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Original article

P63 expression as a biomarker discriminating giant cell tumor of bone from other giant cell-rich bone lesions

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ABSTRACT

Introduction: Giant cell tumor of bone (GCTOB) is a locally aggressive neoplasm that accounts for 5% of all primary bone tumors. This tumor overlaps in histopathologic and radiographic presentations with different malignant, benign, and metabolic giant cell-rich lesions. The purpose of this study is to evaluate p63 expression status in giant cell tumor of bone in comparison with other giant cell-rich lesions.
Materials and methods: In a cross-sectional study we examined immunohistochemical expression of p63 in a series of 100 giant cell-rich bone lesions, including 31 giant cell tumors of bone, 14 osteosarcomas (including 3 giant cell-rich variants), 18 aneurysmal bone cysts (including one solid variant), 8 non-ossifying fibromas, 17 chondroblastomas, 8 tenosynovial giant cell tumors, and 4 brown tumors.
Results: Immunohistochemical analysis showed p63 nuclear expression in 96.8% of giant cell tumors of bone, 14.3% of osteosarcomas, 50% of non-ossifiying fibromas, 22.2% of aneurysmal bone cysts, 68.7% of chondroblastomas, 75.0% of brown tumors and none of the tenosynovial giant cell tumors. Taking into account the intensity of staining, we identified strong staining in 48.4% of giant cell tumors of bone, 35.3% of chondroblastomas and 7.1% of osteosarcomas (in 2 cases which were both giant cell-rich variants). Considering extent of staining, extensive staining was only observed in 58.0% of giant cell tumors of bone, 23.5% of chondroblastomas and 14.3% of osteosarcomas.
Conclusion: A large number of giant cell tumors of bone (96.8%) are positive for p63, which is considerably more than any other giant cell-rich lesion. However, positive staining for p63 is not specific for GCTOB and may be seen in other lesions such as chondroblastoma, non-ossifying fibroma, brown tumor, and giant cell-rich osteosarcoma. P63 is a sensitive (96.8%) and relatively specific marker for discriminating GCTOB from other types of giant cell-rich lesions. We suggest a combined scoring method for p63 IHC staining interpretation in GC-rich lesions, considering both intensity and extent of reaction, with a 2+ cut off as a more accurate marker for the diagnosis of GCTOB within the appropriate clinical context.

1. Introduction

Giant cell tumor of bone (GCTOB) is a benign neoplasm that accounts for 5% of all primary bone tumors and 20% of benign bone tumors [1]. Although generally classified as a benign lesion, GCTOB is well known for its tendency to behave aggressively [2] and rarely evolve into malignant forms, with occasional distant metastases [3]. Eighty percent of patients are between 20 and 50 years of age at the time of presentation. It is very unusual for GCTOB to occur in patients younger than 20 years or older than 50 years old [4]. It most commonly arises at the epiphysis or metaphysis of long bones, with the distal femur, proximal tibia, distal radius and proximal humerus being the most common sites affected [5–8].

GCTOB is histologically characterized by osteoclast-like giant cells in a background of mononuclear stromal cells, which comprise the neoplastic component of the tumor [7,9]. The tumor can show several histologic variations, including spindle-shaped stromal cells with a storiform pattern, paucity of giant cells, fibrosis, hemorrhage, necrosis, xanthomatous change, and secondary aneurysmal bone cyst (ABC) changes. These variations along with the tendency of a wide range of other bone lesions to be rich in giant cells, add to the challenge of differentiating GCTOB from different malignant, benign, and metabolic giant cell-containing lesions,

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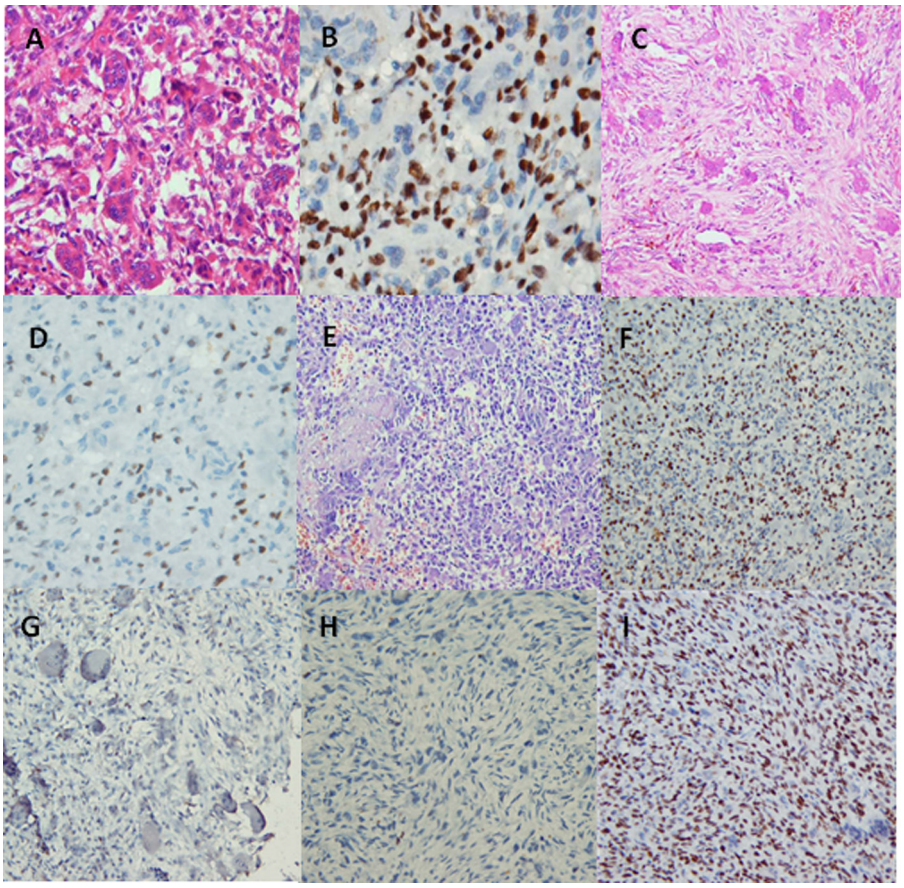

Fig. 1. IHC expression of p63 in different giant cell-rich lesions of bone and soft tissue. (A) H&E (×40) and (B) IHC staining (×40) in GCTOB demonstrates extensive and strong expression of p63 in mononuclear stromal cells. (C) H&E (×20) and (D) IHC stain for p63 (×40) in NOF exhibits focal expression of this marker. (E) H&E (×20) and (F) IHC (×20) slides of chondroblastoma reveal diffuse and strong expression of p63. (G) IHC stains in a case of ABC (×40) and (H) a case of conventional osteosarcoma (×20) with negative reaction for p63 versus (I) a giant cell-rich variant of osteosarcoma (×20) with diffuse and strong p63 expression. *A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM02731.*

especially in limited samplings. Examples of such lesions are osteosarcoma (OSA), non-ossifying fibroma (NOF), ABC, chondroblastoma (CB), tenosynovial giant cell tumor (TSGCT), and brown tumor [10,11]. Although radiological and clinical information helps in the diagnosis of GCTOB, there remain challenging cases that are difficult to differentiate. Due to different prognosis and treatment of GCTOB from the above mentioned lesions, using a biomarker to discriminate this benign aggressively behaving lesion from other morphologically similar entities seems to be essential [11–17]. Furthermore, there is currently no reliable marker available to aid in diagnosis of GCTOB.

P63 is a member of p53 tumor suppressor gene family with nuclear expression in squamous epithelium, urothelium, myoepithelial cells of mammary and salivary glands, and basal cells of prostate [18–21].

Recent studies have demonstrated immunohistochemical and molecular expression of p63 in mononuclear stromal cells of GCTOB [11–16]. The purpose of this study was to investigate p63 expression in GCTOB and to determine whether this biomarker can be used to discriminate it from other giant cell-rich lesions.

2. Materials and methods

2.1. Patients and tissue samples

A total number of 100 giant cell-rich lesions were selected from the archives of Department of Pathology at Shafa Yahyaeyan Orthopedic Hospital in Tehran from 2001 to 2013. These included 31 giant cell tumors of bone, 14 osteosarcomas (Including 3 giant cell rich variants), 18 aneurysmal bone cysts, 8 non-ossifiying fibromas, 17 chondroblastomas, 8 tenosynovial giant cell tumors, and 4 brown tumors. Two pathologists reviewed H&E-stained slides of all cases independently, along with clinical and imaging findings to confirm the diagnoses. All of the cases were surgical resection specimens. Representative formalin-fixed paraffin-embedded blocks with adequate amount of tissue were selected for Immunohistochemistry staining. Clinical and demographic data was ascertained by medical records review.

2.2. Immunohistochemical staining and statistical analysis

Immunohistochemical staining for p63 was carried out on 5 μm-thick sections from formalin-fixed paraffin-embedded tissues. The sections were deparaffinized with xylol, and dehydrated using serial dilutions of ethanol. Antigen retrieval achieved by microwave treatment with concentrated Tris-EDTA (PH = 9) buffer for 20 min. To suppress endogenous peroxidase activity, tissues were blocked with hydrogen peroxide 3%. Then, antibody staining was carried out using monoclonal mouse anti-human p63 antibody (DAKO, Clone DAK-p63, Code M7317) at a dilution of 1:50 for one hour at room temperature. The sections were counterstained with hematoxylin. Positive controls (prostatic adenocarcinoma) were carried out with all samples at the same run with the same procedure. P63 staining, demonstrating a nuclear pattern, was examined by a pathologist with bright field microscope. Any nuclear staining of stromal mononuclear cells was assumed as positive. Intensity

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Table 1
Demographic data and location of giant cell-rich lesions. *GCTOB*, giant cell tumor of bone; *OSA*, osteosarcoma; *NOF*, non-ossifying fibroma; *TSGCT*, tenosynovial giant cell tumor; *ABC*, aneurysmal bone cyst; *CB*, chondroblastoma; *SD*, standard deviation.

Tumor Type	Total	Age	Sex		Location		
	No.	Mean ± SD	Male No. (%)	Female No. (%)	Long Bones	Flat Bones	Small Bones of extremities
GCTOB	31	30.3 ± 9.7	15 (48.4)	16 (51.6)	27 (87.1)	3 (9.7)	1 (3.2)
OSA	14	21.1 ± 12.1	7 (50)	7 (50)	12 (85.7)	2 (14.3)	0 (0)
NOF	8	19.4 ± 4.9	5 (62.5)	3 (37.5)	8 (100)	0 (0)	0 (0)
TSGCT	8	27 ± 13.4	1 (12.5)	7 (87.5)	6 (75)	2 (25)	0 (0)
ABC	18	14.9 ± 5.5	8 (44.4)	10 (55.5)	13 (72.2)	3 (16.7)	2 (11.1)
CB	15	21.3 ± 5.1	11 (73.3)	5 (26.7)	12 (80)	2 (13.3)	1 (6.7)
Brown Tumor	4	40.5 ± 22.2	2 (50)	2 (50)	3 (75)	0 (0)	1 (25)

Table 2
Results of P63 immunohistochemical staining in giant cell-rich lesions.

Diagnosis	Total	p63-positive cases	Intensity of staining% (No.)			Extent of staining% (No.)		
	No.	% (No.)	1+	2+	3+	<10%-	10–50%	>50%
GCTOB	31	96.8 (30)	9.7 (3)	38.7 (12)	48.4 (15)	19.4 (6)	19.4 (6)	58.1 (18)
OSA	14	14.3 (2)	0	7.1 (1)	7.1 (1)	0	0	14.3 (2)
NOF	8	50 (4)	25 (2)	25 (2)	0	37.5 (3)	12.5 (1)	0
ABC	18	22.2 (4)	22.2 (4)	0	0	16.7 (3)	5.6 (1)	0
CB	17	68.7 (11)	5.9 (1)	23.5 (4)	35.3 (6)	41.2 (7)	0	23.5 (4)
TSGCT	8	0	0	0	0	0	0	0
Brown Tumor	4	75 (3)	50 (2)	25 (1)	0	50 (2)	25 (1)	0

of staining was scored on a scale of 0–3+ as follows: no specific staining (0), weak (1+), moderate (2+), and strong (3+). In addition, extent of staining was considered as focal (less than 10% of tumor cells), intermediate (10%–50% of tumor cells), and extensive (more than 50% of tumor cells).

The results are reported as mean ± standard deviation (SD) for the quantitative variables and percentages for the categorical variables. The groups were compared using independent sample *t*-test for normally distributed continuous variables and the chi-square test (or the Fisher exact test) for the categorical variables. A p-value of less than 0.05 was considered statistically significant. All the statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) for Windows.

3. Results

The patients' age ranged between 6 and 70 years with an average of 24.5 ± 11.6 years. No significant gender predominance was noted (49% male, 51% female, sex ratio = 1.1). Comparing mean age of patients with different tumor types showed higher mean age in Brown tumors, but lower mean age in aneurysmal bone cysts. In addition, the highest and the lowest discrete male predominance were found in chondroblastomas (64.7%) and tenosynovial giant cell tumors (12.5%), respectively. The most common locations for various types of tumors were distal femur in giant cell tumors of bone (35.5%), distal femur in osteosarcomas (42.9%), tibia in non-ossifying fibromas (87.5%), knee in tenosynovial giant cell tumors (75.0%), distal femur in aneurysmal bone cysts (16.7%), and proximal humerus (64.7%) and proximal tibia in brown tumors (50.0%) (Table 1).

Immunohistochemical analysis showed p63 nuclear expression in 96.8% of giant cell tumors of bone (Fig. 1), 14.3% of osteosarcomas, 50.0% of non-ossifying fibromas, 22.2% of aneurysmal bone cysts, 68.7% of chondroblastomas, 75.0% of Brown tumors, and none of the tenosynovial giant cell tumors (Fig. 1) (Table 2). There was one solid variant among ABCs, which showed negative immunostaining for p63.

Taking into account the intensity of staining, strong staining was seen in 48.4% of giant cell tumors of bone, 35.3% of chondroblastomas and in 7.1% of osteosarcomas, but not in other types of lesions

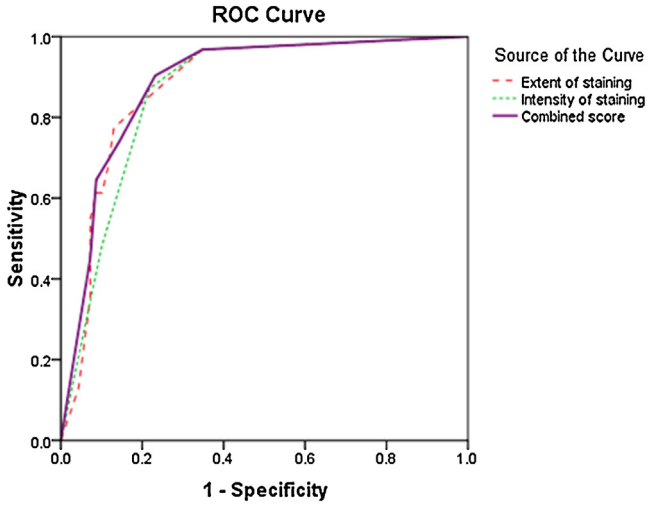

Fig. 2. ROC curve comparing different scoring methods for p63 expression in giant cell-rich lesions.

(Table 2). Considering the extent of staining for p63, diffuse staining was only observed in 58.1% of GCTs of bone, 23.5% of CBs, and 14.3% of OSAs, while intermediate staining was seen in 19.4% of GCTs of bone, 12.5% of non-ossifying fibromas, 5.6% of aneurysmal bone cysts, and 25.0% of brown tumors (Table 2). Focal immunohistochemical reaction was observed in most giant cell rich lesions and is considered less specific for GCTOB.

We used a combined scoring method considering both extent and intensity of staining (Fig. 2). In this scoring method, each tumor with either extensive or strong staining takes a score of 4+. Tumors with both intermediate extent and moderate-intensity take a score of 3. Those with focal and weak staining take a score of 1, and other combinations take a score of 2. This method is as sensitive and specific in discriminating GCTOB from other tumors as using the strength of the staining alone, but it yielded better results with greatest area under the curve based on ROC analysis. A cut off score of 2 in the combined scoring method for p63 is the most sensitive and specific way to distinguish GCTOB from other giant cell-rich lesions.

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Table 3
Specificity, sensitivity, PPV, NPV, and AUC in different interpretation methods of p63 immunohistochemical staining for diagnosing GCTOB. *PPV*, Positive predictive value; *NPV*, Negative predictive value; *AUC*, Area under the Curve.

AUC	NPV	PPV	Specificity	Sensitivity	Cut off value	Scoring system
0.865	97.8%	55.6%	65.2%	96.8%	1+	Intensity of staining
0.827	93.1%	64.3%	78.3%	87.1%	2+	
0.691	79.5%	68.2%	89.8%	48.4%	3+	
0.878	89.5%	72.7%	86.9%	77.4%	10%	Extent of staining
0.763	82.3%	75%	91.3%	58.1%	50%	
0.886	97.8%	55.6%	65.2%	96.8%	Score 1	Combined scoring
0.886	94.6%	63.6%	76.8%	90.3%	Score 2	Combined scoring
0.886	90.2%	64.1%	79.7%	80.6%	Score 3	Combined scoring

Table 3 compares sensitivity, specificity, positive predictive value, negative predictive value, and area under the curve of p63 using different scoring systems in discriminating GCTOB from other giant cell-rich lesions.

4. Discussion

GCTOB is a benign but locally aggressive neoplasm with a high recurrence rate, along with the potential for malignant transformation. Differential diagnoses include a wide spectrum of bone lesions raging from benign lesions, such as ABC and chondroblastoma, to high-grade sarcomas. These giant cell-rich tumors have overlapping histomorphologic features, and clinical and radiological data are needed to reach an accurate diagnosis. Several studies have recently evaluated the role of p63 as a marker to differentiate giant cell tumors of bone from other benign, malignant, and metabolic giant cell-rich lesions. Our study focused on the value of this marker by considering both strength and extent of its expression. We suggest a combined method of scoring for p63, in which weak positive staining in 10%–50% of cells or moderate-intensity reactivity in more than 10% of the cells supports a diagnosis of GCTOB over other differential diagnoses. Maues De Paula et al. proposed a 50% cut off [16]. Our results showed that p63 is expressed in the majority of giant cell tumors of bone (96.8%) which is consistent with findings of previous studies [11–17]. These findings show high sensitivity of p63 for the diagnosis of GCT. In contrast, we showed that p63 is indeed expressed in a broad range of benign giant cell-rich tumors of bone such as CB, NOF, and brown tumor, as claimed by some other authors [12,16]. Hence, to discriminate GCTOB from other giant cell-containing lesions, we need a more specific criterion. Except for 2 (14.3%) OSAs and 6 (35.3%) CBs, none of the other giant cell-rich tumors expressed p63 strongly or extensively, while the majority of GCTOBs (64.5%) showed strong or extensive reactivity for p63. Considering the fact that most false positive results of p63 occur in tumors with weak or focal p63 expression, a stricter cut off compensates the diagnostic pitfall of this marker.

We did not detect p63 expression in any tenosynovial giant cell tumor, which is consistent with data in the recent literature [11,15]. De la Roza [12] reported one TSGCT with p63 expression.

In our study, 68.7% of chondroblastomas expressed p63, 35.5% with a strong, and 23.5% with an extensive reaction. Some authors have similarly reported such a high rate of p63 expression in CBs [12,16], which suggests that chondroblastoma contributes to the greatest part of positive reactions seen in other giant cell-rich lesions other than GCT and negatively affects the specificity of this marker for GCTOB. Immunohistochemical staining for S100 is useful in supporting the diagnosis of CB over GCTOB. Owing to our results, a cocktail immunohistochemical staining panel comprising both p63 and S100 yields an excellent sensitivity (97%) and positive predictive value (70%) in distinction of GCTOB from other giant cell-rich lesions.

The presence of p63 positive cells is useful in supporting a diagnosis of giant cell-rich tumor of bone. However, p63 positivity is not specific for giant cell tumor of bone especially when it is weak or focal. In some other lesions such as chondroblastoma and giant cell-rich osteosarcomas, we may encounter strong staining which limits usefulness of p63 as a discriminating marker for giant cell tumor of bone. A final diagnosis cannot be made without due consideration of all clinical, radiological and pathological data. Future studies with larger sample size (especially for giant cell rich variant of osteosarcoma) are required to investigate the definite role of p63 as a discriminating marker.

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