Modern Pathology (2008) 21, 369–375

www.modernpathology.org

Giant cell tumor of bone express p63

Brendan C Dickson[1], Shu-Qiu Li[1], Jay S Wunder[2], Peter C Ferguson[2], Behnam Eslami[3,*], Joel A Werier[4], Robert E Turcotte[5] and Rita A Kandel[1]

[1]*Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada;* [2]*University Musculoskeletal Oncology Unit, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada;* [3]*Department of Oral Medicine, Infection & Immunity, Harvard School of Dental Medicine, Boston, MA, USA;* [4]*Division of Orthopaedic Surgery, The Ottawa Hospital, Ottawa, ON, Canada and* [5]*Department of Orthopedic Surgery, McGill University Health Centre, Montreal, QC, Canada*

p63 contributes to skeletal development and tumor formation; however, little is known regarding its activity in the context of bone and soft tissue neoplasms. The purpose of this study was to investigate p63 expression in giant cell tumor of bone and to determine whether it can be used to discriminate between other giant cell-rich tumors. Seventeen cases of giant cell tumor of bone were examined to determine the cell type expressing p63 and identify the isoforms present. Total RNA or cell protein was extracted from mononuclear- or giant cell-enriched fractions or intact giant cell tumor of bone and examined by RT-PCR or western blot, respectively. Immunohistochemistry was used to evaluate p63 expression in paraffin embedded sections of giant cell tumor of bone and in tumors containing multinucleated giant cells, including: giant cell tumor of tendon sheath, pigmented villonodular synovitis, aneurysmal bone cyst, chondroblastoma, and central giant cell granuloma. The mononuclear cell component in all cases of giant cell tumor of bone was found to express all forms of TAp63 (α, β, and γ), whereas only low levels of the TAp63 α and β isoforms were detected in multinucleated cells; ΔNp63 was not detected in these tumors. Western blot analysis identified p63 protein as being predominately localized to mononuclear cells compared to giant cells. This was confirmed by immunohistochemical staining of paraffin-embedded tumor sections, with expression identified in all cases of giant cell tumor of bone. Only a proportion of cases of aneurysmal bone cyst and chondroblastoma showed p63 immunoreactivity whereas it was not detected in central giant cell granuloma, giant cell tumor of tendon sheath, or pigmented villonodular synovitis. The differential expression of p63 in giant cell tumor of bone and central giant cell granuloma suggest that these two tumors may have a different pathogenesis. Moreover, p63 may be a useful biomarker to differentiate giant cell tumor of bone from central giant cell granuloma and other giant cell-rich tumors, such as giant cell tumor of tendon sheath and pigmented villonodular synovitis.
Modern Pathology (2008) **21,** 369–375; doi:10.1038/modpathol.2008.29; published online 29 February 2008

Keywords: bone tumor; giant cell tumor of bone; p63

Giant cell tumor of bone is a primary bone neoplasm occurring most frequently in young adults, with a slight female predominance.[1] These tumors can be locally aggressive with a tendency for recurrence.[2] Lung metastases occur infrequently; more rarely, these tumors behave as a sarcoma.[2] In previous studies we observed that the giant cell component of these tumors overexpressed cyclin D1 protein.[3] Compared to the mononuclear component, the giant cells also had higher levels of p21 and more p21 bound to cyclin D1.[4] p21 may explain the cyclin D1 accumulation, but the mechanism leading to increased p21 expression is unknown. A potential mechanism regulating this process might be through p63, which has been shown to activate transcription of p21.[5,6] Further, p63 is also known to induce expression of Jagged1[7] which is involved in Notch signaling and osteoclastogenesis.[8]

p63 is a member of the gene family that includes p53 and p73. It can arise from two different transcriptional start sites, encoding proteins either with (TAp63) or without (ΔNp63) a transactivating domain. The C terminus end can also undergo alternative splicing (α, β, or γ), thereby resulting in six potential isoforms.[9–11] The isoforms differ in their transcriptional activity and function and can be expressed in normal and neoplastic tissues.[9–17] p63 plays a role in skeletal development as mutations in p63 in humans lead to limb abnormalities (limb–mammary syndrome) and null p63 mice

Correspondence: Dr RA Kandel, MD, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Room 6-500, 600 University Avenue, Toronto, Ontario, Canada M5G 1X5.
E-mail: rkandel@mtsinai.on.ca

*Current address: Department of Oral Medicine, Infection & Immunity, Harvard School of Dental Medicine, Boston, MA, USA
Received 06 March 2007; revised 26 April 2007; accepted 07 May 2007; published online 29 February 2008

exhibit skeletal defects.[18] Alterations in p63 expression have been detected in a variety of tumors and have been implicated in tumor pathogenesis. To our knowledge, in tumors of bone and soft tissue p63 expression has only been described in osteosarcoma, where it is postulated to contribute to the development of these tumors.[19]

Bone and soft tissue neoplasms containing multinucleated giant cells represent a heterogeneous group of benign and malignant tumors. Differentiation among these tumors based on morphology alone can be challenging, particularly in instances of limited sampling, such as with needle-core biopsies. The purpose of this study was to determine whether giant cell tumor of bone express p63, and to examine whether p63 can be used as a biomarker to discriminate giant cell tumor of bone from other giant cell-rich tumors.

Materials and methods

Fresh Tissue

Fresh giant cell tumor of bone tissue was obtained during surgery from 17 consecutive patients. Consent was obtained prior to surgery and the use of the tissue had been approved by the individual institutional research ethics boards. In all cases, representative tissue was fixed in 10% buffered formalin, paraffin-embedded, and 5 μm sections cut and stained with hematoxylin and eosin for histological assessment. Tumor tissue was either immediately frozen in liquid nitrogen and stored at -80°C until further analyzed ($n=6$), or processed for cell culture ($n=12$).

Cell Culture

To isolate the cells, tissue was digested in 0.3% collagenase and 0.3% dispase for up to 4 h at 37°C as described previously.[4] The cells were plated in monolayer culture overnight in αMEM supplemented with 10% fetal bovine serum. To separate the mononuclear cells from giant cells, the cultures were subjected to a short period (approximately 3 min) of trypsin digestion (0.2%; Gibco BRL, Burlington, Canada). The majority of the mononuclear cells were collected and processed for RNA or protein extraction as described below. This was considered the mononuclear-enriched fraction. The giant cell-enriched component, which remains attached to the dish, was scraped directly into the appropriate extraction solution.

Preparation of Total Cell Lysates and Western Blot Analysis

Proteins were extracted from the mononuclear or giant cell-enriched fractions ($n=7$ cases) with RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% Na-deoxycholate, 0.1% SDS, 2 mM EDTA, 1 mM Na_3VO_4, 5 mM NaF containing protease inhibitor cocktail tablet (Roche Applied Science, Penzberg, Germany) on ice for 30 min. The extracts were stored at -80°C until further analyzed. Protein concentration was determined using a protein assay kit (Pierce, Rockford, USA) according to the manufacturer's instructions.

In all 30 μg of the cell lysate was separated by electrophoresis on 8% SDS-polyacrylamide gels and electroblotted to nitrocellulose membranes (Pall Gelman Laboratory, Ann Arbor, MI, USA). The blots were incubated with antibody reactive with p63 (1:250, Dakocytomation, Mississauga, Canada) overnight at 4°C. Immunodetection was performed using HRP-conjugated goat anti-mouse IgG secondary antibodies (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) which was visualized using ECL Plus Western Blotting Detection System (Amersham Pharmacia Biotech, Buckinghamshire, UK) according to the manufacturer's instructions. The blots were stripped and re-probed for actin (1:10 000, AC-15, Sigma Aldrich, St Louis, MO, USA) to ensure equal loading. Protein expression was semi-quantified by densitometry using LabWorks 4.0 Image analysis software program (UVP Bioimaging Systems, Upland, CA, USA).

RNA Extraction and RT-PCR

Total RNA was isolated either from tumor or the mononuclear or giant cell-enriched fractions using RNeasy Mini kit (Qiagen) and treated with RNase free DNase I (Qiagen) according to the manufacturer's instructions. This approach was taken because antibodies to all the different isoforms are not available. Reverse transcription of RNA was done in a final volume of 25 μl containing 1 $\times$ RT-PCR buffer, 5.5 mM MgCl, 500 μM of dNTP, 2.5 μM random hexamer, 0.4 U/μl of Rnase inhibitor, 1.25 U/μl of reverse transcriptase and 1 μg of RNA (Gene Amp Gold RNA Core Kit, PE Biosystems, Mississauga, Canada). All PCRs were performed using primers specific to p63 isotypes using conditions previously described to ensure the analysis was performed in the linear range of the reaction.[20] Each PCR was run in 25 μl containing PCR buffer, 1.5 mM $MgCl_2$, 200 μM of dNTP, 300 μM of each primer, 1U Qiagen Hot Start Taq and 1 μl of cDNA. The thermal cycle conditions were an initial 95°C for 15 min, followed by 35 cycles consisting of 95°C for 50 s, 56°C for 1 min, 72°C for 1 min. 18S rRNA served as the housekeeping gene.

Immunohistochemical Staining of Tumors

Cases of giant cell tumor of bone ($n=14$), giant cell tumor of tendon sheath ($n=13$), pigmented villonodular synovitis ($n=9$), chondroblastoma ($n=11$) and aneurysmal bone cyst ($n=7$) were identified

and subsequently retrieved from the files of Mount Sinai Hospital, according to institutional consent. All cases of central giant cell granuloma ($n=12$), were contributed separately by one author (BE). Each of the cases had been fixed in formalin and embedded in paraffin for a period ranging from several months to years. All cases were reviewed to confirm the diagnosis, and excluded if there was disagreement from the initial diagnosis or insufficient tissue was available for immunohistochemical analysis.

Tissue sections were cut from paraffin blocks (5 μm) and incubated, after microwave treatment (10 mM citrate buffer, pH 6.0 for 15 min), with antibody reactive with p63 (dilution 1:1000, LabVision Corp, Freemont, CA, USA), which recognizes all isoforms, for 1 h at room temperature. Immunoreactivity was visualized following sequential incubation with secondary antibody (dilution 1:200; Vector Laboratories, Burlington, Canada), avidin-biotin peroxidase complex and 3,3′diaminobenzidine (Sigma-Aldrich, St Louis, MO, USA). The sections were counterstained with hematoxylin. Lesions exhibiting $\geq 5\%$ of cells positive for p63 were regarded as positive.

Results

Expression of p63 mRNA in Giant Cell Tumor of Bone

Frozen tissue from six cases of giant cell tumor of bone were evaluated for p63 expression. All tumors expressed the TAp63 isoform of p63 and no ΔNp63 isoforms were detected (Figure 1). To ensure that the absence of ΔNp63 was not an artifact of our methodology or the primers selected, we examined for p63 expression in lung cancer cell lines (NCI-H358, ADC; NCI-H520 (SQCC), ATCC) which are known to express this isoform. ΔNp63 expression was detected (data not shown) confirming that giant cell tumor of bone does not express this isoform. The tumor cells expressed all three alternatively spliced variants (α, β, and γ). Similarly when the tumor was fractionated into giant cell-enriched and mononuclear cell-enriched components a similar pattern was observed (Figure 2). The mononuclear cells of all tumors expressed all three of the TAp63 isoforms (α, β, and γ). In contrast the giant cells expressed low levels, relative to the mononuclear cells, of TAp63 α and β, and no γ isoform was detected. No ΔNp63 isoforms were detected in either the giant cell or the mononuclear cell-enriched populations.

Expression of p63 Protein in Giant Cell Tumor of Bone

Total cell protein extracted from either the giant cell-enriched or the mononuclear-enriched fractions were evaluated for p63 expression by western blot analysis. p63 was detected in all the mononuclear

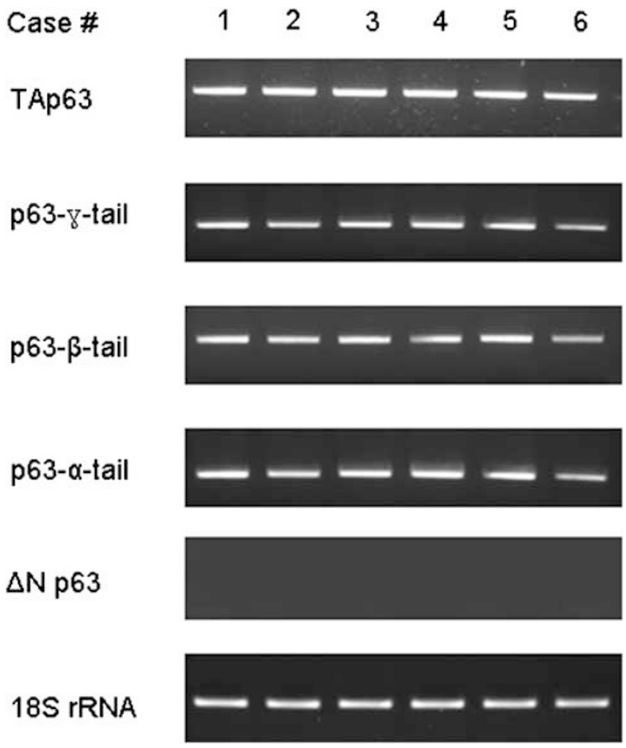

Figure 1 p63 expression in giant cell tumor of bone. RNA was extracted from intact tumors and examined for the expression of all isoforms of p63 by RT-PCR. This is an ethidium bromide-stained gel showing expression patterns in six representative tumors.

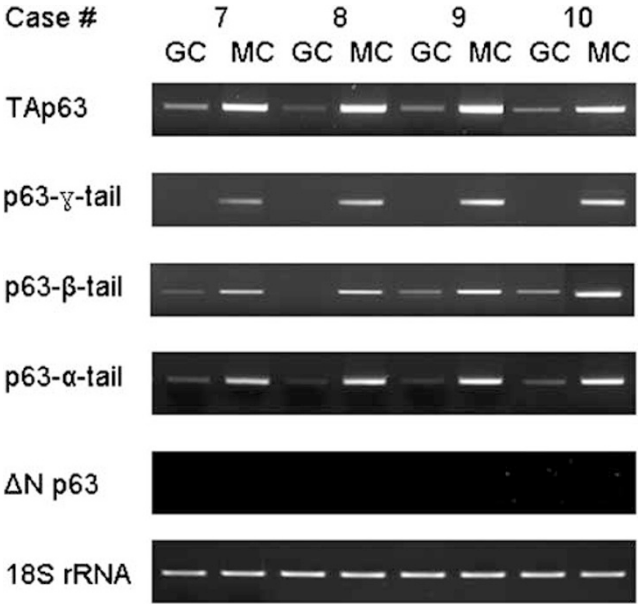

Figure 2 Distribution of p63 expression in giant cell tumor of bone. RNA was extracted from giant cell-rich (GC) and mononuclear-enriched (MC) fractions of giant cell tumor of bone as described under the methods. Each fraction was examined for the expression of all isoforms of p63 by RT-PCR. This is an ethidium bromide-stained gel showing the expression patterns in four tumors. 18S rRNA served as a housekeeping gene to ensure equal loading of DNA.

cell-enriched extracts and the giant cell-enriched fraction expressed none or low levels of p63 protein. Only a single protein band of approximately 80 kDa in size was detected (Figure 3).

p63 Protein Expression in Neoplasms Containing Giant Cells

Immunostaining demonstrated nuclear expression of p63 in the mononuclear component of all (100%) of the cases of giant cell tumor of bone (Figure 4); this ranged from 15–85% of mononuclear cells (mean 43.6%), and staining was predominantly moderate-strong in intensity (Table 1). Three of 10 (30.0%) cases of chondroblastomas expressed p63 within the mononuclear cell component; this ranged from 7–75% of cells (mean 34.0%), and staining was predominantly mild-moderate in intensity. Two of seven (28.6%) cases of aneurysmal bone cyst expressed p63 within the mononuclear cell component; this ranged from 15–30% of cells (mean 22.5%), and staining was predominantly mild-moderate in intensity. Occasional cells exhibited a mild cytoplasmic blush, which was regarded as negative for p63 staining. Among the multinucleated cells isolated nuclei infrequently demonstrated weak staining. No p63 immunoreactivity was detected in any of the cases of central giant cell granuloma ($n = 12$). Other giant cell-containing soft tissue tumors such as pigmented villonodular synovitis ($n = 9$) and giant cell tumor of tendon sheath ($n = 13$) were also negative (Figure 5).

Discussion

In this study, we demonstrate that giant cell tumor of bone express p63. These tumors express all three isoforms (α, β, and γ) of TAp63 but no ΔNp63 isoform was detected. As we were able to detect ΔNp63 in lung cancer cell lines known to express this isoform, this confirmed that its absence in giant cell tumor of bone was genuine and not a methodological artifact. It was further demonstrated, using immunohistochemistry for p63, that this protein may be useful as a biomarker to differentiate giant cell tumor of bone from central giant cell granuloma as well as other giant cell-rich tumors such as giant cell tumor of tendon sheath and pigmented villonodular synovitis, as these did not express p63. Interestingly p63 mRNA was detected predominately in mononuclear cells. Giant cells had low

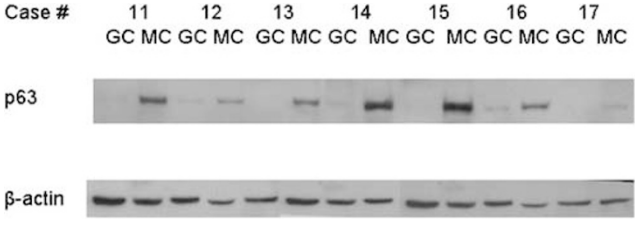

Figure 3 Distribution of p63 protein in giant cell tumor of bone. Total cell protein was extracted from giant cell (GC) and mononuclear-enriched (MC) fractions of giant cell tumor of bone as described under the methods. The protein extracts were separated by SDS-PAGE, electroblotted, and immunoblotted for p63. This western blot shows p63 expression patterns in seven tumors. The blot was stripped and reprobed for β-actin to ensure there was equal loading of proteins.

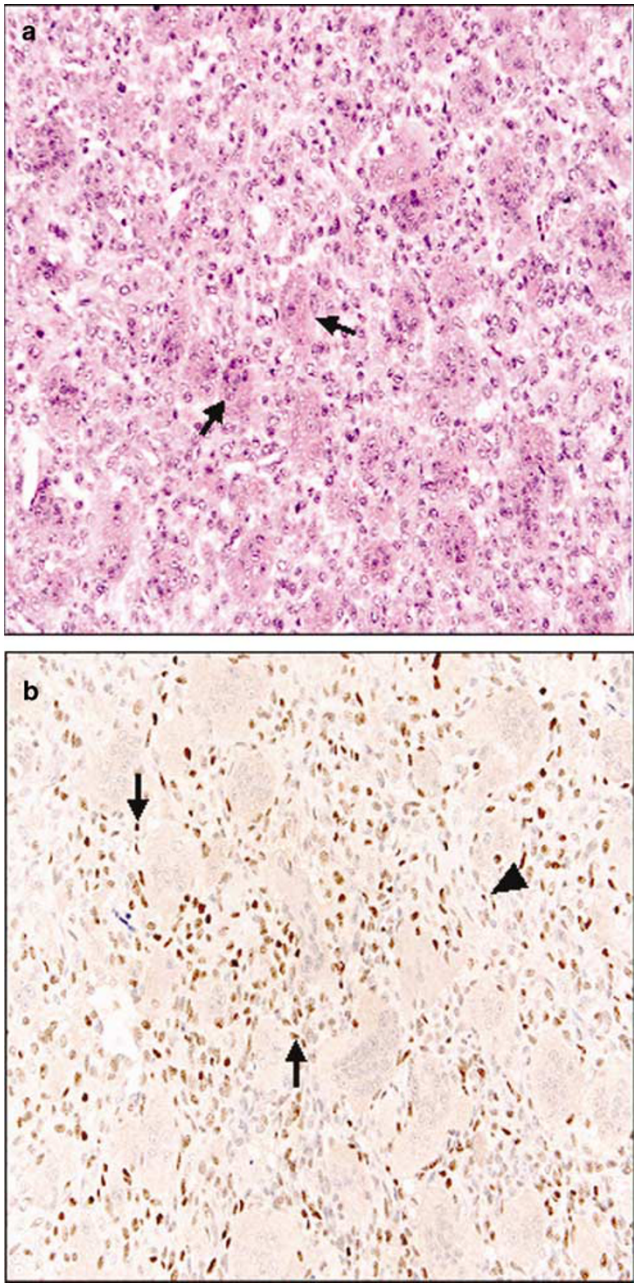

Figure 4 Evaluation of p63 protein expression in giant cell tumor of bone. (**a**) Photomicrograph showing a typical giant cell tumor consisting of numerous giant cells (↑) distributed amongst mononuclear cells. (**b**) Immunostaining for p63 in a paraffin-embedded tissue section of a representative case of giant cell tumor of bone shows expression predominately in mononuclear cells. (↑). Occasionally giant cells show protein overexpression (▲). (**a**)**:** hematoxylin and eosin: (**b**): immunoperoxidase with hematoxylin counterstain; For all images, original magnification × 200).

Table 1 Percentage of positive nuclei and intensity of staining of mononuclear cell component of giant cell tumors

Case no.	*Immunostaining of mononuclear cells*	
	Positive (%)	*Intensity*
1	15	Moderate
2	65	Strong
3	40	Moderate
4	15	Moderate
5	60	Strong
6	60	Strong
7	40	Moderate
8	85	Moderate
9	60	Strong
10	55	Moderate
11	30	Moderate
12	30	Moderate
13	15	Moderate
14	40	Mild-moderate

There was negligible nuclear staining within the multinucleated giant cell component of the tumors.

p63 gene expression levels compared to mononuclear cells and they showed differential isoform expression, as the TAp63γ variant was not detected in these cells. Protein expression reflected the pattern of gene expression, as p63 protein was detected predominately in mononuclear cells when compared to giant cells by both western blot analysis and immunostaining of intact tumor sections. The latter observation confirmed that the differential cell expression of p63 was not an artifact of cell isolation and culture. Although occasional giant cells contained nuclei which were positive for p63, it is likely this represents a nucleus from a mononuclear cell that recently fused with the giant cell especially as the immunoreactivity was usually weak. The presence of p63 in giant cell tumor of bone was not entirely unexpected as it has been detected in osteosarcomas and in bone marrow cells.[19,20] However in those studies as RNA from the entire tissue was examined it is not known if p63 was preferentially expressed in any particular cell type.

The mechanism(s) leading to p63 protein overexpression in giant cell tumor of bone is not known. It is possible that p63 gene amplification may be the cause of p63 over-expression as amplification has been detected in some squamous cell cancers of the head and neck and lung.[21,22] However as giant cells form by fusion of mononuclear cells[23,24] if the p63 gene was amplified it would be expected that the p63 mRNA levels in the giant cells would be similar to those in mononuclear cells. This suggests that gene amplification is likely not the cause of p63 overexpression in these tumors but it will be necessary to confirm this by determining p63 gene copy number. p63 mutation(s) could potentially lead to p63 protein stabilization; however, in contrast to p53, p63 mutations have not been identified to date in human tumors and thus is less likely to be the underlying mechanism leading to protein overexpression. Determination of the exact mechanism regulating p63 expression in giant cell tumor of bone will have to wait until the p63 regulatory mechanisms have been fully delineated.

On the basis of this study it is not clear whether p63 contributes to the pathogenesis of giant cell tumor of bone, or if it is present as just a 'bystander.' Based on its function in the cell, p63 could contribute to tumorigenesis in a number of ways. It is known to bind and in some instances inactivate p53;[9,10] however, in other tumors this effect appears to be mediated by the ΔNp63 isoform, which is not expressed in giant cell tumor of bone. Alternatively p63 could regulate cell death or proliferation. p63 can induce expression of molecules such as CD95 and TRAIL which influence apoptosis,[25–28] or inhibit proliferation by blocking binding of the NF-Y transcription factor which is required for transcription of cyclin B and cdk1 genes involved in regulating the cell cycle.[29] Determination of the functional significance of p63 in giant cell tumor of bone requires further study.

Immunostaining for p63 demonstrated expression in all cases of giant cell tumor of bone examined and a substantial number of cases of aneurysmal bone cyst and chondroblastoma; conversely, it was not detected in central giant cell granuloma, giant cell tumor of tendon sheath, or pigmented villonodular synovitis. The relationship of giant cell tumor of bone and central giant cell granuloma has long been controversial. For example, in a previous study we have shown little difference in terms of cycle markers between these two entities.[30] However, the absence of p63 expression in central giant cell granuloma suggests these tumors may have a pathogenesis that differs from that of giant cell tumor of bone. Interestingly, the giant cell component of giant cell tumor of salivary gland has similar histological features to giant cell tumor of bone, but does not express p63,[31] lending support to suggestions that this tumor is also unrelated to giant cell tumor of bone. The observation that giant cell tumor of tendon sheath and pigmented villonodular synovitis were both negative for p63 expression is consistent with the belief that these lesions have a shared pathogenesis.[32]

It has been suggested central giant cell reparative granuloma is a solid variant of aneurysmal bone cyst.[33,34] In this respect, it is noteworthy that the majority of aneurysmal bone cysts examined failed to express p63 suggesting they may be related tumors. However, the presence of p63 expression in some cases of aneurysmal bone cyst is as yet unexplained. It is possible that the lesions may have had a secondary component of giant cell tumor of bone that was unsampled, as these tumors are often known to co-exist.[35] This is supported by our observation that more tumors which had combined features of giant cell tumor of bone and aneurysmal bone cyst express p63 than tumors composed of

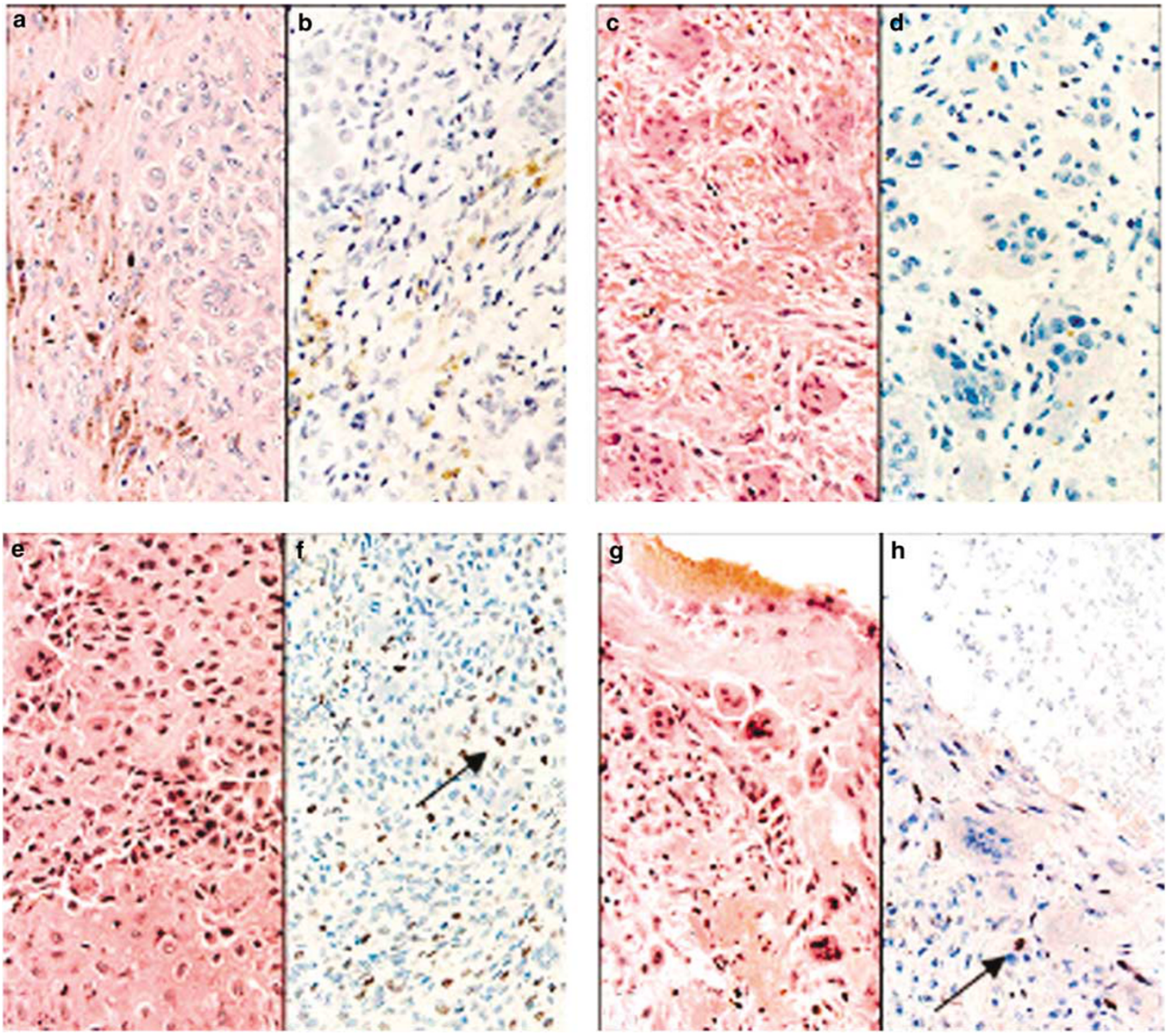

Figure 5 Immunohistochemical staining for p63 in neoplasms containing giant cells. (**a**) Giant cell tumor of tendon sheath, with (**b**) none of the mononuclear cells staining for p63. Refractile cytoplasmic/extracytoplasmic material is hemosiderin pigment. (**c**) Central giant cell granuloma, with none (**d**) of the mononuclear cells staining for p63. (**e**) Chondroblastoma with some (**f**) mononuclear cells showing nuclear expression of p63 **(↑).** (**g**) Aneurysmal bone cyst with a small percentage (**h**) of mononuclear cells showing nuclear expression of p63 **(↑). a**, **c**, **e**, **g:** H&E (left panel), **b**, **d**, **f**, **h:** immunoperoxidase staining for p63. For all images, original magnification $\times 200$.

aneurysmal bone cyst alone (data not shown). Alternatively, it is possible that p63 expression is a feature of some bone lesions, which is why it was not detected in giant cell-rich soft tissue lesions.

In summary, giant cell tumor of bone express TAp63. All isoforms of TAp63 (α, β, and γ) were observed in the mononuclear cells but only low levels of the TAp63 α and β isoforms were detected in giant cells. ΔNp63 isoforms were not detected in any of the cells in these tumors. The lack of p63 expression in central giant cell granuloma suggests that these tumors are different from giant cell tumor of bone and raises the possibility that p63 may be involved in the pathogenesis of giant cell tumor of bone but determination of its exact role requires further investigation. Furthermore, the restricted pattern of p63 expression may have translational application in the clinical setting by aiding distinction between morphologically similar lesions in situations where tissue sampling is limited, such as with needle-core biopsies, since the presence of p63 expression appears to differentiate giant cell tumor of bone from central giant cell granuloma and other giant cell-rich lesions, such as giant cell tumor of tendon sheath and pigmented villonodular synovitis. Further investigation using a larger sample size is necessary to confirm the clinical utility of these observations.

Acknowledgements

This work was supported by a CIHR-IHRT grant. We thank Trish Wegrynowski for assistance with the immunohistochemical studies and Marie Maguire for secretarial assistance.

References

1 Reid R, Banerjee SS, Sciot R. Giant cell tumour. In: Fletcher CDM, Unni KK, Mertens F (eds). Pathology & Genetics: Tumours of Soft Tissue and Bone. IARC Press: Lyon, 2002, pp 310–312.
2 Mendenhall WM, Zlotecki RA, Scarborough MT, *et al.* Giant cell tumor of bone. Am J Clin Oncol 2006;29:96–99.
3 Kauzman A, Li SQ, Bradley G, *et al.* Cyclin alterations in giant cell tumor of bone. Mod Pathol 2003;16:210–218.
4 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.
5 Wu G, Nomoto S, Hoque MO, *et al.* DeltaNp63alpha and TAp63alpha regulate transcription of genes with distinct biological functions in cancer and development. Cancer Res 2003;63:2351–2357.
6 Dietz S, Rother K, Bamberger C, *et al.* Differential regulation of transcription and induction of programmed cell death by human p53-family members p63 and p73. FEBS Lett 2002;525:93–99.
7 Ross DA, Kadesch T. Consequences of Notch-mediated induction of Jagged1. Exp Cell Res 2004;296:173–182.
8 Yamada T, Yamazaki H, Yamane T, *et al.* Regulation of osteoclast development by Notch signaling directed to osteoclast precursors and through stromal cells. Blood 2003;101:2227–2234.
9 Moll UM, Slade N. p63 and p73: roles in development and tumor formation. Mol Cancer Res 2004;2:371–386.
10 Flores ER. The Roles of p63 in cancer. Cell Cycle 2007;6:300–304.
11 Yang A, Kaghad M, Wang Y, *et al.* p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Mol Cell 1998;2:305–316.
12 Koker MM, Kleer CG. p63 expression in breast cancer: a highly sensitive and specific marker of metaplastic carcinoma. Am J Surg Pathol 2004;28:1506–1512.
13 Koga F, Kawakami S, Fujii Y, *et al.* Impaired p63 expression associates with poor prognosis and uroplakin III expression in invasive urothelial carcinoma of the bladder. Clin Cancer Res 2003;9:5501–5507.
14 Pelosi G, Pasini F, Olsen Stenholm C, *et al.* p63 immunoreactivity in lung cancer: yet another player in the development of squamous cell carcinomas? J Pathol 2002;198:100–109.
15 Unger P, Ewart M, Wang BY, *et al.* Expression of p63 in papillary thyroid carcinoma and in Hashimoto's thyroiditis: a pathobiologic link? Hum Pathol 2003;34:764–769.
16 Geddert H, Kiel S, Heep HJ, *et al.* The role of p63 and deltaNp63 (p40) protein expression and gene amplification in esophageal carcinogenesis. Hum Pathol 2003;34:850–856.
17 Di Como CJ, Urist MJ, Babayan I, *et al.* p63 expression profiles in human normal and tumor tissues. Clin Cancer Res 2002;8:494–501.
18 Brunner HG, Hamel BC, Bokhoven Hv H. P63 gene mutations and human developmental syndromes. Am J Med Genet 2002;112:284–290.
19 Park HR, Kim YW, Park JH, *et al.* Low expression of p63 and p73 in osteosarcoma. Tumori 2004;90:239–243.
20 Vincek V, Knowles J, Li J, *et al.* Expression of p63 mRNA isoforms in normal human tissue. Anticancer Res 2003;23:3945–3948.
21 Yamaguchi K, Wu L, Caballero OL, *et al.* Frequent gain of the p40/p51/p63 gene locus in primary head and neck squamous cell carcinoma. Int J Cancer 2000;86:684–689.
22 Massion PP, Taflan PM, Rahman SM, *et al.* Role of p63 amplification and overexpression in lung cancer development. Chest 2004;125:102S.
23 Vignery A. Macrophage fusion: the making of osteoclasts and giant cells. J Exp Med 2005;202:337–340.
24 Hosaka M, Hatori M, Smith R, *et al.* Giant cell formation through fusion of cells derived from a human giant cell tumor of tendon sheath. J Orthop Sci 2004;9:581–584.
25 Atkins GJ, Haynes DR, Graves SE, *et al.* Expression of osteoclast differentiation signals by stromal elements of giant cell tumors. J Bone Miner Res 2000;15:640–649.
26 Lau YS, Sabokbar A, Gibbons CL, *et al.* Phenotypic and molecular studies of giant-cell tumors of bone and soft tissue. Hum Pathol 2005;36:945–954.
27 Kakizoe M. Apoptosis in giant cell tumors of bone. Kurume Med J 2000;47:125–134.
28 Huang L, Xu J, Li K, *et al.* Thapsigargin potentiates TRAIL-induced apoptosis in giant cell tumor of bone. Bone 2004;34:971–981.
29 Jung MS, Yun J, Chae HD, *et al.* p53 and its homologues, p63 and p73, induce a replicative senescence through inactivation of NF-Y transcription factor. Oncogene 2001;20:5818–5825.
30 Kauzman A, Li SQ, Bradley G, *et al.* Central giant cell granuloma of the jaws: assessment of cell cycle proteins. J Oral Pathol Med 2004;33:170–176.
31 Tse LL, Finkelstein SD, Siegler RW, *et al.* Osteoclast-type giant cell neoplasm of salivary gland. A microdissection-based comparative genotyping assay and literature review: extraskeletal ''giant cell tumor of bone'' or osteoclast-type giant cell ''carcinoma''? Am J Surg Pathol 2004;28:953–961.
32 Rubin BP. Tenosynovial giant cell tumor and pigmented villonodular synovitis: a proposal for unification of these clinically distinct but histologically and genetically identical lesions. Skeletal Radiol 2007;36:267–268.
33 Ilaslan H, Sundaram M, Unni KK. Solid variant of aneurysmal bone cysts in long tubular bones: giant cell reparative granuloma. AJR Am J Roentgenol 2003;180:1681–1687.
34 Oda Y, Tsuneyoshi M, Shinohara N. 'Solid' variant of aneurysmal bone cyst (extragnathic giant cell reparative granuloma) in the axial skeleton and long bones. A study of its morphologic spectrum and distinction from allied giant cell lesions. Cancer 1992;70:2642–2649.
35 Rosenberg AE, Nielsen GP, Fletcher JA. Aneurysmal bone cyst. In: Fletcher CDM, Unni KK, Mertens F (eds). Pathology & Genetics: Tumours of Soft Tissue and Bone. IARC Press: Lyon, 2002, pp 338–339.