View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Via Medica Journals

FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 48, No. 4, 2010
pp. 632-636

PTP4A3 (PRL-3) expression correlate with lymphatic metastases in gastric cancer

Anna Pryczynicz[1], Katarzyna Guzińska-Ustymowicz[1], Xiao-Jia Chang[2], Joanna Kiśluk[1], Andrzej Kemona[1]

[1]Department of General Pathomorphology, Medical University of Białystok, Białystok, Poland
[2]Attogen Biomedical Research, 100 Barber Avenue, Worcester, MA 01606, USA

Abstract: Many studies have proved that protein tyrosine phosphatase type IV A member 3 (PTP4A3, PRL-3) plays a major role in the metastasis of gastric cancer, especially to local lymph nodes. The objective of the current study was to assess the expression of PTP4A3 in gastric cancer in correlation with chosen anatomoclinical parameters and patients' survival. A total of 71 patients with gastric carcinomas were divided according to Lauren's, Goseki's, Bormann's and Kubo's classifications. The level of PTP4A3 was determined immunohistochemically using a mouse monoclonal anti-PTP4A3 antibody (clone 3B6, anti-human PTP4A3, Attogen Biomedical Research, USA). A statistically significant correlation was observed between PTP4A3 and Kubo's classifications (p=0.0454) and on the verge of statistical significance with Lauren's classification (p=0.0503). The expression of the protein was associated more with the poorly-differentiated mucoid carcinoma and diffused-type carcinoma (58% of cases). We demonstrated a statistically significant correlation between local lymph node involvement and positive expression of PTP4A3 in the primary tumour (p=0.0000). The current study seems to prove that PTP4A3 may have a significant impact on the lymphatic spread of gastric carcinoma. The protein expression is also significantly associated with gastric carcinomas having a worse prognosis, although patients' survival rate showed lack of correlation with PTP4A3 expression.

Key words: gastric cancer, protein tyrosine phosphatase type IVA member 3, PTP4A3, PRL 3

Introduction

The incidence of gastric carcinoma in Poland is very high. According to the National Cancer Registry, it is the sixth most common malignancy among men and the eighth among women [1]. The stage of the disease is the most important prognostic factor. Gastric cancers frequently metastasize to the nearest lymph nodes, involving retroperitoneal and left supraclavicular lymph nodes as well as distant organs. The ability of cancer cells to disseminate from the primary tumour to lymph nodes and to the nearest and distant tissues and organs is a fundamental feature of malignancies and the major cause of therapeutic failures. Metastasis formation is a complex and multistage process involving proteolysis, mobility and migration of cells, proliferation and angiogenesis. Cancer cells, released from the primary tumour, invade the surrounding tissues, penetrate into lymphatic or blood vessels, then migrate through the vascular walls to the adjacent tissues, where they eventually settle, proliferate, induce angiogenesis, forming metastatic foci. Early detection of metastases greatly improves the patients' prognosis. Recently, it has been found that the protein formerly known as phosphatase of regenerating liver-3 (PRL-3) new named protein tyrosine phosphatase type IV A member 3 (PTP4A3) could be used as a marker for detecting early stage metastases [2].

The PTP family comprises of a large group of enzymes involved in one of the most important cellular reaction, *i.e.* dephosphorylation of tyrosine residues, affecting activation or inactivation of enzymes. PTPs regulate many cellular processes, both physiological and pathological, including cell growth, differentiation, cycle or neoplastic transformation. Non-classic representatives of this family include three closely related proteins phosphatases (PTP4A1-PTP4A3) with a unique COOH terminal group. All

Corresponding: Katarzyna Guzińska-Ustymowicz, MD, Department of General Pathomorphology, Medical University of Białystok, ul. Waszyngtona 13, 15-269 Bialystok, Poland. Tel: +48 85 7485942, Fax: +48-85-7485996, e-mail: kasia.guzinska@gmail.com

©Polish Histochemical et Cytochemical Society
Folia Histochem Cytobiol. 2010:48(4): 632 (632-636)
Doi: 10.2478/v10042-010-0070-7

SOME RIGHTS RESERVED

these proteins share at least 75% of their amino acid sequences. PTP4A1 is localized mainly in the brain and muscles, PTP4A2 is expressed in the skeletal muscles and PTP4A3 predominantly in the myocardium and skeletal muscles [2]. The PTP4A3 molecule, also known as PTP4A3, possesses an enzyme active site labelled with the CX_5R signature, where Cys104 is an enzymatic nucleophil and Arg110 binds the phosphate groups on phosphotyrosine [3]. The PTP4A3 molecule contains a *C*-terminal consensus sequence for prenylation and can be found in membranes and intercellular structures when it is prenylated, whereas in the nucleus when it is non-prenylated. The PTP proteins in which the *C*-terminal group has undergone mutation or is lacking are located in the cytoplasm and/or cell nucleus [4]. The role of PTP4A3 has not been fully elucidated. Matter *et al.* [5] have suggested that PTP4A3 can play a role in the regulation of intercellular calcium transmitters induced by angiotensin II. Wu *et al.* [6] have identified PTP4A3 in cell membrane components during mitosis or metaphase. This location may suggest that PTP4A3 is engaged in the regulation of cell cycle. This protein also plays a significant part in the induction of angiogenesis through recruitment of endothelial cells from the circulating blood and is involved in the formation of microcirculation [7]. However, abnormal regulation of tyrosine phosphorylation and dephosphorylation may result in cancer. Saha *et al.* [8] were the first to show the relationship between PTP4A3 expression and liver metastases of colorectal carcinoma. The protein overexpression was also observed in cancer of the ovaries [9], breasts [10] and stomach [11].

The aim of the current study was to assess the expression of PTP4A3 in gastric carcinoma in correlation with chosen anatomoclinical parameters.

Materials and methods

The study was conducted on a group of 71 patients treated surgically for gastric carcinoma in the Department of Surgery. Sections, 4 μm thick, were cut from paraffin blocks and stained with hematoxylin-eosin (H+E). The routine histopathological assessment covered tumour location, histological type, malignancy grade (G), stage (pTN) and the presence of metastases to local lymph nodes. Gastric carcinomas were divided according to Lauren's [12], Goseki's [13], Bormann's [14] and Kubo's [15] classifications.

Immunohistochemical analysis. Formalin-fixed and paraffin-embedded tissue specimens were cut on a microtome into 4 μm sections. The sections were deparaffinized in xylene and hydrated in alcohol. To visualize the antigen, the sections were heated in a microwave oven for 15 min in a citrate buffer (pH 6.0). They were incubated with 0.5% hydrogen peroxide solution in methanol in order to block endogenous peroxidase. Incubation was performed with mouse monoclonal antibody against human PTP4A3 (monoclonal antibody 3B6, Attogen Biomedical Research, USA – anti-PTP4A3 antibody clone 3B6 has been previously described by Peng *et al*., 2004 [16] over the night at 4°C. The reaction was carried out using biotinylated anti-mouse antibody and streptavidin-conjugated with horseradish peroxidase (LSAB2, DAKO, Poland). A colour reaction for peroxidase was developed with chromogene diaminobenzidine (DAKO, Poland). Protein expression was determined using a semiquantitative method and assessed as positive (reaction visible in >5% of tumour cells) or negative (lack of reaction, or reaction present in <5% of cells). Positive reactions were assessed in at least 500 cancer cells in each tissue specimen under a light microscope (×400).

Statistical analysis. Statistical analysis was conducted using Fisher's test and χ^2. Log-rank test according to Kaplan-Meier survival analysis approach was employed to compare the overall survival rates of patients. A p-value <0.05 was considered statistically significant.

Results

The study group consisted of 71 patients with gastric carcinoma. Twenty-two patients had metastases to lymph nodes, including 9 with metastases to group N1 lymph nodes (nodes 1-6), 12 to group N2 (nodes 5-11) and one to group N3 (nodes 12-14). Positive cytoplasmic PTP4A3 expression in main mass of tumor has been presented in Figure 2. Statistical analysis revealed no correlation between PTP4A3 expression in main mass and clinico-pathological parameters, such as age, gender, location, invasion depth, histological malignancy grade and classifications of Goseki and Bormann (Table 1). An on the verge of statistical significance relationship was noted between PTP4A3 expression and Lauren's classification (p=0.0503). The intestinal type of carcinoma in most cases (31/47 cases) didn't show expression of PTP4A3 protein, but the diffuse type of gastric carcinoma shown this expression (14/24 cases). A statistically significant relationship was also observed between PTP4A3 expression and Kubo's classification (p=0.0454). According to the data presented in the table, positive PTP4A3 expression was associated with poorly differentiated mucoid carcinoma, carcinoma diffusum and carcinoma diffusum anaplasticum. Also a very significant correlation was found between local lymph node involvement and positive PTP4A3 expression (p<0.001). Positive PTP4A3 expression in the main mass of tumour was detected in as many as 95,5% (21/22 cases) of patients with lymph node involvement whereas only 18,4% (9/49 cases) were free of lymph node metastases. Patients' survival rate showed lack of correlation with PTP4A3 expression (p=0.1808) (Figure 1).

Discussion

The study aimed to analyse the role of PTP4A3 protein in gastric carcinoma in correlation with the presence of lymph node involvement. As revealed by statistical analysis, positive PTP4A3 expression is strongly associated with metastases to local lymph nodes and their

©Polish Histochemical et Cytochemical Society
Folia Histochem Cytobiol. 2010:48(4): 633 (632-636)
Doi: 10.2478/v10042-010-0070-7

Table 1. Relationship between PTP4A3 expression in primary lesion of gastric cancer and clinicopathological factors.

Parameters		PTP4A3 expression in main mass		
		absent	present	p
Age	≤50	9 (47.4%)	10 (52.6%)	0.4161
	>50	32 (61.5%)	20 (38.5%)	
Gender	Male	28 (56%)	22 (44%)	0.7935
	Female	13 (61.9%)	8 (38.1%)	
Location	Upper 1/3	2 (33.3%)	4 (66.7%)	0.2700
	Middle 1/3	16 (53.3%)	14 (46.7%)	
	Lower 1/3	23 (65.7%)	12 (34.3%)	
Invasion depth	Mucosa	5 (83.3%)	1 (16.7%)	0.1715
	Muscular coat	11 (68.7%)	5 (31.3%)	
	Serosa	25 (51%)	24 (49%)	
Histological differentiation	G2	20 (66.7%)	10 (33.3%)	0.2297
	G3	21 (51.2%)	20 (48.8%)	
Goseki's classification	I	4 (80%)	1 (20%)	0.4039
	II	5 (41.7%)	7 (58.3%)	
	III	2 (50%)	2 (50%)	
	IV	10 (40%)	15 (60%)	
Lauren's classification	Intestinal type	31 (65.9%)	16 (34.1%)	0.0503
	Diffuse type	10 (41.7%)	14 (58.3%)	
Borrmann's classification	I - polypoid	4 (80%)	1 (20%)	0.1201
	II - fungating	8 (66.7%)	4 (33.3%)	
	III - ulcerated	26 (60.5%)	17 (39.5%)	
	IV - infiltrative	3 (27.3%)	8 (72.7%)	
Kubo's classification	Adenocarcinoma w.d.*	11 (61.1%)	7 (38.9%)	**0.0454**
	Adenocarcinoma m.d.*	13 (76.5%)	4 (23.5%)	
	Adenocarcinoma p.d.*	5 (62.5%)	3 (37.5%)	
	Ca solidum	4 (100%)	0 (0%)	
	Ca mucoides p.d.*	2 (25%)	6 (75%)	
	Ca diffusum mucocellulare	0 (0%)	1 (100%)	
	Ca diffusum anaplasticum	2 (40%)	3 (60%)	
Extent of lymph node metastasis	Absent	40 (81.6%)	9 (18.4%)	**<0.001**
	N1	1 (11.1%)	8 (88.9%)	
	N2	0 (0%)	12 (100%)	
	N3	0 (0%)	1 (100%)	
Lymphatic invasion	Absent	40 (81.6%)	9 (18.4%)	**<0.001**
	Present (N1, N2, N3)	1 (4.5%)	21 (95.5%)	

Relationship is significant at the level of $p<0.05$. Significant relationship is marked in bold. **w.d.* well differentiated, *m.d.* moderately differentiated, *p.d.* poorly differentiated, *Ca* carcinoma.

extent, which seems to confirm earlier findings. Miskad *et al.* [11] in an immunohistochemical study have shown that high PTP4A3 expression is correlated with lymph node metastases and their extent. Moreover, they detected PTP4A3 expression in 92.6% of metastatic lymph nodes. They also observed high protein expression in other metastases, *e.g.* to the liver and peritoneum, and PTP4A3 correlation with pT stage. They additionally performed a study by hybridization method in situ, confirming their earlier results associated with metastases to lymph nodes. They also noted that high PTP4A3 expression was associated with gender, tumour

©Polish Histochemical et Cytochemical Society
Folia Histochem Cytobiol. 2010:48(4): 634 (632-636)
Doi: 10.2478/v10042-010-0070-7

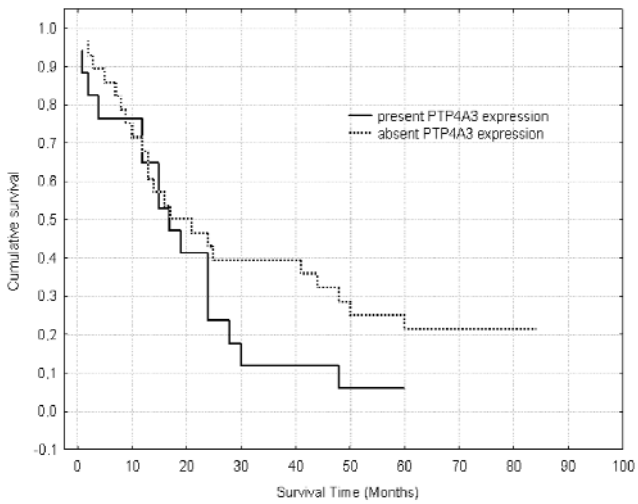

Fig. 1. Survival curve of patients with gastric cancer. There was no significant difference in overall survival rates between the patients with present PTP4A3 expression in primary lesion and those with absent PTP4A3 expression (p=0.1808).

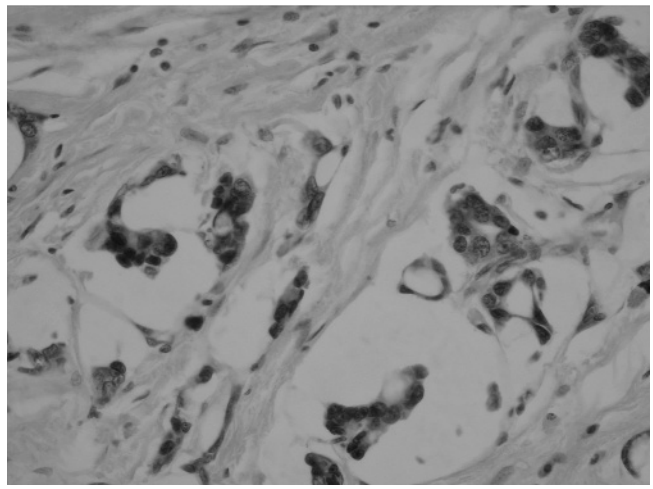

Fig. 2. Expression of PTP4A3 in gastric cancer. Magnification ×400

size and metastasis via blood vessels. Patients with high protein expression had worse prognosis, although their survival did not correlate statistically with the expression [17]. Also Li *et al.* [18] obtained similar results associating the role of PTP4A3 with local lymph node metastases, their extent and T stage. Moreover, they analysed cases of gastric carcinoma with metastases to the peritoneum and found positive PTP4A3 expression in as many as 80.9%. In their study, the protein expression also negatively correlated with patient's prognosis – those with a lack of PTP4A3 expression had better postoperative survival index. Wang *et al.* [19,20] confirmed a significant role of PTP4A3 protein in local lymph node metastases. All these studies definitely indicate that PTP4A3 protein has a major effect on gastric carcinoma spread via the lymphatic pathway as compared to colorectal carcinoma in which, according to different literature sources, this protein has a role rather in distant metastasizing. Most studies have indicated a lack of significant role of PTP4A3 protein in local lymph node metastases of colorectal carcinoma [16,21,22].

Classification of gastric carcinomas according to Goseki, Lauren, Bormann and Kubo allowed us to identify a certain characteristic feature associated with the presence of PTP4A3 expression. The diffuse type of gastric carcinoma (according to the Lauren's microscopic classification) showed a higher PTP4A3 expression (58%) as compared to the intestinal type. Such a correlation, the same statistically insignificant, was also observed in Bormann's classification. The diffuse type of gastric cancer (macroscopic assessment) showed positive PTP4A3 expression in 72.7%, whereas in the remaining types (polypous, fungating and ulcerative) positive expression was noted in a small percentage of cases. The diffuse type infiltrates flatly into the wall of the stomach and narrows its lumen. It grows over a considerable area, is poorly differentiated and gives metastases to lymph nodes. Moreover, a statistically significant correlation was noted between PTP4A3 expression and Kubo's classification. Poorly-differentiated mucoid carcinoma showed positive PTP4A3 expression in 75% (6/8) of cases, mucocellular carcinoma in 100% (1/1 case), anaplastic diffuse carcinoma in 60% (3/5 cases), whereas in other types the protein expression was rather lacking. The analysis of the carcinomas according to Goseki's classification revealed higher PTP4A3 expression in type II (58%) and IV (60%), i.e. in cancers with a large amount of mucus in their cells (a statistically insignificant finding). These two types of gastric cancer are associated with worse prognosis than types I and III.

Thus, PTP4A3 may play a major role in metastasis via the lymphatic pathway. At the same time, as shown by our study, PTP4A3 expression is also connected with increased production of mucus by cancer cells. A question thus arises whether and how the PTP4A3 protein affects this process as well as how it is associated with mucous-producing cancer cells. Cai *et al.* [23] have revealed that the mean survival time of the patients is shorter when PTP4A3 expression is present (18.9 months). Moreover, in their in vitro study, decreased expression of PTP4A3 had an inhibitory impact on the proliferation of cancer cells and tumour growth. As shown in literature reports and our own data, PTP4A3 plays a major role in metastasizing of gastric cancer. Therefore, it seems useful to investigate this protein as an early poorly prognosticating factor in gastric cancer patients.

©Polish Histochemical et Cytochemical Society
Folia Histochem Cytobiol. 2010:48(4): 635 (632-636)
Doi: 10.2478/v10042-010-0070-7

References

[1] Didkowska J, Wojciechowska U, Tarkowski W, Zatoński W. Nowotwory złośliwe w Polsce w 2005 roku, Centrum Onkologii – Instytut, Warszawa, 2007.

[2] Bessette DC, Qiu D, Pallen CJ. PRL PTPs: mediators and markers of cancer progression. *Cancer Metastasis Rev.* 2008;27:231-252.

[3] Kim KA, Song JS, Jee J, *et al*. Structure of human PRL-3, the phosphatase associated with cancer metastasis. *FEBS Lett.* 2004;565:181-187.

[4] Zeng Q, Si X, Horstmann H, Xu Y, Hong W, Pallen C.J. Prenylation-dependent association of protein-tyrosine phosphatases PRL-1, -2 and -3 with the plasma membrane and the early endosome. *J Biol Chemistr*. 2000;275:21444-21452.

[5] Matter WF, Estridge T, Zhang C, *et al*. Role of PRL-3, a human muscle-specific tyrosine phosphatase, in angiotensin-II signaling. *Biochem Biophys Res Commun.* 2001;283:1061-1068.

[6] Wu X, Zeng H, Zhang X, Zhao Y, *et al*. Phosphatase of regenerating liver-3 promotes motility and metastasis of mouse melanoma cells. *Am J Pathol*. 2004;164:2039-2054.

[7] Guo K, Li J, Wang H, *et al*. PRL-3 initiates tumor angiogenesis by recruiting endothelial cells in vitro and in vivo. *Cancer Res.* 2006;66:9625-9635.

[8] Saha S, Bardelli A, Buckhaults P, *et al*. A phosphatase associated with metastasis of Colorectal cancer. *Science*. 2001;294:1343-1346.

[9] Polato F, Codegoni A, Fruscio R, *et al*. PRL-3 phosphatases is implicated in ovarian cancer growth. *Clin Cancer Res.* 2005;11:6835-6839.

[10] Wang L, Peng L, Dong B, *et al*. Overexpression of phosphatase of regenerating liver-3 in breast cancer: association with a poor clinical outcome. *Ann Oncol.* 2006;17:1517-1522.

[11] Miskad UA, Semba S, Kato H, Yokozaki H. Expression of PRL-3 phosphatase in human gastric carcinoma: close correlation with invasion and metastasis. *Pathobiology*. 2004;71:176-184.

[12] Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. *Acta Pathol Microbiol Scand.* 1965;64:31-49.

[13] Goseki N, Takizawa T, Koike M. Differences in the mode of extension of gastric cancer classified by histological type: new histological classification of gastric carcinoma. *Gut*. 1992;33:606-612.

[14] Borrmann R. Geshwulste des Magens und Duodenums. In: Henker F, Lubarsh D. Handbuchder Spezieller Pathologischen, Volume IV, Anatomie und Histolgie Berlin: Springer-Verlag 865; 1926.

[15] Kubo M, Sasako M, Gotoda T, *et al*. Endoscopic evaluation of the remnant stomach after gastrectomy: proposal for a new classification. *Gastric Cancer*. 2002;5:83-89.

[16] Peng L, Ning J, Meng L, Shou C. The association of the expression level of protein tyrosine phosphatase PRL-3 protein with liver metastasis and prognosis of patients with colorectal cancer. *J Cancer Res Clin Oncol.* 2004;130:521-526.

[17] Miskad U.A, Semba S, Kato H, *et al*. High PRL-3 expression in human gastric cancer is a marker of metastasis and gradem of malignancies: an in situ hybidization study. *Virchows Archiv*. 2007;450:303-310.

[18] Li ZR, Wang Z, Zhu BH, *et al*. Association of tyrosine PRL-3 phosphatase protein expresson with peritoneal metastasis of gastric carcinoma and prognosis. *Surgery Today.* 2007;37:646-651.

[19] Wang Z, He YL, Cai SR, *et al*. Expression and prognostic impact of PRL-3 in lymph node metastasis of gastric cancer: its molecular mechanism was investigated using artificial microRNA interference. *Int J Cancer.* 2008;123:1439-1447.

[20] Wang Z, Cai SR, He YL, *et al*. High Expression of PRL-3 can Promote Growth of Gastric Cancer and Exhibits a Poor Prognostic Impact on Patients. *Ann Surg Oncol.* 2008 Nov 14. [Epub ahead of print]

[21] Kato H, Semba S, Miskad UA, Seo Y, Kasuga M, Yokozaki H. High expression of PRL-3 promotes cancer cell motility and liver metastasis in huma colorectal cancer: a predictive molecular marker of metachronous liver and lung metastases. *Clinical Cancer Research*. 2004;10:7318-7328.

[22] Wang Y, Li ZF, He J, *et al*. Expression of the human phosphatases of regenerating liver (PRLs) in colonic adenocarcinoma and its correlation with lymph node metastasis. *Int J Colorectal Dis.* 2007;22:1179-1184.

[23] Cai SR, Chen CQ, Wang Z, *et al*. Expression of phosphatase of regenerating liver-3 in gastric cancer, its relationship with prognosis, and its role in gastric cancer cell proliferation. *Zhonghua Yi Xue Za Zhi.* 2008;88:2326-3230.

Submitted: 8 April, 2010
Accepted after reviews: 15 July, 2010

©Polish Histochemical et Cytochemical Society