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Original Article

Expressions and clinical significance of factors related to giant cell tumor of bone

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Abstract: Background: Giant cell tumor of bone (GCTB) is a relatively rare tumor of bone, characterized by numerous multinucleated cells, severe osteolysis, and local recurrence. Purpose: To explore the role of S-phase kinase-interacting protein 2 (Skp2), cyclin-dependent kinase inhibitor p27, and the transcription factor E2F-1 expression in the development of GCTB, and the relationship of expression of these proteins with tumor recurrence. Methods: Forty-four patients with GCTB were selected and demographic and clinical data were collected. The levels of Skp2, p27, and E2F-1 protein expression were immunohistochemically assessed in surgical specimens. Results: Skp2, p27, and E2F-1 proteins were detected in the nuclei of mononuclear stromal cells. Positive Skp2 expression was observed in 66% (29/44) of GCTB patient samples, and positive p27 expression was found in 39% (17/44) of samples. Within almost all GCTB patients, there was an inverse correlation between Skp2- and p27-positive tumor cells. Positive expression of E2F-1 was present in 28 of 44 (64%) patients. In addition, expression of skp2 and p27, infiltration of soft tissues, and surgical operation were significantly associated with recurrence in patients with GCTB. Conclusion: The immunohistochemical assessment of Skp2, p27 and E2F-1 may be useful in the diagnosis of GCTB and prediction of its prognosis.

Keywords: Skp2, p27, E2F-1, expression, recurrence, GCTB

Introduction

Giant cell tumor of bone (GCTB) has occupied a central stage in musculoskeletal tumor practice because of its relatively common incidence, the striking features of giant cell formation, severe bone destruction (osteolysis), and the risk for joint-related fractures in the femur and tibia [1-3]. The rate of local recurrence following surgical curettage is relatively high (about 20%), and salvage procedures may be required in some recurrent cases. The cyclin-dependent kinase inhibitor p27 is important in the control of mammalian cell proliferation [4, 5]. p27 is destabilized in many types of human cancer, which correlates with the process of tumor aggressiveness and poor prognosis [6-8]. The level of p27 expression is regulated by ubiquitin-dependent degradation promoted by S-phase kinase-interacting protein 2 (Skp2) [9]. Skp2 is the ubiquitin ligase subunit that targets p27Kip1 (p27) for degradation [10-12]. Skp2 is induced in the G1-S transition of the cell cycle, frequently overexpressed in human cancer, and displays transformation activity in experimental models. It has been reported that Skp2 overexpression may lead to accelerated p27 degradation and contribute to tumor progression in human oral [13, 14], colon [15], gastric [16] and prostate cancer [17]. Furthermore, Skp2 has been shown to be involved in the degradation of other cell cycle regulators, including E2F-1 [18], cyclin E [19] and cyclin D1 [20]. E2F-1 (generically referred to as E2F) [21], the first identified member of a family of transcription factors, is the factor triggering p53-dependent or -independent apoptosis [22]. E2F-mediated control of gene expression plays a crucial role in the control of cellular proliferation.

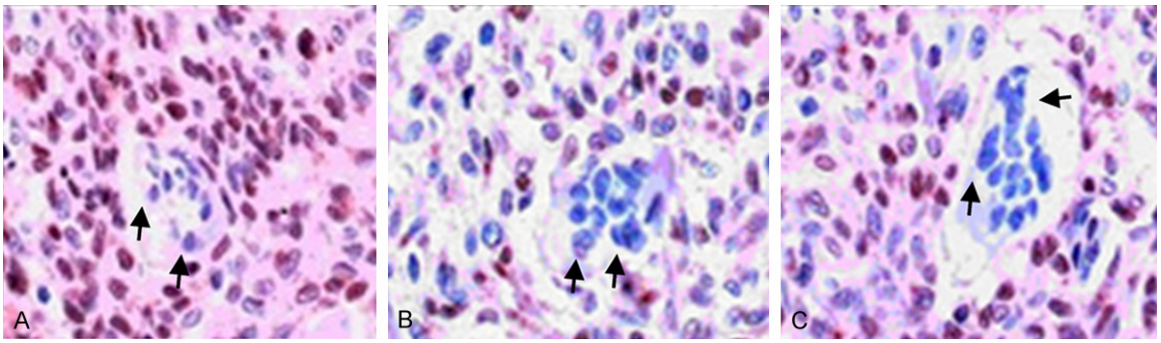

Figure 1. Representative results of immunostaining for Skp2, p27and E2F-1 in GCTB (×200). A: Skp2; B: p27; C: E2F-1. The black arrows indicated the nuclear staining.

Table 1. Correlation of Skp2 expression with p27 and E2F-1 expressions in GCTB patients' samples

Index	Skp2		r_s	P	p27		r_s	P
	+	-			+	-		
p27								
+	8	9	-0.316	0.037				
-	21	6						
E2F-1								
+	19	9	0.054	0.726	11	17		
-	10	6			6	10	0.018	0.091

In GCTB, the relationship between expression of the cyclin-dependent kinase inhibitor p27 and tumor aggression and prognosis is still unclear. Furthermore, the expression of E2F-1, one of the cell cycle regulators of skp2, is not known in GCTB. In this study, we aimed to elucidate the role of Skp2 in tumor progression through the system of ubiquitin-dependent degradation, and the role of p27 and E2F-1 in GCTB, by examining the correlation between Skp2, p27, and E2F-1 expression in GCTB.

Patients and methods

Patients

Tumor specimens from 44 patients with GCTB were obtained from the surgical pathology files of the pathology departments, the Kunshan First People's Hospital and other hospitals in China from 2000 to 2005. There were 25 male and 19 female patients included. The age of the patients ranged from 19 to 68 years, with a mean age of 34.5 years. With respect to tumor location, there were 14 cases in the proximal tibia, 12 cases in the distal tibia, and the remaining 18 cases were from locations outside the tibia. The maximum tumor diameter was greater than 5 cm in 22 (50%) of cases and less than 5 cm in the remaining 22 cases. Similarly, approximately half of the cases involved cortical destruction and soft-tissue invasion (23 cases). Nineteen cases were treated with intralesional curettage and reconstruction and 25 cases were treated with amputation. Tumor stage was classified according to radiological characteristics [23]. There were a total of 12 recurrences within the follow-up time period (27%). This study was approved by the ethics committee of Second Affiliated Hospital of Soochow University. Written informed consent was obtained from all participants.

Immunohistochemical detection of Skp2, p27 and E2F-1

Resected specimens were fixed with 10% formaldehyde and embedded in paraffin blocks. Immunohistochemical staining was performed by the standard avidin-biotin peroxidase complex method, as described previously [24]. Briefly, the sections were incubated with anti-Skp2 polyclonal antibody (Santa Cruz Biotechnology Corporation, Santa Cruz, CA, USA) at a dilution of 1:100 and anti-p27 monoclonal antibody (clone 57, Transduction Laboratories, Lexington, KY, USA) at a dilution of 1:1000. All sections were then incubated with an appropriate biotinylated secondary antibody (Fuzhou Maixin Biotechnology, Inc. Fuzhou, China) and counterstained lightly with hematoxylin-eosin. A negative control was prepared by substituting normal rabbit and mouse serum for each primary antibody. No staining was detected in any control section (figures not shown).

Table 2. Relationship between Skp2, p27 and E2F-1 expression and clinicopathological characteristics in GCTB patients' samples

Parameters	n	Skp2		χ^2	p27		χ^2	E2F-1		χ^2
		+	%		+	%		+	%	
Gender										
Male	25	17	68		10	15		17	8	
Female	19	12	63	0.11	7	12	0.04	11	8	0.48
Age (years)										
<45	34	24	10	1.46	14	20	0.41	23	11	1.04
≥45	10	5	5		3	7		5	5	
Tumor size (cm)										
<5	22	12	10	2.53	9	13	0.10	15	7	0.39
≥5	22	17	5		8	14		13	9	
Tumor location										
Proximal tibia	14	11	3	2.70	6	8	2.10	10	4	8.94
Distal femur	12	8	4		5	7		10	2	
Proximal humerus	8	5	3		3	5		5	3	
Distal radius	7	4	3		3	4		3	4	
Other position	3	1	2		0	3		0	3	
Radiological stage										
Stage 1	20	10	10	7.44*	12	8	7.91*	10	10	3.13
Stage 2	14	9	5		4	10		10	4	
Stage 3	10	10	0		1	9		8	2	
Recurrence										
Yes	12	11	1	4.87*	1	11	6.39*	9	3	0.92
No	32	18	14		6	16		19	13	

*P<0.05.

Assessment of Skp2, p27 and E2F-1 expression

The immunostaining of Skp2, p27 and E2F-1 was independently quantitated and validated by two senior pathologists (Feng Yizhong and Li Hai) in a blindly coded manner. In order to score the degree of expression of each protein, more than 10 high-powered magnification fields were randomly selected and at least 1000 cells were counted. The sections were graded for percentage of positive nuclei. The cut-off value of the scores of tumor cells to distinguish the low and high expression of Skp2, p27 and E2F-1, has been previously described [13, 25]. In brief positive staining of 20% of cells for Skp2 and E2F-1 and 50% of cells for p27 were used as the cut-off points for discrimination of Skp2, p27 and E2F-1 immunostaining.

Statistical analysis

Statistical analyses were conducted using the SPSS 13.0 software package (SPSS Inc., IL, USA). The Chi-square test was used to describe a correlation between Skp2, p27, E2F-1 and clinical features. Spearman rank correlation analysis was used to describe a correlation between Skp2, p27 and E2F-1. Cox regression analysis was applied to evaluate potential risk factors of recurrence for GCTB. P<0.05 was used as the criterion for statistical significance.

Results

General expression profiling of Skp2, p27 and E2F-1 in GCTB

Immunostaining results showed that Skp2, p27, and E2F-1 proteins were detected in the nuclei of mononuclear stromal cells (**Figure 1A-C**). Positive Skp2 expression was observed in 66% (29/44) of GCTB patient samples, and positive p27 expression was found in 3398.6% (17/44) of samples. Within almost all GCTB patients, there was an inverse correlation between Skp2- and p27-positive tumor cells (**Table 1**). Staining for E2F-1 expression was positive in 28 of 44 patients (64%).

Relationship between the expressions of Skp2, p27 and E2F-1 and clinicopathological factors

Based on the radiological classification, positive Skp2 expression was present in 50% 64% and 100% of Stage 1, Stage 2 and Stage 3 GCTB patients' samples, respectively, all of which were statistically significant (P<0.05). Skp2 immunostaining was positive in samples from 11 of 12 patients (92%) who suffered a local recurrence whereas Skp2 immunostaining was positive in only 18 of 32 patients (56%) that did not suffer a local recurrence. This difference was also statistically significant (P<0.05). Positive p27 expressions in the recurrent and no recurrence groups were also statistically significant: 8% (1/12) and 50% (16/32), respectively (P<0.05). In contrast, no signifi-

Table 3. Risk factors of recurrence of GCTB

Index		β	SE	Wald	df	Sig.	Exp(β)	95.0% CI for Exp(β)	
								Lower	Upper
Step 4	X5	-1.770	0.695	6.494	1	0.011	0.170	0.044	0.665
	X6	-1.944	0.639	9.251	1	0.002	0.143	0.041	0.501
	X7	1.836	0.616	8.880	1	0.003	6.272	1.875	20.983
	X8	-1.540	0.597	6.654	1	0.010	0.214	0.067	0.691

B, regression coefficient; SE, standard error; Wald, corresponding to χ^2 value; df, degree of freedom; Sig, *P* value; Exp(β), relative risk; CI, confidence interval; X5, destruction cortical bone or soft tissue invasion; X6, surgical resection type; X7, expression of Skp2; X8, expression of p27.

cant difference in E2F-1 expression was observed between the recurrent and no recurrence groups. With the numbers available, no significant association was identified between Skp2 and other clinicopathological features (gender, age, tumor size and location) (P>0.05; **Table 2**).

Potential risk factors for recurrence of GCTB

In this study, eight potential risk factors were evaluated, including patient gender (X1), patient age (X2), tumor location (X3), tumor size (X4), cortical destruction or soft tissue invasion (X5), surgical resection type (X6), expression of Skp2 (X7) and expression of p27 (X8). The potential risk factors are denoted in **Table 3**. Univariate Cox regression analysis showed that gender (X1), cortical destruction or soft tissue invasion (X5), surgical resection type (X6), expression of Skp2 (X7) and expression of p27 (X8) have a significant effect on recurrence (P<0.05). Further multivariate Cox regression analysis showed that only cortical destruction or soft tissue invasion (X5), surgical resection type (X6), expression of Skp2 (X7) and expression of p27 (X8) were significantly associated with recurrence, in which a high expression of Skp2 (P<0.05), a low expression of p27 (P<0.05), and the bone cortex and soft tissue destruction (P<0.05) were risk factors for recurrence of GCTB (P<0.05). Amputation as the operation mode was a protective factor against recurrence of GCTB.

Discussion

Although the majority of GCTB follows a benign clinical course, a subset can behave in a locally aggressive manner causing destruction of cortical bone with extension into the adjacent soft tissues. The high rate of local recurrence following surgical curettage (15-20%) and the potential for metastases (2% of patients) indicate the need for more research into the treatment of this type of tumor. In many tumors, reduced p27 expression is associated with a worse clinical outcome and greater tumor invasiveness. Likewise, high levels of expression of Skp2 are found associated with tumor progression [19-23]. However, the relationship between p27 expression and tumor aggression in GCTB, and the relationship between Skp2 and p27 expression in these patients have not previously been examined. In this study, our immunohistochemical results demonstrated that Skp2 expression was inversely correlated with p27 expression. Furthermore, multivariate Cox regression analysis showed that a high expression of Skp2, a low expression of p27 and local tumor aggressiveness were risk factors for recurrence of GCTB, with the use of amputation as a protective factor against recurrence of GCTB.

Skp2 is known to be involved in degradation of both the negative cell-cycle regulator p27, and the positive regulators E2F-1 [19]. In this study, we identified an inverse relationship between the levels of expression of Skp2 and p27. However, there was no inverse relationship between the expression levels of Skp2 and those of E2F-1. Thus, our data indicate that a more likely target substrate of Skp2 in GCTB is p27, suggesting that reduction in the rate of Skp2-induced degradation of p27 may influence tumor progression. Further analysis of p27 and Skp2 expression together may help to reveal the causative relationship between p27 and tumor aggression and prognosis in GCTB.

In GCTB, the mechanism of altered expression of Skp2 remains to be determined. Our study revealed that there was an inverse correlation between the level of expression of Skp2 protein and p27 protein. The levels of both Skp2 and p27 expression were thought to be increased in the G1/S-phase; however, staining of both markers could not be obtained in all samples simultaneously as the tumor cells might have been in other phases of the cell cycle. Because the staining scores of Skp2 approximated those

of p27 in tumors at later stages other than those at early stages, we consider that the inverse correlation between these two markers might not be significant at the earlier stages.

We found a statistically significant relationship between Skp2 expression and increasing radiological stage in 44 GCTB patients. In addition, the expression of Skp2 in patients with recurrence was significantly higher than that in patients without recurrence, indicating that Skp2 in tumor cell proliferation may play a role in tumor aggressiveness. Furthermore, it is conceivable that with further study, Skp2 may be a useful predictor of the biologic behavior of GCTB.

In conclusion, this study has demonstrated inversely correlating expression of Skp2 and p27 in GCTB, which adds evidence that p27 may be the target substrate of Skp2 in this tumor. In addition, the expression of Skp2 and p27 are associated with recurrence of GCTB. Further studies are needed to elucidate the participation of other cell cycle regulators such as E2F-1, or other components of the Skp1/Cul1/F-box complex in GCTB.

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Disclosure of conflict of interest

None.

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