Expression of the Ki-67 Antigen in Laryngeal Papillomatosis

There is a wide spectrum of severity in laryngeal papillomatosis. It is difficult to predict the aggressiveness and severity of the disease in most of the cases.

In this study, we examined the pattern of cellular proliferating activity by detecting the expression of the Ki-67 antigen by using Ki-S5 monoclonal antibody immunohistochemistry study in 7 cases of laryngeal papillomatosis, 8 cases of vocal polyp, 12 cases of laryngeal granuloma, and 11 cases of laryngeal squamous cell carcinoma, and 2 cases of laryngeal atypical carcinoid tumor.

The mean value of Ki-67 labeling index in laryngeal papillomatosis was 31.76 (range: 14.65 to 60.00), 11.47 (range 0 to 33.3) in vocal polyp, 10.42 (range 0.2 to 25.00) in laryngeal granuloma; 40.98 (range 8.3 to 98.00) in laryngeal squamous cell carcinoma; 9.65 (range 8.0 to 11.3) in laryngeal atypical carcinoid tumor.

The Ki-67 labeling index was higher in laryngeal papillomatosis and laryngeal squamous cell carcinoma than vocal polyp and laryngeal granuloma. But there was no significant
difference between laryngeal papillomatosis and laryngeal squamous cell carcinoma.

The histologic changes of laryngeal papillomatosis included papillomatous growth, acanthosis and koilocytosis of the epithelium in all cases. There were also basal hyperplasia without loss of basal polarity. There was no epithelial dysplasia beyond moderate dysplasia and the basement membrane was intact in all cases. The Ki-67 antigen signal was detected mostly in parabasal cells and the lower third of epithelium. The higher Ki-67 LI was found in cases with epithelial dysplasia and the Ki-67 antigen signal could be demonstrated in the middle and the upper thirds of epithelium.

Long term follow-up and more cases should be enrolled in further study to deduce the relationship between the clinical behavior and the expression of Ki-67 antigen in laryngeal papillomatosis. However, we obtained much experience in using Ki-S5 monoclonal antibody to detect Ki-67 in formalin-fixed specimens from this study. It is beneficial for studies of the relationship between the cellular proliferating activity and the biobehavior in laryngeal carcinoma and other head and neck cancers.

Keywords: Ki-67, proliferative activity, laryngeal papillomatosis

2. 研究目的

Laryngeal papillomatosis is one of the most difficult and frustrating diseases that otolaryngologist treat. It is aggressive, recurrent, and reluctant to treatment. There is a wide spectrum of severity in laryngeal papillomatosis. Sometimes it is difficult at the first presentation to predict the aggressiveness and severity of the certain case.

Tumor cell proliferative activity provides insights into tumor biology. Many human tumors are characterized by expression of nuclear proteins associated with proliferation, such as proliferating cell nuclear antigen (PCNA) and Ki-67 antigen. Ki-S5, a newly developed monoclonal antibody to the Ki-67 antigen, binds to a formalin-resistant epitope of the Ki-67 antigen. It allows estimation of the fraction of proliferating cells with respect to the total tumor cell population in formalin-fixed and paraffin-embedded specimens and thus makes it applicable to assess proliferating activity throughout a range of surgical pathology specimens by using immunohistochemical study.

The relationship between cellular proliferating activity and clinical aggressiveness of laryngeal papillomatosis is yet to be defined. In this study, we tried to characterize the pattern of expression of the Ki-67 antigen in laryngeal papillomatosis and assessed the relationship between the Ki-67 antigen and the histological pattern in laryngeal papillomatosis, and compared the expression of Ki-67 with other laryngeal lesions. We also intended to elucidate the cellular proliferative activity and the clinical aggressiveness in laryngeal papillomatosis and examined the value of the Ki-67 antigen in assessment of the biological activity of laryngeal papillomatosis.

3. 材料と方法

Seven cases with a diagnosis of laryngeal papillomatosis, 8 cases of vocal polyp, 12 granuloma, 11 cases of laryngeal squamous cell carcinoma, and 2 cases of atypical carcinoid tumor were enrolled in this study. Clinical records were reviewed and pertinent data were abstracted. Information obtained for cases of laryngeal papillomatosis included patient
demographics, onset-age, age at diagnosis, location and number of sites of disease, extension of lesions, type and number of surgical procedures, length of treatment, outcome, complications, regression at puberty, and history of tracheotomy.

Surgical specimens were fixed in 10% formalin and adequately paraffin embedded. Routine histological examination was performed and categorized as benign or atypical on the basis of cellular aberrations such as dyskeratosis, increased nuclear-cytoplasm ratios, nuclear enlargement, nuclear hyperchromatism, loss of transepithelial maturation, and abnormal mitotic figures. The diagnosis of mild, moderate, and severe atypia was made. Representative block from each surgical procedure was used for further immunohistochemical study of the Ki-67 antigen using Ki-S5 monoclonal antibody immunohistochemistry.

**Ki-67 Labeling Index (Ki-67 LI)**

At least five representative areas, each containing over 100 tumor cells, from the most evenly and heavily labeled area were observed under high-power field (objective lenses, X40). All positive cells were counted regardless of the intensity of staining. A minimum of 500 tumor cells was counted in each section. The Ki-67 labeling index (Ki-67 LI) was defined as percentage of the Ki-S5 positively stained cells to all tumor cells.

4. 結果

The mean value of Ki-67 LI in laryngeal papillomatosis was 31.76 (range: 14.65 to 60.00); 11.47 (range 0 to 33.3) in vocal polyp; 10.42 (range 0.2 to 25.00) in laryngeal granuloma; 40.98 (range 8.3 to 98.00) in laryngeal squamous cell carcinoma; 9.65 (range 8.0 to 11.3) in laryngeal atypical carcinoid tumor. The Ki-67 labeling index was higher in laryngeal papillomatosis and laryngeal squamous cell carcinoma than vocal polyp and laryngeal granuloma. But there was no significant difference between laryngeal papillomatosis and laryngeal squamous cell carcinoma, even it seemed to be in higher range for laryngeal SCC.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
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</thead>
<tbody>
<tr>
<td>papilloma</td>
<td>7</td>
<td>14.65</td>
<td>60.00</td>
<td>31.7614</td>
<td>18.7858</td>
</tr>
<tr>
<td>polyp</td>
<td>8</td>
<td>0.00</td>
<td>33.30</td>
<td>11.4750</td>
<td>11.4813</td>
</tr>
<tr>
<td>granuloma</td>
<td>12</td>
<td>0.20</td>
<td>25.00</td>
<td>10.4250</td>
<td>8.6785</td>
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<tr>
<td>SCC</td>
<td>11</td>
<td>8.30</td>
<td>98.00</td>
<td>40.9818</td>
<td>34.0868</td>
</tr>
<tr>
<td>carcinoid</td>
<td>2</td>
<td>8.00</td>
<td>11.30</td>
<td>9.6500</td>
<td>2.3335</td>
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</tbody>
</table>

The table below shows the relationship between the histologic changes of laryngeal papillomatosis and the Ki-67 signal. The histologic changes of laryngeal papillomatosis included papillomatous growth, acanthosis and koilocytosis of the epithelium in all cases. There were also basal hyperplasia without lose of basal polarity. There was no epithelial dysplasia beyond moderate dysplasia and the basement membrane was intact in all cases. The Ki-67 antigen signal was detected mostly in parabasal cells and the lower third of epithelium. The higher Ki-67 LI was found in cases with epithelial dysplasia and the Ki-67 antigen signal could be demonstrated in the middle and the upper thirds of epithelium.
There is a wide spectrum of severity in laryngeal papillomatosis; some with solitary papillomata that is cured after a single surgical procedure while at the other extreme some with multiple papillomata arising from multiple laryngeal sites that require tens if not hundreds of surgical procedures to control spread. Quick et al. (1979) pointed out that an interdependency exists between the grade of cellular atypia and recurrences of papillomata. Nikolaidis et al. (1985) stated, based on histological investigation, that the prognosis was worse when hyperkeratosis, atypia, intracellular keratinization, abnormal mitosis and increased mitosis were observed.

Tumor cell proliferative activity provides insights into tumor biology. Many human tumors are characterized by expression of nuclear proteins associated with proliferation, such as proliferating cell nuclear antigen (PCNA) and Ki-67.

There were rare studies regarding cellular proliferating activity of the laryngeal papillomatosis. The relationship between cellular proliferating activity and clinical aggressiveness of laryngeal papillomatosis is yet to be defined.

<table>
<thead>
<tr>
<th>No.</th>
<th>Ki-67 LI</th>
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<tr>
<td>1</td>
<td>27.5</td>
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<td>yes</td>
<td>yes</td>
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</tr>
<tr>
<td>2</td>
<td>19.4</td>
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<td>yes</td>
<td>no</td>
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<tr>
<td>3</td>
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<td>no</td>
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</tr>
<tr>
<td>4</td>
<td>56.7</td>
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<td>upper,middle</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>upper,middle</td>
</tr>
<tr>
<td>6</td>
<td>14.65</td>
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<td>no</td>
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</tr>
<tr>
<td>7</td>
<td>26.8</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
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</table>

5. 討論

Stem and his colleague evaluated DNA ploidy and cell proliferation in 19 cases of juvenile laryngeal papillomatosis. They concluded that high S-phase fraction, proliferative index, and Ki-67 expression correlated with aggressive clinical course. The severity of their cases was much severe with seven out of nineteen cases that had distal tracheal or bronchial spread of the papilloma. The frequency of surgical excision and recurrence frequency often used, as the indexes of severity of disease, were not included in their study. In addition, Ki-67 was only correlated with clinical remission, which was observed in short follow time. In our study, ki-67 labeling index was found to be higher in cases with more dysplastic epithelium and in cases with Ki-67 labeling signal extending to more superficial layers. Dysplastic epithelium suggests more aggressive behavior of laryngeal papillomatosis, at least in a pathological view. Even though we could not deduce the conclusion that Ki-67 expression was correlated with the severity of the clinical course, it seemed to have the tendency.

Ki-67 score in tumor front was found to have significant correlation with prognosis of laryngeal carcinoma in Welkoborsky and his colleague's study (20). In this study, we also
examined the expression of Ki-67 antigen in laryngeal carcinoma. We found it higher than vocal polyp and laryngeal granuloma. But the Ki-67 LI varied in wide range. It might be due to the location of Ki-67 signal calculated, histological classification, and clinical stages. Pignataro, et al. and Zidar, et al. suggested that the expression of Ki-67 antigen in dysplastic laryngeal mucosa might be useful in prediction of the progression of laryngeal precancerous lesion. Further study is needed to clearly clarify the relationship between the expression of Ki-67 antigen, histologic characteristics, and the clinical course of laryngeal precancerous lesion and laryngeal carcinoma.

6. 記載成果自評

We examined the expression of Ki-69 in laryngeal papillomatosis, vocal polyp, laryngeal granuloma and laryngeal cancer. We also assessed the relationship between Ki-67 expression, histologic characteristics, and the biological activity of laryngeal papillomatosis as the basis for further study of laryngeal papillomatosis. Long term follow-up and more cases should be enrolled in further study to deduce the relationship between the clinical behavior and the expression of Ki-67 antigen in laryngeal papillomatosis. However, we obtained much experience in using Ki-S5 monoclonal antibody to detect Ki-67 in formalin-fixed specimens from this study. It is beneficial for further studies of the relationship between the cellular proliferating activity and the biobehavior in laryngeal carcinoma and other head and neck cancers.

7. 參考文獻


