Results and discussion

In all cases of primary tumor and matched lymph node metastasis, p53 mutations were identical. These results are in agreement with the studies referred to in the Introduction section, in which the same concordance between primary tumor and lymph node metastasis was observed. It appears that in HNSCC carcinogenesis, p53 mutations occur before a lesion metastasizes and are maintained during metastasis. Although in the process of tumor progression, additional mutations may occur or mutations may be lost, these phenomena apparently do not influence the p53 status. Similar findings on consistency in p53 status in the autopsied cases confirm this stability of p53 status during tumor progression and metastasis, In the cases with multiple HNSCCs, all lesions had different mutations. These data support the assumption that different p53 mutations in different HNSCCs are due to an independent origin of these tumors. The alternative monoclonal theory (23) appears to be less likely. This theory states that new tumors could be the result of micrometastatic foci and that the difference in p53 mutations in different lesions can be explained by assuming that p53 mutations are either lost or acquired during metastasis. In this case, similar changes would occur during the development of lymph node metastasis and result in differences between p53 mutations in primary tumor and lymph node which disagrees with our observations. Moreover, p53 mutation analysis to assess cional relationship appears to be superior to LOH studies. In the 15 cases of primary tumor with matched lymph node metastasis, no differences in LOK were observed which suggests that these markers are stabile during tumor progression; however, in this rather limited sample, the same LOH pattern was observed in HNSCCs from different patients. Therefore, the occurrence of similar LOH patterns in anatomically distinct tumors in an individual case may be due to chance and cannot be considered proof for a common clonal origin. The three cases with clinically separate primary SOCs in the head and neck region and the lung serve to illustrate the application of p53 mutation analysis in clinical pathology. Different p53 mutations were observed in one case and identical p53 mutations in the two others, thus suggesting lung metastasis from HNSCC in the two former cases and a second primary tumor in the latter case.

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Laryngeal spindle cell, verrucous and basaloid squamous carcinoma

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Spindle cell carcinoma

This variant of squamous cell carcinoma (SCC) is histologically characterized by a SCC and another underlying or adjacent spindle cell or pleomorphic component (1). Most but not all spindle cell carcinomas grow rapidly, are polypoid and bulky. They constitute approximately 1% of all malignant laryngeal neoplasms, In the upper respiratory tract, the larynx is the most common site, and

within the larynx, the true vocal cords (2). Radiotherapy will not induce an anaplastic carcinoma, Tumors with histology compatible with spindle cell carcinoma are not homogenous but comprise the following: 1) spindle cell carcinoma (SOC associated with malignant spindle cells that are demonstrably epithelial); 2) squamous cell carcinoma with pseudosarcomatous stroma in which a SCC is associated with atypical but non-neoplastic fibroblastic or fibrohistiocytic proliferation (Lane's pseudosarcoma); and 3) true examples of carcinosarcoma as in the three listed below.

i) Spindle cell carcinoma may histologically show a wide spectrum ranging from pleomorphic sarcoma suggestive of rhabdomyosarcoma, or dense fibrosarcoma-like structures, to more loose vascular structures. The spindle-shaped cells may vary in appearance, sometimes being very pleomorphic and bizarre. The spindle cells are of squamous origin, may be closely packed, and may sometimes even have a clear cell appearance. Abundant collagen is a frequent finding, whereas osteoid or cartilaginous tissue are not (2, 3). Mitoses are infrequent. Bizarre giant tumor cells are common while multinucleated giant cells are rare. The surface epithelium is often ulcerated and dysplastic. In many cases, but not all, one can see a diffuse connection with the overlying epithelium. In yet other cases, the lesion is associated with a readily recognized invasive SCC of the overlying epithelium. The blood vessels show no tendency towards a radial arrangement. In most cases the spindle cells stain positively for cytokeratins, and may also have a dual positivity for vimentin (2, 4). The spindle cells are often nondiploid, and have a lower mean per cent MIB-1 staining than the squamous component (4).

ii) The second type is a SCC with pseudosarcomatous stroma (Lane's pseudosarcoma) (5). The spindle cells are atypical and bizarre, but are non-neoplastic. The spindle cells are of fibroblastic or fibrohistiocytic origin, and negative for cytokeratins. There are, however, rare occasions when no SCC is to be found but only *in situ* carcinoma.

ii) The third type, carcinosarcoma, is very rare in the larynx. Laryngeal carcinosarcoma is as carcinosarcoma elsewhere in the body, *i.e.,* a sarcoma coexisting with squamous cell carcinoma.

Verrucous squamous cell carcinoma

Verrucous squamous cell carcinoma (VSCC) is an extremely well differentiated variant of squamous cell carcinoma (1, 6), which may be erroneously diagnosed as it lacks several of the conventional features of epithelial malignant neoplasia. It represents 1-2% of laryngeal carcinomas (7). It is usually superficial, bulky and presents as a warty papillomatous surface. Most tumors are found in the glottis (8). VSCC shows slow growth and eventually local invasion but rarely, if ever, metastases. The low-grade malignancy of this neoplasm allows for conservative therapy and neck dissection is not indicated. Radiotherapy gives good results, and does not induce anaplastic carcinoma (9). Microscopically the tumor consists of a well-differentiated epithelium with keratinized surface, and apparent absence of both dysplasia and stromal invasion. The rete ridges are blunt ended and irregular. Proliferating squamous masses often bulge outwards in a papillary fashion. Superficially abundant keratin is present, often displaying an externally pointed appearance, *i.e.,* the "church spire" effect. Typically, there is a dense, mononuclear cell exudate beneath the tumor processes. Under high power, the basal cells are crowded and composed of prominent cells, often with vesicular nuclei and showing a single eosinophilic nucleolus (9). The cells of the intermediate zone of VSCC appear to be larger than those of the same zone in squamous papilloma (10). Image analysis has demonstrated a significant difference between the sizes of the intermediate cells in these two conditions; a mean cell area greater than 300 μm^2 squared supports a diagnosis of VSCC whereas an area less than 250 μm^2 supports a diagnosis of squamous papilloma (11). Examination of VSCCs and papillomas for the presence of human papilloma virus (HPV), using *in situ* hybridization with DNA probes, has revealed that papillomas may be related to HPV, but VSCCs are likely not (42 of 42 papillomas were positive for HPV 6/11, while none of 11 VSCC contained demonstrable HPV) (12). Verrucous SCC of the larynx should thus be distinguished from squamous papilloma. Discrimination can also be made from papillary squamous cell carcinoma, which is an invasive SCC with an exophytic papillary component (1). This latter distinction is less important because papillary SCC has a clinical behavior similar to verrucous SCC.

Basaloid squamous cell carcinoma

This is a high-grade SCC which is a bimorphic carcinoma consisting of a mixture of basaloid and squamous cell components (1). Basaloid SCC was recognized as a separate entity in the upper respiratory tract only a decade ago (13). Various terms have been used to designate this neoplasm, and when the tumor occurs in the anal canal, it has been called cloacogenic carcinoma, In the upper respiratory tract it is predominantly found in the hypopharynx and base of tongue. Basaloid SCC is a tumor of high-grade malignancy, and should be distinguished from i) adenoid squamous cell carcinoma (low-grade), and ii) adenosquamous cell carcinoma (high-grade), Adenoid squamous cell carcinoma shows pseudoglandular lumina or spaces resulting from acantholysis of the tumor cells. There is no true glandular differentiation and thus no mucin production. Adenosquamous cell carcinoma has histological features of both adenocarcinoma and SCC. Laryngeal basaloid SCC often appears as an exophytic, polypoid and centrally ulcerated mass. Microscopically, the basaloid component is usually the more prominent, and consists of small cells with hyperchromatic nuclei and scanty cytoplasm. The cells are arranged in lobular masses or cords, often with small cystic spaces containing mucoid material, which stains with periodic acid-Schiff stain and/or Alcian blue. There is peripheral palisading, focal squamous differentiation, often numerous mitoses, and areas of necrosis. Stromal hyalinization is a common finding, The squamous component, which may be invasive or in *situ,* can be defined by two or more of the following: i) keratin pearl formation; ii) individual cell keratinization; ii) intracellular bridging; and iv) cells arranged in a pavement or mosaic pattern (13, 14). Basaloid SCC is positive for cytokeratins, often for EMA and CEA, and may also show positivity for, *e.g.,* NSE and S-100 (15, 16). The differential diagnosis is primarily with adenoid cystic carcinoma, but also with neuroendocrine carcinoma (small cell). High molecular weight cytokeratin reactivity is reported to more consistently differentiate basaloid SCC from small cell undifferentiated carcinoma than reactivity with neuroendocrine markers (17).

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Adenocarcinomas and salivary gland neoplasms of the larynx

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Introduction

Minor salivary gland tumors of the larynx are rare; only a few large series have been reported from a single institution. Therefore, details of their clinical and pathological behavior come only through composite analysis of small series. Less than 1% of the epithelial malignancies of the larynx are of salivary gland origin.

Subepithelial and intraepithelial glands

The sites of origin of the salivary gland neoplasms of the larynx follow the anatomical distribution of the larynx subepithelial glands and the intraepithelial mucous glands. Approximately two-thirds of the adenoid cystic carcinomas are in the subglottis, The other carcinomas, in contrast, are rarely subglottic, with supraglottic and transglottic involvement being nearly equal. The lower part of the glottic region shows the greatest differences in density of submucosal glands: 13 glands/cm2 on the vocal cords to 128 glands/cm2 on the false vocal cords and medial wall of Morgagni's sinus. The greatest concentration of glands is in the saccule (139 glands/cm^2). There is a Very low density of glands in the extrinsic laryngeal regions. A typical intraepithelial gland is made up of 15-30 mucus-secreting cells with a structure like that of goblet cells. They extend from the epithelial surface down toward the basement membrane, on which they may rest, but do not penetrate. Irregularly distributed in the larynx, intraepithelial glands are most numerous in the supraglottis and least numerous in the subglottis.

Salivary gland neoplasms

With the exception of adenoid cystic carcinomas, salivary-type carcinomas are rare in the larynx. Even pleomorphic adenomas are almost curiosities in this organ.

Benign neoplasms

Benign laryngeal neoplasms are extremely unusual with exception of oncocytic lesions.

Oncocytic lesions (metaplasia, hyperplasia, oncocytoma

Oncocytic lesions of the larynx occur most often in patients aged 50-80 years. A slight predominance of male patients exists. These lesions are most often located in the false Vocal cord or laryngeal ventricle areas. The microscopic findings in most cases suggest oncocytic metaplasia and cystic hyperplasia rather than neoplasia. Well-defined, columnar, oncocytic epithelium arises from seromucous ducts or acini and subsequently expands into cystic structures. Papillary growths into the cystic spaces are common. The process seems to be multifocal in the large majority of cases, and this contributes to the impression that this is a metaplastic process.

Pleomorphic adenoma

Certainly, the diagnosis of pleomorphic adenoma in this anatomic location should be entertained cautiously, and distinction from the many tumors that occur more frequently *(e.g.,* adenoid cystic carcinomas, mucoepidermoid carcinoma and low-grade chondrosarcoma) should be foremost in the mind of the pathologist.

Other benign tumors

Myoepitheliomas have been rarely reported in the larynx. The morphology and clinical course has been identical to their counter part in the major salivary glands.

Malignant neoplasms

Adenoid cystic carcinomas

Adenoid cystic carcinomas of the larynx comprise only about 0.25% of laryngeal carcinomas. The age range of occurrence is fairly wide, but they are found most often in the fourth to the sixth decades of life. The sex incidence is approximately equal. These laryngeal tumors most often occur in the subglottic area, but a substantial proportion are found supraglottically, Tumors of glottic origin are even less common. The histological features are the same as those seen with adenoid cystic carcinomas found at other sites. Total laryngectomy has generally been employed for treatment. Not unexpectedly, a high incidence (>50%) of local treatment failure does occur; thus postoperative radiotherapy may be beneficial. Because the reported incidence of lymph node metastasis is higher than that for adenoid cystic carcinomas elsewhere, some have recommended elective neck dissection.