Original Paper

Factors Affecting the Recurrence of Giant Cell Tumor of Bone After Surgery: A Clinicopathological Study of 80 Cases from a Single Center

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Key Words

GCTB • CD147 • Surgery • Campanacci grade

Abstract

Background/Aims: This aim of the present study was to identify specific markers determining the recurrence of the giant cell tumor of bone (GCTB). **Methods:** This study involved the clinicopathological analysis of 80 cases. All of the clinical features, pathological fracture, Campanacci grade, histological features and surgical methods were reviewed. Immunohistochemistry was used to detect the expression of Ki-67, CD147, mutant p53 and p63 in GCTB. Comparisons between different groups were performed using the Chi-square test. The risk factors affecting recurrence were analyzed using a binary logistic model. Kaplan-Meier analysis was employed for the survival analysis between the groups. Cell proliferation assays, migration and invasion assays were used to detect the function of CD147 on GCTB *in vitro*. **Results:** The univariate analysis showed that Ki-67 and CD147 expression, pathological fracture, Campanacci grade and surgical method were associated with recurrence. The multivariate analysis revealed that CD147 expression, Campanacci grade and surgical method were the factors affecting GCTB recurrence. In addition, the Kaplan-Meier analysis revealed that these factors affected tumor-free survival time. *In vitro* study revealed that the CD147 knockdown by small interfering RNA (siRNA) technique dramatically reduced the proliferation, migration and invasion of GCTB. **Conclusion:** Our results suggest that CD147 may serve as an adequate marker for GCTB recurrence. Campanacci grade is a risk factor for GCTB recurrence, which is also affected by the surgical method used.

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Introduction

Giant cell tumor of bone (GCTB) is a challenging clinicopathological entity, accounting for 5% of all primary bone tumors [1]. The occurrence of GCTB is primarily observed in individuals between 20 and 40 years old. The site of predilection for GCTB is the extremity of long bone in skeleton of mature adults. Histologically, it is composed of oval and spindle mononuclear cells, uniformly distributed and large multinucleated osteoclast-like giant cells. Although the term "giant cell tumor" implies that the multinucleated giant cells are responsible for the proliferative capacity of this tumor, there is evidence that the stromal cells, the major components of the mononuclear cell population, represent the true neoplastic components of GCTB [2]. Due to its local recurrence and rare pulmonary metastases [3], GCTB is considered to have a low malignant potential [4].

At present, surgery is the primary treatment method for GCTB. When GCTB is unresectable, or when surgery is likely to result in severe morbidity, denosumab, a receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitor can be used [5]. Denosumab functions by inhibiting osteoclastogenesis. GCTB patients can survive for a long time after proper treatment, therefore the impact of surgical treatment on limb function should be fully considered. The choice of surgical method depends on many factors, such as pathological fracture, tumor size, bone cortex damage, soft tissue involvement, and articular cartilage damage [6, 7]. Nowadays, curettage and prosthetic replacement are the main methods to treat GCTB.

To date, defining cellular and molecular characteristics of more aggressive GCTB has been difficult to elucidate. Studies have shown that mutations in p53 mutation are associated with local GCTB recurrence, and the overexpression of C-myc oncogene has been associated with GCTB metastasis [8, 9]. Telomeric reduction and telomerase activity may act as oncogenic events, promoting and sustaining the transformed GCTB phenotype [10]. Some studies have shown that a high Ki-67 proliferative index is associated with the malignancy of bone tumors [11]. In some tumors, CD147 acts as a prognostic marker and therapeutic target [12]. It has been suggested that mutations in p53 are correlated with the lung metastasis in GCTB [13]. In addition, the overexpression of p63 has been implicated in GCTB progression [14]. Thus, these proteins might play a regulatory role in the progression of GCTB. However, the prognostic value of these proteins in GCTB has not been evaluated.

In this study, we detected the expression and localization of Ki-67, CD147, mutant p53, and P63 in 80 cases of GCTB and examined the relationship of these results to the recurrence of GCTB. In addition, we observed and followed-up these patients, evaluating their corresponding clinical features, pathological fracture, Campanacci grade and the used surgical methods to explore the factors associated with the recurrence of GCTB. *In vitro* study was performed to investigate the function of CD147 on GCTB. We hope to elucidate the factors influencing the recurrence of GCTB.

Patients and Methods

Patients

From 2005 to 2009, 96 cases of GCTB were treated at Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Complete clinical and pathological data was generated for each patient, and 80 patients had complete follow-up data. Two Chief Pathologists confirmed all pathological diagnoses after surgery. GCTB was most commonly located in the femur (38 cases), followed by the tibia (25 cases), the radius (7 cases), the humerus (5 cases), the fibula (3 cases) and some rare sites, such as the talus (1 case) and the ischium (1 case). 30 cases of pathological fracture occurred in this series. A total of 15 cases of recurrence were observed at the time of final consultation. The specimens from all cases were fixed with 10% formalin, decalcified when necessary, and embedded in paraffin. The imaging classification was determined according to Campanacci's grading system [15]. The patients were followed up at 3-month intervals for the first 3 years, every 6 months until the 5th year, and every year until the time of final consultation.

Immunohistochemistry

A standard immunohistochemistry staining procedure was used in the present study. The paraffin-embedded sections were cut at 4 μ m, dewaxed in xylene and treated with microwave heating at 60°C for 20 min in a EDTA buffer (pH 9.0) for antigen retrieval. Each slide was blocked for endogenous peroxidase activity through incubation in 0.3% H₂O₂ for 10 min, followed by overnight incubation at 37°C with a mAb Ki-67 (1:200, DAKO), CD147 (1:100, Santa Cruz, USA), mutant p53 (1:200, DAKO) or p63 (1:80, DAKO). The slides were subsequently rinsed three times in PBS, incubated for 30 min with Dako REAL™ EnVision™ (DAKO, USA), followed by three washes in PBS and color development for 3-10 minutes in a moist chamber at room temperature using DAB. The slides were counterstained with hematoxylin and dehydrated in graded ethyl alcohol (70%, 90%, and 100%). In the sections used as negative controls, the primary antibody was substituted with PBS.

Evaluation of Immunohistochemistry

A semi-quantitative counting standard was used to measure Ki-67 proliferation. Two blinded observers determined the labeled cell count (Ki-67 proliferation index) in ten high-power fields. The Ki-67 proliferation index was defined as the ratio of labeled cells to total cells, and the median of Ki-67-positive cells was calculated. When the number of CD147-positive cells was $\leq 5\%$, the tissue was considered negative; 6-25%, weak; 26-50%, moderate; and $\geq 51\%$, strong [16]. When the number of nuclei positively stained for p63 or mutant p53 was $>10\%$, the tissue was considered positive, otherwise the tissue was considered negative [17].

Establishment of GCTB Cell Line and Cell Culture

Mak et al [18, 19] established primary cell cultures of GCTB stromal tumor cells from fresh GCTB tissue. According to their method, we got GCTB stromal tumor cells from the fresh specimens. They were cultured in Dulbecco's modified Eagle's medium and supplemented with 10% fetal bovine serum (Biowest, South America Origin), 100 U/ml penicillin (Sigma-Aldrich, St Louis, MO, USA) and 100 mg/ml streptomycin (Sigma-Aldrich).

siRNA Transfection and Quantitative Real-time PCR Analysis (qRT-PCR)

CD147-specific siRNA (sense, 5-UUC UCC GAA CGU GUC ACG UTT-3, antisense, 5-ACG UGA CAC GUU CGG AGA ATT-3) and negative control siRNA (sense, 5-UUC UCC GAA CGU GUC ACG UTT-3, antisense, 5-ACGU GAC ACG UUA GAA TT-3) were selected and synthesized by GenePharma. A total of 5×10^4 cells were seeded into each well of a 6-well plate. The next day, the cells were transfected with the siRNAs using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. For Western blot assays, migration and invasion, the cells were collected 48h after transfection. The mRNA expression levels were quantified using a 7500 Real-Time PCR System with 7500 software, version 2.0.5 (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. β -Actin was used as the endogenous control for quantifying mRNA levels. Three independent assays were performed.

Cell Proliferation Assays

5000 cells were seeded into each well of a 96-well plate and incubated after 48 h transfection. A 10- μ L aliquot of CCK-8 (Dojindo, Japan) was added to quintuplicate wells and incubated for 2 h. To calculate the number of viable cells in each well, the absorbance at 450 nm was measured. Each measurement was performed in quintuplicate, and the experiments were repeated twice.

In vitro migration and invasion assays

Cell migration and invasion assays were performed in a 24-well plate with 8-mm pore size chamber inserts (Corning, New York, NY, USA). For the migration assays, after transfection with siRNA, 5×10^4 cells per well were placed into the upper chamber on an uncoated membrane. For the invasion assays, 1×10^5 cells per well were placed into the upper chamber on a Matrigel-coated membrane. The cells were diluted with serum-free culture medium. In both assays, when the cells were seeded into the upper chamber, they were suspended in 200 μ L of Dulbecco's modified Eagle's medium without fetal bovine serum. The lower chambers contained 800 μ L of medium with 10% FBS. The cells were incubated at 37°C in 5% CO₂ for 18 h and 20 h for the migration and invasion assays, respectively. Then, the membrane inserts were removed

and non-invading cells were removed from the upper surface of the membrane. Cells that had moved to the bottom of the chamber were fixed with 100% methanol for 30 min and stained with 0.1% crystal violet for 30 min. Finally, the cells in at least 10 random fields were imaged and counted using a CKX41 inverted microscope (Olympus, Tokyo, Japan). The assays were independently conducted three times.

Ethics Statement

The study was approved by the Ethics Committees of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Ethics Committee specifically approved that not informed consent was required because data were going to be analysed anonymously.

Statistical Evaluation

The data were compiled and analyzed using SPSS software (version 21.0, Chicago, IL, USA). Comparisons between different groups were performed using the Chi-square test. The risk factors affecting recurrence were analyzed using a binary logistic model. The Kaplan-Meier analysis was employed for the survival analysis between the groups. The tumor-free survival time is the period from surgery to the presence of new lesions. A significant result was considered at $P < 0.05$.

Results

Basic Patient Information

A total of 49 males and 31 females were included in this study. The median age was 32 years old (range: 16-74 years). The follow-up period ranged from 32 to 86 months, and the median time was 58 months. According to Campanacci's grading system, 19 cases were stage 1, 37 cases were stage 2, and 24 cases were stage 3. The time to recurrence ranged from 1 to 48 months. The recurrence rate in the series was 18.75%. The recurrence occurs in the femur (7/38 cases), the tibia (5/25 cases), the radius (2/7 cases) and the humerus (1/5 cases). No significant difference was found between the different locations. A total of 57 patients received curettage and blurring and the remaining 23 patients received tumor resection and prosthesis replacement. Curettage was performed through a large cortical window to remove the entire visible tumor. The cavity was subsequently blurred using

Fig. 1. Immunohistochemical analysis of recurrent (A, a, B, b, C, c and D, d) and non-recurrent GCTB (E, e, F, f, G, g and H, h). The Ki-67 proliferation index of recurrent GCTB (A) was higher than that of non-recurrent GCTB (E). The Ki-67 (%) in Fig. 1A is 20%, while the Ki-67 (%) in Fig. 1 is 8%. CD147 immunostaining for recurrent GCTB (B) was stronger than that for non-recurrent GCTB (F). The expression of mutant p53 (C and G) and p63 (D and H) was similar in GCTB with or without recurrence. A, B, C, D, E, F, G and H = 2×; a, b, c, d, e, f, g and h = 20×.

Table 1. Univariate analysis of prognostic factors in GCTB. The result of Chi-square analysis revealed that no significant statistical effect on the local recurrence rate was observed for gender, age, mutant p53, p63, and tumor diameter. Ki-67 ($P=0.042$), CD147 ($P=0.010$), pathological fracture ($P=0.002$), Campanacci grade ($P=0.000$) and surgical method ($P=0.030$) were the factors influencing the recurrence of GCTB. * $P<0.05$

Variable	Parameter	With recurrence	Without recurrence	Recurrence rate	χ^2 value	p value
Gender	Female	5	26	16.13%	0.23	0.443
	Male	10	39	20.41%		
Age	≤ 40 years	11	52	17.50%	0.32	0.398
	> 40 years	4	13	23.50%		
Mutant P53	Negative	11	48	18.64%	0.00	0.599
	Positive	4	17	19.05%		
P63	Negative	2	17	10.53%	1.11	0.244
	Positive	13	48	21.31%		
Ki-67	$\leq 15\%$	4	36	10.00%	4.02	0.042*
	$> 15\%$	11	29	27.50%		
CD147	Negative+weak	3	37	7.50%	6.65	0.010*
	Moderate+strong	12	28	30.00%		
Tumor diameter	$\leq 4.5\text{cm}$	11	37	22.92%	1.37	0.191
	$> 4.5\text{cm}$	4	28	12.50%		
Surgical method	Curettage and blurring	14	43	24.56%	4.40	0.030*
	Tumor resection and prosthesis replacement	1	22	4.35%		
Campanacci's grade	Grade 1	0	19	0.00%	13.32	0.000*
	Grade 2	5	32	13.51%		
	Grade 3	10	14	41.67%		
Pathological fracture	With	11	19	36.70%	10.11	0.002*
	Without	4	46	8.00%		

Table 2. The binary logistic regression analysis of prognostic factors in GCTB. Binary logistic regression with forward Wald method considering Ki-67, CD147, pathological fracture, surgical method and Campanacci grade confirmed that the variables that influence the recurrence include CD147, surgical method and Campanacci grade

Variable	B	$S.E$	Wals	P value	OR	95% CI
CD147	1.695	0.788	4.631	0.031	5.446	1.163~25.498
Surgical method	-1.333	0.575	5.376	0.02	0.264	0.085~0.814
Campanacci grade	1.895	0.62	9.337	0.002	6.653	1.973~22.435

a high-speed bone drill and washed with distilled water until all pathological tissue was removed. Lastly, the cavity was filled with autologous bone, allogeneic bone or bone cement. Indications for prosthesis replacement rather than curettage included pathological fractures with joint invasion, a large tumor with soft tissue extension or unstable fracture pattern.

Immunohistochemistry and Univariate Analysis Results

p63, Ki-67 and mutant p53 were observed only in the nuclei of mononuclear stromal cells, and no staining was present in the multinucleated giant cells. CD147 was detected in the multinuclear osteoclast-like giant cells and stromal cells, with strong immunostaining on cell membrane. The immunohistochemical staining of two typical cases of GCTB with or without recurrence is shown in Fig. 1. The experimental data also revealed that the recurrence rate was higher in the Ki-67 $>15\%$ group than that in the Ki-67 $\leq 15\%$ group, consistent with the result obtained in previous reported literature [11]. The mean Ki-67 index in non-recurrent group is 8.73%, while the mean Ki-67 index in recurrent group

Fig. 2. Tumor-free survival by the surgical method used in 80 cases of GCTB.

Fig. 3. Tumor-free survival by the Campanacci grade in 80 cases of GCTB.

is 24.17%, which is much higher than that of non-recurrent group. Similarly, there was a significant difference in the recurrence rate between the CD147-positive and CD147-negative groups ($\chi^2=6.65$, $P=0.010$). The local recurrence rate was 4.35% (1/23 cases) in patients treated through tumor resection and prosthesis replacement. Conversely, the local recurrence rate was higher in patients treated through tumor curettage and blurring, with a 24.56% incidence. The recurrence rate was improved with improvements in the Campanacci grade, and a significant difference was observed ($\chi^2=13.32$, $P=0.000$). Similarly, the local recurrence rate was higher in patients with pathological fracture ($\chi^2=10.11$, $P=0.002$). No significant statistical effect on the local recurrence rate was observed for gender, age, mutant p53, p63, and tumor diameter. The results of the univariate analysis are shown in Table 1.

Multivariate Analysis

Independent factors associated with the recurrence of GCTB were assessed using a logistic regression model. The following factors were assessed in the model: Ki-67, CD147, surgical method and Campanacci grade. The factors independently associated with GCTB recurrence through binary logistic regression were CD147 (OR=5.446, 95% CI 1.163-25.498,

Fig. 4. Tumor-free survival by CD147 in 80 cases of GCTB.

Fig. 5. *In vitro* study of CD147 function on GCTB. (A), (B) Primary GCTB stromal tumor cells were established from fresh GCTB tissue. Multinucleated giant cells could also be seen in the field (magnification: 100×). (C) qRT-PCR assay revealed that CD147 was down-regulated after transfection for 48h. (D) Cell proliferation assay revealed that knockdown of CD147 dramatically reduced the proliferation of GCTB. (E) The migration and invasion assay showed that inhibition of CD147 could inhibit the migration and invasion of GCTB. The left panel shows photos of representative fields (magnification: 100×) of invasive cells and the right panel shows histograms of the results. The statistical analysis was performed using Student's t-test ($n = 3$). The Error bars represent the SEM, * $P < 0.05$.

$P=0.031$), surgical method (OR=0.246, 95% CI 0.085-0.814, $P=0.02$) and Campanacci grade (OR=6.653, 95% CI 1.973-22.435, $P=0.002$). Ki-67 was not significantly associated with recurrence in the multivariate analysis. Therefore, CD147 and Campanacci grade were the risk factors for GCTB recurrence, while the surgical method was a protective factor. The results of the multivariate analysis are shown in Table 2.

Tumor-free Survival Time

As shown in Fig. 2, the Kaplan-Meier analysis revealed a significant difference in tumor-free survival time between the curettage and blurring group and prosthesis replacement

group ($\chi^2=4.150$, $P=0.042$). Local recurrence occurred in stage 2 or 3 tumors, with a significant difference between the three stages of the tumor, as shown in the Kaplan Meier curves of survival to local recurrence ($\chi^2=13.885$, $P=0.001$) (Fig. 3). A similar result was observed for the tumor-free survival time between the CD147-negative and CD147-positive groups ($\chi^2=7.362$, $P=0.007$) (Fig. 4).

In Vitro Study of CD147 Function

We established primary GCTB stromal tumor cells from fresh GCTB tissue (Fig. 5A and 5B). siRNA technique was used to knockdown CD147. The experimental data revealed that CD147 was down-regulated after transfected for 48 h (Fig. 5C). Cell proliferation assays revealed that knockdown of CD147 dramatically reduced the proliferation of GCTB (Fig. 5D). Moreover, the migration and invasion assays showed that inhibition of CD147 could inhibit the migration and invasion of GCTB *in vitro*, as shown in Fig. 5E. In conclusion, CD147 plays an important role in the progression of GCTB *in vitro*.

Discussion

GCTB is a primary bone tumor that predominantly occurs in young adults. According to 2013 WHO classification of the bone tumors, GCTB is considered to be intermediate (locally aggressive, rarely metastasizing) tumor. To date, no prognostic marker for the risk of GCT recurrence has been identified. To identify a prognostic marker for the risk of GCTB recurrence, we detected the expression of Ki-67, mutant p53, p63 and CD147 using immunohistochemistry in 80 cases. Univariate analysis revealed that Ki-67 and CD147 affected recurrence, while CD147 expression was independently associated with recurrence of GCTB in the multivariate analysis. Notably, p63 and mutant p53 were not associated with the recurrence of GCTB in this study.

CD147, also called extracellular matrix metalloproteinase inducer (EMMPRIN) or Basigin, is a transmembrane glycoprotein of the immunoglobulin superfamily. The relative molecular mass of CD147 is 50-60 kDa, and this protein is widely present in human tissues and organs [20]. The gene encoding CD147 is located on chromosome 19p13.3 [21]. CD147 is a multifunctional membrane protein involved in many malignant tumors, such as lung and breast cancers [22, 23]. CD147 can induce MMP synthesis in stromal cells, resulting in tumor progression and extracellular matrix protein degradation to regulate tumor cell biological behaviors, including invasion and metastasis [24]. These findings suggest that CD147 plays an important role in tumorigenesis and might be a potential target for anti-tumor therapy. In the present study, the results of the Chi-square and Kaplan-Meier analyses showed that the level of CD147 expression was positively correlated with the recurrence of GCTB and negatively correlated with the tumor-free survival period of GCTB patients. In addition, *in vitro* study showed that knockdown of CD147 dramatically reduced the proliferation, migration and invasion of GCTB. Therefore, CD147 might be a risk factor for the recurrence of GCTB and might be a novel therapeutic target for the treatment of this tumor.

It has been suggested that the Campanacci classification is associated with GCTB recurrence, and the higher the grade, the higher the rate of recurrence [25]. However, other studies have shown that the recurrence of GCTB is not associated with the Campanacci grade, and the surgical method is the primary factor influencing postoperative recurrence. Prosser et al retrospectively analyzed the factors influencing the recurrence of GCTB in 193 patients. These authors showed that the recurrence of GCTB was closely associated with the involvement of the cortical bone. When the tumor did not break through the cortical bone, the recurrence rate was only 7%; however, when the tumor broke through the cortical bone, the rate was as high as 29% [25]. Balke et al reported that the involvement of soft tissue was an independent predictor for GCTB recurrence. When the soft tissue surrounding the tumor was not affected, the recurrence rate was 16.2%, but when the soft tissue was affected through tumor invasion, the recurrence rate was nearly twice as large as the former (29.7%).

These authors suggested that before determining the surgical method for the treatment of GCTB patients, the destruction of cortical bone and the involvement of soft tissue should be evaluated as predictive values for tumor recurrence [26]. In the present study, we observed that the Campanacci grade was an independent factor influencing recurrence. The recurrence rate in the Campanacci grade 1 group was 0, whereas that in grades 2 and 3 was 13.51% and 41.67%, respectively.

The influence of surgical method on GCTB recurrence remains unclear. The recurrence rate of simple tumor curettage with or without bone graft is approximately 17% to 31% [27, 28]. The recurrence rate observed in the present study was 18.75%. For Campanacci grade 3 GCTB, where both the bone and soft tissue are affected, conventional tumor curettage and bone grafting are difficult to guarantee the complete removal of lesions, resulting in a high rate of recurrence after operation. For these patients, tumor resection and prosthesis replacement can resolve the problem. Some studies have shown that after tumor-type prosthesis replacement, GCTB could be thoroughly treated, and the survival index was significantly improved, showing an adequate curative effect [29]. Therefore, the use of tumor type prosthesis replacement to treat GCTB has received much attention. However, the unavoidable clinical complications of prosthesis replacement (prosthetic loosening, allergy and secondary infection) and the limited service life of the prosthesis (10-15 years), makes prosthesis replacement inappropriate for young patients with a Campanacci grade of 1-2. For these patients, tumor curettage with inner wall blurring and repeated hypotonic distilled water washing is used to reduce postoperative GCTB recurrence [30].

In conclusion, CD147 might be an adequate marker for GCTB recurrence. Campanacci grade is a risk factor for the recurrence of GCTB. An appropriate surgical method should be decided for patients with a Campanacci grade of 2 or 3 to both remove the tumor and preserve joint function.

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Disclosure Statement

The authors declared that they have no conflicts of interest to this work.

References

- 1 Balke M, Campanacci L, Gebert C, Picci P, Gibbons M, Taylor R, Hogendoorn P, Kroep J, Wass J, Athanasou N: Bisphosphonate treatment of aggressive primary, recurrent and metastatic Giant Cell Tumour of Bone. *BMC Cancer* 2010;10:462.
- 2 Wuelling M, Engels C, Jesse N, Werner M, Kaiser E, Delling G: Histogenesis of giant cell tumors. *Pathologie* 2002;23:332-339.
- 3 Bertoni F, Bacchini P, Staals EL: Malignancy in giant cell tumor of bone. *Cancer* 2003;97:2520-2529.
- 4 Ghert MA, Rizzo M, Harrelson JM, Scully SP: Giant-cell tumor of the appendicular skeleton. *Clin Orthop Relat Res* 2002;400:201-210.
- 5 Lewin J, Thomas D: Denosumab: a new treatment option for giant cell tumor of bone. *Drugs Today (Barc)* 2013;49:693-700.
- 6 van der Heijden L, Dijkstra PD, Campanacci DA, Gibbons CL, van de Sande MA: Giant cell tumor with pathologic: should we curette or resect? *Clin Orthop Relat Res* 2013;471:820-829.
- 7 van der Heijden L, van de Sande MA, Dijkstra PD: Soft tissue extension increases the risk of local recurrence after curettage with adjuvants for giant-cell tumor of the long bones. *Acta Orthop* 2012;83:401-405.

- 8 Papanastassiou I, Ioannou M, Papagelopoulos PJ, Arealis G, Mihas C, Iakovidou I, Demertzis N: P53 expression as a prognostic marker in giant cell tumor of bone: a pilot study. *Orthopedics* DOI: 10.3928/01477447-20100329-15.
- 9 Gamberi G, Benassi MS, Böhling T, Ragazzini P, Molendini L, Sollazzo MR, Merli M, Ferrari C, Magagnoli G, Bertoni F, Picci P: Prognostic relevance of C-myc gene expression in giant-cell tumor of bone. *J Orthop Res* 1998;16:1-7.
- 10 Zheng MH, Siu P, Papadimitriou JM, Wood DJ, Murch AR: Telomeric fusion is a major cytogenetic aberration of giant cell tumors of bone. *Pathology* 1999;31:373-378.
- 11 Antal I, Sápi Z, Szendrői M: The prognostic significance of DNA cytophotometry and proliferation index (Ki-67) in giant cell tumors of bone. *Int Orthop* 1999;23:315-319.
- 12 Nabeshima K, Iwasaki H, Koga K, Hojo H, Suzumiya J, Kikuchi M: Emmprin (basigin/CD147): matrix metalloproteinase modulator and multifunctional cell recognition molecule that plays a critical role in cancer progression. *Pathol Int* 2006;56:359-367.
- 13 Masui F, Ushigome S, Fujii K: Giant cell tumor of bone: A clinicopathologic study of prognostic factors. *Pathol Int* 1998;48:723-729.
- 14 Lau CP, Ng PK, Li MS, Tsui SK, Huang L, Kumta SM: p63 regulates cell proliferation and cell cycle progression-associated genes in stromal cells of giant cell tumor of the bone. *Int J Oncol* 2013;42:437-443.
- 15 Campanacci M, Baldini N, Boriani S, Sudanese A: Giant-cell tumor of bone. *J Bone Joint Surg Am* 1987;69:106-114.
- 16 Lu Q, Lv G, Kim A, Ha JM, Kim S: Expression and clinical significance of extracellular matrix metalloproteinase inducer, EMMPRIN/CD147, in human osteosarcoma. *Oncol Lett* 2013;5:201-207.
- 17 Lee CH, Espinosa I, Jensen KC, Subramanian S, Zhu SX, Varma S, Montgomery KD, Nielsen TO, van de Rijn M, West RB: Gene expression profiling identifies p63 as a diagnostic marker for giant cell tumor of the bone. *Mod Pathol* 2008;21:531-539.
- 18 Mak IW, Turcotte RE, Popovic S, Singh G, Ghert M: AP-1 as a regulator of MMP-13 in the stromal cell of giant cell tumor of bone. *Biochem Res Int* DOI: 10.1155/2011/164197.
- 19 Mak IW, Cowan RW, Popovic S, Colterjohn N, Singh G, Ghert M: Upregulation of MMP-13 via Runx2 in the stromal cell of giant cell tumor of bone. *Bone* 2009;45: 377-386.
- 20 Wang B, Xu YF, He BS, Pan YQ, Zhang LR, Zhu C, Qu LL, Wang SK: RNAi-mediated silencing of CD147 inhibits tumor cell proliferation, invasion and increases chemosensitivity to cisplatin in SGC7901 cells *in vitro*. *J Exp Clin Cancer Res* 2010;29:61.
- 21 Kanekura T, Chen X, Kanzaki T: Basigin (CD147) is expressed on melanoma cells and induces tumor cell invasion by stimulating production of matrix metalloproteinases by fibroblasts. *Int J Cancer* 2002;99:520-528.
- 22 Kong LM, Liao CG, Fei F, Guo X, Xing JL, Chen ZN: Transcription factor Sp1 regulates expression of cancer-associated molecule CD147 in human lung cancer. *Cancer Sci* 2010;101:1463-1470.
- 23 Zhou S, Liao L, Chen C, Zeng W, Liu S, Su J, Zhao S, Chen M, Kuang Y, Chen X, Li J: CD147 mediates chemoresistance in breast cancer via ABCG2 by affecting its cellular localization and dimerization. *Cancer Lett* 2013;337:285-292.
- 24 Nabeshima K, Iwasaki H, Koga K, Hojo H, Suzumiya J, Kikuchi M: Emmprin (basigin/CD147): matrix metalloproteinase modulator and multifunctional cell recognition molecule that plays a critical role in cancer progression. *Pathol Int* 2006;56:359-367.
- 25 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;435:211-218.
- 26 Balke M, Schremper L, Gebert C, Ahrens H, Streithuerger A, Koehler G, Harges J, Gosheger G: Giant cell tumor of bone: treatment and outcome of 214 cases. *J Cancer Res Clin Oncol* 2008;134:969-978.
- 27 Khan MT, Gray JM, Carter SR, Grimer RJ, Tillman RM: Management of the giant-cell tumours of the distal radius. *Ann R Coll Surg Engl* 2004;86:18-24.
- 28 Lim YW, Tan MH: Treatment of benign giant cell tumours of bone in Singapore. *Ann Acad Med Singapore* 2005;34:235-237.
- 29 Khan SA, Kumar A, Inna P, Bakhshi S, Rastogi S: Endoprosthetic replacement for giant cell tumour of the proximal femur. *J Orthop Surg (Hong Kong)* 2009;17:280-283.
- 30 Siddiqui MA, Seng C, Tan MH: Risk factors for recurrence of giant cell tumours of bone. *J Orthop Surg (Hong Kong)* 2014;22:108-10.