cyclin-dependent kinase inhibitors (CKIs), such as p21WAF1. It is widely accepted that malignant transformation of squamous epithelium progresses through a number of steps, some of which can be morphologically recognized, such as the so-called

precancerous lesions (2). Alterations in oncogenes and tumor suppressor genes participate in the development of neoplastic transformation and progression.

Several approaches have allowed for the identification of the genes actually involved in these lesions, or at least, of the chromosomal regions involved. The roles of genes such as p53, p16INK4a, and p21WAF1 as tumor suppressors have been investigated in laryngeal carcinoma. The participation of oncogenes such as *ras* or cyclin D1 is also of interest. Finally, molecules involved in invasiveness, such as matrix metalloproteinases, participate in progression of carcinomas. p53 mutation is the most frequent genetic alteration identified in human cancer (3). Loss of p53 function appears as one of the limiting steps for neoplastic transformation. p53 mutations have been detected in preneoplastic lesions (4). Moreover, p53 protein overexpression, a supposed marker for p53 mutation, is a common phenomenon in premalignant laryngeal tissues (5, 6). Loss of heterozygosity (LOH) at 17p13, where p53 is located, is already detected in hyperplastic lesions, with the number of cases with LOH increasing with progression to overt dysplasia and/or carcinoma *in situ*, but no longer progressing once invasive carcinoma is developed (7). About 50% of carcinomas harbor p53 alterations, although no association is seen with stage of disease, suggesting that it does not represent any further advantage for tumor progression once initiated (1, 5).

The role of p21WAF1 as a tumor suppressor in laryngeal carcinoma was suggested by its regulation, at least in part, by wild-type but not mutant p53 (8) as well as its ability to inhibit cyclin-dependent kinase activity (9). However, in squamous cells, p21WAF1 expression occurs apparently by p53-independent pathways (as can be seen in normal squamous tissue samples). p21WAF1 expression increases in preneoplastic lesions and can be detected throughout the entire thickness of the epithelium. Most of carcinomas express high levels of both mRNA and protein. Only poorly differentiated carcinomas have low mRNA levels and appear negative by immunohistochemistry. Even within moderately differentiated carcinomas, areas with better squamous differentiation are more strongly positive than less differentiated areas (1). Use of p21WAF1 for gene therapy in squamous cell carcinoma of the head and neck, however, has so far proven unsuccessful (10).

p16INK4a is the first member of a family of CKIs with known tumor suppressor activity (11). It is located at 9p21, another chromosome region that is frequently deleted in hyperplasia and more frequently in carcinoma *in situ*, suggesting a progression in preinvasive lesions. The frequency of LOH at 9p21 is the same in both carcinoma *in situ* and invasive carcinoma, similar to what is seen for p53 (7). There are several ways of inactivating p16INK4a, including mutation or promoter hypermethylation associated with LOH or homozygous deletion (12, 13), all of which are found in a number of carcinomas (14), but the finding of LOH in preinvasive lesions suggests that inactivation of p16INK4a can precede invasiveness. However, the INK4a locus has a complex structure, coding for two different transcripts, both with tumor suppressor activity, but through different pathways (15). In fact, p19ARF (p14ARF in humans), exerts its tumor suppressor activity through sequestering and promoting degradation of MDM2, a cellular protein that binds to, and promotes degradation of, p53. Therefore, the exact role of the two genes in tumor suppression by INK4a locus is still unknown.

Some other tumor suppressor genes are likely to participate in laryngeal carcinogenesis. These are known to be at 3p21 or near-

by, although they are still far from fully characterized. Activation of proto-oncogenes to oncogenes is the other main feature present in neoplastic transformation. Although theoretically many genes could be involved in squamous cell transformation, very little is known about the actual *in vivo* role of most of the oncogenes. An important event in many malignancies is the participation of the *ras* family, which represent the largest number of oncogenic alterations in human cancers. However, *ras* activation (usually by point mutation) appears as a key event in glandular, but not squamous transformation. Most of reports agree that there is an extreme rarity of *ras* mutations in head and neck cancer (16), although *Ras* protein overexpression is a common finding in carcinomas, independent of gene mutation, which, surprisingly, appears associated to a favorable patient outcome (17). On the other hand, the participation of cyclin D1 in laryngeal carcinomas has been well established. Cyclin D1 is the regulatory subunit that activates cyclin-CDK complexes, and it is associated mainly with CDK4 (18). Amplification leading to overexpression of cyclin D1 is associated with tumor progression in invasive carcinomas (19). Whether this is due to higher aggressiveness of the tumors or because it is a late event in neoplastic progression remains unexplained.

The key feature that differentiates preinvasive and invasive lesions is the progression through basal membrane. There, neoplastic cells progress according to their ability to detach from surrounding cells, to attach to stromal elements, promote degradation of stromal macromolecules and move on. All these features are necessary and must be tightly coordinated to be effective. Loss of adhesion molecules has been reported as well as analysis of different proteolytic enzymes. Most of the enzymes show an increase in its production or activity in tumors when compared to non-neoplastic tissues. However, production of some enzymes appears specific to neoplastic tissue. This is the case of collagenase-3, a member of the matrix metalloproteinase family (20) that is detected in invasive tumors but not in normal laryngeal mucosa. Moreover, expression of collagenase-3 is limited to advanced tumors, suggesting that these tumors have an invasive advantage. Collagenase-3 is activated by proteolysis of procollagenase-3. This is efficiently achieved by other members of the family, gelatinase A and the membrane-type matrix metalloproteinase, which are coordinately overexpressed in cases with collagenase-3 expression (16). In summary, loss of tumor suppressor function is implicated in the first steps of malignant transformation, although it does not confer additional advantages to invasive tumors. Activation of oncogenes participates in later steps, near the acquisition of the invasive phenotype, where a collaboration with rearrangement of adhesion molecule expression and modulation of proteolytic enzyme production is needed for further tumor progression.

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white to red, and are delicate, granular, polypoid structures which vary from 1-10 mm in diameter, most being less than 5 mm. In florid cases the papillomas form a solid field of mucosal thickening without invasion deep to mucosa. Under magnification, small individual papillae can be discerned as blunt finger-like processes with branches, which never become long and filiform. Microscopically, the papillary processes are seen as cylindrical projections with smaller offshoots of squamous-cell-covered epithelium cut in various planes, being second or even third order branching of the papillary structures. In a minority of cases there is keratosis in which layers of completely keratinized anucleate cells are seen on the surface of the papillae.

Sometimes cells of the squamous epithelial covering of the papillae show atypical change, a situation which is frequently related to the presence of koilocytosis. Epithelial atypia has been alleged to be associated with rapid recurrence of papillomas and increased risk of progression to carcinoma. Koilocytosis is frequently seen in the upper, intermediate and superficial zones of the squamous epithelium of laryngeal papillomas. It consists of a spherical enlargement of the cells of the lesion, accompanied by perinuclear vacuolation, so that either no stained cytoplasm is seen in the cell at all, or there is but a thin rim of cytoplasm around the cell periphery. The nucleus is central and often enlarged, angular or wrinkled. It may exhibit moderate degrees of atypical change. Infection of the cells of squamous papillomas of the larynx by human papillomavirus is frequent (see below), and has been closely correlated with the presence of koilocytosis. In a few cases of papillomatosis some of the material shows a papillary transformation not only of squamous cell epithelium, but also of respiratory epithelium. The latter comprises nonmalignant respiratory epithelium featuring both ciliated cells and goblet cells, the appearances being reminiscent of those of the entity of cylindrical cell papilloma of the nose and paranasal sinuses. Papillomas showing respiratory epithelial hyperplasia have a decided tendency to recur. Difficulty may be experienced in distinguishing two other types of neoplastic lesion, which occur particularly often on the vocal cords, from squamous papilloma: i) keratotic plaque with dysplasia; and ii) carcinoma of both verrucous squamous and regular types.

Raised plaques composed of thickened squamous epithelium with a keratinized surface are seen quite frequently in biopsy. They show a mild to severe degree of dysplasia of the deeper layers of their squamous cells. It is important to separate these lesions from squamous papillomas because the former have a malignant potential which is not possessed by the latter. A careful examination of the whole biopsy for evidence of cylinders of papilloma formation and the branching that is associated with them will usually suffice to distinguish this lesion. The degree of dysplasia exhibited by the plaque is often more severe than would be expected in a papilloma.

Squamous carcinoma may exhibit papilloma formation and should not be mistaken for benign squamous papilloma. The pattern is not, however, as symmetrical as in papillomas and branching of papillae is unusual. Verrucous squamous carcinoma may display very long papillae, usually without branching. The rete ridges are irregular and the basement membrane is rarely hyalinized, a feature which is frequent in papillomas. Squamous cells of the intermediate layer in verrucous carcinomas and some papillary areas of regular squamous carcinoma are larger than those of the corresponding layer in squamous papillomas, showing a mean area of more than 300 μm^2 on image analysis.

Squamous papilloma of the larynx

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Papilloma is a benign exophytic neoplasm of epithelium on a connective tissue core. In the larynx the stratified squamous variety is the commonest form of papilloma. They are found in both adults and children, but, in the latter, because of the much narrower diameter of the airway, the symptoms are more serious and treatment is more urgent and difficult. It is thus customary to divide the condition on the basis of the age of the patient into juvenile and adult types. In some juvenile cases the papillomas persist into adult life. The histopathological appearances are similar at all ages. By far the commonest site of occurrence of squamous papilloma in the larynx is the vocal cord, usually in the anterior half. Multiple papillomas may, particularly in children, spread upwards to the supraglottis, pharynx and soft palate. Squamous papillomas range from