PGP9.5 as a Prognostic Factor in Pancreatic Cancer

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ABSTRACT
The expression of PGP9.5 was evaluated using immunohistochemistry in 69 resected ductal carcinomas of the pancreas and in normal pancreatic tissue. Overexpression did not seem to differ with histological type or pathological stage. A significant negative correlation was found between overexpression of PGP9.5 and postoperative survival. Multivariate analysis also suggested PGP9.5 along with tumor stage and extrapancreatic plexus invasion as strong predictors of the outcome. This study suggests that PGP9.5 expression may be used as a marker for predicting the outcome of resection-treated pancreatic cancer patients.

INTRODUCTION
Pancreatic carcinoma is the fifth leading cause of cancer death in the United States (1). Despite recent advances in diagnosis and treatment, this disease has a dismal 5-year survival rate of as much as 20% after resection of the tumor (2). Molecular cell biology studies have revealed that pancreatic cancer is a disease with multiple changes in cancer-related genes (3). One of the most common genetic changes identified in pancreatic cancer involves the K-ras oncogene (4). Point mutations in codons 12 or 13 have been found in 80–100% of pancreatic cancers (3–5). The p16 tumor suppressor gene is inactivated in more than 80% of pancreatic cancers, p53 is inactivated in more than 80% of pancreatic cancers, p53 is inactivated in 50–75% of pancreatic cancers, and DPC4 is inactivated in 50% of pancreatic cancers (1, 3). There are other factors associated with the neoplastic transformation of pancreatic duct cells (6).

PGP9.5, a ubiquitin hydrolase, is widely expressed in neuronal tissues and has been suggested as a NE3 marker (7, 8). The ubiquitin-proteasome pathway degrades cytosolic and nuclear proteins via an ATP- and ubiquitin-dependent mechanism, which involves the regulation of cell cycle genes (9, 10). Using the serial analysis of gene expression method, Hibi et al. (11) recently found that the PGP9.5 transcript was highly expressed in lung cancer. They further examined PGP9.5 status in lung cancer and found that PGP9.5 expression is closely associated with advanced stages of lung cancer (12).

This result prompted us to examine PGP9.5 status in pancreatic cancer. To evaluate its role in pancreatic cancer, we retrospectively investigated the expression of PGP9.5 in 69 resected pancreatic cancers and in normal pancreatic tissues from age-matched autopsy cases using IHC. As expected, 26 pancreatic cancers showed PGP9.5 expression that was independent of NE status. We then investigated for a possible correlation of PGP9.5 expression in tumors with the clinicopathological features. Strikingly, patients with PGP9.5-negative pancreatic cancer had significantly better survival rates than those who were PGP9.5 positive. Therefore, PGP9.5 may be a novel marker for indicating the prognosis of pancreatic cancer patients.

MATERIALS AND METHODS
Tissue Specimens. Pancreatic cancer specimens from 69 patients who underwent surgical resection in the Department of Surgery II, Nagoya University Hospital (Nagoya, Japan) and 2 normal pancreatic tissues from aged-matched autopsy cases with unrelated diseases were included in this study. The patient population consisted of 44 males and 25 females, and their mean age was 61.8 years (age range, 38–79 years; SD, 8.8 years). Of 69 cases, 54 were moderately differentiated adenocarcinoma, 7 were well differentiated adenocarcinoma, and 2 were poorly differentiated adenocarcinoma. The remaining cases consisted of papillary (n = 3), adenosquamous (n = 2), and mucinous cystic (n = 1) carcinomas. The specimens were fixed with 10% formalin, embedded in paraffin, and cut into several sections, each of which was 3 μm thick.

Immunohistochemical Analysis. The sections were deparaffinized by xylene and rehydrated in graded alcohol. Endogenous peroxidase was blocked with methanol containing 3% H2O2 for 5 min. Microwave treatment was performed for 4 min in Antigen Retrieval Glyca solution (Biogenex, San Ramon, CA). After blocking with normal goat serum for 20 min, the slides were incubated with polyclonal rabbit anti-serum against PGP9.5 (Biogenesis) at 1:1000 dilution for 1 h at room temperature. PGP9.5 protein was visualized by using the Vectastain ABC kit and diaminobenzidine tetrahydrochloride containing 0.03% H2O2. Nuclei were counterstained with Mayer’s hematoxylin. A monoclonal antibody against CGA (Dako, A/S, Denmark) was applied in 40 cases according to the manufacturer’s instructions. Immunohistochemical staining was evaluated by an experienced pathologist (T. N.) who was blinded to any clinical data. In all cases, intrapancreatic nerves, ganglion cells, and islet cells served as a positive internal control for PGP9.5 and CGA staining. Only cytoplasmic staining above the background level was re-
positively stained tumors.

...regarded as specific staining. Tumors that had more than 50% (original magnification, \(3\)) and intrapancreatic nerves arrow PGP9.5 was seen mainly in islet cells (\(\text{arrow}\)) and intrapancreatic nerves (original magnification, \(\times200\)).

Fig. 1  PGP9.5 expression in normal pancreatic tissue. Expression of PGP9.5 was seen mainly in islet cells (\(\text{arrow}\)) and intrapancreatic nerves (original magnification, \(\times200\)).

...tumors.

**Statistical Evaluation.** Statistical analysis was performed by \(\chi^2\) and Spearman’s rank correlation tests for group differences using Statview software (Version 5; 1998; SAS Institute, Inc.). Survival rates were calculated by the Kaplan-Meier method, and statistical significance was examined using the log-rank test. Prognostic factors were evaluated by univariate and multivariate analyses (proportional hazard regression model).

**RESULTS**

We first examined the expression of PGP9.5 in normal pancreatic tissues using IHC and found PGP9.5 staining mainly in islet cells and intrapancreatic nerves (Fig. 1). This finding is consistent with the fact that PGP9.5 is a NE peptide that is widely expressed in neuronal and NE tissues (7, 8).

Subsequently, we examined PGP9.5 expression in pancreatic cancer and found varying numbers of tumor cells expressing PGP9.5 mainly in their cytoplasm (Fig. 2A). Of 69 pancreatic cancer samples examined, 26 (37.7%) cases showed positive staining with PGP9.5. To confirm that PGP9.5 expression is independent of NE status, CGA staining was performed using the same cancer samples (Fig. 2B). CGA expression was mainly seen in islet cells and was seen in <1% of cancer cells in 11 of 40 samples of pancreatic cancer (Fig. 3). Although 5 of these 11 cases also expressed PGP9.5, more than 50% of tumor cells expressed PGP9.5, suggesting that PGP9.5 expression was independent of NE status.

We next examined the correlation of PGP9.5 status to the clinicopathological features to evaluate the role of PGP9.5 in pancreatic cancer. There was no significant difference in the distribution of patients positive or negative for PGP9.5 expression in terms of age, tumor histology, tumor differentiation, tumor size, and the extent of tumor or lymph node metastasis (data not shown).

...for survival analysis.

DISCUSSION

NE differentiation in pancreatic carcinomas has been reported in various studies using IHC (13–15). However, the expression of PGP9.5 and its relation to other NE markers have not been studied in pancreatic cancer to date. In this study, CGA was used as a marker for NE differentiation because it has been demonstrated to be a well-characterized marker for NE differentiation (16). We found that tumor cells immunoreactive to CGA monoclonal antibody accounted for <1% of the tumor cell population. On the other hand, PGP9.5 was expressed in 26 of 69 (37.7%) pancreatic cancers. These results suggested that the expression of PGP9.5 in pancreatic cancer is independent of NE differentiation.

Subsequently, we investigated a possible correlation of PGP9.5 status with clinicopathological features. Although PGP9.5 expression was not associated with tumor histology or the extent of the tumor, pancreatic cancer patients with PGP9.5 expression had significantly shorter survival times than those without PGP9.5 as assessed by the Kaplan-Meier method after other observations were censored (\(P = 0.0006\)). This finding further supports the concept that PGP9.5 status might be an important predictor of prognosis.

To date, little is known about the role of PGP9.5 in pancreatic cancer. PGP9.5 belongs to the ubiquitin carboxyl-terminal hydrolase family. Currently accumulating data suggest that these enzymes play an important role in the cellular proteolytic pathway that regulates many cellular processes including the cell cycle and cell death (17). Ubiquitin carboxyl-terminal hydrolases are \(M_r 25,000\) enzymes involved in the translational processing of pro-ubiquitin gene products as well as in the release of ubiquitin from tagged proteins, i.e., de-ubiquitination, by which the degradation of cyclins decreases, which could contribute to the overexpression of these molecules in tumors (10, 17–19).

It has recently been reported that the expression of PGP9.5 in lung cancer may play a causative role in the oncogenic transformation of human lung epithelial cells (12).
Our findings in pancreatic cancer support this hypothesis because (a) PGP9.5 was expressed in pancreatic cancer cells independently of NE status, (b) PGP9.5 expression was not found in the normal duct epithelium but became activated during the course of neoplastic transformation, and (c) its expression was closely associated with tumor aggressiveness.

This study provides solid evidence for additional studies on the molecular mechanism of PGP9.5 overexpression in pancreatic cancer cells. Pancreatic cancer is one of the most aggressive cancers in the world. Therefore, the pancreatic cancer patients without PGP9.5 expression still do not have better survival rates than other common cancer patients. Although we may not be able to change the overall survival using information about

Fig. 2 A, PGP9.5 expression in pancreatic cancer. Varying numbers of tumor cells expressed PGP9.5 mainly in the cytoplasm (arrow; original magnification, ×200). B, this case did not express CGA (original magnification, ×200).

Fig. 3 CGA expression was seen mainly in islet cells (long arrow) and seen in <1% of cancer cells (short arrow; original magnification, ×100).

Fig. 4 The cumulative survival of patient groups according to PGP9.5 status. PGP9.5-negative cases had a significantly better survival rate than PGP9.5-positive cases (P = 0.0006).
PGP9.5 status in pancreatic cancer, PGP9.5 expression could be used as a marker for predicting the outcome of resection-treated pancreatic cancer patients.

ACKNOWLEDGMENTS

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REFERENCES


Table 1  Risk factors for overall survival rate determined by univariate or multivariate analysis in 68 cases of pancreatic cancer

<table>
<thead>
<tr>
<th>Independent factor</th>
<th>Univariate</th>
<th>Multivariate</th>
<th>RR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrapancreatic nerve plexus invasion (positive or negative)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.311</td>
<td>0.174–0.556</td>
</tr>
<tr>
<td>PGP9.5 (positive or negative)</td>
<td>0.0009</td>
<td>0.0006</td>
<td>0.368</td>
<td>0.208–0.652</td>
</tr>
<tr>
<td>Tumor stage (pTNM; stage IVa, IVb or I, II, III)</td>
<td>0.0012</td>
<td>0.0097</td>
<td>2.360</td>
<td>1.231–4.524</td>
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<sup>a</sup> RR, relative risk.
<sup>b</sup> CI, confidence interval.
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