Expression of the Tumor Suppressor Gene Maspin in Human Pancreatic Cancers

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ABSTRACT

The tumor suppressor gene maspin, a unique member of the serpin superfamily, inhibits cell motility, invasion, and metastasis in breast and prostate cancers. Maspin is expressed in normal human mammary and prostate epithelial cells but down-regulated during cancer progression. In this study, we analyzed the expression of maspin in various human cancer cells by means of Northern blot and immunohistochemistry. Maspin gene expression proved to be up-regulated in pancreatic cancer. Maspin expression was not detected in any of 6 gastric cancers, 4 melanomas, or 6 of 7 breast cancer cell lines examined. In contrast, 5 of 9 pancreatic cancer cell lines showed maspin expression, although maspin expression was not detected in normal pancreatic tissue. Furthermore, maspin was expressed in 23 of 24 tumor specimens obtained from pancreatic cancer patients as well as all high-grade precancerous lesions (PanIN3 and intraductal carcinoma extension). In contrast, no expression was observed in normal and low-grade precancerous lesions. Our results show that maspin is a new factor associated with pancreatic cancer. In addition, the detection of maspin in pancreatic tumor tissues and its lack of expression in all normal pancreatic tissues suggests that maspin may be a useful marker of primary human pancreatic cancer.

INTRODUCTION

Maspin (mammary serpin) is a serine protease inhibitor related to the serpin family (1). The maspin gene was originally identified in normal mammary epithelium by subtractive hybridization on the basis of its expression at the mRNA level (1). It was shown to have tumor suppressive activity attributable to inhibition of breast cancer cell motility, invasion, and metastasis (2–4). Maspin is a M, 42,000 protein with sequence homology to other inhibitory serpins (2, 5). Maspin, which is located at the cell membrane and the extracellular matrix, does not act as a classical inhibitory serpin with antiprotease activity against trypsin-like serine proteases (6–8).

Maspin is expressed in normal human mammary and prostate epithelial cells but down-regulated during cancer progression. The loss of maspin gene expression with increasing malignancy is regulated at the transcriptional level (9). Recent publications have discussed the participation of cytosine methylation and chromatin condensation in the down-regulation of maspin expression during neoplastic progression (10).

Although at present the molecular and biological mechanisms of the function(s) of maspin remain unknown there is evidence that maspin interacts with the p53 tumor suppressor pathway and may function as an inhibitor of angiogenesis in vitro and in vivo (11, 12). Using Northern blot analysis, reverse transcription PCR and immunohistochemistry, we found further evidence of decreasing maspin expression with increasing malignancy in human breast cancer tissues (13). Pemberton et al. (14) demonstrated the presence of maspin in the epithelium of several normal human organs (such as prostate, thymus, testis, small intestine, and colon) and particularly in the myoepithelium of the breast, where it is localized and probably functions both intra- and extracellularly. Because the maspin gene is expressed in the epithelium of other glands, it is conceivable that it may play a similar role in the pancreas as well. For this reason, we were interested in determining whether the tumor suppressor function described for maspin in mammary carcinomas can also be detected in pancreatic cancers. Interestingly, our data revealed a different pattern of maspin gene expression from that in breast cancer cells. Maspin was not expressed in normal human pancreatic cells but showed strong expression in pancreatic cancer cells as well as a weaker but detectable expression in precancerous pancreatic lesions. These results suggest that maspin is a new factor associated with pancreatic cancer.

MATERIALS AND METHODS

Cell Culture and Clinical Specimens. The following human cancer cell lines were studied: breast cancer (MCF-7, ZR-75–1, SK-BR-3, BT-20, T47D, MDA-MB-231, MDA-MB-468), pancreatic cancer (BxPC-3, AsPC-1, MIAPaCa-2, CAPAN-1, CAPAN-2, PANC-1, PSN-1, KP2, FA-6), gastric cancer (MKN-1, MKN-7, MKN-28, MKN-45, MKN-74, KATO III), and melanoma (SEKI, G361, A375, MeWo). All of the cancer cell lines were cultured in RPMI 1640 supplemented with 10% FCS. HMECs were purchased from Clonetics (San Diego, CA), maintained according to supplier’s instructions, and
assessed at early passages. Total RNA was extracted from cells when cultures reached 80% confluence, as described previously (15, 16).

Whipple resection specimens were obtained from 24 patients [14 female and 10 male; mean age, 69.6 years (range 46–76)] with ductal adenocarcinoma of the pancreas head from a series of 70 pancreatic resections performed in the years 1996–1999 in the Department of Surgery, University of Kiel (Kiel, Germany). Histological classification and grading were performed according to the criteria of WHO 1996 and Lüttges et al. (17, 18). Of the carcinomas, one was classified as grade 1, 15 as grade 2, and 8 as grade 3. One tumor was staged as stage I disease, 6 as stage II, 14 as stage III, and 3 as stage IV. Ductal lesions were classified according to the recently proposed PanIN classification (19–21). These samples were obtained from different areas of the same surgical specimens as the carcinomas. In addition, three surgical specimens of normal pancreas (from 1 male and 2 female patients) were also investigated and served as control tissues. The staining of the carcinomas, normal pancreatic tissues, intraductal tumor extensions, and hyperplastic duct epithelia was evaluated. The cytoplasmic staining intensity was scored as follows: 0, no staining; 1, faint; 2, moderate; and 3, strong. The cytospin specimens were scored in the same manner.

RNA Isolation and Northern Blot. Maspin cDNA was kindly provided by Dr. Ming Zhang (Baylor College of Medicine, Houston, TX). Total cell RNA was isolated from the cancer cell lines using the RNeasy Mini kit (QIAGEN, Hilden, Germany). A 2.5-kb EcoRI/XhoI fragment from the maspin cDNA plasmid (pMZ-32) was labeled with 32P using a Rediprime DNA labeling system (Amersham Life Science, Arlington Heights, IL) and used in Northern analyses of total RNA as described previously (15, 16). For standardization, membranes were stripped and reprobed with the probe 36B4 under similar conditions to assess RNA loading and transfer efficiency (22, 23). The human multiple tissue Northern (MTN) blots (Clontech, Palo Alto, CA) were used to determine the tissue distribution.

Immunohistochemistry. A mouse antihuman maspin monoclonal antibody was purchased from PharMingen International (San Diego, CA). For the staining of HMECs and cancer cell lines, cells were cultured in chamber slides for 24 h to 60–70% confluency, fixed with 4% paraformaldehyde in PBS and permeablized with methanol/3% H2O2 before blocking with 10% fetal bovine serum for 30 min. Cells were incubated with antihuman maspin antibody (diluted 1:75) according to the manufacturer’s instructions. Peroxidase-conjugated sheep antimouse IgG was used as secondary antibody at a dilution of 1:75 and was color-developed using diaminobenzidine. Cells were then counterstained with hematoxylin, dehydrated, and mounted. In addition, 5-μm sections of formalin-fixed, paraffin-embedded tissue samples from pancreatic cancers, normal pancreatic tissues, and precancerous pancreatic lesions were analyzed. After microwave-based antigen retrieval with 0.05 M Tris buffer (pH 9.0) for 15 min, the sections were incubated with the antihuman maspin monoclonal antibody (diluted 1:75) for 12 h. Bound antibodies were detected using the avidin-biotin complex technique. New Fuchsin/Naphtol AS-Bi phosphate was used as a substrate and hematoxylin was used for counterstaining.

RESULTS

Expression of Maspin in Human Pancreatic Cancer Cell Lines. To evaluate the expression of maspin in cancer cells several human cancer cell lines and normal tissues were investigated by means of Northern blot. Maspin gene expression was not detected in any of the six gastric cancer, four melanoma and seven breast cancer cell lines with the exception of the MDA-MB-468 breast cancer cell line (Fig. 1, A, C, and D). In contrast, maspin mRNA expression was observed in five of nine
human pancreatic cancer cell lines (Fig. 1B). Maspin was highly expressed in BxPC-3 and AsPC-1, whereas low expression was found in CAPAN-2, KP2, and FA-6. In the normal tissues, high expression of maspin mRNA was observed in mammary epithelial cells (Fig. 1A), whereas none of eight other normal tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas) showed expression of maspin mRNA (Fig. 1E). It should be noted that, although a variety of pancreatic cancer cell lines exhibited maspin mRNA expression, maspin was not detected in normal pancreatic tissues on Northern blot analysis.

The expression of maspin in pancreatic cancer cells was confirmed by immunohistochemistry. Representative staining results in mammary epithelial cells and pancreatic cancer cell lines are shown in Fig. 2. Positive staining for maspin was observed in HMECs (Fig. 2D), but no staining was seen in the MCF-7 breast cancer cell line (data not shown). Of the pancreatic cancer cell lines, BxPC-3 and AsPC-1 showed strong maspin staining (Fig. 2, A and B), and a weak signal was observed in KP2 (data not shown). As shown in Fig. 2C, the pancreatic cell line PANC-1 lacked maspin expression. These data are consistent with Northern blot analysis.

Expression of Maspin in Surgical Specimens. Acinar cells and ductal epithelia from the tumor-associated pancreatic tissues as well as the normal pancreas (control cases) stained negatively (Fig. 3A; Table 1). A strong cytoplasmic reaction of all tumor cells was observed in 23 of 24 ductal adenocarcinomas (Fig. 3B; Table 1), which was diffusely distributed throughout the tumors. The staining intensity was generally strong and varied only a little, except that cells with a broad clear cytoplasm showed a faint positivity (Fig. 3C; Table 1). The only case that did not stain positively was a rare type of clear-cell ductal adenocarcinoma (24). Intraductal non-clear-cell areas of this case, however, showed faint nuclear and cytoplasmic staining. Some cells also exhibited nuclear staining that was always accompanied by strong cytoplasmic staining. Intraductal extensions of the carcinomas (17/17) and lesions of PanIN grade 3 (7/7) stained positive also but with lower intensity. In contrast, ductal hyperplasia without dysplasia and low-grade dysplasia (Table 1, 8/8; PanIN 1A, 1B, and 2) such as mucinous cell hypertrophy or papillary hyperplasia stained negative (Fig. 3D; Table 1). In addition, foci of squamous intraductal metaplasia (6/6) showed cytoplasmic staining (Table 1). There was no correlation between the staining intensity and the histological grade or stage of the tumors.

DISCUSSION

Maspin was originally described as a tumor suppressor gene that affects cell motility and invasion (1). Recent findings suggest that maspin is part of the p53 tumor suppressor pathway (12). Maspin expression is high in normal human mammary and prostate epithelial cells but is decreased in breast and prostate cancers and lost in metastatic cells. We examined the expression
of maspin in various cancer cells using Northern blot analysis and immunohistochemistry.

Only a few reports of maspin expression in cancer cells have been published (12, 25, 26). However, our Northern blot analysis revealed that more than one-half of the pancreatic cancer cell lines examined expressed maspin mRNA. Immunohistochemical staining using a monoclonal maspin antibody yielded identical expression patterns, indicating that up-regulated maspin mRNA expression is translated into protein in pancreatic cancer cells. Previously presented data of maspin

Fig. 3 Maspin staining in pancreatic cancer tissues. A, normal pancreatic tissue of the control cases with negatively stained acinar and ductal cells. B, invasive ductal adenocarcinoma of the pancreas with strong cytoplasmic and nuclear staining of the tumor cells and negative hyperplastic duct epithelium. C, invasive ductal adenocarcinoma showing strong cytoplasmic staining of tumor cells with dense cytoplasm and faint staining of those with clear and vacuolated cytoplasm. D, ductal papillary hyperplasia without nuclear atypia (PanIN1b, bottom half) without expression of maspin and PanIN3 lesion (strong nuclear abnormalities, top half) showing moderate cytoplasmic and nuclear staining.
expression in normal pancreatic tissue showed conflicting results: Pemberton et al. (14) could not detect maspin mRNA expression by Northern blot but did detect maspin-like protein expression in glandular epithelia of the pancreas by immunostaining using a polyclonal antibody. The discrepancy may be attributable to different characteristics of the antimaspin antibodies used, such as reaction with distinct epitopes or different specificity. A monoclonal antimaspin antibody was used in our study. On the other hand, reduced or lacking mRNA expression has been reported in breast and prostate tumor cells, whereas corresponding normal cells exhibited high expression (1–4, 20). Although this difference between the pancreas and other organs needs further analysis, maspin appears to be an interesting factor associated with pancreatic cancer.

Although cell lines often develop artificial gene changes during long-term culturing, our studies showed that maspin expression occurs not only in pancreatic cancer cell lines but also in clinical pancreatic cancer tissues. Interestingly, we observed maspin staining in 23 of 24 pancreatic cancer tissues, which suggests that maspin expression is a common event in pancreatic cancer cells. Unlike the cancer tissues, no or faint expression was observed in corresponding normal tissues and low-grade precancerous lesions. Furthermore, maspin expression seems to increase with increasing malignancy from normal pancreas tissue via precancerous lesions to invasive carcinomas. These findings indicate that maspin expression is of biological relevance in vivo for the development of pancreatic cancers. Although at present, the molecular and biological mechanisms of maspin’s function are unknown, several authors adhere to the hypothesis that maspin functions at the level of invasion and metastasis by blocking tumor cell migration and proliferation (2–4). Our findings, which show the up-regulation of maspin in pancreatic cancer, provide new information about factors that regulate tumor cell development.

It has been shown repeatedly that distinct genes such as the K-ras oncogene and the tumor suppressor genes p53, p16, DCC, and DPC4/SMAD4 are frequently altered in pancreatic cancer and may be essential for its genesis (27, 28). Interestingly, the maspin gene is mapped on chromosome 18q21.3 in close proximity to the DCC and DPC4/SMAD4 genes. Losses of chromosome 18q including the loci for the genes DCC and DPC4/SMAD4 are the most frequently identified genetic alterations in pancreatic cancer (29). Although the regulatory mechanism of maspin expression remains unknown, our study has added the maspin gene to the list of possible genes involved in pancreatic carcinogenesis. The biological role of maspin expression in pancreatic cancer should be determined by further investigation.

Carcinoma of the pancreas is the fourth highest cause of cancer-related death and shows the highest mortality rate of all cancers in most Western countries (30). Several tumor-associated antigens, such as CEA, CA125, and CA19–9, are used to monitor pancreatic cancer patients (31). However, they are not tumor specific and are commonly expressed in normal and benign conditions (32, 33). The fact that maspin was detected in pancreatic cancer but was not expressed in normal pancreas tissues suggests that maspin could serve as a useful marker for primary human pancreatic cancer.

In conclusion, we have demonstrated that maspin may play an important role in the carcinogenesis of pancreatic cancer, in addition to its tumor suppressor activity in breast and prostate cancer. We have shown that maspin gene expression is up-regulated in pancreatic cancer at the RNA and protein level, in contrast to its down-regulation in breast and prostate cancers. The function of maspin as a tumor suppressor gene involved in tumor invasion, metastasis, and angiogenesis may not be limited to breast and prostate cancer. Its relationship to carcinoma of the pancreas opens a new angle to the discussion on its function in cancer.

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