Int J Clin Exp Pathol 2016;9(2):2147-2152
www.ijcep.com /ISSN:1936-2625/IJCEP0018181

Original Article

Relationship between RECK/matrix metalloproteinase and recurrence/metastasis of giant cell tumor of spine

Zhanpeng Luo[1,2*], Yi Yang[3*], Liang Wang[2], Xiaobo Luo[2], Yuanzheng Ma[1,2]

[1]Southern Medial University, Guangzhou 510515, Guangdong, China; [2]Department of Orthopedics, 309 Hospital of PLA, Beijing 100091, China; [3]Department of Dermatology, The General Hospital of PLA, Beijing 100036, China. [*]Equal contributors.

Received October 20, 2015; Accepted November 28, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: Giant cell tumor of spine (GCTS) is one common benign tumor with high recurrence rate after surgery. The finding of novel index for predicting recurrence is thus of critical importance. Matrix metalloproteinase (MMP) can work as prognostic indexes for various tumors, and is shown to be negatively related with RECK gene expression. This study thus examined the levels of MMP-2, MMP-9 and RECK in GCTS patients, in order to elucidate the correlation between gene expression and recurrent tumors. Tumor tissues were collected to examine the expressional profiles of MMP-2, MMP-9 and RECK genes, along with post-operative follow-ups on all patients. The correlation between gene expression and clinical features was analyzed by SPSS 13.0 software. There was a significant correlation between Enneking stage or Campanacci grade with the expression of MMP-2, MMP-9 and RECK genes ($P<0.05$). Those genes were unrelated with patients' age or sex ($P>0.05$). The recurrence of tumor is correlated with expression of RECK, MMP-2 and MMP-9 in tumor tissues ($P<0.05$). Using Logistic regression, enhanced RECK expression can decrease the risk of GCTS recurrence while MMP-9 up-regulation increased the risk of recurrence ($P<0.05$). No significant correlation existed between MMP-2 and recurrence. RECK/MMP-9 expressions are closely correlated with the recurrence of GCTS, and can works as important indexes predicting tumor prognosis.

Keywords: Giant cell tumor of bone, Matrix metalloproteinase-2, matrix metalloproteinase-9, RECK

Introduction

Giant cell tumor of bone (GCTB), also named as osteoclastoma, is one common primary benign tumor in bone tissues, occupying 4%~5% of all primary bone tumors. Due to its high recurrence [1] and invasive nature [2], and certain pulmonary metastasis, GCTB is believed to be one potential malignant tumor. GCTB is mostly common in females between 20~45 years old, with higher incidence in Asian people compared to Western population [3]. GCTB is mostly occurred in epiphysis of upper and lower tibia, and frequently leads to pathological bone fracture due to lytic bone lesion. Giant cell tumor of spine (GCTS) occupies about 3%~6% of total cases of GCTB [4]. Due to its special location of occurrence, GCTS may cause dysfunctions to certain extents following compression of spinal cord or nerves, or even causing paralysis. Surgical resection is the major approach in treating GCTS, but having a post-operative rate as high as 30%~50% [3]. Therefore the prediction of recurrence after GCTS surgery is of critical importance.

Matrix metalloproteinases (MMPs) is a family of zinc ion-dependent endonuclease family with highly conserved sequence. They are able to degrade most proteins within basal membrane and extracellular matrix (ECM). MMPs, including gelatinase, collagenase and matrilysin, are produced in the form of inactive zymogen, and need to be activated for exerting proteinase activity. Certain tumor-inhibitory gene fragments exert their functions by interacting with MMPs [5]. MMPs thus play a crucial role in the invasion and migration of tumors. As one important membrane of MMPs family, MMP-2 is also called gelatinase A, and is secreted by various cells to degrade multiple ECM. In a cascade reaction initiated by MMPs, MMP-2 is key

enzyme in the activation. Tumor cells can potentiate their invasion and metastatic function via over-expressing MMP-2, which, therefore, can work as prognostic factor for multiple tumors including breast cancer [6, 7], endometrium carcinoma [8] and oral squamous cell carcinoma [9] Previous study has indicated the expression of MMP-2 in basal cells of GCTB [10]. Microarray analysis also revealed elevated MMP-2 expression in GCTB patients [11]. On the other hand, gelatinase B (MMP-9) is also related with multiple tumors including colorectal cancer [12] and gastric carcinoma [13]. Other studies have also suggested potent expression of MMP-9 in GCTB tissues and the relation with tumor invasion/metastasis [14, 15]. Therefore, MMP-2 and MMP-9 may be both related with recurrence and/or metastasis of GCTB.

Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) were firstly identified in 1998 for inhibiting MMPs expression and activity. RECK expression level is negatively correlated with that in MMPs, and can inhibit tumor's invasion and metastasis [5, 16, 17]. We thus hypothesized that RECK gene expression was correlated with occurrence/progression of RECK. We thus investigated the expression profiles of RECK, MMP-2 and MMP9 on GCTs patients, in order to illustrate its correlation with GCTS recurrence, and to guide the clinical treatment by establishing more sensitive indexes, as well as lowering GCTS recurrent rate.

Materials and methods

Patients

A cohort of 42 GCTS patients (19 males and 23 females, aging between 13 and 66 years old) from the department of orthopedics from January 2004 to December 2009 were recruited in The 309th Hospital of Chinese PLA. Inclusive criteria: (1) With confirmed GCTS diagnosis; (2) Primary tumor patients; (3) No metastasis at the primary diagnosis; (4) Having undergone surgical resection; (5) Full medical history; and (6) Available post-operative samples for immunohistochemical (IHC) examinations.

In an Enneking staging system, there were 10 stage I, 13 of stage II and 19 of stage III patients. By Campanacci grading under X-ray, there were 11 of grade I, 14 patients at grade II, and 17 of grade III patients. This study has been pre-approved by the ethical committee of The 309th Hospital of Chinese PLA and has obtained consents from all participants. With permission, tumor tissues were collected during the surgery.

Post-operative follow-ups

Follow-ups persisted for 12~60 months until the endpoint or patient death. All cases have successfully finished the follow-up. There were 17 cases of recurrence, 2 patients having pulmonary metastasis, and 23 patients without recurrence.

IHC staining

All tumor samples were fixed in 10% formalin and were embedded in paraffin. After sectioning into consecutive slices with 4 μm thickness, IHC staining was performed using SP method following manual instruction of test kits including MMP-2, MMP-9 and SP (Maixin, Fuzhou, China). Parallel negative controls were also performed using PBS instead. Positive staining was determined as brown-yellow granules in the cytoplasm. Five high-magnification fields were randomly selected to count 100 cells. The staining grade was measured by both percentage score and intensity score as previously documented [18]. Percentage score was scaled as 1, 2 or 3 for those fields with less than 10%, 10%~50% and more than 50% of positive cells, respectively. Staining intensity score was given from 0 to 3 including negative staining, light yellow, moderate, and dark-brown color. The total score (= percentage score + staining intensity score) was divided as negative (equal or less than 4) or positive (larger than 4).

Statistical analysis

SPSS 13.0 software was used to process all collected data. Enumeration data were compared by chi-square test. Logistic regression analysis was used for multi-factor. The significance level was determined as 0.05.

Results

Gene expression and clinical features

IHC staining images of RECK, MMP-2 and MMP-9 genes were shown in **Figure 1**. Among

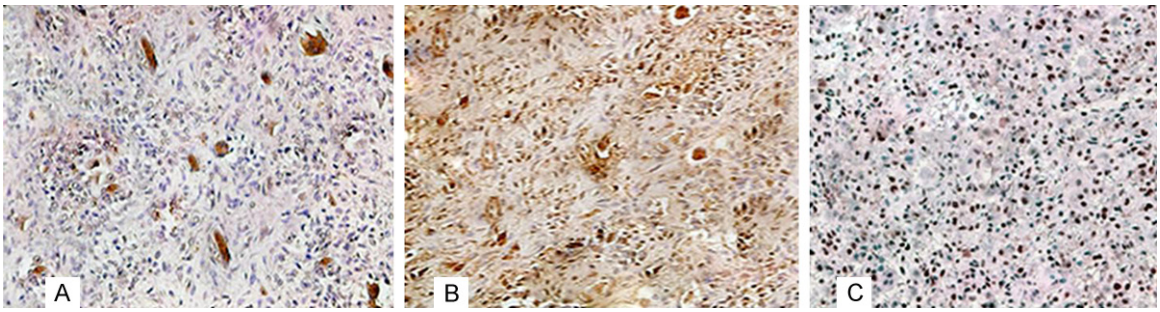

Figure 1. IHC staining image of MMP-2 (left), MMP-9 (middle) and RECK (right).

Table 1. Clinical features and expression levels of RECK, MMP-2 and MMP-9 gene

Clinical index	N	RECK Positive	*P* value	MMP-2 Positive	*P* value	MMP-9 Positive	*P* value
Sex							
Male	19	10	0.55	10	0.55	13	0.11
Female	23	10		10		10	
Age							
<30 years	19	7	0.37	10	0.55	11	0.71
≥30 years	23	13		10		12	
Enneking stage							
Stage I & II	23	15	0.01*	7	0.01*	8	<0.01*
Stage III	19	5		13		15	
Campanacci grade							
Grade I & II	25	16	<0.01*	8	0.01*	10	0.02*
Grade III	17	4		12		13	

Note: *, P<0.05.

Table 2. Clinical features and GCTS recurrence/metastasis

Clinical index	N	No recurrent group	Recurrence-metastasis group	χ^2 value	*P* value
Sex					
Male	19	9	10	0.77	0.38
Female	23	14	9		
Age					
<30 years	19	10	9	0.06	0.80
≥30 years	23	13	10		
Enneking stage					
Stage I & II	23	17	6	7.53	<0.01*
Stage III	19	6	13		
Campanacci grade					
Grade I & II	25	17	8	4.37	0.04*
Grade III	17	6	11		

Note: *, P<0.05.

all 42 patients, there were 20 positive cases for RECK (47.62%), 20 MMP-2 positive cases (47.62%) and 23 patients with MMP-9 positive expression (54.76%). Chi-square revealed no significant relation between patients' age and sex with gene expression level. Enneking stage and Campanacci grade, however, is significantly correlated with gene expression levels. (P<0.05, **Table 1**).

GCTS recurrence and clinical features

In all 42 patients undergone follow-ups, there were 17 recurrent cases and 2 cases of pulmonary metastasis, both of which were classified in recurrent group (N=19). Chi-square analysis revealed no significant relation between patients' age and sex with recurrence/metastasis. Enneking

Table 3. Gene expression and GCTS recurrence/metastasis

	N	No recurrent group	Recurrence-metastasis group	χ^2 value	*P* value
RECK					
Positive	20	5	15	6.31	0.01
Negative	22	14	8		
MMP-2					
Positive	20	13	7	6.02	0.01
Negative	22	6	16		
MMP-9					
Positive	23	15	8	8.19	<0.01
Negative	19	4	15		

Table 4. Multi-variate analysis of GCTS recurrence

	OR	95% CI	*P* value
RECK	0.17	0.04~0.83	0.028*
MMP-2	4.05	0.97~16.91	0.055
MMP-9	5.19	1.12~24.04	0.035*

Note: *, P<0.05.

stage and Campanacci grade, however, is significantly correlated with recurrence/metastasis. (P<0.05, **Table 2**).

Gene expression and GCTS recurrence/metastasis

Within all 20 patients with RECK-positive expression, there were 3 cases of recurrence or metastasis. Such ratio was 14/20 for MMP-2 and 16/23 for MMP-9. Chi-square analysis revealed the correlation between GCTS recurrence/metastasis with expression of RECK, MMP-2 or MMP-9 gene expression (P<0.05, **Table 3**).

Multi-factor analysis of GCTS recurrence/metastasis

To rule out possible interfering effects on GCTS recurrence, we employed Logistic regression analysis using sex, age, Enneking stage, Campanacci grade, and expressions of RECK, MMP-2 and MMP-9 as independent variables. Results showed the decreased risk of GCTS recurrence/metastasis by up-regulating RECK, which thus plays as one protective factor (OR=0.17, P=0.028, **Table 4**). MMP-9 overexpression, however, elevated risk of recurrence (OR=5.19, P=0.035, **Table 4**). No significant correlation existed between MMP-2 and GCTS recurrence (OR=4.05, P=0.055, **Table 4**).

Discussion

Surgical resection is still the major approach for treating GCTB, whose major postoperative complication is recurrence [1]. GCTB can be classified as grade I to grade III based on Jaffe system according to the ratio of mononuclear mesenchymal cells against osteoclast cells, and heteromorphism of mononuclear mesenchymal cells [19]. Clinical follow-ups, however, revealed the inability of Jaffe grade system to predict the recurrence of GCTB after surgery. Campanacci further classified GCTB into three grades based on X-ray change [20]. Some studies have higher recurrence rate of GCTB in those patients with advanced Campanacci grades [21, 22]. This study obtained similar results as Campanacci grade III patients had higher recurrent rate compared to grade I and II patients. Some studies, however, rejected the significant correlation between Campanacci grade and GCTB recurrence [23, 24]. This issue thus requires more systematic illustration. Enneking also classified GCTB into three stages based on clinical features, X-ray and pathology [25]. Some scholars suggested elevated risks of pulmonary metastasis in those GCTB patients at stage III [26], a result that occurred also in our study, suggesting the close correlation between Enneking stage and GCTB prognosis.

Current study about MMPs and GCTB mainly focused on the correlation between MMPs expression and GCTB occurrence, but lacked the potency of MMPs as prognostic indicators. This study thus performed IHC staining on GCTS tumor tissues for quantifying MMP-2 and MMP-9 expression, in parallel with follow-ups to elucidate patients' prognosis. Results showed MMP-9 but not MMP-2 as one risk factor for GCTS recurrence. In addition, RECK gene expression was also negatively correlated with MMPs expression. We thus tested the expression of RECK in tumor tissues and found RECK gene as one protective factor preventing GCTS

recurrence or metastasis. Previous report has mentioned higher MMP-9 expression in recurrent GCTB tissues [15], indicating the correlation between MMP-9 overexpression and GCTB recurrence. One recent study has also suggested the close correlation between MMP-9 and GCTB prognosis [27], in addition to the involvement of MMP-2 in GCTB recurrence. This is inconsistent with our results perhaps due to the including of malignancy transformation and different population selected in two studies. No direct study regarding RECK and GCTB has been reported. RECK, although RECK has been suggested to be involved in the progression of other tumors. For example, RECK can inhibit the invasion/metastasis of neuroblastoma and hepatoblastoma via inhibiting MMPs expression [28]. Other study has revealed the modulation on tumor progression by RECK via modulating the expression of vascular endothelial growth factor to regulate tumor angiogenesis, making RECK as one important prognostic indicator for tumors [29].

In summary, this study for the first time revealed the protective function of RECK against GCTS recurrence or metastasis, in addition to the correlation between MMP-9 and GCTS recurrence. Our results provide new insights regarding the evaluation of GCTS prognosis. However, due to the limited sample size, the promotion of RECK and MMP-9 as effective clinical indexes for GCTS needs further validation by multi-centered, large sample study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yuanzheng Ma, Department of Orthopedics, 309 Hospital of Chinese PLA, 17 Montenegro Hu Lu, Haidian Distric, Beijing 100091, China. Tel: +86-10-66775961; Fax: +86-10-66775961; E-mail: myuanzhengl@sina.com

References

[1] Bergovec M, Petković M, Smerdelj M, Seiwerth S, Brkić L, Robert K, Orlić D. [Giant cell tumorof bone: results and treatment complications]. Acta Med Croatica 2014; 68: 405-10.

[2] Balke M, Schremper L, Gebert C, Ahrens H, Streitbuerger A, Koehler G, Hardes J, Gosheger G. Giant cell tumor of bone: treatment and outcome of 214 cases. J Cancer Res Clin Oncol 2008; 134: 969-78.

[3] Sung HW, Kuo DP, Shu WP, Chai YB, Liu CC, Li SM. Giant-cell tumor of bone: analysis of two hundred and eight cases in Chinese patients. J Bone Joint Surg Am 1982; 64: 755-61.

[4] Suit H and Spiro I. Radiation treatment of benign mesenchymal disease. Semin Radiat Oncol 1999; 9: 171-8.

[5] Takahashi C, Sheng Z, Horan TP, Kitayama H, Maki M, Hitomi K, Kitaura Y, Takai S, Sasahara RM, Horimoto A, Ikawa Y, Ratzkin BJ, Arakawa T, Noda M. Regulation of matrix metalloproteinase-9 and inhibition of tumor invasion by the membrane-anchored glycoprotein RECK. Proc Natl Acad Sci U S A 1998; 95: 13221-6.

[6] Sivula A, Talvensaari-Mattila A, Lundin J, Joensuu H, Haglund C, Ristimäki A, Turpeenniemi-Hujanen T. Association of cyclooxygenase-2 and matrix metalloproteinase-2 expression in human breast cancer. Breast Cancer Res Treat 2005; 89: 215-20.

[7] Ren F, Tang R, Zhang X, Madushi WM, Luo D, Dang Y, Li Z, Wei K, Chen G. Overexpression of MMP Family Members Functions as Prognostic Biomarker for Breast Cancer Patients: A Systematic Review and Meta-Analysis. PLoS One 2015; 10: e0135544.

[8] Yuan Y, Shen N, Yang SY, Zhao L, Guan YM. Extracellular matrix metalloproteinase inducer and matrix metalloproteinase-2 overexpression is associated with loss of hormone receptor expression and poor prognosis in endometrial cancer. Oncol Lett 2015; 10: 342-348.

[9] Mishev G, Deliverska E, Hlushchuk R, Velinov N, Aebersold D, Weinstein F, Djonov V. Prognostic value of matrix metalloproteinases in oral squamous cell carcinoma. Biotechnol Biotechnol Equip 2014; 28: 1138-1149.

[10] Rabinovich A, Mak IW, Cowan RW, Turcotte RE, Colterjohn N, Singh G, Ghert M. Matrix Metalloproteinase Activity in the Stromal Cell of Giant Cell Tumor of Bone. Open Bone J 2009; 1: 46-52.

[11] Jakobs M, Häupl T, Krenn V, Guenther R. [MMP- and FAP-mediated non-inflammation-related destruction of cartilage and bone in rheumatoid arthritis]. Z Rheumatol 2009; 68: 683-94.

[12] Zheng CG, Chen R, Xie JB, Liu CB, Jin Z, Jin C. Immunohistochemical expression of Notch1, Agged1, NF-kappaB and MMP-9 in colorectal cancer patients and the relationship to clinicopathological parameters. Cancer Biomark 2015; 15: 889-97.

[13] Zhang Q, Wang P, Shao M, Chen SW, Xu ZF, Xu F, Yang ZY, Liu BY, Gu QL, Zhang WJ, Li Y. Clinicopathological correlation of keratinocyte growth factor and matrix metalloproteinase-9 expression in human gastric cancer. Tumori 2015; 101: 566-71.

[14] Ueda Y, Imai K, Tsuchiya H, Fujimoto N, Nakanishi I, Katsuda S, Seiki M, Okada Y. Matrix metalloproteinase 9 (gelatinase B) is expressed in multinucleated giant cells of human giant cell tumor of bone and is associated with vascular invasion. Am J Pathol 1996; 148: 611-22.
[15] Kumta SM, Huang L, Cheng YY, Chow LT, Lee KM, Zheng MH. Expression of VEGF and MMP-9 in giant cell tumor of bone and other osteolytic lesions. Life Sci 2003; 73: 1427-36.
[16] Trombetta-Lima M, Winnischofer SM, Demasi MA, Astorino Filho R, Carreira AC, Wei B, Assis-Ribas Td, Konig MS,Bowman-Colin C, Oba-Shinjo SM, Marie SK, Stetler-Stevenson W, Sogayar MC. Isolation and characterization of novel RECK tumor suppressor gene splice variants. Oncotarget 2015; 6: 33120-33.
[17] Takagi S, Simizu S and Osada H. RECK negatively regulates matrix metalloproteinase-9 transcription. Cancer Res 2009; 69: 1502-8.
[18] Meng N, Li Y, Zhang H, Sun XF. RECK, a novel matrix metalloproteinase regulator. Histol Histopathol 2008; 23: 1003-10.
[19] Gupta A, Nath R and Mishra M. Giant cell tumor of bone: Multimodal approach. Indian J Orthop 2007; 41: 115-20.
[20] Campanacci M, Baldini N, Boriani S, Sudanese A. Giant-cell tumor of bone. J Bone Joint Surg Am 1987; 69: 106-14.
[21] Rock M. Curettage of giant cell tumor of bone. Factors influencing local recurrences and metastasis. Chir Organi Mov 1990; 75 Suppl: 204-5.
[22] Prosser GH, Baloch KG, Tillman RM, Carter SR, Grimer RJ. Does curettage without adjuvant therapy provide low recurrence rates in giant-cell tumors of bone? Clin Orthop Relat Res 2005; 211-8.
[23] Xu H and Niu X. [Analysis of the risk factors for local recurrence of giant cell tumor of long bone]. Zhonghua Zhong Liu Za Zhi 2014; 36: 465-8.
[24] Siddiqui MA, Seng C and Tan MH. Risk factors for recurrence of giant cell tumours of bone. J Orthop Surg (Hong Kong) 2014; 22: 108-10.
[25] Enneking WF, Spanier SS and Goodman MA. A system for the surgical staging of musculoskeletal sarcoma. 1980. Clin Orthop Relat Res 2003; 4-18.
[26] Blackley HR, Wunder JS, Davis AM, White LM, Kandel R, Bell RS. Treatment of giant-cell tumors of long bones with curettage and bone-grafting. J Bone Joint Surg Am 1999; 81: 811-20.
[27] Han YH, Gao B, Huang JH, Wang Z, Guo Z, Jie Q, Yang L, Luo ZJ. Expression of CD147, PCNA, VEGF, MMPs and their clinical significance in the giant cell tumor of bones. Int J Clin Exp Pathol 2015; 8: 8446-52.
[28] Xu M, Wang HF and Zhang HZ. Expression of RECK and MMPs in Hepatoblastoma and Neuroblastoma and Comparative Analysis on the Tumor Metastasis. Asian Pac J Cancer Prev 2015; 16: 4007-11.
[29] Alexius-Lindgren M, Andersson E, Lindstedt I, Engström W. The RECK gene and biological malignancy--its significance in angiogenesis and inhibition of matrix metalloproteinases. Anticancer Res 2014; 34: 3867-73.