IMMUNOHISTOCHEMICAL IDENTIFICATION OF PROLYL 4-HYDROXYLASE IN GASTRIC CANCER TISSUES

Hideo Matsui¹, Kiyoshi Kubochi³, Keiichi Yoshino¹, Isao Okazaki², Kyuya Ishibiki¹ and Masaki Kitajima¹

¹) Department of Surgery and 2) Department of Public Health, School of Medicine, Keio University, Tokyo, Japan
³) Department of Surgery, National 2nd Tokyo Hospital, Tokyo, Japan

To elucidate the fibrotic mechanism in gastric cancers, we investigated the tissue localization of prolyl 4-hydroxylase (PH), an enzyme which plays an important role in collagen biosynthesis, by means of immunoperoxidase staining.

MATERIALS AND METHODS
Antibodies: Monoclonal antibodies to human placental PH (anti-α and β-subunit) were used for this study.
Specimens: The frozen sections, 4μm thick, were from gastric cancer tissues of 51 cases (13 scirrhous type and 38 non-scirrhous type) and were fixed in cold acetone at 4°C for 5 min.
Immunohistochemistry: Immunoperoxidase staining was performed by the avidin-biotin-peroxidase complex (ABC) method(1).

RESULTS AND DISCUSSION
Staining pattern of PH in gastric cancer tissues:
Cytoplasmic staining of PH was observed in either fibroblasts or cancer cells (Fig.1). The α-subunit positive rate was more frequent in fibroblasts. On the other hand, the β-subunit positive rate was more frequent in cancer cells. The differences between scirrhous and non-scirrhous type were not statistically significant.

These results suggested that the same collagen biosynthesis mechanism worked in both scirrhous and non-scirrhous types, and as the reason why the stroma formation was poor in non-scirrhous gastric cancer, the collagenolytic enzyme activities might be predominant.
Distribution of PH positive cells in gastric cancer tissues:
In the scirrhous type, the PH positive rate in fibroblasts tended to be low in the central zone and high in the invading zone (Table 1). However there was no difference between the zones with regard to the positive rate in
Prolyl 4-hydroxylase in gastric cancer cells.

These results suggested that fibroblasts participated in collagen biosynthesis in the invading zone and that the collagen biosynthesis from fibroblasts in the central zone might be decreased by the abundant deposition of collagens around fibroblasts.

Fig.1. Immunoreactive β-subunit staining in scirrhou type gastric cancer tissue. In the central zone(a), only cancer cells (arrowheads) are stained. In the invading zone(b), both fibroblasts (arrow) and cancer cells are stained. (x400)

Table 1. Distribution patterns of proly 4-hydroxylase positive fibroblasts in the central and the invading zones of gastric cancer tissues.

<table>
<thead>
<tr>
<th>Histological classification</th>
<th>PH&lt;sup&gt;1)&lt;/sup&gt;</th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Central zone</td>
</tr>
<tr>
<td>Scirrhous type</td>
<td>α&lt;sup&gt;2)&lt;/sup&gt;</td>
<td>2/9</td>
</tr>
<tr>
<td></td>
<td>β&lt;sup&gt;3)&lt;/sup&gt;</td>
<td>6/13 * 11/13</td>
</tr>
<tr>
<td>Non-scirrhous type</td>
<td>α</td>
<td>11/37</td>
</tr>
<tr>
<td>type</td>
<td>β</td>
<td>21/38</td>
</tr>
</tbody>
</table>

1): prolyl 4-hydroxylase positive cases/total cases
2): α-subunit *
3): β-subunit *: p<0.05 by the Chi<sup>2</sup> test

REFERENCE