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Expression of matrix metalloproteinase 9 in pancreatic ductal carcinoma is associated with tumor metastasis formation

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Abstract: The objective of the current study was to assess the expression of matrix metalloproteinase 9 (MMP-9) in pancreatic ductal carcinoma and to examine its correlation with chosen clinico-anatomical parameters. The study group consisted of 36 patients with pancreatic ductal carcinoma. Tumors were stained using immunohistochemical method (NCL - MMP-9, Novocastra). No correlation was found between tumor MMP-9 expression and age, gender or grade of histological malignancy. However, statistical analysis revealed a relationship between tumor MMP-9 expression and histological type (adenocarcinoma mucinosum) of pancreatic carcinoma. The expression was strongly correlated with lymph node involvement and occurrence of distant metastases ($p<0.00001$). The results indicate a correlation between the expression of MMP-9 in pancreatic ductal carcinoma and worse prognosis (shown by lymph node involvement and distant metastases).

Key words: Pancreatic carcinoma - Matrix metalloproteinase-9 (MMP-9) - Immunohistochemistry

Introduction

Metalloproteinases play a significant role both in physiological and pathological processes in the human body, acting as mediators in remodelling and degradation of extracellular space components. Much attention has been paid to their involvement in neoplasia, mechanisms of invasion and formation of metastases. An increase in the level of metalloproteinases initiates spread of cancer cells, thus causing tumor invasion and formation of distant metastases [14]. It has been shown that MMP-8 is secreted by squamous epithelial carcinoma cells, MMP-7 is produced by cancer cells of the stomach, breast and lungs, while MMP-2, MMP-9 and MMP-14 by epithelial cells of various cancers [10]. Bramhall *et al.* [2] performed comparative analysis of the expression of metalloproteinases (MMP-2, MMP-3, MMP-7 and MMP-11) in pancreatic ductal carcinoma and healthy pancreas (intended for transplantation) using Northern blot method and in situ hybridization Their results clearly indicated an increase in the expression of metalloproteinases in cancer specimens as compared to healthy tissues.

Matrix metalloproteinase 9 (MMP-9) is produced by neoplastic cells as well as by host cells that form the connective tissue stroma indispensable for tumor growth. In neoplastic invasion, MMP-9, involved in degradation and remodelling of the extracellular matrix, and in breakdown of the basement membrane of vessels and ducts, helps overcome the physical barriers and contributes to the formation of distant metastases. It also promotes tumor neovascularization. Numerous studies have been conducted on its role in the devolopment of carcinomas of the breast, the bladder, the pancreas, the colon, the prostate or hypophysial adenomas [4]. A relationship has been described between MMP-9 activity and tumor metastasis formation and patients' survival. For instance, it has been demonstrated in patients with advanced breast cancer that the higher the MMP-9 activity at the time of tumor diagnosis, the shorter overall survival [18]. Similarly, MMP-9 expression has been found to be an unfavorable prognostic marker in non-small cell lung cancer [17].

Therefore, the current study objective was to assess the expression of MMP-9 in pancreatic ductal cancer, and to analyse the correlation of MMP-9 with chosen

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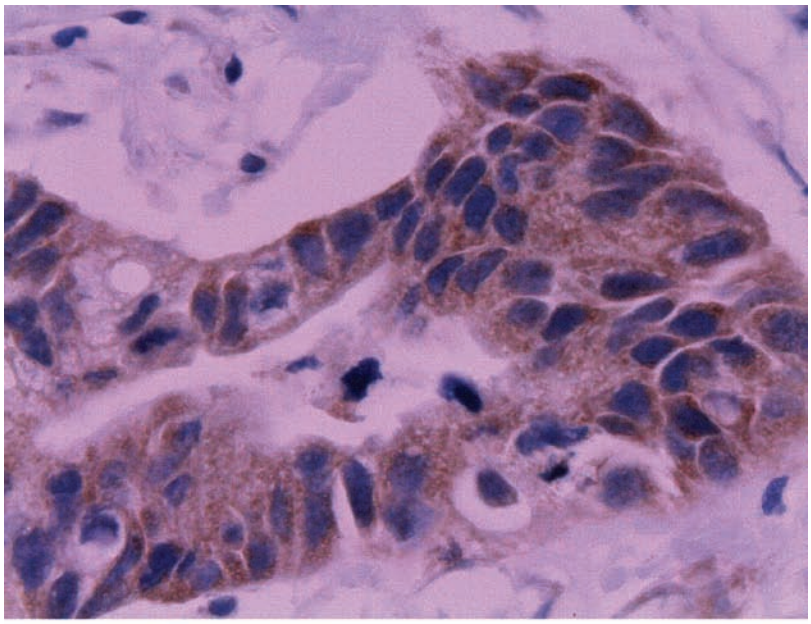

Fig. 1. Expression of MMP-9 in cancer cells (magn. × 40).

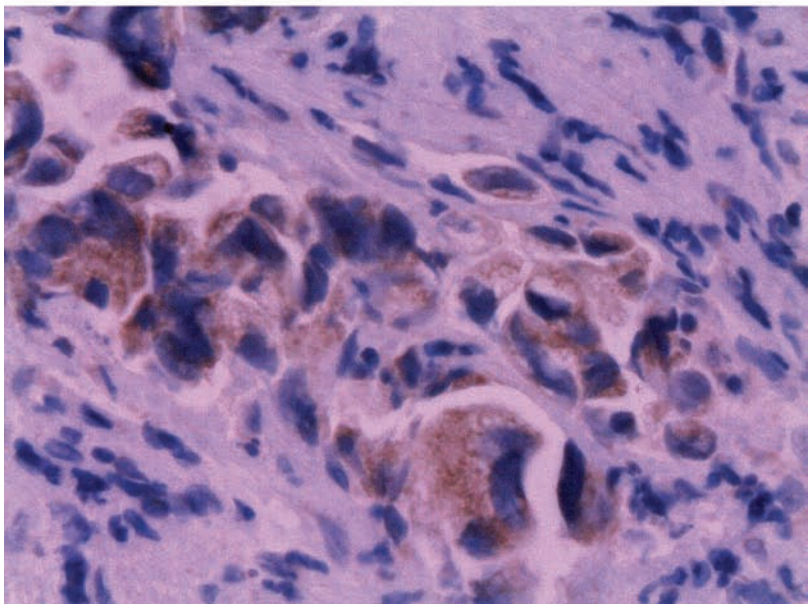

Fig. 2. Cytoplasmic expression of MMP-9 (magn. × 40).

anatomo-clinical parameters: age and gender, histological type of cancer, grade of histological malignancy, lymph node involvement and formation of distant metastases.

Materials and methods

Material. The study involved a group of 36 pancreatic ductal carcinoma patients (8 men, 28 women; of them 17 aged <60 and 19 aged 60 or above) surgically treated at the Department of Gastroenterological Surgery, Medical University of Białystok, in the years 1999-2004. Hematoxylin-eosin -stained section were examined according to the TNM classification. Each tumor was sectioned parallel to the longest axis and at least one total oblong-section (2-3 mm thick) was obtained. It was then divided into small blocks, 1-1.5 cm in diameter, to obtain 4-8 sections consisting of tumor and adjacent macroscopically unchanged tissues.

Immunohistochemistry. Immunohistochemical investigations were performed using monoclonal antibody (Novocastra/NCL-MMP-9/clone 2C3) directed against human matrix metalloproteinase 9. Slides of 4 μm-thick serial sections of the primary tumor were prepared from each patient. A standard avidin-biotin immunoperoxidase method (Novostain Super ABC Kit Universal) was used for the detection of MMP-9. Briefly, the slides were dewaxed using xylene, transferred to alcohol, placed in citric acid buffer (pH=6.0) and heated in a microwave oven (700 W) for 10 min to expose antigens. Endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxide in methanol for 10 min. The slides were then washed 3 times in phosphate-buffered saline (PBS) and incubated in normal horse serum for 15 min to reduce nonspecific antibody binding. After washing with PBS, the slides were incubated overnight at room temperature with monoclonal antibody (Novocastra/NCL-MMP9/clone 2C3; dilution 1:40, Biokom, Poland) was used. Nonspecific mouse IgG was used as a negative control. The reaction products were visualized with diaminobenzidine DAB (DAKO S3000, Dako, Poland).

Evaluation of samples. Cytoplasmic immunostaining and poor stromal immunostaining (Fig. 1 and 2) was observed. Expression

Table 1. Expression of MMP-9 in pancreatic ductal adenocarcinoma in association with chosen anatomoclinical parameters

Variable	Expression MMP-9, n (%)		*p*
	negative	positive	
Gender			
male	6 (75%)	2 (25%)	NS
female	17 (60.7%)	11 (39.3%)	
Age			
<60	11 (64.7%)	6 (35.3%)	NS
≥60	12 (63.2%)	7 (36.8%)	
Histological type of cancer			
adenocarcinoma	23 (69.7%)	10 (30.3%)	0.04
adenocarcinoma mucinosum	0 (0%)	3 (100%)	
Grade of histological malignancy			
G2	22 (68,7%)	10 (31,3%)	NS
G3	1 (25%)	3 (75%)	
Lymph node involvement			
absent	23 (95.8%)	1 (4.2%)	0.00001
present	0 (0%)	12 (100%)	
Distant metastases			
absent	23 (85.2%)	4 (14.8%)	0.00001
present	0 (0%)	9 (100%)	

was semi-quantitatively assessed in neoplastic cells of the primary tumor and was defined as follows:

- negative - indicating lack of reaction to the presence of MMP-9 or reaction in <30% of cells,
- positive - reaction to MMP-9 visible in >30% of cells.

The percentage of MMP-9 positive cells was calculated in 500 cancer cells in each preparation, at a magnification of 400 x. The results were subjected to statistical analysis using exact Fisher test and χ^2 test. The value $p<0.05$ was accepted as the level of significance.

Results and discussion

In recent years, literature reports have described the relationship of the degradation of the extracellular matrix, particularly of the basement membrane, as the basic mechanism facilitating invasion and formation of metastases by cancer cells. The extracellular matrix, which consists of various types of collagens, laminin, fibronectin, elastin and proteoglycans, is degraded by proteinases, such as metalloproteinases. The expression of metalloproteinases is variously regulated and correlates with invasiveness and formation of metastases in carcinomas of the thyroid, the prostate, the ovaries, the stomach and the lungs [16].

We analyzed the expression of matrix metalloproteinase-9 (MMP-9) in correlation with chosen anatomo-clinical parameters (Table 1). No statistically significant relationship was found with age and gender of the studied patients.

The studied group of tumors consisted of adenocarcinoma-type and adenocarcinoma mucinosum neoplasms. The MMP-9 expression strongly correlated with the presence of mucus, previously shown to facilitate cancer spread. Therefore, adenocarcinoma mucinosum is more aggressive than ordinary adenocarcinoma and more difficult to treat. However, no literature data are available on the relationship between MMP-9 expression and secreting or non-secreting histological type of carcinoma. Gress *et al.* [5] have noticed that matrix metalloproteinase 9 can be associated with the process that leads to strong desmoplastic reaction i.e. proliferation of the connective tissue stroma components observed in pancreatic carcinomas.

The role of matrix metalloproteinase 9 has been the subject of many current studies revealing that MMP-9 expression in tumor correlates with shorter survival time. Tumor size, lymph node involvement or distant metastases significantly correlate with shorter survival [7]. Moreover, MMP-9 has been found to contribute to the aggressive behaviour of pancreatic carcinoma and thus lies behind the poor prognosis [6].

In our study, the expression of matrix metalloproteinase 9 was observed in all patients with lymph node involvement. Migration of cancer cells via the lymphatic system plays an essential role in tumor invasion and in the formation of metastases in lymph nodes. Degradation of extracellular matrix by metalloproteinases is the necessary prerequisite for cell migration. Migration of cancer cells can be enhanced through MMP-9 overexpression and is reduced through overexpression of TIMP (tissue inhibitor of metalloproteinase) or application of MMP-9 inhibitors [1,3]. Yamamoto *et al.* [19] have confirmed that both cancer cells and stromal cells are the major source of matrix metalloproteinase 9 in pancreatic ductal carcinoma tissues (among 70 cases, 93% of carcinoma cells and 60% of stroma cells were MMP-9 positive). However, MMP-9 expression was insignificantly correlated with such pathological factors as tumor size, lymph node involvement, formation of distant metastases or tumor stage. Nevertheless, each tumor shows different expression of metalloproteinase 9 and it is likely that MMP-9 differently affects invasiveness and formation of metastases of the respective tumors. According to Maatt *et al.* [11], matrix metalloproteinase 9 does not have a special role in pancreatic ductal carcinoma. The authors analyzed the expression

of MMP-9 in 8 cases of pancreatic tumor. The reaction was pronounced only in tumor epithelial cells, but it was negative in stromal cells in most cases (only sporadic fibroblasts and endothelial cells were positive). Zucker *et al.* [20] observed MMP-9 expression only in 19 out of 45 (43%) pancreatic ductal carcinomas but not in healthy pancreas specimens (p=0.0009). Six of 19 (32%) pancreatic tumors showed either moderate or strong expression. No expression of MMP-9 was found in adjacent tissues, stromal cells of the cancer or in the normal pancreas. No correlation was observed between MMP-9 expression with histological differentiation of the cancer, tumor size, lymph node involvement or survival time.

We found MMP-9 expression in all patients with distant metastases. Numerous reports seem to confirm that matrix metalloproteinase 9 has a significant effect on the formation of metastases of pancreatic ductal carcinoma. Matsuyama *et al.* [12] have proved that pancreatic ductal carcinomas with metastases show much higher MMP-9 expression than metastasis-free carcinomas. Similarly, Nagakawa *et al.* [13], who analyzed 32 cases of pancreatic ductal carcinoma, found distant metastases in 31 of them and demonstrated a major role of matrix metalloproteinase 9 in cancer cell infiltration of blood vessels, which is a risk factor of tumor spread. Angiogenesis, i.e. neovascularization, also plays an outstanding role in the formation of distant metastases. It has been shown that tumor without additional blood vessels can reach 1-2 mm in diameter and needs nutrients supplied by blood vessels to grow and proliferate. Angiogenesis is strictly regulated by the system of stimulators and inhibitors, starting with the release of proteases that decompose the basement membrane and the extracellular matrix. Metalloproteinase 9 helps cancer cells pass through the extracellular matrix barrier and participates in the modulation of signals that affect cell transformation, growth factors, angiogenesis and apoptosis [8,9,15]. The results of our study indicate a correlation between the expression of MMP-9 in pancreatic ductal carcinoma and worse prognosis (shown by lymph node involvement and distant metastases).

References

[1] Bodey B, Bodey B Jr, Siegel SE, Kaiser HE. Immunocytochemical detection of the expression of members of the matrix metalloproteinase family in adenocarcinomas of the pancreas. In Vivo, 2001; 15: 71-76

[2] Bramhall SR, Neoptolemos JP, Stamp GW, Lemoine NR. Imbalance of expression of matrix metalloproteinases (MMPs) and tissue inhibitors of the matrix metalloproteinases (TIMPs) in human pancreatic carcinoma. J Pathol, 1997; 182: 347-355

[3] Bruns CJ, Harbison MT, Kuniyasu H, Eue I, Fidler IJ. In vivo selection and characterization of metastatic variants from human pancreatic adenocarcinoma by using orthotopic implantation in nude mice. Neoplasia, 1999; 1: 50-62

[4] French DL, Ramos-Desimone N, Rozinski K, et al. Matrix metalloproteinase-9 in tumor cell invasion. Ann NY Acad Sci, 1994; 732: 324-334

[5] Gress TM, Muller-Pillasch F, Lerch MM, Friess H, Buchler M, Adler G. Expression and in-situ localization of genes coding for extracellular matrix proteins and extracellular matrix degrading proteases in pancreatic cancer. Int J Cancer, 1995; 62: 407-413

[6] Gurevich LE. Role of matrix metaloproteinases 2 and 9 in determination of invasive potential of pancreatic tumors. Bull Exp Biol Med, 2003; 136: 494-498

[7] Harvey SR, Hurd TC, Markus G, Martinick MI, Penetrante RM, Tan D, Venkataraman P, DeSouza N, Sait SN, Driscoll DL, Gibbs JF. Evaluation of urinary plasminogen activator, its receptor, matrix metalloproteinase-9, and von Willebrand factor in pancreatic cancer. Clin Cancer Res, 2003; 9: 4935-4943

[8] Iki K, Takeo T, Kubozoe T, Aoki S, Hayashi J, Tsunoda T. Detection of serum MMPs in tumor-bearing hamsters. J Hepatobiliary Pancreat Surg, 2002; 9: 478-484

[9] Kuniyasu H, Ellis LM, Evans DB, Abbruzzese JL, Fenoglio CJ, Bucana CD, Cleary KR, Tahara E, Fidler IJ. Relative expression of E-cadherin and type IV collagenase genes predicts disease outcome in patients with resectable pancreatic carcinoma. Clin Cancer Res, 1999; 5: 25-33

[10] Łapińska J. Matrix metalloproteinases in tumor invasion. Współcz Onkol, 1999; 3: 120-122

[11] Maatta M, Soini Y, Liakka A, Autio-Harmainen H. Differential expression of matrix metalloproteinase MMP-2, MMP-9, and membrane type 1-MMP in hepatocellular and pancreatic adenocarcinoma: implications for tumor progression and clinical prognosis. Clin Cancer Res, 2000; 6: 2726-2734

[12] Matsuyama Y, Takao S, Aikou T. Comparison of matrix metalloproteinase expression between primary tumors with or without liver metastasis in pancreatic and colorectal carcinomas. J Surg Oncol, 2002; 80: 105-110

[13] Nagakawa Y, Aoki T, Kasuya K, Tsuchida A, Koyanagi Y. Histologic features of venous invasion, expression of vascular endothelial growth factor and matrix metalloproteinase-2 and matrix metalloproteinase-9, and the relation with liver metastasis in pancreatic cancer. Pancreas, 2002; 24: 169-178

[14] Nagase H, Woessner JF. Matrix metalloproteinases. J Biol Chem, 1999; 274: 21491-21494

[15] Qian X, Rothman VL, Nicosia RF, Tuszynski GP. Expression of thrombospondin-1 in human pancreatic adenocarcinomas: role in matrix metalloproteinase-9 production. Pathol Oncol Res, 2001; 7: 251-259

[16] Ray JM, Stetler-Stevenson WG. The role of matrix metalloproteinases and their inhibitors in tumor invasion, metastasis and angiogenesis. Eur Respir J, 1994; 7: 2062-2072

[17] Szostakiewicz B, Dziadziuszko R, Jassem J. Perspektywy zastosowania inhibitorów angiogenezy w leczeniu niedrobnokomórkowego raka płuca. Współcz Okol, 2003; 7: 668-674

[18] Śliwowska I, Kopczyński Z. Matrix metalloproteinases - bichemical characteristics and clinical value determination in breast cancer patients. Współcz Onkol, 2005; 9: 327-335

[19] Yamamoto H, Itoh F, Iku S, Adachi Y, Fukushima H, Sasaki S, Mukaiya M, Hirata K, Imai K. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human pancreatic adenocarcinomas: clinicopathologic and prognostic significance of matrilysin expression. J Clin Oncol, 2001; 19: 1118-1127

[20] Zucker S, Moll UM, Lysik RM, DiMassimo EI, Stetler-Stevenson WG, Liotta LA, Schwedes JW. Extraction of type-IV collagenase/gelatinase from plasma membranes of human cancer cells. Int J Cancer, 1990; 45: 1137-1142

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