Pancreatic Duct Cell Carcinomas Express High Levels of High Mobility Group I(Y) Proteins

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Abstract

The high mobility group I (HMGI) family of proteins in mammals belongs to a group of nonhistone nuclear proteins known as architectural transcriptional factors. They function in vivo as both structural components of chromatin and auxiliary gene transcription factors. In an earlier study (N. Abe et al., Cancer Res., 59: 1169–1174, 1999), we demonstrated that the expression level of the HMGI(Y) gene/proteins was significantly increased in colorectal adenocarcinoma and colorectal adenoma with severe cellular atypia. In the current study, we analyzed HMGI(Y) expression in several human pancreatic lesions to investigate (a) whether HMGI(Y) overexpression is also observed in pancreatic carcinomas, and (b) the role of HMGI(Y) in the diagnosis of pancreatic neoplasms. To this end, HMGI(Y) expression was determined at the protein level by immunohistochemistry using a HMGI(Y)-specific antibody in 6 surgically resected specimens of nonneoplastic tissue (4 specimens of normal pancreatic tissue and 2 specimens of chronic pancreatitis tissue), 8 pancreatic cystic neoplasms (5 intraductal papilliferous mucinous adenomas, 1 serous cystadenoma, and 2 solid pseudopapillary tumors), and 15 duct cell carcinomas of the pancreas. Immunohistochemical analysis revealed intense nuclear staining in the pancreatic carcinoma cells, whereas only very faint nuclear staining was seen in the nonneoplastic cells. There was a strong correlation between HMGI(Y) protein overexpression and a diagnosis of carcinoma (P = 0.0000018). Thus, an increased expression level of the HMGI(Y) proteins was clearly associated with the malignant phenotype in pancreatic tissue. In addition, a low level of protein expression was also apparent in two of the cystic neoplasms that exhibited cellular atypia, but not in those that did not exhibit cellular atypia. Based on these findings, we propose that the HMGI(Y) proteins could be closely associated with tumorigenesis in the pancreas and that HMGI(Y) could serve as a potential diagnostic molecular marker for distinguishing pancreatic malignancies unambiguously from normal tissue or benign lesions.

Introduction

The HMGI family of proteins in mammals belongs to a group of nonhistone nuclear proteins known as architectural transcriptional factors (1). The HMGI family is known to be composed of three known proteins: (a) HMGI; (b) HMGY; and (c) HMGI-C; the first two, which differ from each other by 11 amino acids (2), are generated from a single functional gene, whereas the third is the product of a separate gene (3, 4). The important structural feature of the HMGI proteins is the presence of three DNA-binding domains called AT hooks, which enable these proteins to bind to the narrow minor grooves of AT-rich sequences in the DNA helix (5). Although the cellular functions of HMGI and HMGY [HMGI(Y)] proteins remain to be determined, these proteins have been implicated in both positive and negative transcriptional regulation of a number of human genes in vivo (6–8), although they themselves have no transcriptional activity (8). The HMGI(Y) proteins have been shown to be essential components of the enhancesome (9), a higher order transcription enhancer complex that is formed when several distinct transcription factors assemble on DNA in a stereospecific manner (10). Thus, they function in vivo as both structural components of chromatin and auxiliary gene transcription factors.

Previous studies have demonstrated an increased expression of the HMGI(Y) proteins during embryogenesis. In contrast, the proteins are undetectable or expressed at very low levels in normal adult tissues in both rodents and humans (11, 12), indicating the critical role(s) of the HMGI(Y) proteins in cell proliferation and/or differentiation during normal development. In fact, inactivation of HMGI-C by knocking out the HMGI-C gene in mice results in a pygmy phenotype (11).

It has also been suggested that alterations in the HMGI gene play an important role in the generation of benign and malignant tumors. Rearrangements of the HMGI(Y) and HMGI-C genes, for example, have been found frequently in benign tumors of mesenchymal origin in humans (13). In the reported cases, the gene rearrangements were caused by chromosomal translocation involving regions 12q13–14 or 6p21, where the HMGI-C and HMGI(Y) genes, respectively, are located. Alterations in the expression level of the HMGI(Y) proteins are also associated with many human neoplasms originating from a variety of tissues, including the thyroid (14), prostate (15), uterus (16), and colorectum (17, 18). We have demonstrated that the expression level of the HMGI(Y) gene/proteins is significantly increased in colorectal adenocarcinoma and in colorectal adenoma with severe cellular atypia (compared with that seen in adenoma with a low degree of atypia and normal mucosa) and that the expression level of the HMGI(Y) proteins is significantly correlated with parameters known to indicate a poor prognosis in patients with colorectal cancer (18). A significant correlation between increased HMGI(Y) mRNA expression and poor prognosis has also been found in patients with prostatic cancer (15). These previous reports suggest that the expression level of the HMGI(Y) proteins/mRNA could be a potential clinicopathological marker with prognostic implications for a wide range of cancers. To test this possibility, we examined the HMGI(Y) expression level in pancreatic neoplasms in the present study and investigated (a) whether HMGI(Y) overexpression is observed in pancreatic duct cell carcinoma (pancreatic carcinoma) and (b) the significance of HMGI(Y) in the diagnosis of pancreatic neoplasms. To this end, immunohistochemical detection of HMGI(Y) proteins using a specific antibody was attempted. Although relatively simple and easy to perform, immunohistochemistry is a potential way to examine whether the expression of a certain protein is specific to tumor cells because it allows precise correlation of the protein expression with the phenotype of the cells on an individual cell basis. In this sense, immuno-
histochemistry can provide more useful information than other assays by which proteins and/or mRNAs are extracted from tumors, possibly including a mixture of proteins and/or mRNAs from normal and irrelevant tissues in the analysis. Based on the above-mentioned considerations, we determined HMGI(Y) protein expression immunohistochemically on surgically resected specimens including normal pancreatic tissue, chronic pancreatitis tissue, various pancreatic cystic neoplasms (intraductal papillary-mucinous adenomas, serous cystadenoma, and solid pseudopapillary tumors) and duct cell carcinomas of the pancreas.

Materials and Methods

Tissue Samples. The tissue samples were obtained at the time of surgery at the First Department of Surgery, Kyorin University Hospital between October 1996 and August 1999. Specimens from 15 pancreatic carcinomas (9 primary carcinomas, 3 liver metastases, 2 peritoneal metastases, and 1 lymph node metastasis), 8 pancreatic cystic neoplasms (5 intraductal papillary mucinous adenomas, 1 serous cystadenoma, and 2 solid pseudopapillary tumors), and 6 nonneoplastic tissues (4 normal pancreatic tissues and 2 chronic pancreatitis tissues) were obtained. We obtained the normal pancreatic tissue specimens from pancreatic tissue that had been surgically resected due to neoplasia (the sample was obtained from a macroscopically healthy region distant from the neoplasm) or from patients with gastric cancer who had undergone distal pancreatectomy for lymph node dissection. All patients gave their informed consent before their inclusion in the study. All of the tissue specimens were fixed as soon as possible after surgical resection in 4% paraformaldehyde in PBS at 4°C for 14 h and treated for cryoprotection in a graded concentration series of sucrose in PBS. The specimens were embedded in OCT compound and then frozen and stored at −80°C until analysis. All of the tissue specimens were histologically examined, and the pathological diagnoses were confirmed.

Immunohistochemical Analysis. Immunohistochemical analysis was performed as reported previously (18). In brief, frozen sections (5 μm) were cut, transferred onto poly-L-lysine-coated slides, air-dried, and then washed in PBS, followed by quenching of endogenous peroxidase activity with 0.3% hydrogen peroxide in methanol. After being further rinsed with PBS, the sections were incubated with normal goat serum for 20 min at room temperature to block nonspecific binding and then incubated with primary anti-HMGI(Y) antibody at a 1:75 dilution for 14 h at 4°C. After being washed in 0.2% Triton X-100 in PBS, the sections were further incubated with biotinylated anti-rabbit IgG for 30 min at room temperature and then washed in 0.2% Triton X-100 in PBS. The intensity of immunoreactivity in these specimens was lower than that seen in carcinomas; the percentage of HMGI(Y)-positive cells also appeared to be lower than that seen in carcinomas (Fig. 1, C–F), although no precise quantification was performed. Histological examination revealed that these HMGI(Y)-positive adenomas exhibited cellular and structural atypia (Fig. 2, E and F), whereas the other cystic neoplasms, which were HMGI(Y) negative, did not exhibit any significant atypia (Fig. 2, A–D). Thus, the expression level of the HMGI(Y) proteins was significantly increased in cystic neoplasms exhibiting cellular atypia. This finding was confirmed in a section in which hyperplastic and atypical cells are next to each other (Fig. 3); whereas tumor cells exhibiting atypia clearly show HMGI(Y) immunoreactivity, neighboring hyperplastic glands not exhibiting atypia do not. The results of the immunohistochemical analysis are summarized in Table 1.

Discussion

Overexpression of the HMGI(Y) gene/proteins has been demonstrated in many types of human malignancies, suggesting that HMGI(Y) may play a vital role in the oncogenic transformation of cells (14–18). Consistent with these data, inhibition of HMGI protein synthesis has been shown to prevent the transformation of rat thyroid cells by murine transforming retrovirus (19). To evaluate the stringency of the association between HMGI(Y) and a diagnosis of malignancy in human pancreatic neoplasms, we investigated the expression of HMGI(Y) proteins in duct cell carcinoma, cystic neoplasm, chronic pancreatitis, and normal tissue of the pancreas. In the HMGI(Y) immunohistochemical analysis, whereas the ductal epithelial cells in the nonneoplastic tissue specimens showed only trace nuclear and cytoplasmic staining, carcinoma cells showed intense nuclear staining. In fact, a strong correlation between HMGI(Y) overexpression and a diagnosis of carcinoma was noted (Table 1; P = 0.000018).

Having shown that an increased expression of the HMGI(Y) proteins was a consistent feature of pancreatic carcinoma, we then examined the expression of these proteins in pancreatic cystic neoplasms. No significant nuclear immunostaining was found in either serous cystadenoma or solid pseudopapillary tumors (Fig. 2, A and B). However, two specimens of intraductal papillary mucinous adenoma of the five specimens examined revealed HMGI(Y) nuclear immunoreactivity (Fig. 2, E and F). The intensity of immunoreactivity in these specimens was lower than that seen in carcinomas; the percentage of HMGI(Y)-positive cells also appeared to be lower than that seen in carcinomas (Fig. 1, C–F), although no precise quantification was performed. Histological examination revealed that these HMGI(Y)-positive adenomas exhibited cellular and structural atypia (Fig. 2, E and F), whereas the other cystic neoplasms, which were HMGI(Y) negative, did not exhibit any significant atypia (Fig. 2, A–D). Thus, the expression level of the HMGI(Y) proteins was significantly increased in cystic neoplasms exhibiting cellular atypia. This finding was confirmed in a section in which hyperplastic and atypical cells are next to each other (Fig. 3); whereas tumor cells exhibiting atypia clearly show HMGI(Y) immunoreactivity, neighboring hyperplastic glands not exhibiting atypia do not. The results of the immunohistochemical analysis are summarized in Table 1.

Results

Immunohistochemical analysis using HMGI(Y)-specific antibodies revealed intense HMGI(Y) immunoreactivity in all of the pancreatic carcinoma specimens examined. Intense and diffuse nuclear staining was characteristically observed in the carcinoma cells (Fig. 1, C–F). Although the HMGI(Y) immunoreactivity was localized mainly in the nuclei, faint staining was also observed within the cytoplasm. HMGI(Y)-positive cells were observed regardless of the degree of differentiation (data not shown) and were basically distributed homogeneously throughout the carcinoma lesion. When the staining intensity and distribution were compared between primary carcinoma cells and metastatic carcinoma cells, no significant differences were observed (Fig. 1, C–F), although we did not examine primary and metastatic lesions from the same cases. In contrast, very faint staining was seen in the nucleus and cytoplasm of the ductal epithelial cells in the six specimens of nonneoplastic tissue (Fig. 1, A and B), whereas the negative control sections did not show any corresponding staining. Because the signal intensity in nonneoplastic tissue specimens was remarkably weaker than that observed in the pancreatic carcinoma specimens or remained almost at the basal level, the HMGI(Y) immunoreactivity level in the nonneoplastic tissue specimens was scored as negative. Under this criterion, a strong correlation between HMGI(Y) overexpression and a diagnosis of carcinoma was noted (Table 1; P = 0.000018).
normal adult pancreatic tissue as assessed by Northern blot analysis using polyadenylated RNA. These findings indicate that an increased expression level of the HMGI(Y) proteins is closely associated with the malignant phenotype in the pancreas, as has been observed previously in other types of human malignancies (14–18), and also that HMGI(Y) could serve as a potential diagnostic molecular marker for distinguishing pancreatic malignancies from normal tissues or benign lesions.

Having shown that the expression level of the HMGI(Y) proteins was consistently increased in pancreatic carcinoma, we then examined the expression levels of the proteins in rare pancreatic cystic neoplasms. Low levels of nuclear HMGI(Y) immunoreactivity were detected in the cell nuclei in two specimens of intraductal papillary mucinous adenoma of the eight cystic neoplasms examined. It is

Table 1  HMGI(Y) protein expression in pancreatic neoplasms

<table>
<thead>
<tr>
<th>Histological type of pancreatic specimens</th>
<th>No. of positive* specimens/ no. of specimens analyzed by immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonneoplastic tissue (n = 6)</td>
<td></td>
</tr>
<tr>
<td>Normal pancreas</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>Neoplastic cystic tumor (n = 8)</td>
<td></td>
</tr>
<tr>
<td>Intraductal papillary mucinous adenoma</td>
<td>2/5 (40%)b</td>
</tr>
<tr>
<td>Serous cystadenoma</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Solid pseudopapillary tumor</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>Duct cell carcinoma (n = 15)</td>
<td>15/15 (100%)</td>
</tr>
</tbody>
</table>

*a The immunostained slides were scored as positive for immunohistochemistry when HMGI(Y) nuclear immunoreactivity was detected in more than 20% of the cells.

*b Histological examination revealed that these HMGI(Y)-positive adenomas exhibited cellular and structural atypia, whereas the other cystic neoplasms did not exhibit any significant atypia.
noteworthy that these particular HMGI(Y)-positive tumors showed cellular and structural atypia, unlike the other cystic neoplasms examined. This may indicate that the adenoma-carcinoma sequence could also be applicable to pancreatic carcinogenesis, as suggested in hamster models of pancreatic carcinogenesis (20). Clinically, intraductal papillary mucinous adenomas are thought to have malignant potential (21), whereas serous cystadenoma and solid pseudopapillary tumor are not considered to have malignant potential (22, 23). Although the intensity and distribution of the immunoreactivity were weaker in the HMGI(Y)-positive intraductal papillary mucinous adenomas than in any of the duct cell carcinomas, our findings may support the view that HMGI(Y)-positive intraductal papillary mucinous adenomas exhibiting cellular atypia are precancerous lesions with malignant potential. Thus, alterations in HMGI(Y) expression could be a general feature of precancerous lesions; increased expression levels of the HMGI(Y) proteins have been observed in intraepithelial cellular dysplasia of the uterine cervix (16) and in colorectal adenoma with severe cellular atypia (18). It may be possible that some events involving alterations in the expression levels of the HMGI(Y) proteins take place in severely dysplastic adenomas, presumably preceding the morphological changes associated with carcinogenic transformation of these cells. However, it remains to be determined whether and how such alterations in the expression levels of the HMGI(Y) proteins are related to pancreatic carcinogenesis and to the development of carcinomas originating from other tissues.

Clinically, differential diagnosis between pancreatic carcinoma and benign pancreatic lesions such as adenoma, hyperplasia, and pancreatitis remains a major problem for clinicians. It has been pointed out that mass-forming pancreatitis masquerades as duct cell carcinoma both in its clinical presentation and in diagnostic imaging (24). We have also demonstrated the difficulty in preoperative differential diagnosis between intraductal papillary mucinous adenoma and intraductal papillary mucinous adenocarcinoma (25). Potential methods for circumventing these difficulties would be either cytodiagnosis or
quantitative analysis of tumor markers in pancreatic juice collected at the time of endoscopic retrograde pancreatography. Although several tumor markers in pancreatic juice, such as carcinoembryonic antigen (26) or CA19–9 (27), have been demonstrated to be useful in the differential diagnosis of pancreatic carcinoma from benign lesions, there appears to be considerable overlap in their levels between patients with and without carcinoma. Recently, several investigators have demonstrated that K-ras gene mutations in cells in pancreatic juice obtained by endoscopic retrograde pancreatography may serve as clinical markers for the diagnosis of carcinoma (28). However, K-ras gene mutations are also known to be present in benign lesions (29) and are therefore considered to be of limited value as a clue to the diagnosis of pancreatic carcinoma. The results of the present study suggest that determination of the expression levels of the HMGI(Y) gene/proteins within the pancreatic juice and/or the cells contained in it by a sensitive and quantitative method, such as competitive reverse transcription-PCR or immunoassay, could contribute to the detection of even a small number of cancer cells.

In conclusion, this study has clearly demonstrated that an increased expression level of the HMGI(Y) proteins is closely associated with the malignant phenotype in pancreatic tissue, suggesting that these proteins could play a vital role in tumorigenesis in the pancreas. The strong correlation between HMGI(Y) overexpression and a histological diagnosis of carcinoma indicates that the determination of the expression level of HMGI(Y) can be of great value in the diagnosis of pancreatic neoplasms.

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References


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