Pathol. Oncol. Res. (2014) 20:467–473
DOI 10.1007/s12253-013-9718-x

RESEARCH

Expression of Cyclophilin A in Gastric Adenocarcinoma Patients and Its Inverse Association with Local Relapses and Distant Metastasis

Evgeniya S. Grigoryeva · Nadezhda V. Cherdyntseva · Mikhail S. Karbyshev · Viktor V. Volkomorov · Ivan V. Stepanov · Marina V. Zavyalova · Vladimir M. Perelmuter · Mikhail A. Buldakov · Sergey G. Afanasjev · Sergey A. Tuzikov · Yulia A. Bukurova · Nikolai A. Lisitsyn · Sergey F. Beresten

Received: 25 July 2013 / Accepted: 29 October 2013 / Published online: 24 November 2013

Abstract The aim of this study was to identify new protein markers of the intestinal and diffuse type gastric adenocarcinoma and to determine their relation to local relapses and distant metastasis. Using two-dimensional gel electrophoresis, we searched for proteins that are overexpressed in the intestinal and/or diffuse type gastric adenocarcinoma, as compared to matched normal mucosa samples with further change confirmation by Western blot. Expression of the selected proteins was further assessed by immunohistocemistry in a large panel of gastric adenocarcinoma with various clinicopathological features. Expression level of cyclophilin A measured with western blot appeared to be increased on average ten times in 63 % of gastric adenocarcinoma vs. paired samples of normal mucosa. The frequency of immunihistochemistry detected cyclophilin A protein expression was found to be equal in tumor of both histotypes, but staining intensity was higher in intestinal versus diffuse types of gastric adenocarcinoma. cyclophilin A protein expression appeared to be lower in deeply invading glandular and cribriform structures of intestinal tumors, as well as in discretely placed groups of the intestinal tumor cells. Local relapses as well as distant metastases registered within 3 year follow up were observed to occur much less frequently in patients with positive cyclophilin A immunostaining in gastric tumors. Analysis of cyclophilin A expression has a potential value for prognosis of gastric adenocarcinoma recurrence and distant metastasis.

Keywords Stomach cancer · Two-dimensional gel electrophoresis · Proteomics · Mass spectrometry · Cancer markers

This work was supported by the International Scientific and Technical Center (#3909), Russian Fund for Basic Research (08-04-13705, 09-04-99072) and Federal Target Program "Research and Educational Personnel of Innovative Russia" (№ 02.740.11.0769).

E. S. Grigoryeva · N. V. Cherdyntseva · M. S. Karbyshev · V. V. Volkomorov · V. M. Perelmuter · M. A. Buldakov · S. G. Afanasjev · S. A. Tuzikov
Cancer Research Institute of Siberian Branch of RAMS, Tomsk, Russian Federation

I. V. Stepanov · M. V. Zavyalova
Siberian State Medical University, Tomsk, Russian Federation

Y. A. Bukurova · N. A. Lisitsyn · S. F. Beresten
Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation

M. S. Karbyshev
International Biotechnology Center "Generium", Moscow, Russian Federation

E. S. Grigoryeva (✉)
5 Kooperativny Street, 634050 Tomsk, Russia
e-mail: grigorieva@oncology.tomsk.ru

Introduction

Despite a decreasing incidence of gastric cancer in Europe and North America, it remains the second most common cause of cancer-related deaths worldwide affecting more than 800 000 individuals annually [1]. As opposed to countries with state supported screening programs for gastric cancer [2], the percentage of deaths during the first year after gastric cancer diagnosis in Russian Federation amounts to 55 %. Gastric cancer is generally diagnosed at advanced stages, when curative treatment is impossible, whereas less than 20 % of gastric tumors are diagnosed at stages I or II, when treatment can result in the increase the 5-year survival rate up to 80 % [3].

This calls for studies aimed at the development of efficient and cheap immunoassays for serological diagnosis and prognosis of gastric cancer, as well as design of more efficient cancer therapies. However, despite the obvious importance of protein marker identification for gastric cancer, only about 150 reports devoted to its comparative proteomics are listed in PubMed database.

In our previous study we identified genes that are differentially expressed in gastric tumors using analysis of miRNA databases [4]. The current study was directed to the search of new potential gastric cancer markers using comparative proteomics of protein extracts obtained from primary tumors and adjacent normal tissues. Using of two-dimensional gel electrophoresis proteomic approach allowed identifying five proteins with an increased expression level in gastric tumors. One of these proteins – cyclophilin A (CypA), was overexpressed in all gastric adenocarcinoma samples compared to paired normal samples.

Cyclophilin A, a member of the immunophilin family that has cytoprotective activity, was originally identified as the intracellular receptor for cyclosporin A (CsA) [5]. Despite the intracellular localization of CypA, some cells acquire the ability to secrete it in response to inflammation [6]. Cyclophilin A is a multifunctional chaperone with peptidylprolyl cis-trans isomerase activity that takes part in immune response and cell signaling in response to a wide variety of stress factors. CypA plays an important role in protein folding, assembly, and trafficking and contributes to the maintenance of correct conformation of nascent or partially denatured proteins during environmental insults [7]. Overexpression of CypA was previously reported in small cell lung carcinoma [8], pancreatic cancer [9], hepatocellular carcinoma [10], renal cell carcinoma [11] and breast cancer [12,13]. This is a first report that demonstrates cyclophilin A overexpression in gastric adenocarcinoma and association of intracellular CypA cytoplasmic expression in tumor cells with clinicopathological features of gastric tumors.

Materials and Methods

Clinical Specimens

Fresh and paraffin-embedded gastric adenocarcinoma samples and paired adjacent normal tissues were obtained from gastric cancer patients, who underwent surgical resection at the Thoracic and Abdominal Department of Tomsk Cancer Research Institute in years 2004–2009 (Table 1). The procedures followed in this study were in accordance with the Helsinki Declaration (1964, amended in 1975 and 1983). This study was approved by Ethical Committee of the Cancer Research Institute, and all patients signed an informed consent for voluntary participation. Clinical features of the cases were extracted from hospital records.

Four fresh paired samples of gastric adenocarcinoma and adjacent normal mucosa were analyzed by 2D electrophoresis (2DE) to search protein with differential expression in tumor tissue. Thirty two fresh paired tissue specimens (16 samples of intestinal and samples of 16 diffuse type tumors) were tested by western blot analysis to confirm protein expression differences in tumor and normal mucosa that have been found using 2DE.

Forty six paraffin-embedded gastric adenocarcinoma samples (29 sections with intestinal and 17 with diffuse type) were used for cyclophilin A immunohistochemical analysis in order to assay its prognostic value. All 46 patients were under follow up in a period extending from 1 to 6 years to register local tumor relapses or distant metastases. The clinicopathological variables were evaluated, including age, sex, the primary tumor, lymph node involvement, histological type and grade (Table 1). Twenty-five patients (54.4 %) were female, and 21 (45.6 %) were male. Tumors were categorized according to Lauren's type as: intestinal type in 29 (63 %), diffuse type in 17 (37 %) out of 46 cases. Five (10.9 %) tumors were well differentiated, 17 (37 %) moderately differentiated, 18 (39.1 %) poorly differentiated and 6 (13 %) tumors were undefined. Mucosa invasion, submucosa invasion and serous invasion were determined in 4 (8.7 %), 15 (32.6 %) and 27 (58.7 %) of the tumors, respectively. Lymph node metastasis was observed in 30 (65.2 %) cases. Distant metastases were observed in 9 (22 %) patients.

Two-dimensional Gel Electrophoresis (2DE)

Four pairs of frozen tumor and histologically normal tissues were homogenized and extracted with Tris-buffered saline (0, 12 M NaCl, 20 mM Tris (pH 7.5), 20 mM KCl, 2 mM MgCl2, 0,1 mM EDTA, 1 mM PMSF) in order to remove major structural proteins [14]. After centrifugation of cellular debris the supernatant was precipitated with cold acetone and the pellet was dissolved in O'Farrell loading buffer (9.5 M urea, 2–4 % CHAPS, 1 % dithiothreitol, and 2 % [v/v] carrier ampholytes). One hundred μg of each soluble protein fraction were subjected to 2DE and gels were fixed and silver stained as previously described [15]. The images of eight gels were scanned by Epson Perfection V750 Pro. The 2D gel image of each tumor sample was compared with the corresponding normal sample using Phoretix v.2003.02 and spots with 2.5-fold or more changes in intensity in two or more sample pairs were selected for the mass-spectrometry identification.

Mass Spectrometry

Protein spots of interest were excised, destained twice with 0.1 ml of 100 mM NH_4HCO_3 in 40 % aqueous acetonitrile

Table 1 Baseline gastric cancer patient characteristics (n =46)

Characteristics		Mean ± standard deviation (range)	n (%)
Age (yr)	<60	49,4±9,04	25 (54.4 %)
	≥60	67,2±5,77	21 (45.6 %)
Gender	Male		21 (45.6 %)
	Female		25 (54.4 %)
Invasion of tumor depth	pT1 (mucosa/submucosa)		4 (8.7 %)
	pT2 (muscle/subserosa)		15 (32.6 %)
	pT3/4 (serosa/organ inv.)		27 (58.7 %)
Lauren type	Intestinal		29 (63 %)
	Diffuse		17 (37 %)
Lymph node involvement	Negative		16 (34.8 %)
	Positive		30 (65.2 %)
Distant metastasis	Negative		46 (100 %)
	Positive		0 (0 %)
Differentiation	Well differentiated		5 (10.9 %)
	Moderately differentiated		17 (37 %)
	Poorly differentiated		18 (39.1 %)
	Undefined		6 (13 %)

solution for 30 min at 37 °C, and dehydrated in 100 % acetonitrile for 5 min. The spots were incubated with 3 μl of 15 μg/ml Trypsin Gold (Promega) at 37 °C overnight and 7 μl of 0.5 % mM TFA in 10 % solution of aqueous acetonitrile was added. 2 μl of the peptide eluate was mixed with an equal volume of 2.5-Dihydroxybenzoic acid matrix on the stainless MALDI target and allowed to air-dry.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) was performed on the Ultraflex II instrument (Bruker Daltonics, Germany). Trypsin autolysis products and keratin-derived precursor ions were automatically excluded. Data from the MS/MS, as PKL (peak list) files acquired by the FlexAnalysis 2.0 (Bruker Daltonics), were used for protein identification in the National Center for Biotechnology Information non-redundant (NCBInr) protein sequence database using the MASCOT search algorithm. The MS/MS data were retrieved against the Homo sapiens subset of the sequences with the parameters set as follows: enzyme (trypsin); allowance of up to one missed cleavage peptide; mass tolerance, ±0.1 Da; and MS/MS tolerance, ±0.05 Da. Fixed modifications of cysteine carbamidomethylation and variable modifications of methionine due to air oxidation were allowed. The threshold used was $p < 0.05$, indicating the identification at the 95 % confidence interval for matched peptides, and MASCOT scores (based on combined MS and MS/MS spectra) over 70 were considered statistically significant.

Western Blotting

Thirty two pairs of normal and tumor tissue samples were ground in liquid nitrogen, lysed in Laemmli sample buffer and loaded onto 10–22 % SDS polyacrylamide gel, according to standard protocol. Proteins were transferred to polyvinylidene fluoride membranes (Millipore) and incubated with rabbit anti-human cyclophilin A antibody (Santa Cruz Biotechnology, Inc.). Blots were stained with horseradish peroxidase-conjugated secondary antibody and visualized by chemiluminescence detection using Amersham ECL Plus™ Western Blotting Detection Reagents (GE Healthcare). CypA expression level was normalised to B-actin level.

Immunohistochemistry

Paraffin-embedded tumor tissue 4 μm sections were probed with primary rabbit anti-human CypA antibody (Sigma-Aldrich), stained with secondary antibody conjugated to horseradish peroxidase and visualized by BioGenex Super Sensitive Non-Biotin HRP Detection System (BioGenex, San Ramon, CA). For each section, ten representative fields with well preserved carcinoma tissue were examined at×400 magnification and 100 carcinoma cells were counted for each field. Immunostaining intensity was classified as negative in case of the absence of cells with cyclophilin A cytoplasmic immunoreactivity in ten fields and positive in cases of the presence of carcinoma cells with cyclophilin A cytoplasmic staining in at least one of ten fields. The frequency of CypA positive staining tumor cells was estimated in all tumor specimens. Percentage of CypA expression in cells of different parenchymal structures (glandular, cribriform, trabecular, solid) and in signet ring cells, for each invasion depth into the stomach wall layers was determined [16].

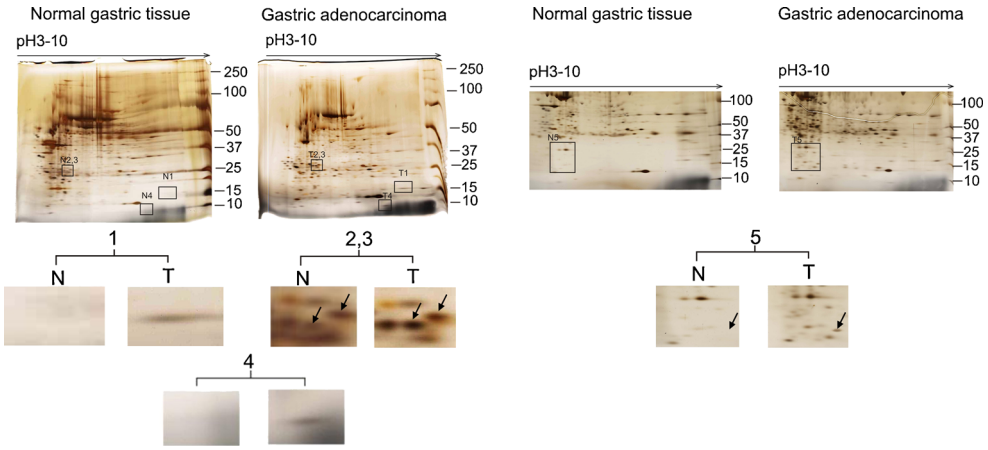

Fig. 1 2D gel images of a matched pair of gastric adenocarcinoma and normal gastric tissues (showed on *top*). Individual spots with differences in staining are shown on bottom. 1 – CypA, 2 – tropomyosin 3, 3 – cathepsin D, 4 – S100 calcium binding protein A9, 5 – thymosin beta 10

Statistical Analysis

Statistical analysis was performed using the software package «Statistica 6.0 for Windows» (v.6.1, series 1203d, StatSoft Inc.) (significance level $P<0.05$). Spearman's rank correlation test was used to examine the association between CypA expression and tumor type as well as depth of tumor invasion. The $\chi 2$ test was used for comparison between qualitative variables.

Results

Using 2DE analysis of two intestinal and two diffuse type samples of gastric adenocarcinoma and matched normal tissues, we have identified five protein spots that were at least 2.5-fold more intensive in at least two tumor tissue extracts, as compared to paired normal mucosa controls (Fig. 1). MALDI-TOF led to robust identification of thymosin beta-10, cathepsin D, S100A9, tropomyosin 3, and cyclophilin A. Overexpression of the first three proteins has been previously reported in gastric tumors [17–19], as well as in other tumor types [20–22]. We focused our attention to analysis of CypA, since its expression changed most significantly in three out of four adenocarcinoma samples versus normal paired samples analyzed by 2DE.

Determination of CypA expression in paired samples of 32 gastric cancer patients by western blot analysis demonstrated that CypA protein amount is on average ten times higher in tumors, as compared to normal tissues in 63 % of patients (Fig. 2). The overexpression frequencies were found to be 64 % and 53 % in intestinal and diffuse tumor types, correspondingly. While analyzing clinicopathological factors, including age, sex, the primary tumor, nodal, the TNM stage group, tumor site and histological grade, no correlation with CypA expression in tumor was found (data not shown).

We analyzed CypA expression by immunohistochemical staining in paraffin-embedded tumor tissue of gastric cancer patients. The expression of CypA, appeared as fine granules and diffused cytoplasmic staining, was stronger in tumor tissues versus normal (Fig. 3). CypA expression in normal mucosa samples was detected in 37.5 % whereas tumor cells were positive in 87.5 % of patients ($P=0.01$). There is no difference in the frequency of CypA protein expression found between intestinal and diffuse types of gastric adenocarcinoma. Positive CypA expression was detected in 88 % of intestinal and 90 % of diffuse type adenocarcinoma.

Immunostaining intensity was classified as mild staining, moderate staining and strong staining. It should be clarified that staining intensity was definitely different: moderate staining was predominant in intestinal type gastric adenocarcinoma, while mild staining was characteristic for diffuse tumor type. The number of CypA-positive cells per 100 cells on a section of intestinal and diffuse tumors was 24.6 and 7.7, respectively ($P=0.002$) (Table 2).

The important prognostic factors of gastric cancer are the depth of tumor invasion and the status of lymph node

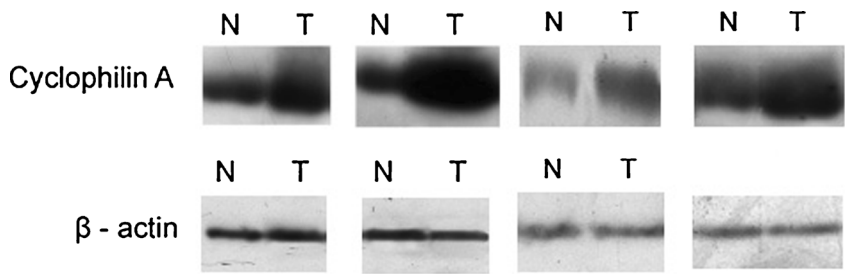

Fig. 2 Western blotting of CypA in matched pairs of tumor and normal tissue samples. (β-actin is used as internal control)

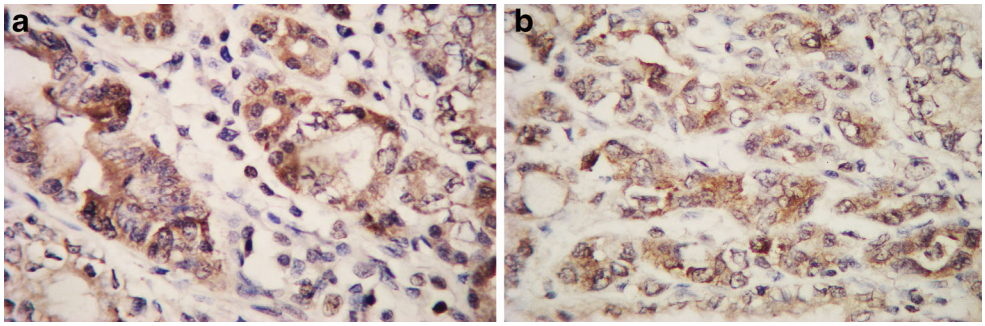

Fig. 3 Immunohistochemical analysis of CypA in: **a** Cytoplasmic expression of CypA in tumor cells of intestinal gastric adenocarcinoma. Diaminobenzidine and eosin stain, ×400 magnification. **b** Cytoplasmic expression of CypA in tumor cells of diffuse type of gastric cancer. Diaminobenzidine and eosin stain, ×400 magnification

metastasis [23]. Kim et al. [24] reported that the deepness of the invasion is related to the lowering of the survival rate, reporting 5-year survival rates of 93.4 %, 89.8 %, 77.2 %, 60.5 %, 39.7 %, and 8.7 % in patients with tumor invasion into lamina propria or muscularis mucosae, submucosa, muscularis propria, subserosa, tumor perforation of serosa, and invasion to adjacent organs, respectively. In order to evaluate potential prognostic significance of CypA expression we studied its association with depth of invasion, local relapses and distant metastasis in 46 patients: 29 with intestinal and 17 with diffuse type of gastric adenocarcinoma. Patients were observed from 1 to 6 years after specific treatment, occurrence of local relapses and distant metastases within follow-up period were fixed.

The evaluation of CypA expression in tumor cells revealed no association with the depth of invasion. Considering the fact that stomach tumor tissue is heterogeneous and represented by several histological structures, we evaluated the CypA expression in each of them at different depth of invasion. In the case of intestinal type adenocarcinoma, we revealed a gradual decrease of CypA expression in glandular and cribriform structures in deeply invading layers of the stomach wall. Also, CypA expression in discretely placed small groups of the tumor cells was significantly lower than in cells forming well-differentiated glandular structures (Table 3). There was no any correlation for diffuse type tumors.

As for postoperative follow-up, local relapses were found to occur more frequently in patients with negative CypA immunostaining. In intestinal type of adenocarcinoma with negative CypA expression, local relapses observed in 67 % of cases, while with positive expression in 12 % ($P=0.01$) In patients with diffuse type of tumor, distant metastases were detected in all cases with negative expression of CypA (Table 4). An incidence of distant metastases in intestinal type of gastric adenocarcinoma was not associated with the expression of CypA.

Thereby, our data clearly demonstrated the potential value of CypA detection for gastric cancer prognosis and postoperative monitoring differential for diffuse and intestinal type.

Table 2 CypA expression in different histological types of gastric adenocarcinoma

Histological type of gastric adenocarcinoma	CypA expression, % (M±S.D.)
Intestinal	24,6±19,3 ($n=26$)
Diffuse	7,7±7,0 ($n=15$)
$P=0,002$	

M ± S.D. – values were recorded as mean ± standard deviation

Discussion

Comparative proteomics hold promise for identification of protein markers for cancer diagnosis and prognosis, but only a few markers are routinely used for gastrointestinal malignancies (mainly carcinoembryonic antigen, CA19-9, and DR70). This is a consequence of insufficient immunoassay sensitivity and specificity that lead to a large quantity of false positive and false negative results [25]. In order to find new

Table 3 CypA expression in parenchymal structures of intestinal tumors at different depths of tumor invasion

Histological structures		CypA expression in each layer, % (M±S.D.)			
		Mucosa a	Submucosa b	Muscular c	Serous d
Glandular structures	1	48.0±33.9 ($n=23$)	28.7±25.6 ($n=22$) $P_a=0.01$	13.5±13.9 ($n=11$) $P_a=0.001$	-
Cribriform structures	2	33.7±33.3 ($n=12$)	29.4±25.7 ($n=10$)	–	–
Groups of cells	3	15.8±16.7 ($n=15$) $P_1=0.0008$	9.5±9.6 ($n=18$) $P_a=0.09$; $p_1=0.002$	9.0±9.5 ($n=14$) $P_a=0.09$	10.0±7.5 ($n=3$)

M ± S.D. mean value ± standard deviation, P_a difference of CypA expression between the indicated tumor structure and glandular structures in the mucosa, P_1 difference of CypA expression between the indicated tumor structure and groups of cells in the mucosa

Table 4 Association of CypA expression in tumor cells with distant metastases in diffuse type of gastric adenocarcinoma

CypA expression	Distant metastasis (number of patients, %)	
	M0	M+
negative	0/2 (0 %)	2/2 (100 %)
positive	12/15 (80 %)	3/15 (20 %)
$P=0,01$.		

potential gastric cancer markers we compared the protein profile of matched pairs of gastric adenocarcinoma and normal gastric tissue. Proteomics approach led to identification of CypA, a new candidate protein marker of gastric cancer that has been previously shown to be overexpressed in several other tumor types [9,12,26]. Our first previous evidence for CypA overexpression in gastric adenocarcinoma was published in 2009 [27]. In current study we confirmed CypA protein overexpression in tumor samples and found the association between CypA expression and occurrence of local relapses and distant metastasis in follow-up period.

Data concerning the increase of CypA expression in tumor tissues is ambiguous. As example Ruy et al. reported about seven overexpressed proteins in gastric adenocarcinoma among which there is no mention of CypA [28]. On the other hand, recently, Bai et al. has demonstrated increased expression of CypA in the tumor tissue of the stomach compared to histologically normal tissue [29]. In this study we

Although the exact molecular mechanisms for CypA overexpression in cancer cells have not been fully studied, it was found that CypA overexpression is associated with tumor cell proliferation, block of apoptosis, increased resistance to hypoxia, as well as with higher tumor aggressiveness and ability to metastasis [29–31]. However, the evaluations of prognostic significance of CypA overexpression performed for different tumor types were controversial. Thus Lim et al. shows that CypA overexpression is a poor prognostic factor because of its association with decreased patient's survival [26]. In contrast, Howard et al. performed tissue microarray immunostaining of CypA, but their study show no statistically significant correlation of immunostaining intensity and overall score with survival [32]. In our study immunohistochemical analysis of CypA expression in tumor subtypes clearly demonstrated much lower content or even complete absence of CypA in diffuse tumors, which are known to display more aggressive behavior versus intestinal tumors, and Cyp A decrease in poorly differentiated elements in the intestinal tumors.

Besides tumors invading into the deep layers of the stomach wall, local relapses, and hematogenous metastasis incidences have been shown to be associated with no CypA expression in tumor cells. Relapse of gastric cancer, in contrast to the primary tumor, characterized by greater biological activity which is expressed in the tendency towards infiltrative growth, a high degree of invasiveness and frequent invasion into surrounding organs. There is some data showing that patients who have had extensive lymph-node dissection conducted are characterized by significant increase in survival rates. Thus, the occurrences of local relapses present to be unfavorable prognostic factor of clinical course of disease.

Lack of CypA expression may assume an unfavorable prognostic factor regarding to tumor progression differentially for patients with diffuse and intestinal type of gastric adenocarcinoma. We can predict low risk of hematogenous metastases in patients with the diffuse type of gastric adenocarcinoma with positive expression of CypA in tumor cells. Loss of CypA expression in advanced tumors can be explained by several reasons: 1) elimination of oxidative stress after formation of the tumor vasculature; 2) loss of tumor cell communication with adjacent stromal elements during epithelial-mesenchymal transition; 3) loss of the necessity of CypA-mediated regulation of immune cell functioning in inflammatory cell infiltrate, when tumors reach a substantial size [7].

Obtained data for the first time demonstrated the potential value of CypA for local relapses and distant metastasis prognosis of gastric cancer.

References

1. Jemal A, Center M, DeSantis C, Ward E (2010) Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev 19(8):1893–1907
2. Mizoue T, Yoshimura T, Tokui N, Hoshiyama Y, Yatsuya H, Sakata K, Kondo T, Kikuchi S, Toyoshima H, Hayakawa N, Tamakoshi A, Ohno Y, Fujino Y, Kaneko S (2003) Prospective study of screening for stomach cancer in Japan. Japan Collaborative Cohort Study Group Int J Cancer 106(1):103–107
3. Washington K (2010) 7th Edition of the AJCC Cancer Staging Manual: Stomach. Ann Surg Oncol 17:3077–3079
4. Volkomorov V, Grigoryeva E, Krasnov G, Litviakov N, Tsyganov M, Karbyshev M, Zavyalova M, Afanasyev S, Cherdyntseva N, Lisitsyn N, Beresten S (2013) Search for potential gastric cancer markers using miRNA databases and gene expression analysis. Exp Oncol 35(1):2–7
5. Obchoei S, Weakley SM, Wongkham S, Wongkham C, Sawanyawisuth K, Yao Q, Chen C (2011) Cyclophilin A enhances cell proliferation and tumor growth of liver fluke-associated cholangiocarcinoma. Mol Cancer 10:102
6. Nigro P, Satoh K, O'Dell M, Soe N, Cui Z, Mohan A, Abe J, Alexis J, Sparks J, Berk B (2011) Cyclophilin A is an inflammatory mediator that promotes atherosclerosis in apolipoprotein E-deficient mice. J Exp Med 208(1):53–66
7. Lee J (2010) Role of Cyclophilin A during Oncogenesis. Arch Pharm Res 33:181–187
8. Campa MJ, Wang MZ, Howard B, Fitzgerald MC, Patz EF (2003) Protein expression profiling identifies macrophage migration inhibitory factor and cyclophilin A as potential molecular targets in nonsmall-cell lung cancer. Cancer Res 63:1652–1656

9. Cecconi D, Astner H, Donadelli M, Palmieri M, Missiaglia E, Hamdan M, Scarpa A, Righetti PG (2003) Proteomic analysis of pancreatic ductal carcinoma cells treated with 5-aza-2′-deoxycytidine. Electrophoresis 24:4291–4303
10. Lim SO, Park SJ, Kim W, Park SG, Kim HJ, Kim YI, Sohn TS, Noh JH, Jung G (2002) Proteome analysis of hepatocellular carcinoma. Biochem Biophys Res Commun 291:1031–1037
11. Yang J, Li A, Yang Y, Li X (2011) Identification of cyclophilin A as a potential prognostic factor for clear-cell renal cell carcinoma by comparative proteomic analysis. Cancer Biol Ther 11(5):535–546
12. Zheng J, Koblinski JE, Dutson LV, Feeney YB, Clevenger CV (2008) Prolyl isomerase cyclophilin A regulation of Janus-activated kinase 2 and the progression of human breast cancer. Cancer Res 68:7769–7778
13. Hathout Y, Riordan K, Gehrmann M, Fenselau C (2002) Differential protein expression in the cytosol fraction of an MCF-7 breast cancer cell line selected for resistance toward melphalan. J Proteome Res 1: 435–442
14. Krasnov GS, Oparina NY, Hankin SL, Mashkova TD, Ershov AN, Zatsepina OG, Karpov VL, Beresten SF (2009) Identification of proteins with altered expression in colorectal cancer by means of 2D-proteomics. Mol Biol (Mosk) 43:321–328
15. Mortz E, Krogh T, Vorum H, Gorg A (2001) Improved silver staining protocols for high sensitivity protein identification using matrix-assisted laser desorption/ionization-time of flight analysis. Proteomics 1:1359–1363
16. Stepanov IV, Zavizlova MV, Grigor'eva ES, Bukurova YA, Afanas'ev SG, Cherdyntseva NV (2010) Clinico-morphological and genetics features of diffuse and intestinal gastric adenocarcinomas. Siberian journal of oncology 40:55–66 (in Russian)
17. El-Rifai W, Moskaluk CA, Abdrabbo MK, Harper J, Yoshida C, Riggins GJ, Frierson HF Jr, Powell SM (2002) Gastric Cancers Over express S100A Calcium-binding Proteins. Cancer Research 62:6823–6826
18. Oien KA, Vass KJ, Downie I, Fullarton G, Keith NW (2003) Profiling, comparison and validation of gene expression in gastric carcinoma and normal stomach. Oncogene 22:4287–4300
19. Ikeguchia M, Fukudaa K, Okaa S, Yamaguchia K, Hisamitsua K, Tsujitania S, Sakatanib T, Uedac T, Kaibaraa N (2001) Clinicopathological Significance of Cathepsin D Expression in Gastric Adenocarcinoma. Oncology 61:71–78
20. Sribenja S, Li M, Wongkham S, Wongkham C, Yao Q, Chen C (2009) Advances in thymosin beta10 research: differential expression, molecular mechanisms, and clinical implications in cancer and other conditions. Cancer Invest 27(10):1016–1022
21. Liaudet-Coopman E, Beaujouin M, Derocq D, Garcia M, Glondu-Lassis M, Laurent-Matha V, Prébois C, Rochefort H, Vignon F (2006) Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. Cancer Lett 237(2):167–179
22. Salama I, Malone PS, Mihaimeed F, Jones JL (2008) A review of the S100 proteins in cancer. Eur J Surg Oncol 34(4):357–364
23. Adachi Y, Yasuda K, Inomata M, Sato K, Shiraishi N, Kitano S (2000) Pathology and prognosis of gastric carcinoma: well versus poorly differentiated type. Cancer 89:1418–1424
24. Kim JP, Lee JH, Kim SJ, Yu HJ, Yang HK (1998) Clinicopathologic characteristics and prognostic factors in 10783 patients with gastric cancer. Gastric Cancer 1:125–133
25. Yoshihara T, Kadota Y, Yoshimura Y, Tatano Y, Takeuchi N, Okitsu H, Umemoto A, Yamauchi T, Itoh K (2006) Proteomic alteration in gastic adenocarcinomas from Japanese patients. Molecular Cancer 5: 75
26. Li Z, Zhao X, Bai S, Wang Z, Chen L, Wei Y, Huang C (2008) Proteomics Identification of Cyclophilin A as a Potential Prognostic Factor and Therapeutic Target in Endometrial Carcinoma. Mol Cell Proteomics 7:1810–1823
27. Grigoryeva E, Bukurova Y, Cherdyntseva N, Afanasyev S, Komarova T, Rodicheva N, Beresten S (2009) 2Dproteomics of gastric cancer: identification of proteins with increased synthesis in tumor tissue. Siberian journal of oncology 5:37–42 (in Russian)
28. Ryu JW, Kim HJ, Lee YS, Myong NH, Hwang CH, Lee GS, Yom HC (2003) The proteomics approach to find biomarkers in gastric cancer. J Korean Med Sci 18(4):505–509
29. Bai Z, Ye Y, Liang B, Xu F, Zhang H, Zhang Y, Peng J, Shen D, Cui Z, Zhang Z, Wang S (2011) Proteomics-based identification of a group of apoptosis-related proteins and biomarkers in gastric cancer. Int J Oncol 38(2):375–383
30. Choi KJ, Piao YJ, Lim MJ, Kim JH, Ha J, Choe W, Kim SS (2007) Overexpressed cyclophilin A in cancer cells renders resistance to hypoxia- and cisplatin-induced cell death. Cancer Res 67:3654–3662
31. Qi YJ, He QY, Ma YF, Du YW, Liu GC, Li YJ, Tsao GS, Ngai SM, Chiu JF (2008) Proteomic identification of malignant transformation-related proteins in esophageal squamous cell carcinoma. J Cell Biochem 104:1625–1635
32. Howard BA, Zheng Z, Campa MJ, Wang MZ, Shama A, Haura E, Herndon JE II, Fitzgerald MC, Bepler G, Patz EF (2004) Translating biomarkers into clinical practice: prognostic implications of cyclophilin A and macrophage migratory inhibitory factor identified from protein expression profiles in non-small cell lung cancer. Lung Cancer 46:313–323