Progress in laryngeal pathology

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Laryngeal precancerous lesions: Current diagnostic and prognostic considerations

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The diagnosis, treatment and prognosis of laryngeal epithelial changes, which are referred to here as epithelial hyperplastic laryngeal lesions (EHLL), depend almost entirely on their histological abnormalities. EHLL cover the whole spectrum of histomorphological changes with more or less expressed cellular and structural abnormalities with a preserved basement membrane (1). However, the lack of uniformity and inconsistency of the terminology, and the fact that the histological features and the biological behavior of these lesions are not always in accordance, have frustrated attempts to gain an internationally accepted classification of EHLL. It is not surprising that more than 20 classifications of EHLL can be found in the literature within the last three decades, however none has entirely fulfilled our expectations for daily practice in predicting evolution, particularly of high risk lesions (2-5). The majority of EHLL classifications have followed similar criteria to those in common use for cervical lesions, now most frequently called cervical intraepithelial neoplasia, for more than 25 years, does not follow the three-grade criteria used for classifying cervical lesions, but was devised to cater to specific clinical and histological laryngeal problems (3, 13).

The Working Group on EHLL of the European Society of Pathology reevaluated and further formulated the histological criteria of the Ljubljana classification in November 1997 in London, UK. The system is divided into four grades as follows: simple (SH) and abnormal (AbH) hyperplasia form a benign group, atypical hyperplasia (AtH) is potentially malignant, and carcinoma in situ (CIS) a malignant lesion. SH is characterized by an augmented spinous cell layer while the basal layer remains unchanged. In AbH, basal and parabasal cells are augmented to the midportion of the epithelial thickness with a perpendicular orientation to the basement membrane. AtH, or risky epithelium, is characterized by a still preserved stratification of epithelial cells, which are enlarged and augmented with basal, and on rare occasions spindled form, differentiation. The nuclear/cytoplasmic ratio is increased and the nuclei of these cells are diagnostically important: loss of epithelial stratification as a whole, marked cellular alterations, and many mitotic figures, more than five per high power field (5). The results of malignant transformation of individual grades of EHLL justify our proposal for dividing the lesions into benign and potentially malignant, according to their histological features. Simple and abnormal hyperplasias are considered benign forms with 0.7% (3/380 patients within 15 years) and 1% (4/414 patients within the same period) of malignant transformation, respectively. AtH is a precancerous lesion in the essential meaning of the word, with 9.5% (101105 patients) of malignant alteration within 15 years (3).

According to other studies of EHLL, patients with AtH or severe dysplasia present the most threatening group associated with the highest risk of cancer development ranging from 19-28% (7, 14).

Laryngeal precancerous lesions have no specific macroscopic appearance, and are variously referred to as chronic laryngitis, leukoplakia, keratosis, erythroplakia, dysplasia, etc. The surface morphology and keratin layer formation of these lesions has no specific meaning, nor any significant relationship with their malignant potential (3, 4). Histopathological diagnosis informs a clinician well of how to treat patients with benign, potentially or actually malignant lesions. On the other hand, reliable evidence is still lack-
ing on how to separate high-risk from low-risk precancerous lesions. Such information would assist in predicting which ATx 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.

References

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 with 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 stable 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.