Expression of Cytokeratine 8 in Laryngeal Lesions

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Abstract

Background: Cytokeratins are structural proteins, which form intermediate filaments within the cytoplasm of simple epithelial cells. Cytokeratin 8 is a tumor-associated antigen, which is shown to be over-expressed in a variety of premalignant and malignant lesions in different head and neck areas.

Objective: Our study presented differential expression of cytokeratin 8 in squamous epithelia of the vocal cords, including normal mucosa, non neoplastic lesions and carcinomas. Also we studied whether cytokeratin 8 is a reliable marker for dysplasia and malignancy of vocal cords.

Methods: Our study comprised 50 subjects; they were classified into two groups. A control group comprised 25 samples of normal mucosa were obtained from patients suffering from chronic laryngitis (n=13), laryngeal carcinomas (n=12). The second group comprised 25 samples of different laryngeal lesions, 7 samples of laryngeal leucoplakia, 3 samples of laryngeal polyps, 4 samples of vocal nodules, 4 samples of chronic hyperplastic laryngitis and 7 samples of laryngeal cancer. All specimens had been confirmed by routine clinical diagnosis and histopathologic examination. Cytokeratin 8 expression was assessed upon immunohistochemistry with specific antibodies in sections of different laryngeal lesions.

Results: Cytokeratin 8 expression was evident in early stages of disease, i.e. dysplastic or neoplastic, but not in normal or hyperplastic epithelium.

Conclusion: Cytokeratin 8 is a reliable marker for dysplasia and malignancy of vocal cords.


Introduction

HNSCC represents 4% of all human cancers in the western world and the incidence of this type of malignancy is expected to increase in the future [1]. The incidence of cancer larynx varies from 2-10 per 100000 populations [2]. The morbidity and mortality from HNSCC are significant and current therapeutic approaches have not increased survival [3,4]. The five year survival rates can be improved by early diagnosis.

Cytokeratins are intermediate filament keratins found in the intracytoplasmic cytoskeleton of epithelial tissue. There are two types of cytokeratins: the low weight, acidic type I cytokeratins and the high weight, basic or neutral type II cytokeratins [4,5]. The high molecular weight cytokeratins, which are the basic or neutral cytokeratins, comprise subtypes CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8 and CK9. The low molecular weight cytokeratins, which are the acidic cytokeratins, comprise subtypes CK10, CK12, CK13, CK14, CK16, CK17, CK18, CK19 and CK20 [5]. Expression of these cytokeratins is frequently organ or tissue specific [6-8]. The expression of cytokeratins depends mainly on the type of epithelium, the moment in the course of terminal differentiation and the stage of development [9,10]. Thus this specific cytokeratin fingerprint allows the classification of all epithelia upon their cytokeratin expression profile. Furthermore this applies also to the malignant counterparts of the epithelia (carcinomas), as the cytokeratin profile tends to remain constant when an epithelium undergoes malignant transformation [11-13]. The main clinical implication is that the study of the cytokeratin profile by immuno-histochemistry techniques is a tool of immense value widely used for tumor diagnosis and characterization in surgical pathology [14-17].

Here, we present a comprehensive study of CK8 expression in normal mucosa and hyperplastic lesions of the vocal cord. We have used immuno-
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hypothesis and we examined the expression of CK8 in histological sections of normal, premalignant lesions and cancer larynx patients, to evaluate their role as biomarkers of histopathological progress in vocal cord carcinogenesis.

**Patients and Methods**

**Tissue samples:**

Our study was conducted in Otolaryngology department Zagazig University Hospital with collaboration of pathology department, from 2005-2008. All human samples were obtained after informed consent. 25 samples of normal mucosa were obtained from patients suffering from chronic laryngitis (n=13), laryngeal carcinomas (n=12). 25 samples of different laryngeal lesions were obtained, 7 samples of laryngeal leukoplakia, 3 samples of laryngeal polyps, 4 samples of vocal nodules, 4 samples of chronic hyperplastic laryngitis and 7 samples of laryngeal cancer. All specimens had been confirmed by routine clinical diagnosis and histopathologic examination. Tissue specimens were shock-frozen in liquid nitrogen and embedded in tissue-tek (Sakura, Fintek NL) to generate 4 gm non-consecutive sections.

**Immunohistochemistry:**

The mouse anti-human CK8 clone 35ßH11 primary antibody, mouse was used (Dako, Glostrup, DK), (diluted 1:100). Immunostaining was performed using the avidin-biotin-peroxidase complex method (Vectastain, Vector laboratories, Burlingame, CA, USA) according to the manufacturer's protocol. Briefly, after fixation in acetone (10 min), endogenous peroxidase activity was inhibited upon treatment with 0.03% H2O2/PBS (10 min). Before specific staining, unspecific antigenic sites were blocked with normal goat serum or normal horse serum. Sections were then incubated with the respective primary antibody for 1 hour at room temperature (RT) followed by incubation with biotinylated anti-rabbit or anti-mouse immunoglobulins and then with avidin-biotin-peroxidase complex (30 minutes at RT for each step). After each step, sections were washed with PBS. Specific peroxidase activity was visualized with 0.05% 3-amino-9-ethylcarbazol as a substrate (Sigma, Deisenhofen, Germany) and 0.02% H2O2/0.1 M Na-acetate buffer pH5.5). Counterstaining was performed with Mayers hematoxylin. Control staining was performed in the absence of primary antibody. Immunostained sections were evaluated upon light microscopy. Double immunostainings were performed with a monoclonal anti Ki67 antibody (Dako, Glostrup, DK) using the avidin-biotin-peroxidase method (ABC, red-brown staining), together with the CK8-specific 35ßH11 antibody using alkaline phosphatase-anti-alkaline phosphatase method and fast Blue BB salt (Sigma, Deisenhofen, Germany) as a chromogenic substrate (deep blue staining). Negative controls were conducted in the absence of primary antibodies for every detection system. Sample evaluation was performed according to criteria of staining intensities (0-++++).

**Results**

Our study comprised 50 patients (40 males and 10 females), with a mean age 45 years. Patients were classified into two groups.

Control group comprised 25 samples of normal mucosa were obtained from patients suffering from chronic laryngitis (n=13), laryngeal carcinomas (n=12). The second group comprised 25 samples of different laryngeal lesions, 7 samples of laryngeal leukoplakia, 3 samples of laryngeal polyps, 4 samples of vocal nodules, 4 samples of chronic hyperplastic laryngitis and 7 samples of laryngeal cancer (Table 1).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Number</th>
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<tbody>
<tr>
<td>Normal mucosa</td>
<td>25</td>
</tr>
<tr>
<td>Chronic laryngitis</td>
<td>4</td>
</tr>
<tr>
<td>Vocal nodule</td>
<td>4</td>
</tr>
<tr>
<td>Vocal polyp</td>
<td>3</td>
</tr>
<tr>
<td>Leukoplakia</td>
<td>7</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>7</td>
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All sections of normal mucosa of patients suffering from either chronic laryngitis or laryngeal carcinoma were devoid of CK 8 expression (Fig. 1-A).

All sections of vocal polyps, nodules, and diffuse hyperplastic laryngitis were devoid of CK8 expression.

All sections of leukoplakia and cancer larynx showed CK8 expression (Table 2). Different intensities of CK8 expression was found in dysplasia and vocal cord malignancy (Table 3) (Fig. 1-B,C, D).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>CK8 expression</th>
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<tbody>
<tr>
<td>Normal mucosa</td>
<td>- ve</td>
</tr>
<tr>
<td>Chronic laryngitis</td>
<td>- ve</td>
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<tr>
<td>Vocal nodule</td>
<td>- ve</td>
</tr>
<tr>
<td>Vocal polyp</td>
<td>- ve</td>
</tr>
<tr>
<td>Leukoplakia</td>
<td>+ ve</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>+ ve</td>
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Fig. (1): CK8 expression in laryngeal specimen after anti CK8 staining at x100 magnification in various representative stages of normal (A), minimal epithelial dysplasia (B), severe dysplasia (C) and carcinoma specimens (D).

Table (3): Differential expression of CK8 in the tissue samples.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Differential expression of CK8</th>
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<tbody>
<tr>
<td>Leucoplakia (mild dysplasia)</td>
<td>+</td>
</tr>
<tr>
<td>Leucoplakia (moderate dysplasia)</td>
<td>++</td>
</tr>
<tr>
<td>Leucoplakia (sever dysplasia)</td>
<td>+++</td>
</tr>
<tr>
<td>carcinoma</td>
<td>++++</td>
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Discussion

Reliable markers for pre-malignant lesions retained their paramount importance, as they are believed to bring about significant improvements of patients’ care and overall survival. This notion was best exemplified by the use of prostate-specific antigen PSA for the early diagnosis of prostate carcinoma.

Clearly, the earlier a malignancy of the head and neck area is diagnosed, the better the prognosis for the patient. With these prerequisites in mind it is interesting to retrieve from the present study that CK8 is absent in normal mucosa composed of squamous epithelium; CK8 expression differentiates dysplastic lesions, carcinomas in situ and small established carcinomas from normal tissue and hyperplastic lesions within the larynx. These features and the de novo expression of qualify CK8 as a worth candidate for the early detection of pre-malignant lesions, which might progress to overt malignancies with significantly enhanced probability.

The findings presented in this study of CK8 expression are in accordance with and complementing previous data of Magin et al. [1], Tao et al. [3] and Gires et al. [13] that demonstrated de novo synthesis of CK8 in dysplastic lesions as well as in head neck carcinomas. Vocal cords carcinomas were characterized by 100% of samples expressing strong levels of K8. Dysplastic leucoplakias were also characterized by 100% of samples expressing from intermediate to strong levels.

Taken together our data qualify CK8 as an excellent marker for premalignant and malignant laryngeal lesions.

Conclusion:

The intermediate filament protein CK8 is known as a tumor-associated antigen. We present a survey of CK8 expression in laryngeal epithelia that demonstrates the specific staining of CK8 in pre-malignant versus normal cells. Dysplastic cells expressed CK8 to strong levels. Hence, CK8 is an excellent marker for the visualization and diagnosis of pre-malignancies and malignant lesions in the larynx.

References

4- TOIVOLA D.M., KU N.O., RESURRECCION E.Z., NELSON D.R., WRIGHT T.L. and OMARY M.B: Keratin 8 and 18 hyperphosphorylation is a marker of progression


