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Original Article

Expression of *POSTN* mRNA is associated with osteosarcoma prognosis

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Abstract: Aim: This study aimed to investigate the effect of *POSTN* mRNA expression on osteosarcoma prognosis. Methods: The carcinoma and adjacent tissues were collected from 81 primary osteosarcoma patients. The expression level of *POSTN* mRNA in bone tissues was detected by real-time polymerase chain reaction (RT-PCR) method and the differences between two groups were compared. The relationships of *POSTN* mRNA expression with clinical indexes and the survival rate of osteosarcoma patients during follow-up were also analyzed. Results: The over-expression level of *POSTN* mRNA in carcinoma tissues was significantly higher than that in adjacent tissues ($P<0.05$). The clinical stages, soft tissue infiltration and preoperative metastasis of osteosarcoma patients were related to *POSTN* mRNA expression. Postoperative follow-up results displayed that with different *POSTN* mRNA expression levels, the survival time of patients was significantly different, and that the higher the *POSTN* mRNA over-expression was, the shorter the patients survived, with statistically significant differences ($P<0.05$). The clinical stages, soft tissue infiltration, preoperative metastasis and *POSTN* mRNA expression were proved by Cox regression analysis to be the prognosis-related factors of osteosarcoma ($P<0.05$). Conclusions: *POSTN* mRNA may be an important factor for osteosarcoma prognosis, which needs to be further investigated in terms of the protein level.

Keywords: Osteosarcoma, *POSTN*, RT-PCR, prognosis

Introduction

Osteosarcoma (OS), a common tumor of orthopedics, ranks first in primary malignant bone tumors [1]. Characterized by spindle neoplastic cells in osteoid tissues, osteosarcoma belongs to mesenchymal malignant tumors [2]. OS mainly occurs in adolescents aged 10~20 with metaphysis of limb bones as the primary position, and its annual incidence is 4~5 per 1 million people [3]. With high malignancy, strong invasion and high local recurrence rate as well as rapid progression, osteosarcoma severely hazards physical health of adolescents [4, 5]. Surgery assisted with neoadjuvant chemotherapy is the main clinical treatment of osteosarcoma. However, the 5-year survival rate of patients developing tumor metastasis after limb salvage surgery is only 20% or so.

New techniques for prognosis and treatment of osteosarcoma are continually improved along with the development of science and the deepening study on the pathogenesis of osteosarcoma. Gene prognosis and molecular targeted therapy based on the development of modern tumor molecular biology and molecular genetics have opened up a new way for prognosis and treatment of osteosarcoma [6-8]. Previous studies have indicated that the occurrence and development of osteosarcoma are closely correlated with the abnormal activation of protooncogenes or the abnormal inactivation of antioncogenes [9-11]. Therefore, it is very important for the prognosis of osteosarcoma to explore new marker genes.

In recent years, the expression of *POSTN* has been found by numerous studies to have significant differences between normal tissues and tumor tissues, which suggests that *POSTN* may be closely associated with the occurrence, development and prognosis of malignant tumors [12-14]. And for this reason, the present study detected the role of *POSTN* gene in diagnosis and identification of patients with osteosarcoma.

Table 1. Expression levels of *POSTN* in case group and control group

Item	Number	POSTN expression intensity			Positive rate	χ^2	*P*
		-	+	++			
Cases	81	15	27	39	81.48%	18.923	<0.01
Controls	81	36	26	19	55.6%		

Materials and methods

Objects of the study

81 osteosarcoma patients were selected into the case group from The First Affiliated Hospital of Chongqing Medical University during July, 2009 and July, 2012. They all diagnosed with primary osteosarcoma by pathological diagnosis, X ray and CT scan. The cases were aged 16~40 with an average age of 28.6±7.8, and did not get radiotherapy or chemotherapy before operation. The osteosarcoma tissue samples without inflammation or necrosis were collected from the primary origin during surgery, put into liquid nitrogen for quick-freeze and preserved at -80°C in ultra cold storage freezer.

The control group contained 12 benign osteochondroma patients from the same hospital during the same period. Specimen collection and operation were the same as the case group. The ethics committee of The First Affiliated Hospital of Chongqing Medical University gave permission to this study. The tissue collection was performed under the informed consent of all the subjects and complied with the provisions of ethics.

POSTN mRNA expression tested with RT-PCR

Total RNA extraction: appropriate-quality bone tissues, added with pro rata Trizol (Invitrogen company), was made into homogenate using homogenizer (OV5 type, Sino instrument Co., Ltd.). Then chloroform, isopropanol and ethanol were added into the homogenate following steps to extract the total RNA. RNA was examined its concentration and purity by ultraviolet spectroscopy method and its integrity by electrophoresis in 1% agarose gel (containing EB).

Synthesis of cDNA by reverse transcription

5 μg of total RNA was added with 0.5 μg Oligo (dT), undergone degeneration at 75°C for 5 min and added to 12.5 μl with DEPC water. Then the operation was as follows: ice-bath for 30 s, adding 40 U RNA Inhibitor, 10 nmol dNTP Mix and First-Strand Buffer, incubation at 42°C for 5 min, adding 200 U SuperScript™II and reaction for 50 min. Finally the reaction was finished after enzyme deactivation at 70°C for 15 min.

RT-PCR

The primers and *Taq* Man probes were designed and synthesized by Shanghai Sangon Biotech Co., Ltd.. The forward and reverse primer sequences of *POSTN* gene were 5'-AATGGAAGGAATGAAAGGCTG-3' and 5'-CCTCGATeTceTeecTeAGT-3' respectively. The *Taq*Man probe was 5'-(FAM)-AGCAGTTTTGCCCATTGACCATGTT (TAMRA)-3'. Glyceraldehyde 3 phosphate dehydrogenase (*GAPDH*) was the reference gene, and its forward and reverse primer sequences were 5'-GAAGGTGAAGGTCGGAGTC-3' and 5'-GAAGATGGTGATGGGATTTC-3' respectively, and its *Taq*Man probe was 5'-(FAM)-CAAGCTTCCCGTTCTCAGCC(TAMRA)-3'.

The RT-PCR solution contained 5.0 μL cDNA, 0.75 μL forward primer, 0.75 μL reverse primer, 12.5 μL SYBGREEN Master Mix (Roche Company) and 6.0 μL DEPC water. The PCR conditions were: initial denaturation at 95°C for 10 min; followed by 45 cycles of degeneration at 95°C for 45 s, annealing at 65°C for 1 min and extension at 72°C for 1 min; at last extension at 72°C for 10 min. Represented by the expression level ratio of *POSTN* to corresponding *GAPDH*, the relative expression levels of *POSTN* mRNA from 0 to the maximum were averagely divided into three equal parts, namely negative (-), weak positive (+) and strong positive (++). Weak and strong positive were collectively referred to as positive. Positive rate = (Weak positive cases + Positive cases)/Total cases ×100%.

Statistical methods

All statistical tests were performed by SPSS-18.0 software. Data comparison between groups was conducted by χ^2 test. Survival data were analyzed by Kaplan-Meier method. The Cox regression model was used for analysis of prognosis-related factors. Statistical significance existed when $P<0.05$.

Table 2. Correlation of *POSTN* mRNA expression levels of osteosarcoma patients with clinical indexes

Item	Cases (n=81)	POSTN expression		Overexpression rate	χ^2	*P*
		Low (n=15)	Over (n=66)			
Gender						
Male	42	9	33	78.57%	0.490	0.573
Female	39	6	33	84.62%		
Age (year)						
≤25	33	4	29	87.87%	1.510	0.258
>25	48	11	37	77.08%		
Tumor size (cm)						
≤3	36	7	29	80.56%	0.037	1.000
>3	45	8	37	82.22%		
Clinical stage						
I+II	30	10	20	66.67%	6.930	0.016*
III	51	5	46	90.20%		
Invasion site						
Distal femur	18	3	16	88.89%	0.087	0.668
Proximal tibia	33	5	28	84.85%		
Other sites	30	7	23	76.67%		
Soft tissue infiltration						
Yes	48	5	43	89.58%	5.125	0.040*
No	33	10	23	69.70%		
Preoperative metastasis						
Yes	33	2	31	93.94%	5.728	0.020*
No	48	13	35	72.92%		

*stands for statistical significance.

Table 3. Association of *POSTN* mRNA expression with survival time of osteosarcoma patients

Duration of follow-up/month	Initial cases	Death cases during follow-up	Cases lost in follow-up
0~12	81	14	1
13~24	66	9	1
25~36	56	8	2
37~48	46	8	3
49~60	35	2	4

Results

Comparison of POSTN mRNA expression levels

The *POSTN* mRNA expression levels of the two groups are shown in **Table 1**. There were 15 negative cases, 27 weak positive cases and 39 strong positive cases in 81 osteosarcoma cases with positive rate of 81.48%. The control group had 36 negative cases, 26 weak positive cases and 19 strong positive cases, and its positive rate was 55.6%. The result showed that the *POSTN* expression levels and positive rate in case group were higher than those in control group, and that the differences between two groups had statistical significance (χ^2 = 18.923, *P*< 0.01).

Correlation of POSTN mRNA expression levels with clinical indexes of osteosarcoma

Further analysis of the relationship of *POSTN* mRNA expression levels with the clinical indexes of the cases manifested that the differences of *POSTN* expression levels in age, gender, tumor size and invasion site of cases had no statistical significance (*P*>0.05).

According to the malignant degree, osteosarcoma was divided into 3 levels in this study: Level I and II were in the low-grade osteosarcoma group while Level III in the high-grade group. The *POSTN* expression positive rate of the above two groups was 66.67% and 90.20% respectively and this difference was statistically significant (*P*<0.05). The soft tissue infiltration and preoperative metastasis analyses of osteosarcoma patients with different *POSTN* mRNA expression levels proved that the positive rate of *POSTN* mRNA expression was significantly higher in osteosarcoma patients with soft tissue infiltration than those without infiltration (89.58% vs. 69.70%) and statistical significance existed in the difference (*P*<0.05); the positive rate in patients with preoperative metastasis (93.94%) was obviously higher than that in patients without metastasis (72.92%), and the difference was statistically significant (*P*<0.05). The results are listed in **Table 2**.

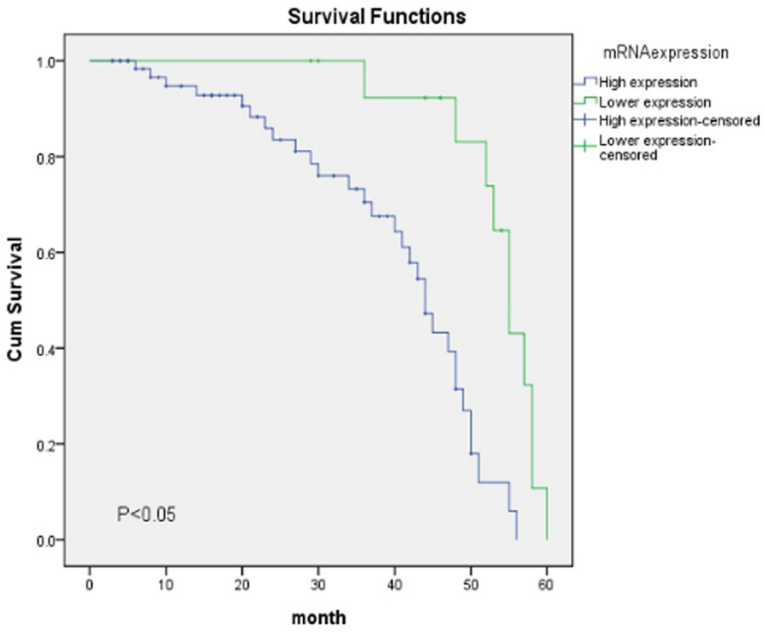

Figure 1. It showed the relationship between *POSTN* mRNA expression levels and survival time of osteosarcoma patients.

Table 4. Cox regression multivariate analysis of prognosis of 81 osteosarcoma patients

Factor	*P*	OR (95% CI)
Clinical stages	0.034	2.103 (1.060-4.175)
Soft tissue infiltration	0.042	2.224 (1.029-4.808)
Preoperative metastasis	0.010	3.040 (1.307-7.072)
POSTN mRNA overexpression	0.000	6.478 (2.463-17.039)

Relationship between POSTN mRNA expression levels and survival time of patients

Table 3 shows the relationship between *POSTN* mRNA expression levels and the follow-up results. In the 60-month follow-up, there were 41 death cases and 11 missing cases among a total of 81 osteosarcoma subjects. During the follow-up, the survival time of patients with low *POSTN* mRNA expression levels was remarkably longer than that of patients with high levels and the statistically significant difference in survival time was observed ($P<0.05$) (**Figure 1**).

Cox regression analysis

Cox regression model was used to investigate osteosarcoma-related factors, such as gender, age, tumor size and position, clinical stages, soft tissue invasion and preoperative metastasis. We found that the prognosis of osteosarcoma was related to clinical stages, soft tissue infiltration, preoperative metastasis and *POSTN* mRNA expressions. The results can be found in **Table 4**.

Discussion

POSTN gene located on human chromosome 13q13.3 is about 36 kb long and encodes periostin (PN) of 836 amino acids [15]. As a member of fasciclin FASI superfamily, POSTN is also known as osteoblast-specific factor 2 (OSF-2) [16]. Periostin, specifically secreted by interstitial cells, mainly distributes in extracellular matrix. It works together with many extracellular matrix proteins such as type W collagen, fibronectin and type I collagen to maintain the stability of the extracellular matrix [17]. Moreover, periostin takes part in the recruitment, adhesion and migration of cells, and regulates cell proliferation, differentiation and apoptosis through multiple signal transduction pathways, which is closely correlated with the occurrence of many tumors [18, 19].

POSTN gene and its encoded periostin have been proved by multiple researches to be closely related to the occurrence, development and metastasis of a variety of malignant tumors in clinic [20, 21]. Sasaki H pointed out that the serum periostin levels of patients of non-small cell lung cancer and thymic carcinoma were significantly high and had much to do with the development and prognosis of tumors. In addition, the prognosis was worse in patients with high *POSTN* mRNA expression levels than those with low expression levels [22, 23]. Shao et al. confirmed the positive expression of periostins in breast carcinoma was correlated with low degree of tumor cell differentiation, malignant tumor progression and the prognosis of patients [24]. Similar results were obtained on head and neck cancer and on pancreas intraductal papillary mucinous tumor [25, 26]. At present, no research has been reported about the association between *POSTN* mRNA expres-

sion and osteosarcoma, therefore, the present study chose to discuss this association.

The present study found that the expression level of *POSTN* mRNA was low in control group yet high in case group. The overexpression rate of *POSTN* mRNA in case group was obviously higher than that in control group (χ^2 = 18.923, P<0.01). The clinical data of osteosarcoma patients, such as age, gender, tumor size and invasion site, had no significant correlation with *POSTN* mRNA expression levels. As for tumor grade, the positive rate of *POSTN* mRNA expression was remarkably higher in high-grade osteosarcoma group than in low-grade group (89.58% vs. 69.7%). Additionally, among patients with soft tissue infiltration or preoperative metastasis, the positive expression rate of *POSTN* mRNA was comparatively higher, and the differences had statistical significance (P<0.05). The follow-up results demonstrated the survival rate was higher in patients with low expression levels than those with high levels. With different *POSTN* mRNA expression levels, patients had notably different survival times. Moreover, the higher the *POSTN* mRNA overexpression was, the shorter the patients survived, and the differences were statistically significant (P<0.05). In addition to *POSTN* mRNA expression levels, the clinical stages, soft tissue infiltration and preoperative metastasis were also served as the indicators of osteosarcoma prognosis shown by Cox regression multivariate analysis.

To sum up, *POSTN* gene may have great influence on the occurrence, development and prognosis of osteosarcoma. However, functional proteins play a direct role in living organisms, and genes are likely to change during the transcription process. Thus, the association between *POSTN* gene and osteosarcoma needs to be further investigated in terms of the protein level.

Disclosure of conflict of interest

None.

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References

[1] Song JG. [Current treatment of primary and metastatic osteosarcoma]. Zhonghua Zhong Liu Za Zhi 2012; 34: 881-884.

[2] Papagelopoulos PJ, Galanis EC, Vlastou C, Nikiforidis PA, Vlamis JA, Boscainos PJ, Fragiadakis EG, Stamos KG, Pantazopoulos T and Sim FH. Current concepts in the evaluation and treatment of osteosarcoma. Orthopedics 2000; 23: 858-867; quiz 868-859.

[3] Qiu Z and Liao Q. [Progress of osteosarcoma therapy]. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2010; 24: 1469-1475.

[4] Zhang FY, Tang W, Zhang ZZ, Huang JC, Zhang SX and Zhao XC. Systematic review of high-dose and standard-dose chemotherapies in the treatment of primary well-differentiated osteosarcoma. Tumour Biol 2014; 35: 10419-10427.

[5] Geetha N, Kumar A, Ramachandran K, Abraham E and Joseph F. Osteosarcoma of the sella. Australas Radiol 1999; 43: 517-519.

[6] Herold T, Metzeler KH, Vosberg S, Hartmann L, Rollig C, Stolzel F, Schneider S, Hubmann M, Zellmeier E, Ksienzyk B, Jurinovic V, Pasalic Z, Kakadia PM, Dufour A, Graf A, Krebs S, Blum H, Sauerland MC, Buchner T, Berdel WE, Woermann BJ, Bornhauser M, Ehninger G, Mansmann U, Hiddemann W, Bohlander SK, Spiekermann K and Greif PA. Isolated trisomy 13 defines a homogeneous AML subgroup with high frequency of mutations in spliceosome genes and poor prognosis. Blood 2014; 124: 1304-1311.

[7] Vidula N and Rugo HS. Translating the molecular message of triple-negative breast cancer into targeted therapy. Clin Cancer Res 2015; 21: 1511-1513.

[8] Kim KH and Park KK. Small RNA- and DNA-based gene therapy for the treatment of liver cirrhosis, where we are? World J Gastroenterol 2014; 20: 14696-14705.

[9] Somers GR, Ho M, Zielenska M, Squire JA and Thorner PS. HER2 amplification and overexpression is not present in pediatric osteosarcoma: a tissue microarray study. Pediatr Dev Pathol 2005; 8: 525-532.

[10] Yi C, Li X, Xu W and Chen A. Relationship between the expression of MTA-1 gene and the metastasis and invasion in human osteosarcoma. J Huazhong Univ Sci Technolog Med Sci 2005; 25: 445-447.

[11] Martin JW, Chilton-MacNeill S, Koti M, van Wijnen AJ, Squire JA and Zielenska M. Digital expression profiling identifies RUNX2, CDC5L, MDM2, RECQL4, and CDK4 as potential predictive biomarkers for neo-adjuvant chemotherapy response in paediatric osteosarcoma. PLoS One 2014; 9: e95843.

[12] Karlan BY, Dering J, Walsh C, Orsulic S, Lester J, Anderson LA, Ginther CL, Fejzo M and Slamon D. POSTN/TGFBI-associated stromal

signature predicts poor prognosis in serous epithelial ovarian cancer. Gynecol Oncol 2014; 132: 334-342.

[13] Forsti A, Jin Q, Altieri A, Johansson R, Wagner K, Enquist K, Grzybowska E, Pamula J, Pekala W, Hallmans G, Lenner P and Hemminki K. Polymorphisms in the KDR and POSTN genes: association with breast cancer susceptibility and prognosis. Breast Cancer Res Treat 2007; 101: 83-93.

[14] Tian B, Zhang Y and Zhang J. Periostin is a new potential prognostic biomarker for glioma. Tumour Biol 2014; 35: 5877-5883.

[15] Hong LZ, Wei XW, Chen JF and Shi Y. Overexpression of periostin predicts poor prognosis in non-small cell lung cancer. Oncol Lett 2013; 6: 1595-1603.

[16] Nuzzo PV, Buzzatti G, Ricci F, Rubagotti A, Argellati F, Zinoli L and Boccardo F. Periostin: a novel prognostic and therapeutic target for genitourinary cancer? Clin Genitourin Cancer 2014; 12: 301-311.

[17] Snider P, Hinton RB, Moreno-Rodriguez RA, Wang J, Rogers R, Lindsley A, Li F, Ingram DA, Menick D, Field L, Firulli AB, Molkentin JD, Markwald R and Conway SJ. Periostin is required for maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. Circ Res 2008; 102: 752-760.

[18] Wong GS, Habibollahi P, Heidari P, Lee JS, Klein-Szanto AJ, Waldron TJ, Gimotty P, Nakagawa H, Taylor PR, Wang TC, Mahmood U and Rustgi AK. Optical imaging of periostin enables early endoscopic detection and characterization of esophageal cancer in mice. Gastroenterology 2013; 144: 294-297.

[19] Lv Y, Wang W, Jia WD, Sun QK, Huang M, Zhou HC, Xia HH, Liu WB, Chen H, Sun SN and Xu GL. High preoparative levels of serum periostin are associated with poor prognosis in patients with hepatocellular carcinoma after hepatectomy. Eur J Surg Oncol 2013; 39: 1129-1135.

[20] Li M, Li C, Li D, Xie Y, Shi J, Li G, Guan Y, Zhang P, Peng F, Xiao Z and Chen Z. Periostin, a stroma-associated protein, correlates with tumor invasiveness and progression in nasopharyngeal carcinoma. Clin Exp Metastasis 2012; 29: 865-877.

[21] Wang Z and Ouyang G. Periostin: a bridge between cancer stem cells and their metastatic niche. Cell Stem Cell 2012; 10: 111-112.

[22] Sasaki H, Dai M, Auclair D, Fukai I, Kiriyama M, Yamakawa Y, Fujii Y and Chen LB. Serum level of the periostin, a homologue of an insect cell adhesion molecule, as a prognostic marker in nonsmall cell lung carcinomas. Cancer 2001; 92: 843-848.

[23] Sasaki H, Dai M, Auclair D, Kaji M, Fukai I, Kiriyama M, Yamakawa Y, Fujii Y and Chen LB. Serum level of the periostin, a homologue of an insect cell adhesion molecule, in thymoma patients. Cancer Lett 2001; 172: 37-42.

[24] Shao R, Bao S, Bai X, Blanchette C, Anderson RM, Dang T, Gishizky ML, Marks JR and Wang XF. Acquired expression of periostin by human breast cancers promotes tumor angiogenesis through up-regulation of vascular endothelial growth factor receptor 2 expression. Mol Cell Biol 2004; 24: 3992-4003.

[25] Kudo Y, Ogawa I, Kitajima S, Kitagawa M, Kawai H, Gaffney PM, Miyauchi M and Takata T. Periostin promotes invasion and anchorage-independent growth in the metastatic process of head and neck cancer. Cancer Res 2006; 66: 6928-6935.

[26] Fukushima N, Kikuchi Y, Nishiyama T, Kudo A and Fukayama M. Periostin deposition in the stroma of invasive and intraductal neoplasms of the pancreas. Mod Pathol 2008; 21: 1044-1053.