Int J Clin Exp Pathol 2017;10(3):3272-3278
www.ijcep.com /ISSN:1936-2625/IJCEP0043948

Original Article

PTP4A3 expression is associated with the clinical features and prognosis of cervical cancer

Li Tang[1*], Hongjin Yu[1,2*], Wenbing Zhong[1], Fubing Yu[1]

[1]*Department of Gynecology, Dongguan Maternal & Children Health Care Hospital, Dongguan, China;* [2]*Department of Gynecology, Dongguan General Hospital, Dongguan, China.* [*]*Equal contributors.*

Received November 9, 2016; Accepted January 6, 2017; Epub March 1, 2017; Published March 15, 2017

Abstract: Background: Protein tyrosine phosphatase 4A3 (PTP4A3) is overexpressed in different cancer types and is involved in tumor progression. However, the clinical significance of PTP4A3 in cervical cancer is still unclear. Methods: Real-time PCR was used to examine the mRNA expression levels of PTP4A3 in 8 pairs of cervical cancer tissues, and immunohistochemistry was used to investigate PTP4A3 protein expression pattern in 144 archived cervical cancer specimens. The correlation of PTP4A3 expression with clinicopathologic features was analyzed. Results: The expression of PTP4A3 was increased in all 8 cervical cancerous tissues compared with that in adjacent normal cervical tissues. PTP4A3 overexpression in patients was correlated with tumor stage (P = 0.004), tumor size (P = 0.024), and lymph node involvement (P = 0.025). Furthermore, patients with high PTP4A3 expression showed shorter overall and recurrence-free survival time after surgery (P = 0.012 and P = 0.005, respectively). Multivariate Cox regression analysis showed that PTP4A3 expression had a predictive value for overall survival of cervical cancer patients (P = 0.029). Conclusion: The expression of PTP4A3 was increased in cervical cancer and it plays an important oncogenic role in tumor progression of cervical cancer.

Keywords: PTP4A3, progression, cervical cancer, prognosis

Introduction

Cervical cancer is among the most prevalent gynecological malignancies, with nearly 530,000 new cases and 275,000 deaths reported annually [1]. Despite the improvement of diagnostic and treatment strategies, most cervical cancer patients diagnosed at advanced stages are not eligible for potential curative therapies. The three-year survival and disease-free survival rate of patients with locoregional recurrent cervical cancer were 55.6% and 54.3% after concomitant chemo-radiation therapy [2]. Recently, more and more studies have been made in understanding the genetic alterations and biological processes in cervical cancer preogression. However, the search for specific molecular and genetic alterations in cervical cancer that have clinicopathologic significance is limited.

Protein tyrosine phosphatase 4A3 (PTP4A3), also named as phosphatase of regenerating liver-3 (PRL-3), is a member of the protein tyrosine phosphatases (PTPs) family. The PTP4A3 gene is located in chromosome 8q24.3 and is composed of 173 amino acids and is a monomer with a complex structure [3]. PTP4A3 has been shown to participate in many fundamental physiological processes. For example, PTP4A3 provokes a tyrosine phosphoproteome to drive prometastatic signal transduction [4]. In vitro study found that PTP4A3 is an important contributor to endothelial cell function and as a multimodal target for cancer therapy and mitigating VEGF regulated angiogenesis [5]. Using yeast two-hybrid screening, Peng et al. found that PTP4A3 interacted with integrin α1, downregulated tyrosine phosphorylation of integrin β1, enhanced the phosphorylation of ERK1/2, indicating its critical role in integrin β1-ERK1/2-MMP2 signaling and cell motility [6]. Accumulating evidence suggests that PTP4A3 is involved in the proliferation, growth regulation, increased metastasis, and invasion in different types of cancers. It has been reported that culture carcinoma associated fibroblasts trigger PTP4A3 overexpression in cancer

cells, and a higher expression of PTP4A3 correlates with tumor progression and its severity [7]. It has been demonstrated that PTP4A3 promotes tumor cell migration and angiogenesis by increasing extracellular oncogenic pathways [8, 9]. In contrast, PTP4A3 inhibitors blocked the migration and invasion of metastatic cancer cells [10]. Likewise, RNAi-mediated knockdown of PTP4A3 inhibits cell invasion and downregulates ERK1/2 expression in cancer cells [11].

Although both PTP4A3 has been shown to be overexpressed in squamous cell carcinoma of the cervix [12], the prognostic role of PTP4A3 in cervical cancer patients has not been investigated. Therefore, we evaluated the expression levels of PTP4A3 in human cervical cancer tissues and analyzed the clinical significance of PTP4A3 expression in cervical cancer patients. Our data demonstrate that PTP4A3 expression is significantly correlated with cervical cancer progression and clinical prognosis. Thus, PTP4A3 may serve as a new prognostic marker and therapeutic target for cervical cancer therapy.

Materials and methods

Patients and tissue specimens

A total of 144 cervical cancer patients who underwent surgical resection from January 2004 to December 2009 at Dongguan General Hospital were analyzed. The records of patients were reviewed in the context of clinicopathological and follow-up information. The tumor stage was classified according to the latest International Federation of Gynecology and Obstetrics criteria. The overall survival (OS) and recurrence-free survival (RFS) was calculated starting from the date of the initial surgery to the time of death and recurrence, respectively. After surgery, resected specimens were processed routinely for macroscopic pathological assessment. In addition, 8 pairs of snap-frozen cervical cancer and normal cervical samples were collected for Real-time PCR. Informed consent was obtained from each patient and this study was approved by the Research Ethics Committee of Dongguan General Hospital.

Quantitative real-time polymerase chain reaction (RT-PCR)

A total of 2 μg of tissue RNA was reverse transcribed to cDNA with a high-capacity (Thermo, USA) according to the manufacturer's instructions. Gene expression was assayed with 2X SYBR Green Mastermix (Bio-Rad) with real-time detection performed with the iCycler thermocycler (Bio-Rad). The primers used are as follows: PTP4A3, sense, 5'-CGGGATGAAGTACGAGGACG-3', and anti-sense, 5'-GCGTGTGTGGGTCTTTGAAC-3'; β-actin, sense, 5'-TCATGAAGTGTGACGTTGACATCCGT-3', and anti-sense, 5'-CCTAGAAGCATTTGCGGTGCACGATG-3'. The $2^{-\Delta\Delta CT}$ method was used to calculate relative expression of PTP4A3 mRNA.

Immunohistochemistry

The formalin-fixed, paraffin-embedded archival specimens were cut in four-μm sections, and were mounted on poly-L-lysine-coated slides. They were then deparaffinized in xylene and rehydrated through graded alcohol to distilled water. Endogenous peroxidase activity was then blocked by incubation in 3% hydrogen peroxide-methanol for 10 min. After washing with phosphate-buffered saline, the slides were blocked with 1% BSA and then incubated overnight at 4°C with anti-PTP4A3 antibody (Abcam, Cambridge, USA; 1:200), followed by incubation with prediluted secondary antibody and streptavidin-horseradish peroxidase complex.

Semiquantitative immunohistochemical scoring

Evaluation of PTP4A3 immunoreactivity was carried out by two independent pathologists. The analysis was assessed according to both the proportion of positively stained tumor cells and the intensity of staining. The area of staining was stratified into 5 scoring groups: 0, not detected; 1, <10% positive cells; 2, 11-50% positive cells; 3, 51-80% positive cells; and 4, >80% positive cells. Intensity of stained cells was graded semi-quantitatively into four levels: 0: no staining; 1: weak staining; 2: positive staining; and 3: strong staining. Using this method of assessment, the expression of PTP4A3 was evaluated by the staining index (scored as 0, 1, 2, 3, 4, 6, 8, 9 or 12). Staining index score ≥6 was identified as high expression, while score <6 was low expression.

Statistical analysis

All statistical analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, USA). A standard chi-squared test was performed to

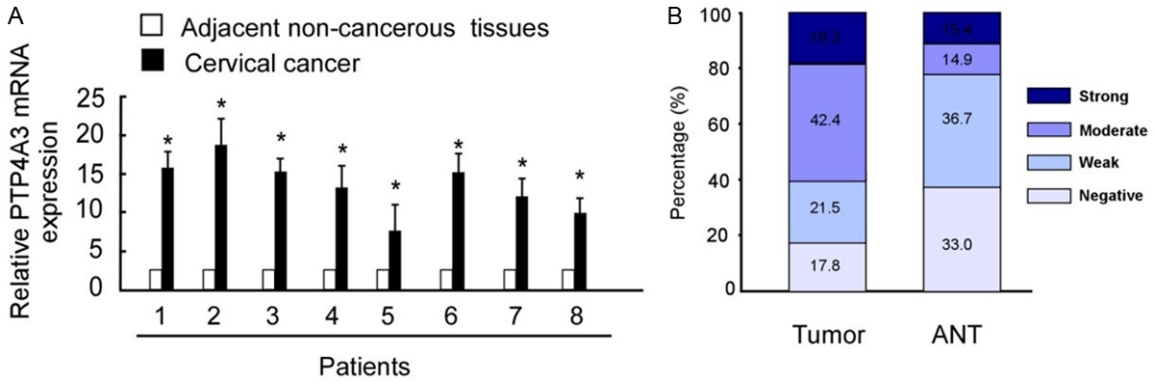

Figure 1. Expression pattern of PTP4A3 in cervical cancer. A. PTP4A3 mRNA expression in 8 pairs of cervical cancer and adjacent normal cervical tissues. B. Bar graph showed the percentage of different staining intensity for PTP4A3 in 144 cervical cancer patients.

assess the association between PTP4A3 expression and the clinicopathological characteristics. Overall survival (OS) and recurrence-free survival (RFS) curves were obtained by the Kaplan-Meier method and were compared with the log-rank test. Multivariate analysis was performed using the Cox regression model. A two tailed *P*-value of <0.05 was considered statistically significant.

Results

PTP4A3 is upregulated in cervical cancer

Analysis of the expression of PTP4A3 by real-time PCR determined that PTP4A3 was significantly upregulated in cervical cancer tissues compared with that in adjacent normal tissues (**Figure 1A**). Additionally, PTP4A3 staining intensity was scored as strong or moderate intensity in 60.7% of tumor tissues, whereas only 30.3% of corresponding adjacent normal cervical tissues were scored as strong or moderate intensity (**Figure 1A**, **1B**). Our results showed that PTP4A3 was frequently overexpressed in human cervical cancer.

Association of PTP4A3 expression and clinicopathological factors

PTP4A3 expression in 144 primary cervical cancer specimens was determined by immunohistochemistry. The representative immunostaining of PTP4A3 in cervical cancer tissues was shown in **Figure 2**. PTP4A3 protein mainly localized at cell membrane and cytoplasm. Statistical analysis further showed positive associations of PTP4A3 expression with tumor stage (P = 0.004), tumor size (P = 0.024), and lymph node involvement (P = 0.025) (**Table 1**). No significant difference between high and low levels of PTP4A3 was observed in terms of patient age, tumor differentiation, or histological type.

PTP4A3 expression predicted worse overcome in cervical cancer

Statistical analysis also revealed a correlation between PTP4A3 expression and patients' clinical prognosis. Kaplan-Meier analysis showed that higher PTP4A3 expression led to shorter overall survival and recurrence-free survival time, while lower PTP4A3 expression resulted in longer survival (P = 0.012 and P = 0.005, respectively; **Figure 3**). Multivariable Cox regression analysis showed that PTP4A3 expression might serve as an independent predictor of OS in patients with cervical cancer prognosis (P = 0.029; **Table 2**).

Discussion

In the present study, we investigated the expression patterns of PTP4A3, by Real-time PCR and IHC, using fresh and paraffin-embedded cervical cancer tissues. Real-time PCR assay revealed that up-regulation of PTP4A3 was detected in cervical carcinoma tissues, when compared with their adjacent normal cervix tissues. Moreover, overexpression of PTP4A3 was also correlated to advanced tumor stage, tumor size, lymph nodes metastasis, and poor clinical prognosis. These data provided evidence that the elevated expression of PTP4A3 might play an important role in tumori-

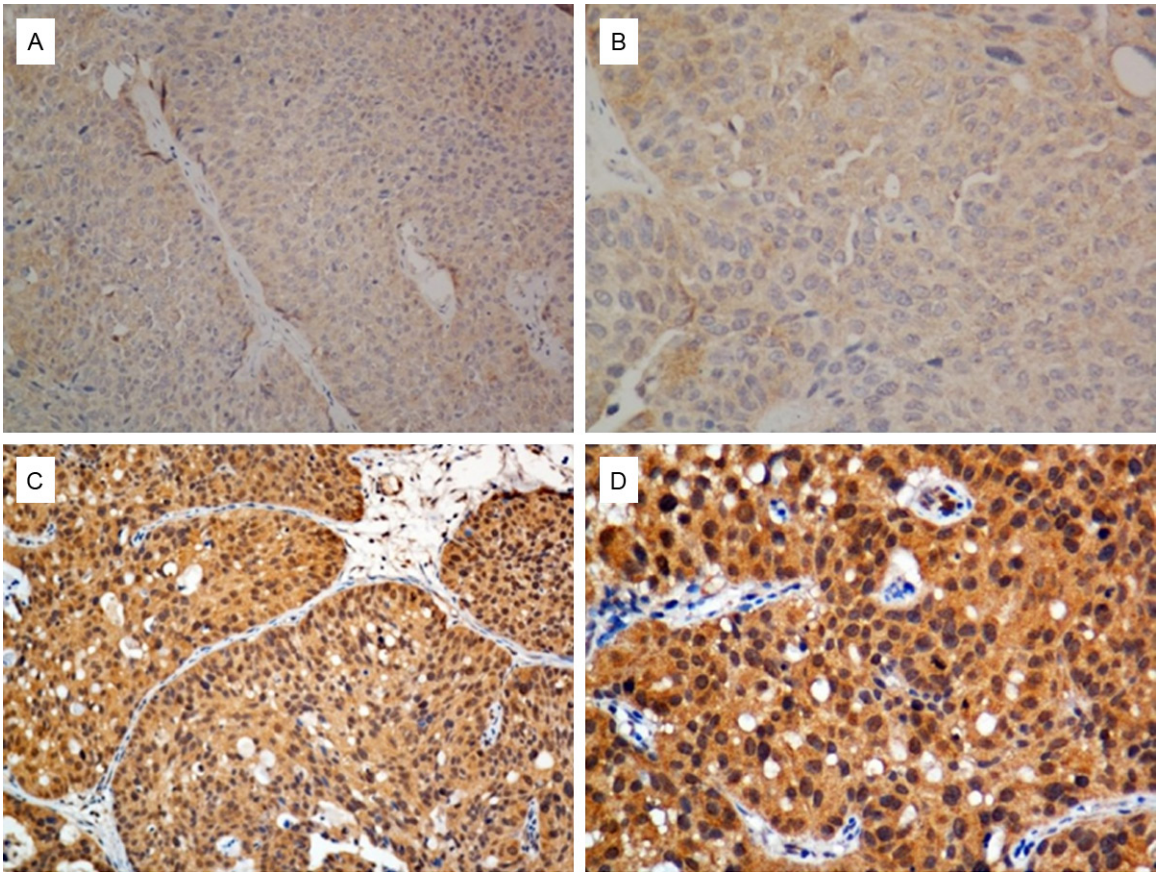

Figure 2. Representative immunostaining pictures of PTP4A3 in cervical cancer. A, B. Low expression of PTP4A3; C, D. High expression of PTP4A3 in cervical cancer tissues. A and C: 200×; B and D: 400×.

Table 1. Association between clinical parameters with PTP4A3

Features		Total	PTP4A3		*P*
			Low	High	
Age	≤45 y	90	35	55	0.978
	>45 y	54	27	27	
Tumor stage	IB1+IB2	95	49	46	0.004
	IIA1+IIA2	49	13	36	
Tumor size	≤4 cm	87	44	43	0.024
	>4 cm	57	18	39	
Differentiation	1/2	108	43	65	0.174
	3	36	19	17	
Histological type	SCC	89	36	53	0.422
	AC	55	26	29	
LN Metastasis	No	110	53	57	0.025
	Yes	34	9	25	
		144	62	82	

SCC: squamous cell cancer; AC: Adenocarcinoma.

genic process and progression of cervical carcinoma.

PTP4A3 belongs to a small class of PTPs, which are cell signaling molecules that contain a PTP domain and a characteristic C-terminal prenylation motif. Together with tyrosine kinases, PTPs play regulatory roles in a variety of important signaling molecules and cellular processes [13]. The expression of PTPs is strictly tissue specific, and most cells express 30% to 60% of all the PTPs, while neuronal and hematopoietic cells express higher number of PTPs in compared with other cell types [14]. Studies of this class of PTPs demonstrated that dysregulation of PTP activity is involved in various diseases, including cancer, and that prenylation of PTPs is correlated with cancer development and metastasis [15]. As a prenylated PTP, PTP4A3 participates in many fundamental physiological processes and emerges as potential biomarkers and therapeutic targets for various types of malignancy. Lian et al. demonstrated that PTP4A3 promotes tumor invasion, metastasis and cell adhesion by interacting with JAM2 in colon cancer [16]. Maacha et al. reported that

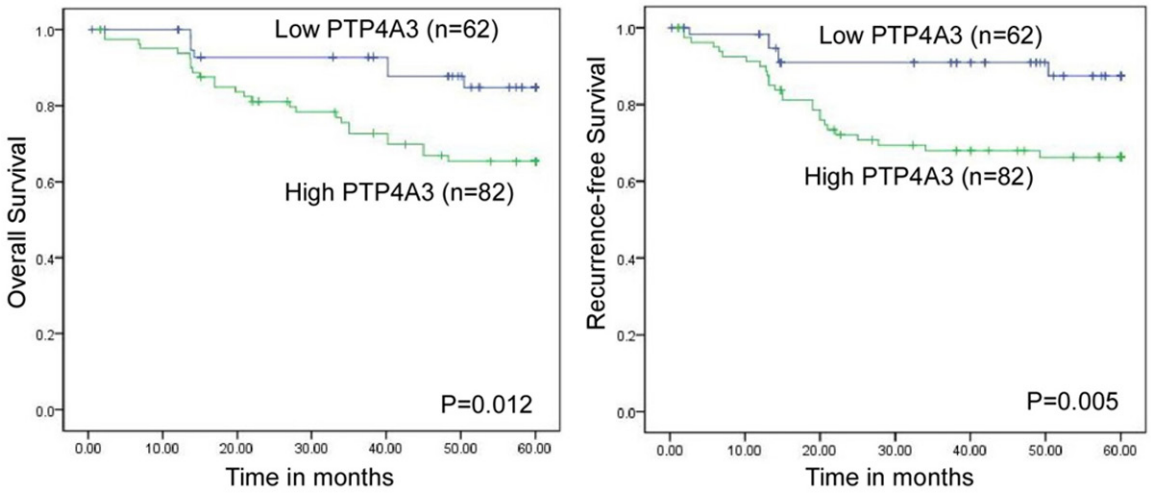

Figure 3. Kaplan-Meier curves for overall survival (OS) and recurrence-free survival (RFS) based on PTP4A3 expression in cervical cancer patients.

Table 2. Multivariate cox proportional hazards regression models for estimating OS and RFS

Prognostic variables	OS		RFS	
	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)	*P*
Age (>45 y vs ≤45 y)				
Tumor Stage (IIA vs IB)	4.483 (1.774-9.344)	0.015	3.181 (1.164-8.216)	0.034
Tumor size (>4 cm vs ≤4 cm)			2.106 (1.819-7.314)	0.026
Tumor grade (Grade 3 vs 1/2)				
Histological type (SCC vs AC)				
LN Metastasis (+ vs -)	2.724 (2.013-6.516)	0.045		
PTP4A3 expression (high vs low)	3.152 (2.278-7.674)	0.029		

PTP4A3 promotes human uveal melanoma aggressiveness through membrane accumulation of matrix metalloproteinase 14 (MMP14), while inhibition of MMP14 expression in uveal melanoma cells expressing PTP4A3 impairs their migration in vitro and invasiveness [17]. Liu et al. proposed that PTP4A3 plays a critical role in ovarian cancer tumorigenicity and maintaining the malignant phenotype of ovarian cancer, and could be used as a promising therapeutic target and potential early biomarker in ovarian cancer progression [18]. However, the roles of PTP4A3 in cervical cancer remain largely unknown. In our studies, PTP4A3 was highly expressed in cervical cancer tissues in comparison to that in adjacent normal cervix. We also found significantly positive correlations between PTP4A3 expression and tumor stage, tumor size, and lymph nodes status, indicating the specific involvement of PTP4A3 protein in cervical cancer development.

Several recent studies have demonstrated the PTP4A3 positive expression has a significant worse overall survival compared with those do not express. Yeh et al. showed that PTP4A3 overexpression was significantly associated with higher tumor stage, lymph nodal metastasis, vascular invasion and unfavorable prognosis in bladder cancer patients [19]. den Hollander et al. revealed that high expression of PTP4A3 could serve as an independent prognostic indicator for worse overall survival of patients with triple-negative breast cancer [20]. Xing et al. suggested that PTP4A3 may serve as a potential prognostic biomarker and an indicator of lymph node metastasis and vascular invasion in human gastric cancer [21]. Our results are consistent with some previous studies, which found that patients with overexpression of PTP4A3 have a significant worse overall and recurrent-free survival than that with low PTP4A3 expression. These facts may suggest

that PTP4A3 could serve as a prognostic factor for predicting poorer outcome.

In light of the evidence discussed here, we propose that overexpression of PTP4A3 may play crucial roles in cervical cancer development. In addition, our study introduces PTP4A3 protein expression as a new independent prognostic marker in cervical carcinomas. Our future studies will be aimed to more fully dissect the molecular mechanism underlying PTP4A3 promotion of cervical cancer cell growth, migration and progression.

Disclosure of conflict of interest

None.

Address correspondence to: Fubing Yu, Department of Gynecology, Dongguan Maternal & Children Health Care Hospital, Dongguan, China. Tel: +86 133-1668-0799; Fax: +86 769-2223-8375; E-mail: fubingyu0769@126.com

References

[1] Peiretti M, Zapardiel I, Zanagnolo V, Landoni F, Morrow CP and Maggioni A. Management of recurrent cervical cancer: a review of the literature. Surg Oncol 2012; 21: e59-66.

[2] Cerrotta A, Gardan G, Cavina R, Raspagliesi F, Stefanon B, Garassino I, Musumeci R, Tana S and De Palo G. Concurrent radiotherapy and weekly paclitaxel for locally advanced or recurrent squamous cell carcinoma of the uterine cervix. A pilot study with intensification of dose. Eur J Gynaecol Oncol 2002; 23: 115-119.

[3] Guzinska-Ustymowicz K and Pryczynicz A. PRL-3, an emerging marker of carcinogenesis, is strongly associated with poor prognosis. Anticancer Agents Med Chem 2011; 11: 99-108.

[4] Walls CD, Iliuk A, Bai Y, Wang M, Tao WA and Zhang ZY. Phosphatase of regenerating liver 3 (PRL3) provokes a tyrosine phosphoproteome to drive prometastatic signal transduction. Mol Cell Proteomics 2013; 12: 3759-3777.

[5] Zimmerman MW, McQueeney KE, Isenberg JS, Pitt BR, Wasserloos KA, Homanics GE and Lazo JS. Protein-tyrosine phosphatase 4A3 (PTP4A3) promotes vascular endothelial growth factor signaling and enables endothelial cell motility. J Biol Chem 2014; 289: 5904-5913.

[6] Peng L, Jin G, Wang L, Guo J, Meng L and Shou C. Identification of integrin alpha1 as an interacting protein of protein tyrosine phosphatase PRL-3. Biochem Biophys Res Commun 2006; 342: 179-183.

[7] Mollevi DG, Aytes A, Berdiel M, Padulles L, Martinez-Iniesta M, Sanjuan X, Salazar R and Villanueva A. PRL-3 overexpression in epithelial cells is induced by surrounding stromal fibroblasts. Mol Cancer 2009; 8: 46.

[8] Fiordalisi JJ, Dewar BJ, Graves LM, Madigan JP and Cox AD. Src-mediated phosphorylation of the tyrosine phosphatase PRL-3 is required for PRL-3 promotion of Rho activation, motility and invasion. PLoS One 2013; 8: e64309.

[9] Krndija D, Munzberg C, Maass U, Hafner M, Adler G, Kestler HA, Seufferlein T, Oswald F and von Wichert G. The phosphatase of regenerating liver 3 (PRL-3) promotes cell migration through Arf-activity-dependent stimulation of integrin alpha5 recycling. J Cell Sci 2012; 125: 3883-3892.

[10] Min G, Lee SK, Kim HN, Han YM, Lee RH, Jeong DG, Han DC and Kwon BM. Rhodanine-based PRL-3 inhibitors blocked the migration and invasion of metastatic cancer cells. Bioorg Med Chem Lett 2013; 23: 3769-3774.

[11] Cao Y, Tu Y, Mei J, Li Z, Jie Z, Xu S, Xu L, Wang S and Xiong Y. RNAimediated knockdown of PRL3 inhibits cell invasion and downregulates ERK1/2 expression in the human gastric cancer cell line, SGC7901. Mol Med Rep 2013; 7: 1805-1811.

[12] Bardelli A, Saha S, Sager JA, Romans KE, Xin B, Markowitz SD, Lengauer C, Velculescu VE, Kinzler KW and Vogelstein B. PRL-3 expression in metastatic cancers. Clin Cancer Res 2003; 9: 5607-5615.

[13] Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J and Mustelin T. Protein tyrosine phosphatases in the human genome. Cell 2004; 117: 699-711.

[14] Wang WQ, Sun JP and Zhang ZY. An overview of the protein tyrosine phosphatase superfamily. Curr Top Med Chem 2003; 3: 739-748.

[15] Ostman A, Hellberg C and Bohmer FD. Protein-tyrosine phosphatases and cancer. Nat Rev Cancer 2006; 6: 307-320.

[16] Lian S, Meng L, Xing X, Yang Y, Qu L and Shou C. PRL-3 promotes cell adhesion by interacting with JAM2 in colon cancer. Oncol Lett 2016; 12: 1661-1666.

[17] Maacha S, Anezo O, Foy M, Liot G, Mery L, Laurent C, Sastre-Garau X, Piperno-Neumann S, Cassoux N, Planque N and Saule S. Protein tyrosine phosphatase 4A3 (PTP4A3) promotes human uveal melanoma aggressiveness through membrane accumulation of matrix metalloproteinase 14 (MMP14). Invest Ophthalmol Vis Sci 2016; 57: 1982-1990.

[18] Liu H, Al-aidaroos AQ, Wang H, Guo K, Li J, Zhang HF and Zeng Q. PRL-3 suppresses c-Fos and integrin alpha2 expression in ovarian cancer cells. BMC Cancer 2013; 13: 80.

[19] Yeh HC, Huang CN, Li CC, Chang LL, Lin HH, Ke HL, Huang AM, Liang PI, Li CF and Wu WJ. Overexpression of PTP4A3 is associated with metastasis and unfavorable prognosis in bladder cancer. World J Urol 2016; 34: 835-846.

[20] den Hollander P, Rawls K, Tsimelzon A, Shepherd J, Mazumdar A, Hill J, Fuqua SA, Chang JC, Osborne CK, Hilsenbeck SG, Mills GB and Brown PH. Phosphatase PTP4A3 promotes triple-negative breast cancer growth and predicts poor patient survival. Cancer Res 2016; 76: 1942-1953.

[21] Xing X, Lian S, Hu Y, Li Z, Zhang L, Wen X, Du H, Jia Y, Zheng Z, Meng L, Shou C and Ji J. Phosphatase of regenerating liver-3 (PRL-3) is associated with metastasis and poor prognosis in gastric carcinoma. J Transl Med 2013; 11: 309.