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PCNA - A Cell Proliferation Marker in Vocal Chord Cancer. Part I: Premalignant Laryngeal Lesions

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Abstract. *Laryngeal hyperkeratotic lesions can progress to fully developed malignant carcinoma in some cases. These premalignant lesions are proliferative disorders whose potential for further tumour progression is perhaps difficult to assess by mere histology. Immunostaining with PCNA, a protein correlated with cell proliferation, has been used to study tissue behavior in 30 cases of premalignant laryngeal vocal chord lesions treated by epithelial stripping in microlaryngoscopy, 15 of whom had no progression and 15 had recurrence and final development of full malignancy. The results showed a statistically significantly higher PCNA-index in the cases which underwent further tumour progression towards malignancy. PCNA testing may thus be suggested as a marker for tumour progression potential and help in determining clinical treatment choices.*

The hyperplastic hyperkeratotic proliferative mucosal changes, frequent in the human larynx (1), form a heterogeneous group of pathological lesions with different potential of progression and clinical course. It has been known since the beginning of the century (2) that precancerous lesions can undergo progressive cellular changes towards malignancy through several steps of progressive cellular atypia (3,4,5). This process can be favoured by carcinogenic agents (6,7,8) or be apparently spontaneous (9). A clear retrospective evaluation of the published data concerning the progression of vocal chord premalignancies is made difficult due to the impossibility of clear matching of the same entity in different and sometimes quite generic classifications (10), and due to different orientations as regards treatment. Up to now a definitive classification of premalignant lesions has not been agreed upon. The most recent agreement is to use the LIN (laryngeal intraepithelial neoplasia) classification (11), which has been derived from the classification of premalignant lesions of the uterine cervix, substituting the previous classification by Kleinsasser and Hellquist (12,13) .

These premalignant lesions show a low mitotic index and most of them behave quite benignly; stripping of the vocal chord epithelium in microlaryngoscopy (14) is all that is needed for definitive treatment (15). Left alone, perhaps some of them will eventually progress to epithelial carcinoma, and this justifies the surgical treatment. In some cases the treatment may be insufficient and after some time, from a month up to several years, hyperkeratosis will recur and may progress to regular epithelial carcinoma.

This great variability in the progression potential of otherwise apparently equal lesions documents a still incomplete understanding of the intimate mechanisms that make the cells progress towards malignancy and the prognostic factors used today still remain subjective and uncertain.

In order to assess further the progressive potential of premalignant lesions, many markers of cell behaviour have been studied. Cell proliferation is considered a general marker of the degree of malignant deviation of tissue, as well as oncogene expression (16), specific cytokeratines (17) and DNA changes seen by flowcytometry (18). Perhaps no truly reliable index has been found up to now. Specifically in premalignant hyperkeratotic lesions and laryngeal intraepithelial neoplasms classified as LIN II and LIN III, which show a large variability in their potential to develop in full vocal chord cancer, no parameter has proven to be a reliable predictive marker for the potential of further progression.

The problem of prediction of further cell changes towards greater invasiveness extends also to the already malignant lesions. Once carcinoma has established itself on the vocal chord, its further potential for recurrence after radical surgery is so far not predictable. While in most cases subperichondral

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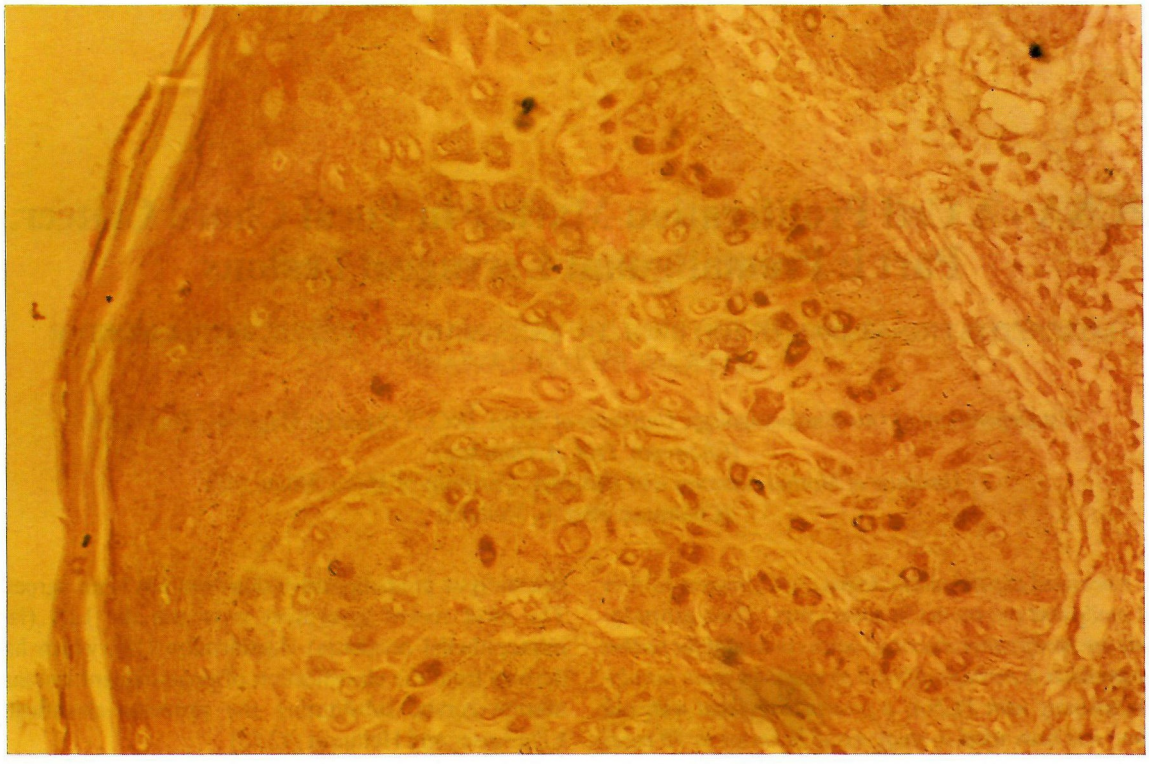

Figure 1. *Tissue positivity to PCNA/Cyclin.*

chordectomy (19), or-according to some protocols-radiotherapy (20,21), alone prove to be curative in some cases, recurrence can be seen. In these cases the prognosis drops dramatically and the tumours which show themselves able to recur once demonstrate a high potential for further recurrence and final death of the patient (22).

The possibility of testing the potential of a malignant or premalignant tissue to progress further could help to determine differentiated treatment protocols, more aggressive than the standard in selected cases of high malignant potential. Further markers for the mitotic potential of the cells could fill this void. Recently, proliferating cell nuclear antigen (PCNA), a 36kDalton protein of delta-DNA-polymerase (23) expressed mainly in the S-phase of cell replication (24), has been studied for this purpose and specific antigens for immunohistochemistry are now commercially available.

Premalignant laryngeal vocal chord tissue has been subjected to analysis for this nuclear antigen, in order to assess the prognostic value of PCNA in this lesion.

Materials and Methods

From the LIN lesions treated in the 1st ENT Department of Milan University 30 cases of intraepithelial LIN II vocal chord lesions were selected and examined. All cases were treated surgically by epithelial stripping in microlaryngoscopy according to Kleinsasser with complete removal of the pathologic tissue. The patients were subjected to a 5 year follow up. 15 cases that did not show any further problem and 15 which developed recurrence with full vocal chord carcinoma were examined.

Table I. *Analysis of PCNA-reactivity percentages of hypercheratosis and vocal chord carcinoma.*

	Range	Mean	Median
non evolution	2.50-7.20	5.05	4.73±1.58
with evolution	3.09-28.0	13.59	15.98±8.59
Variance analysis (F-test):	F=15.3	p<0.0001	

Table II. *Comparison between reactivity in premalignancies that would finally develop in carcinoma and vocal chord carcinoma.*

	Range	Mean	Median
premalignancy with evol.	3.09-28.0	13.59	15.98±8.59
carcinoma	8.10-36.80	25.58	24.18±8.14
Variance analysis (F-test):	F=5.76	p<0.025	

The stripped tissue was fixed in 10% buffered formalin. Paraffin sections of 4 micrometers from the same block used for routine histological examination were air-dried overnight at room temperature and immunostained with the monoclonal antibody PC10 (Dakopatts UK Ltd.) at a dilution of 1:200, using an immunoperoxidase method (ABC complex) with light hematoxylin counterstaining. All immunostained sections were examined using a x 100 objective. A minimum of 1000 cells in random fields were counted in every case. Control samples were

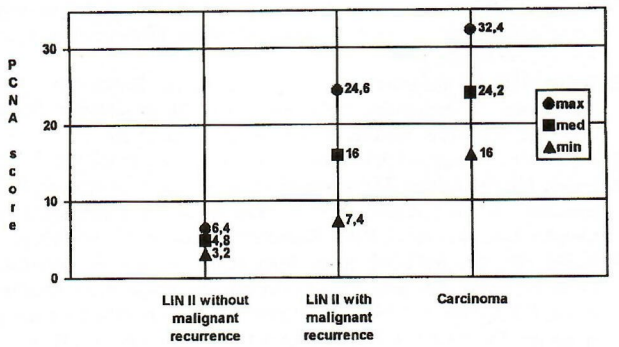

Figure 2. *Variance test analysis (F-test) for PCNA reactivity in non evolutive premalignancy, evolutive premalignancy and carcinoma.*

obtained with the same technique on normal tonsillar tissue, obtained by biopsy.

The PC10-index was defined as the number of cells with strong unequivocal nuclear staining, corresponding to cells in their S-phase and the results were expressed as percentage of the total number of cells counted. The specimens were blind tested, no clinical data were known to the pathologist and immunohistochemistry was done without knowledge of the results of routine histology. The results were subjected to statistical evaluation with the F variance test (25).

Results

All examined fields showed PCNA immunoreactivity (Figure 1). PCNA/cyclin reactivity lay in a range between 2.5% and 28.O0%. The immunoreactivity from the 15 specimens coming from patients who later developed full carcinoma was significantly greater than that from the other 15, who did not show any further degeneration (Table I). For comparison, the PCNA test was repeated on the tissue of the epithelial neoplasms that arose in the 15 patients who showed tumor progression, and the index was again significantly greater (Table II) than in the original premalignant lesion. Overall, the PCNA-index was significantly lower in the hypercheratotic lesions which did not progress to malignancy compared to those which did and to carcinoma that arose from these lesions.

Discussion

Tumor progression is an extremely important field in evaluation of treatment of precancerous lesions. It has always been known that the so-called precancerous lesions of the lining epithelia can show a very large array of progressive behaviour, ranging from no progression at all to highly aggressive invasive neoplasia (26,27,28,29,30). Local recurrence of the premalignant lesion is a frequent event, resembling the first step toward local malignancy (31,32,33,34). Progression towards fully expressed carcinoma can be seen in the remaining premalignant tissue as well as in the surrounding, apparently normal, mucosa, presenting itself directly as a malignant neoplasm at recurrence (35). Comparative oncology shows us even more clearly that the passage from a premalignant lesion towards carcinoma is a continuous phenomenon in which many intermediate steps are presen (36,37,38). While treatment of carcinoma as a lifethreatening disease offers a large array of options, from conservative to highly destructive, the treatment of precancerous lesions, and especially those which do not show a great tendency to tumor progression, must be more conservative.

At the moment no proven index is available to predict the progression of a precancerous lesion to malignant neoplasia. We know only that the probability of degeneration rises with the degree of cellular abnormalities. The percentages shown in the literature depend not only on the accuracy of histology, but also on follow-up and types of treatment of the primary non malignant lesion. Furthermore, the persistence of the primary risk factors may favor further progression of the remaining epithelia towards malignancy.

A reliable index for the progressive potential of these lesions would be of great importance. Our results point in this direction, by suggesting a reliable marker for tumor progressive behaviour in laryngeal intraepithelial neoplasia. A high PCNA-index in otherwise equal hypercheratotic LIN lesions was shown to be linked to a high probability of further malignant progression. This findings may justify a differentiated clinical treatment with more aggressive protocols in selected cases.

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