ing on how to separate high-risk from low-risk precancerous lesions. Such information would assist in predicting which AtH will regress, remain stable, or progress to invasive cancer, and would thus influence the mode of treatment.

The studies presented here, based on the light microscopic level, may be considered to be clinically feasible methods for daily routine practice in determining the risk level for laryngeal cancer development.

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Clonality studies in multiple head and neck cancers: p53 mutations compared with LOH at 3p, 9p and 17p loci

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Introduction

Patients wjth head and neck squamous cell carcinoma (HNSGC) are at risk of developing additional tumors in the head and neck (1). However, HNSCC patients also frequently develop local reCurrences or locoregional metastases (2). Differentiation between metastasis or recurrence of the primary tumor *versus* second tumor may be difficult as all lesions have the histological appearance of squamous cell carcinoma. Differentiation between these possibilities, however, carries important differences in therapeutic and prognostic consequences. Therefore, diagnostic modalities other than histopathological ones are needed to distinguish between local recurrence and metastasis on the one hand and second tumor on the other. Molecular biological detection techniques may be useful in these cases. Several genetic markers have been used for assessing the clonal relationship between separate HNSCCs occurring in individual patients. Among these are loss of heterozygosity (LOH) patterns at loci 3p and 9p. These changes have been shown to occur early in carcinogenesis (3, 4), but as it has been demonstrated that they may differ among primary tumors and their matched lymph node metastases, it is obvious that they do not meet the criterion of stability during tumor progression and metastasis (5, 6). Furthermore, p53 mutations have been employed as a clonal marker. This seems to be promising, p53 being mutated in a high percentage of HNSCCs and showing a huge variability in its mutations (7). However, p53 will only be useful as a clonal marker in HNSCC when mutations develop before metastasis has occurred and they are not lost during tumor progression. This can be demonstrated by investigating whether a specific p53 mutation is consequently found in a primary tumor and its matched lymph node metastasis. Literature on this issue yields conflicting data. In some studies, complete concordance was observed (8-17), but different p53 mutations in primary tumor and lymph node metastasis have also been reported (11-13, 18, 19). Tumors may contain different clones, with different expression of metastatic potential. Discordancies in p53 mutations between primary tumor and lymph node metastasis mean that studies in which different p53 mutations in different neoplastic lesions are supposed to indicate an independent origin of those lesions (8, 10, 11, 15, 17, 20, 21) are at least premature: the possibility that a tumor has changed its p53 mutation status during progression by acquiring new mutations or losing initially present mutations cannot be excluded.

Materials and methods

We recently developed a p53 mutation analysis strategy consisting of direct sequencing full-length p53 mRNA as well as DNA from the mutated exon followed by screening for mutations already identified by DNA sequencing. This strategy proved to be very sensitive and resulted in a p53 mutation percentage of almost 100% in an unselected sample of HNSCCs (22). In the present study, we applied this technique to study 15 primary HNSCCs with matched lymph node metastasis to determine whether p53 mutations are stabile during metastasis. We also compared loss of heterozygosity at loci 3p, 9p and 17p in this same series to confirm or refute differences in LOH patterns at these loci emanating during metastasis and tumor progression. Furthermore, tumor tissue from eight cases with multiple primary HNSCCs, from four autopsied HNSCC cases with disseminated disease and from three cases with surgically treated HNSCC as well as lung SCC were subjected to the same p53 mutation analysis to obtain a full impression of the usefulness of p53 mutations when investigating the clonal relationship between several HNSCC manifestations in an individual case. Studies concerning 3p and 9p LOH for the three latter groups are still in progress and will be presented at the symposium.

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