CLINICO-PATHOLOGICAL AND HISTOCHEMICAL STUDIES ON HYPERTROPHIC DUCT EPITHELIUM IN HUMAN NON-ENDOCRINE PANCREAS CANCER

BY

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ABSTRACT

The hypertrophic duct epithelium of the pancreas, including the pyloric gland metaplasia, mucous cell hypertrophy and ductal papillary hyperplasia were studied clinico-pathologically and histochmically to examine their precancerous character.

A total of 180 surgical and autopsy specimens (90 pancreata with cancer and 90 pancreata without cancer) were analysed.

The overall incidence of these three types of hypertrophic epithelium in the pancreas cancer was much higher than that in the pancreas without cancer. These hypertrophic lesions appeared most frequently in the interlobular duct.

The histochemical study revealed the presence of a new type of glycoprotein in these hypertrophic duct epithelia, however, this substance was not detected in the cancer cells nor in the normal epithelium. This suggests that these hypertrophic lesions may not be the precursors of cancer but rather the coexistent lesions of pancreas cancer.

INTRODUCTION

The histopathology of the human pancreatic duct epithelium in the normal and neoplastic pancreas was reported by several investigators. However, no detailed report concerning the relationship between the hypertrophic duct epithelium, patient age, duct size and histological type of pancreatic cancers has appeared in the literature. In the present study, the hypertrophic duct epithelium of the normal and cancerous pancreas was studied clinico-pathologically and histochemically to elucidate its precancerous character.

The hypertrophic duct epithelium in the human pancreas cancer was classified according to Cubilla and Fitzgerald into three main histological types:

1. Pyloric gland metaplasia, with columnar duct epithelium, resembling the pyloric gland of the stomach.
2. Mucous cell hypertrophy, with mucin-producing cells, almost invariably confined to the intramural glands of the large or intermediate-size duct.
3. Ductal papillary hyperplasia, consisting of high columnar cells showing intraductal papillary growth into the ductal lumen.

Cubilla and Fitzgerald suggested that the ductal papillary hyperplasia may represent a precancerous lesion, while pyloric gland metaplasia and mucous cell hypertrophy are benign. These pancreatic epithelial duct changes can also be observed occasionally in association with other benign lesions. The pathological significance of the

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histological types of hypertrophic duct epithelium is still uncertain.

Robert and Spicer\textsuperscript{11} were the first to conduct a histochemical study on the human pancreatic duct epithelium. They differentiated sialomucin and sulphomucin by means of the high iron-diamine method of Spicer.\textsuperscript{13} In the normal pancreatic duct epithelium, Roberts and Burns\textsuperscript{12} found four types of mucus-secreting epithelial cells and confirmed the presence of both the sulphated mucin and sialomucin. They also demonstrated the presence of the sulphated glycoprotein and neutral glycoprotein in the epithelium of the duct of various sizes. In their investigation, the histochemical reaction of the tumor cells was qualitatively identical to that of the goblet cells.

**Materials and Methods**

*Materials*

A total of 180 cancerous and non-cancerous specimens were obtained (Tokyo Medical and Dental University, 102 autopsy specimens; Cancer Institute, 53 autopsy and 15 surgical specimens; Japan Red Cross Medical Center, 10 autopsy specimens).

In 90 primary, non-endocrine cancer cases, up to 6 sections were taken from the head, body or tail of the pancreas. For the control, one section was taken from the head, body or tail of the pancreas of 90 non-cancerous specimens.

All tissues were fixed in 10% formalin and embedded in paraffin, and the specimens were sectioned to a thickness of 4 micrometers.

*Methods*

For visualization of the mucousubstances, the concanavalin A-horseradish peroxidase method (Con A-HRP) of Katsuyama and Spicer\textsuperscript{10,11} was used. This method is comprised of the following procedures:

a) The non-modified Con A-HRP method (Con A-HRP)

b) Sixty-minute oxidation with 1% periodic acid before Con A-HRP staining (PA-Con A-HRP)

c) Oxidation and reduction before Con A-HRP staining (PA-Red-Con A-HRP)

In procedure a), the deparaffinized sections were rinsed with phosphate-buffered saline (PBS) and immersed for 30 minutes at room temperature in a solution containing 0.1% Con A in PBS. The sections were washed 3 times with PBS and made to react for 10 minutes in 10 ml of 0.5 M tris-buffer (pH 7.0) containing 3 mg of DAB tetrahydrochloride (Sigma, USA) and one drop of hydrogen peroxide. Subsequently, the sections were rinsed in tap water, dehydrated and mounted on the glass slides.

In procedure b), the deparaffinized sections were rinsed in distilled water, oxidized in 1% aqueous periodic acid for 30 minutes and then rinsed in tap water for 5 minutes. Subsequently, the sections were stained by the Con A-HRP technique.

In procedure c), the deparaffinized sections were rinsed in distilled water, oxidized in 1% aqueous periodic acid for 30 minutes and rinsed in tap water for 5 minutes. Subsequently, the sections were stained by the Con A-HRP technique.

Periodic acid Schiff (PAS),\textsuperscript{7} Alcian blue\textsuperscript{6,8} and the high iron-diamine method by Spicer\textsuperscript{13} were used for histochemical staining (Table 1). The stained specimen were observed under a light microscope.

**Result**

In the pyloric gland metaplastic epithelium, which resembled the pyloric gland of the stomach, columnar and cuboidal cells with abundant cytoplasm were noted. The slender nuclei were located at the cell base (Fig. 1).
In the mucous cell hypertrophic epithelium, columnar, occasionally stratified, cells with uniformly increased cytoplasm were noted. However, the cells did not form papillary projections (Fig. 1, 2).

In the ductal papillary hyperplastic epithelium, columnar cells with papillary projections and stalks were noted. The projections contained vascular and supporting tissue. In some cases the epithelium showed small projections while in the others the ductal lumen was filled with extensively desquamated papillae (Figs. 3, 4).

The incidence of hypertrophic duct epithelium in the pancreas cancer cases was four times higher than that in the control cases (Table 2). Epithelial changes similar to the pyloric gland metaplastic epithelium and ductal papillary hyperplastic epithelium appeared more frequently in the pancreas cancer than in the control cases.

The relationship between the incidence of hypertrophic duct epithelium and histological types of pancreas cancer is shown in Table 3.

Ductal papillary hyperplastic epithelium was found only in the cases with identified tubular or papillary adenocarcinoma. On the other hand, pyloric gland metaplastic epithelium and mucous cell hypertrophic epithelium were noted in the identified tubular and papillary adenocarcinoma and colloid and anaplastic carcinoma. In a case with acinar cell carcinoma, neither pyloric gland metaplastic epithelium, mucous cell hyperplastic epithelium, nor ductal papillary hyperplastic epithelium was noted.

The pancreatic ductal system was divided into the main duct, the interlobular (medium-sized) duct and the intra-acinar duct.
Table 3. Histological Types of Non-endocrine Cancer and Distribution of Hypertrophic Duct Epithelium

<table>
<thead>
<tr>
<th>Histological type</th>
<th>No. of cases</th>
<th>Pyloric gland metaplastic epithelium</th>
<th>Mucous cell hypertrophic epithelium</th>
<th>Ductal papillary hyperplastic epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular adenocarcinoma</td>
<td>67</td>
<td>10</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Papillary adenocarcinoma</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Colloid carcinoma</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Acinar cell carcinoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

Table 4. Distribution of Hypertrophic Duct Epithelium

<table>
<thead>
<tr>
<th></th>
<th>Hypertrophic duct epithelium in pancreas cancer</th>
<th>Hypertrophic duct epithelium in pancreas without cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-acinar</td>
<td>Inter-lobular</td>
</tr>
<tr>
<td>Pyloric gland metaplastic epithelium</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Mucous cell hypertrophic epithelium</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Ductal papillary hyperplastic epithelium</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Total (cases)</td>
<td>9</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 5-a. Age Distribution of Hypertrophic Duct Epithelium in Pancreas Cancer

<table>
<thead>
<tr>
<th>Patient age</th>
<th>39-40</th>
<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
<th>70-80</th>
<th>Total (cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyloric gland metaplastic epithelium</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Mucous cell hypertrophic epithelium</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>Ductal papillary hyperplastic epithelium</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Total (cases)</td>
<td>0</td>
<td>10</td>
<td>18</td>
<td>12</td>
<td>7</td>
<td>47</td>
</tr>
</tbody>
</table>

Table 5-b. Age Distribution of Hypertrophic Duct Epithelium in Pancreas Without Cancer

<table>
<thead>
<tr>
<th>Patient age</th>
<th>30-40</th>
<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
<th>70-80</th>
<th>80-90</th>
<th>Total (cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyloric gland metaplastic epithelium</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Mucous cell hypertrophic epithelium</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Ductal papillary hyperplastic epithelium</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total (cases)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>
The three types of hypertrophic duct epithelium were most frequently observed in the interlobular duct, in cancer as well as in the control cases. Ductal papillary hyperplastic epithelium was not found in the intra-acinar duct (Table 4).

Tables 5a and 5b show the relationship between the incidence of hypertrophic duct epithelium and patient age.

The incidence of hypertrophic duct epithelium was the highest in the pancreas cancer patients between 50 and 60 years of age.

The results of histochemical staining of the hypertrophic duct epithelium are shown in Table 6. The mucous substance of normal epithelium was strongly positive for staining with PAS, AB pH 1.0, AB pH 2.5 and HID, but negative for staining with Con A-HRP, PA-Con A-HRP and PA-Red-Con A-HRP.

Three types of hypertrophic duct epithelium were negative for staining with AB pH 1.0 and HID (Fig. 7, 8). The pyloric gland metaplastic epithelium and mucous cell hypertrophic epithelium were strongly positive for staining with PA-Red-Con A-HRP (Fig. 5). The ductal papillary hyperplastic epithelium was negative for staining with AB pH 1.0, HID, Con A-HRP and PA-Con A-HRP, and negative or weakly positive for staining with PA-Red-Con A-HRP (Fig. 5). The hypertrophic duct epithelium was strongly positive for staining with PAS and AB pH 2.5 (Fig. 6).

The cells of non-endocrine pancreas cancer were strongly positive for staining with PAS, AB pH 1.0 and AB pH 2.5, but were negative for staining with HID and Con A-HRP sequence.

**DISCUSSION**

Changes in the pancreatic duct epithelium and hypertrophic patterns were described by Nakamura\(^6\) in 1924.

Yotsuyanagi\(^20\) reported that epithelial metaplasia, according to his classification, was found in 64% of normal pancreas and that intraductal changes were found in 7 of 19 cases (39%) with different diseases affecting the pancreas. Sommers *et al.*\(^16\) noted ductal papillary hyperplasia in 40% of the cancer cases, whereas adenosomatous hyperplasia was seen in about 9% of the non-cancer cases and in 28% of the diabetes mellitus cases. Regarding the biological significance of hypertrophic duct epithelium, Cubilla and Fitzgerald\(^2\) suggested that ductal papillary hyperplasia is a precursor lesion of pancreas cancer. They noted that the incidence of ductal papillary hyperplasia in the pancreas cancer group was two to three times higher than that in the control group. On the other hand, in their study,
the incidence of mucous cell hypertrophy and pyloric gland metaplasia in pancreas cancer was not significantly higher than that in the control group.

No detailed study on the incidence of hypertrophic duct epithelium associated with patient age and duct size has been reported to date. Our findings indicate that the incidence of the 3 types of hypertrophic duct epithelium may be age-related. The 3 types of hypertrophic duct epithelium were observed primarily in the interlobular duct, indicating that the interlobular duct is the main site of the hypertrophic change in the epithelial cells of the pancreatic duct.

Robert and Spicer\textsuperscript{[11]} were the first to perform a histochemical study on the pancreatic duct epithelium in normal and hypersecre-atory states including cystic fibrosis. They used various histochemical methods and detected sulphomucin and sialomucin in the secretory mucin by the use of the high iron-diamine method of Spicer.\textsuperscript{[13]} McMinn and Kugler\textsuperscript{[9]} used various histochemical methods as well as autoradiography of $^{35}$SO$_4$ in vivo and in vitro to investigate the histochemical properties of the normal pancreatic duct epithelium. They found that except in the rats the duct epithelium of all species had a secretory activity. The secretory material contained a sulphated mucous substance. Roberts and Burns\textsuperscript{[2]} studied the histochemical properties of the pancreas cancer cell and identified 4 types of mucus-secreting cells among the normal epithelial cells. These cells, confirmed by histochemical methods, such as Biebrich scarlet, PAS, Alcian blue, HID, PAD and its variants, were cuboidal cells in the intralobular duct. The results of that study indicated that the secreted mucous substance in the smaller duct was sulphated glycoprotein and that a smaller amount of sulphated mucin was secreted. However, particularly neutral glycoprotein and sialomucin appeared with the increase in the duct size. High columnar cells (type III cell in their study) were negative for staining with AB pH 1.0 and HID/ AB pH 2.5. These investigators pointed out that the mucin of pancreas cancer was qualitatively identical to the mucosubstance produced by the goblet cells. Saitoh and Uzman\textsuperscript{[14]} suggested that the rate of production and secretion of total sulphated mucopolysaccharides and their proportion were different among the established mammalian cell lines.

Concanavalin A (Con A) selectively binds to mannose, glucose and fructose (Goldstein\textsuperscript{[22]}). Although the precise sugar unit which binds with Con A in glycoprotein has not yet been determined, the Con A-HRP sequence can reveal the existence of a particular carbohydrate complex in the glycoprotein.

Based on the present histochemical study, the present findings can be summarized as follows:

(a) All mucosubstances containing hexoses and deoxyhexoses, neutral mucosubstances, sulphated as well as some non-sulphated mucosubstances, are produced in the 3 types of hypertrophic duct epithelium in human pancreas cancer.

(b) A glycoprotein of unknown sugar unit structure is produced characteristically in the pyloric gland metaplastic epithelium and mucous cell hypertrophic epithelium.

(c) Ductal papillary hyperplastic epithelium which was weakly positive or negative for staining with PA-Red. Con A-HRP may be a different type of hypertrophic duct epithelium than the pyloric gland metaplastic epithelium and mucous cell hypertrophic epithelium.

(d) The histochemical reactions of non-endocrine pancreas cancer cells were identical to those of the normal duct epithelium.
except staining reaction with HID.

c) Both the negative staining reaction with AB pH 1.0 and HID and the positive
staining reaction with PA-Red-Con A-HRP in the 3 types of hypertrophic epithelium
indicated that the sugar unit conjugated abnormally with the peptide chain and a new
type of glycoprotein was produced.

(f) Negative staining reaction with HID
and AB pH 1.0 indicated a sulphated muco-
substance deficiency in the hypertrophic
duct epithelium.

A comparison between the incidence of
hypertrophic duct epithelium in the cases
with and without pancreas cancer suggests
that these hypertrophic epithelia may possi-
ably represent precancerous lesions in pan-
creas cancer. However, the present histo-
chemical findings suggest that these hyper-
trophic lesions may not be the precursor of
cancer, but rather the coexistent lesions of
pancreas cancer.

More fundamental and biochemical
analyses of cell kinetics in the pancreatic
duct epithelium are necessary for the eluc-
idation of the relationship between the
hypertrophic cells and their malignant
changes.

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EXPLANATION OF FIGURES

Plate 1.

Fig. 1. Mucous cell hypertrophic epithelium, accompanied by pyloric gland metaplastic epithelium in the interlobular duct. Occasional stratification of columnar cells appears in the ductal lumen. Slender nuclei of the small pyloric gland metaplastic epithelium situated at the base of the cell. (Hematoxylin-Eosin ×100)

Fig. 2. Mucous cell hypertrophic epithelium consisting of uniformly increased cytoplasm of columnar cells of the interlobular duct. (Hematoxylin-Eosin ×100)

Fig. 3. Ductal papillary hyperplastic epithelium of the interlobular duct, showing papillary projections with stalks, composed of vascular and supporting tissue. (Hematoxylin-Eosin ×40).

Fig. 4. Desquamated papillae filling the ductal lumen in the interlobular duct. (Hematoxylin-Eosin ×200).

Plate 2.

Fig. 5. Mucous cell hypertrophic epithelium, pyloric gland metaplastic epithelium and mucus are stained brown by the PA-Red-Con A-HRP staining method. However, ductal papillary hyperplastic epithelium and normal epithelium are not stained. (PA-Red-Con A-HRP ×100)

Fig. 6. Mucous cell hypertrophic epithelium and mucus are stained with magenta by periodic acid Schiff (PAS) in the interlobular duct. (PAS ×100)

Fig. 7. Mucous cell hypertrophic epithelium is negative for staining with Alcian blue pH 1.0 in the interlobular duct. (AB pH 1.0)

Fig. 8. Mucous cell hypertrophic epithelium is negative for staining with the high iron-diamine method in the interlobular duct. (HID ×100).