Original paper

Significance of β-catenin expression for the incidence of pathological fractures in giant cell tumors of bone

Jelena Sopta[1], Nenad Lujic[2], Relja Kovacevic[1], Radoslav Davidovic[3]

[1]Institute for Pathology, Medical Faculty, University Belgrade, Serbia
[2]Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia
[3]Institute of Nuclear Sciences "Vinča", University of Belgrade, Serbia

Aim of the study is to determine the possible roles of p53, cyclin D1, B-catenin and Ki-67 in the increase in risk of fractures in patients with giant cell tumor of bone.
The study included a total of 164 patients with giant cell tumor of bone (GCTB), 21 (12.8%) with and 143 (87.2%) without fracture. The samples were analyzed immunohistochemically for expression of Ki-67, p53, cyclin D1 and β-catenin.
According to the immunohistochemical expression of p53 and Ki-67 in mononuclear stromal cells, as well as of cyclin D1 in multinuclear giant cells, there was no significant association with immunopositivity and risk of fractures. However, our research revealed that patients with cytoplasmic expression of β-catenin in stromal cells had three times more frequent occurrence of pathological fractures, which was highly statistically significant ($\chi^2 = 7.065$; $p = 0.008$). Moreover, a highly statistically significant correlation between the nuclear expression of β-catenin in giant cells and the incidence of pathological fractures was also found ($\chi^2 = 8.824$; $p = 0.003$).
The study showed that β-catenin expression highly correlates with the incidence of pathological fractures in patients with GCTB. Taking into account that β-catenin is closely linked to activation of the Wnt signaling pathway in GCTB pathogenesis, one could postulate that activation of the Wnt pathway is one of the contributing factors to locally destructive behavior of this tumor, as well as to the incidence of pathological fractures.

Key words: giant cell tumor of bone, pathologic fracture, β-catenin, Wnt pathway.

Introduction

Giant cell tumor of bone (GCTB) is a primary bone tumor composed of two types of cells: mononuclear and osteoclast-like giant multinuclear cells [1, 2]. It usually involves the end of a long bone in middle-aged patients. GCTB most commonly occurs in patients 20-40 years of age, and can be found in many sites of the body; however, a half of GCTBs occur around the knee [3, 4]. Taking into consideration its unclear biological behavior, high recurrence rate and possible pulmonary metastasizing, GCTB is one of the most controversial and widely discussed bone tumors. Pathological fractures occur at first presentation in 9.0% to 30.0% of all patients with giant cell tumor of bone. The importance of understanding the incidence of pathological fractures in GCTB is evidenced by the fact that its presence determines the therapeutic approach, primarily the type and extent of surgical intervention [5, 6, 7].

Our research involved determining the possible role of p53, cyclin D1, B-catenin and Ki-67 in the

increase in risk of fractures in patients with giant cell tumor of bone.

Material and methods

The analysis included a total of 164 GCTB samples diagnosed at the Reference Center for Bone and Joint Tumors within the Institute for Pathology of the Belgrade Faculty of Medicine. All samples belong to patients registered in the Bone Tumor Registry and treated at the Institute for Orthopedic Surgery "Banjica".

Pathological fractures were confirmed on the basis of relevant radiological and surgical data.

Immunohistochemistry

Serial sections, 5 μm thick, were cut and immunohistochemical techniques were carried out using the avidin-biotin-peroxidase complex method with an LSAB2 kit (Dako, Glostrup, Denmark). The primary antibodies used in this study were: p53 (M3629Clone 318-6-11, dilution 1 : 25; Dako), β-catenin (M3539 Clone β-catenin1, dilution 1 : 200; Dako), Ki-67 (M7240 Clone MIB-1, dilution 1 : 100; Dako), and cyclin D1 (EPR2241, ab134175, dilution 1 : 200; Abcam).

Tumor cells with nuclear staining were considered positive in immunohistochemistry for p53, cyclin D1 and Ki-67. Giant cells with nuclear and stromal cells with cytoplasmic staining were considered positive in immunohistochemistry for β-catenin. The labeled-cell count of all antibodies was determined in ten high-power fields at 200× magnification, by two blinded independent observer-pathologists. The p53 expression was evaluated qualitatively in mononuclear tumor cells. For cyclin D1 and β-catenin we observed qualitative positivity separated in giant cells to less than 15 nuclei (GC < 15) and cells to 15 or more than 15 nuclei (GC ≥ 15). The percentage of positive cells for Ki-67 was recorded semi-quantitatively and used to obtain a score on a scale of 0-3 as follows: score 0 if no positive cells; score 1 if ≤ 5% cells were positive; score 2 if 6-19% of cells were positive; score 3 if ≥ 20% positive cells. In statistics, we used uni- and multivariate analyses, and a p-value of less than 0.05 was accepted as statistically significant.

Table I. Basic demographic and clinical characteristics of patients

Parameter		N	%
Gender	male	76	46.3
	female	88	53.7
Age (years)		30.8 ±13.2; 28 (5-64)	
Duration of symptoms (months)		6.2 ±6.3; 4 (1-38)	
Pain	no	20	12.4
	yes	141	87.6
Swelling	no	28	17.4
	yes	133	82.6
Localization	femur	70	42.7
	tibia	34	20.7
	humerus	12	7.3
	scapula	2	1.2
	radius	17	10.4
	ulna	9	5.5
	pelvis	3	1.8
	spine	6	3.7
	foot	2	1.2
	hand	3	1.8
	fibula	6	3.7
Pathological fracture	no	143	87.2
	yes	21	12.8

Results

Demographic characteristics

Out of 164 patients with primary GCTB, 76 were men and 88 were women, making the gender ratio of 19 : 22. The mean age of first diagnosis was 30.8 ±13.2 years. Duration of symptoms varied from 1 day (in patients with sudden pathological fractures) to 12.6 months (Table I).

Symptoms and localization

The main symptoms were pain and swelling. The most frequent localization was the knee region – 63.4% (distal femur 42.7% and proximal tibia 20.7%) (Table I).

Fractures

Our study included 21 patients with pathological fractures and typical microscopic tumor presentation (Fig. 1A, 2). They represent 12.8% of all patients (Table I).

Immunohistochemistry

According to the immunohistochemical expression of p53 and Ki-67 in mononuclear stromal cells, as well as of cyclin D1 in multinuclear giant cells, there was no significant association with immunopositivity or risk of fractures (Tables II-IV).

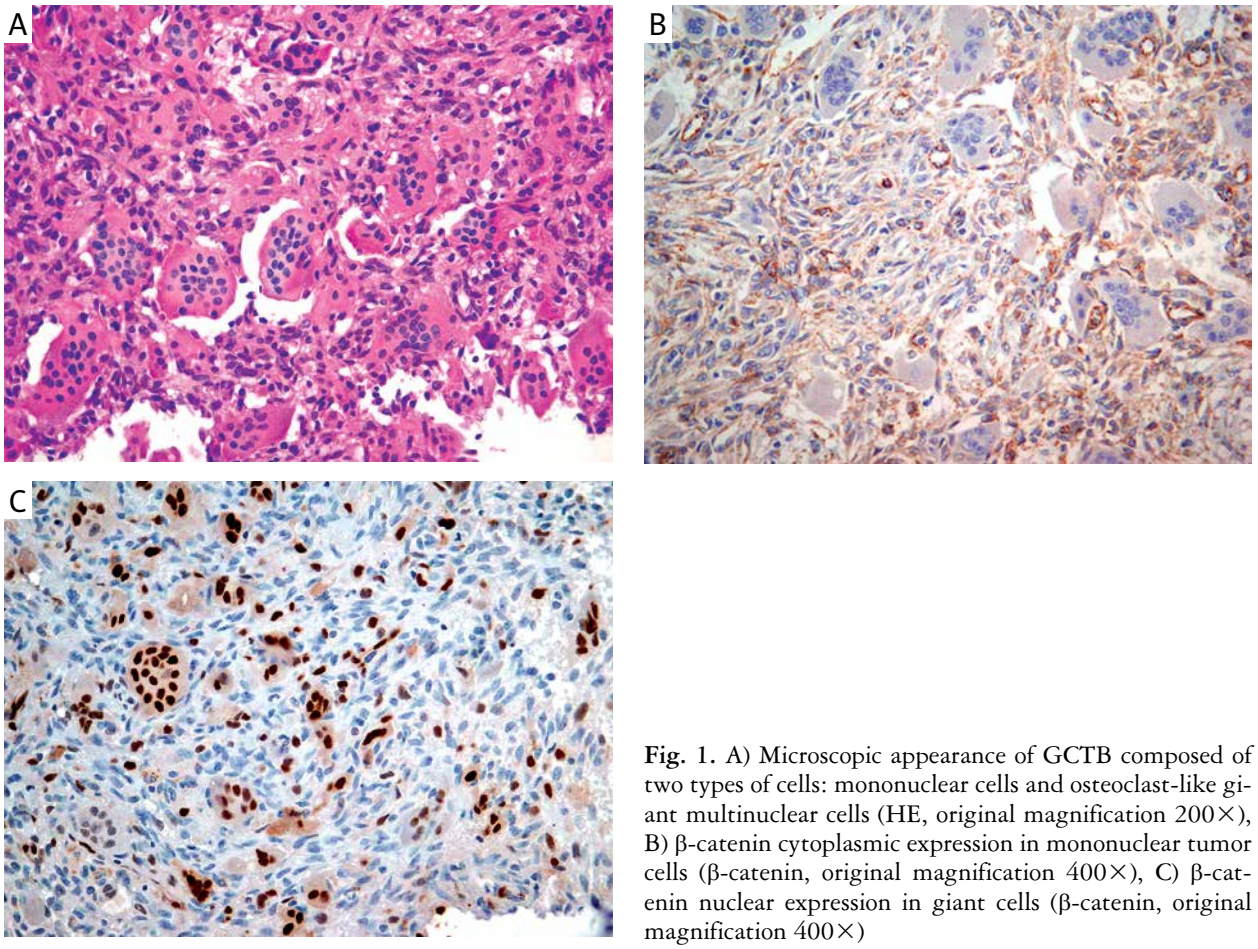

Fig. 1. A) Microscopic appearance of GCTB composed of two types of cells: mononuclear cells and osteoclast-like giant multinuclear cells (HE, original magnification 200×), B) β-catenin cytoplasmic expression in mononuclear tumor cells (β-catenin, original magnification 400×), C) β-catenin nuclear expression in giant cells (β-catenin, original magnification 400×)

Our sample confirmed a twice higher frequency of pathological fractures in patients with p53 expressed in stromal cells, but statistical analysis showed no significance of this difference ($\chi^2 = 2.705$; $p = 0.100$) (Table II).

Distributions of patients with pathological fractures were extremely similar, almost identical in each of the categories of Ki-67 positivity ($\chi^2 = 0.026$; $p = 0.872$) (Table III).

Table II. Association of pathological fractures with p53 expression

			Pathological fracture		Total
			no	yes	
p53 in stromal cells	no	N	61	5	66
		%	92.4	7.6	100.0
	yes	N	82	16	98
		%	83.7	16.3	100.0
Total		N	143	21	164
		%	87.2	12.8	100.0

$\chi^2 = 2.705$; $p = 0.100$

Cyclin D1 expression in giant cells with less than 15 nuclei was most commonly associated with pathological fractures (in 16.5% of patients). Patients with cyclin D1 positivity in giant cells with more than 15

Table III. Association of pathological fractures with Ki-67 expression

			Pathological fracture		Total
			no	yes	
Ki-67	0	N	28	4	32
		%	87.5	12.5	100.0
	≤ 5%	N	93	14	107
		%	86.9	13.1	100.0
	6-19%	N	12	2	14
		%	85.7	14.3	100.0
	≥ 20%	N	10	1	11
		%	90.9	9.1	100.0
Total		N	143	21	164
		%	87.2	12.8	100.0

$\chi^2 = 0.026$; $p = 0.872$

Table IV. Cyclin D1 as compared to pathological fractures

			Pathological fracture		Total
			no	yes	
Cyclin D1 in giant cells	0	N	37	3	40
		%	92.5	7.5	100.0
	< 15 nuclei	N	91	18	109
		%	83.5	16.5	100.0
	15+ nuclei	N	15	0	15
		%	100.0	0.0	100.0
Total		N	143	21	164
		%	87.2	12.8	100.0

$\chi^2 = 0.007$; $p = 0.933$

nuclei had no pathological fractures. Nevertheless, the difference was not statistically significant ($\chi^2 = 0.007$; $p = 0.933$) (Table IV).

By means of correlating β-catenin expression with the incidence of pathological fractures, we found a highly statistically significant correlation between these two features in our sample (Table V).

Our research revealed that patients with cytoplasmic expression of beta-catenin in stromal cells (Fig. 1B) had three times more frequent incidence of pathological fractures, which was highly statistically significant ($\chi^2 = 7.065$; $p = 0.008$).

Moreover, a highly statistically significant correlation between the nuclear expression of β-catenin in giant cells (Fig. 1C) and the incidence of pathological fractures was also found ($\chi^2 = 8.824$; $p = 0.003$).

Discussion

GCT is a locally aggressive and purely osteolytic skeletal lesion [1, 3, 4, 7]. Pathological fractures can be caused by tumor invasion and associated with stress. They represent a relatively frequent complication found in 9-30% of patients with GCTB [4, 8]. The prevalence of pathological fractures in this study was 12.8%, which was in compliance with the findings of other authors.

Causes of pathological fractures are multifactorial and still insufficiently known. An additional possible parameter leading to the incidence of pathological fractures is the activation of the Wnt pathway of carcinogenesis described in GCTB [9, 10, 11, 12]. Individual genes included in the Wnt pathway influence the destructivity of GCTB and promote cell growth. It is widely known that β-catenin has an important role in the Wnt signaling pathway, as well as in the incidence of numerous primary mesenchymal tumors of bone and soft tissue [11, 12, 13, 14].

Our research reaffirmed that β-catenin expression in the cytoplasm of mononuclear cells, as well as in giant cell nuclei, was associated with a high risk of fracture incidence with GCTB. Tumors with stromal, mononuclear cells displaying cytoplasmic positivity to β-catenin were three times more frequently manifested, both clinically and radiologically, by the incidence of pathological fractures. Nuclear expression of β-catenin in giant multinuclear, osteoclast-like cells was highly statistically correlated with the incidence of fractures. Positivity to β-catenin in GCTB cells indicated activation of the Wnt pathway in GCTB, as well as the possible importance of this signaling path-

Table V. Association of β-catenin expression in stromal and giant cells with the occurrence of pathological fractures

			Pathological fractures		
			yes	no	Total
β-catenin in stromal cells	yes	*N*	85	6	91
		%	93.4	6.6	100.0
	no	*N*	58	15	73
		%	79.5	20.5	100.0
0		*N*	92	8	100
		%	92.0	8.0	100.0
β-catenin in giant cells	< 15 nuclei	*N*	43	8	51
		%	84.3	15.7	100.0
	15+ nuclei	*N*	8	5	13
		%	61.5	38.5	100.0
Total		*N*	143	21	164
		%	87.2	12.8	100.0

way, both for the pathogenesis and for locally aggressive behavior of this primary bone tumor.

Bearing in mind that Shuxin *et al*. [15] confirmed that the JUN gene, which is a part of the Wnt pathway activated in GCTB, was in charge of promoting stromal cell growth and increasing the destructivity of this tumor, this could be used for explaining the association with pathological fractures. It is also known that the JUN gene plays a very important role in the canonical Wnt signaling pathway, primarily by interacting with β-catenin – TCFs transcriptional complex. Therefore, β-catenin expression indicates increased transcription in GCTB, as well as possible hyperactivation of the JUN gene [14, 15, 16].

In addition, this gene, which is closely linked to β-catenin, correlates with positive regulation of matrix metalloproteinases, which have a great significance for dyscoherence of tumor cells, and therefore locally aggressive biological behavior. The following matrix metalloproteinases are highly positive in GCTB: MMP-2, MMP-13 and MMP-9. The role of MMP-13 is to optimize bone resorption by giant osteoclast-like cells, whereas its high expressivity in GCTB could be responsible for the occurrence of osteolysis and pathological fractures [15, 16, 17, 18].

Both the JUN gene and β-catenin could also impact certain physiological processes in extracellular matrix, by promoting growth and emphasizing destructivity in GCTB.

Conclusions

The importance of understanding the occurrence of pathological fractures in GCTB is evident in the fact that its presence determines the therapeutic approach, primarily the type and extent of surgical intervention. The study showed that β-catenin expression highly correlates with the incidence of pathological fractures in GCTB patients. Taking into account that β-catenin is closely linked to activation of the Wnt signaling pathway in GCTB pathogenesis, one could postulate that activation of the Wnt pathway is one of the contributing factors for locally destructive behavior of this tumor, as well as for the incidence of pathological fractures.

All procedures followed were in accordance with the ethical standards and approved by the Ethics Committee (Belgrade University; School of Medicine), number of solution: 29/V-1 (11.10.2012.)

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The authors declare no conflict of interest.

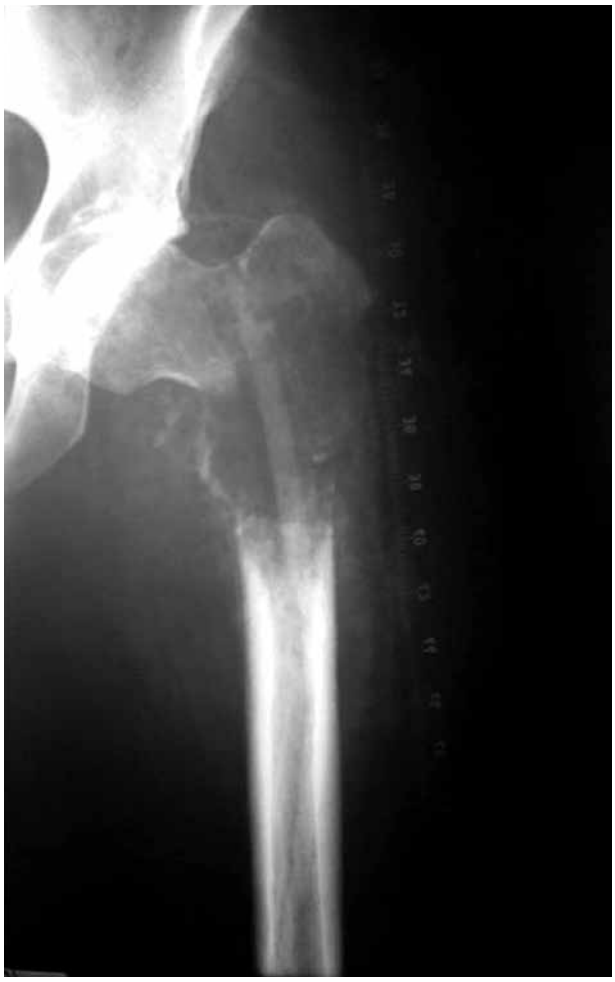

Fig. 2. Native radiography – GCTB in proximal femur with pathologic fracture

References

1. Raskin KA, Schwab JH, Mankin HJ, et al. Giant cell tumor of bone. J Am Acad Orthop Surg 2013; 21: 118-126.
2. Wulling M, Engels C, Jesse N, et al. The nature of giant cell tumor of bone. J Cancer Res Clin Oncol 2001; 127: 467-474.
3. Errani C, Ruggieri P, Asenzio MA, et al. Giant cell tumor of the extremity: a review of 349 cases from a single institution. Cancer Treat Rev 2010; 36: 1-7.
4. van der Heijden, Dijkstra S, Campanacci D, et al. Giant cell tumor with pathologic fracture: should we curette or resect? Clin Orthop Relat Res 2013; 471: 820-829.
5. Balke M, Schremper L, Gebert C et al. Giant cell tumor of bone: treatment and outcome of 214 cases. J Cancer Res Clin Oncol 2008; 134: 969-978.
6. Alberghini M, Kliskey K, Krenacs T, et al. Morphological and immunophenotypic features of primary and metastatic giant cell tumour of bone. Virchows Arch 2010; 456: 97-103.
7. Dongqi L, Jinlei Z, Yi L at al. Surgery methods and soft tissue extension are the potential risk factors of local recurrence in giant cell tumor of bone. World J Surg Oncol 2016; 14: 114-121.
8. Niu X, Zhang Q, Hao L, et al. Giant cell tumor of the extremity: retrospective analysis of 621 Chinese patients from one institution. J Bone Joint Surg Am 2012; 94:461-467.
9. Jiang HL, Jiang LM, Han WD. Wnt/β-catenin signaling pathway in lung cancer stem cells is a potential target for the development of novel anticancer drugs. J BUON 2015; 20: 1094-1100.

10. Kandel R, Li SQ, Bell R, et al. Cyclin D1 and p21 is elevated in the giant cells of giant cell tumors. J Orthop Res 2006; 24: 428-437.
11. Matsubayashi S, Nakashima M, Kumagai K, et al. Immunohistochemical analyses of beta-catenin and cyclin D1 expression in giant cell tumor of bone (GCTB): a possible role of Wnt pathway in GCTB tumorigenesis. Pathol Res Pract 2009; 205: 626-633.
12. Cowan RW, Singh G. Giant cell tumor of bone: a basic science perspective. Bone 2013; 52: 238-246.
13. de Souza PE, Paim JF, Carvalhais JN, Gomez RS. Immunohistochemical expression of p53, MDM2, Ki-67 and PCNA in central giant cell granuloma and giant cell tumor. J Oral Pathol Med 1999; 28: 54-58.
14. Steensma MR, Tyler WK, Shaber AG, et al. Targeting the giant cell tumor stromal cell: functional characterization and a novel therapeutic strategy. Plos One 2013; 8: e69101.
15. Shuxin C, Chunquan L, Bingli W, et al. Identification of differentially expressed genes and their subpathways in recurrent versus primary bone giant cell tumors. Int J Oncol 2014; 45: 1133-1142.
16. Barham W, Frump A, Sherrill T, et al. Targeting the Wnt pathway in synovial sarcoma models. Cancer Discov 2013; 3: 1286-1301.
17. Ng TL, Gown AM, Barry TS, et al. Nuclear beta-catenin in mesenchymal tumors. Mod Pathol 2005; 18: 68-74.
18. Haydon RC, Deyrup A, Ishikawa A, et al. Cytoplasmic and/or nuclear accumulation of the beta-catenin protein is a frequent event in human osteosarcoma. Int J Cancer 2002; 102: 338-342.

Address for correspondence

Jelena Sopta
Institute for Pathology
Medical Faculty
University Belgrade
Dr Subotica 1
11000 Belgrade, Serbia
e-mail: jslabic@yahoo.com