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ORIGINAL ARTICLE

Smooth muscle actin expression in primary bone tumours

F. Hemingway · T. G. Kashima · G. Mahendra · A. Dhongre · P. C. W. Hogendoorn · F. Mertens · N. A. Athanasou

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Abstract Alpha isoform of smooth muscle actin (SMA) expression has been reported in giant cell tumour of bone (GCTB) and other benign and malignant bone tumours, but the pattern of SMA expression and the precise nature of SMA-expressing cells in these lesions is uncertain. We determined by immunohistochemistry the expression of SMA and other muscle and vascular markers in normal bone, GCTB and a wide range of primary benign and malignant bone tumours. Cultured stromal cells of GCTB, chondroblastoma (CB), and aneurysmal bone cyst (ABC) were also analysed for SMA expression. SMA was only noted in blood vessels in normal bone. SMA was expressed by mononuclear stromal cells (MSC) cultured from GCTB, ABC and CB. SMA was strongly and diffusely expressed by MSC in non-ossifying fibroma, fibrous dysplasia, and "brown tumour" of hyperparathyroidism. SMA expression was also noted in GCTB, ABC, CB, chondromyxoid fibroma, malignant fibrous histiocytoma of bone and osteosarcoma. Little or no SMA was noted in Langerhans cell histiocytosis, simple bone cyst, Ewing's sarcoma, osteoblastoma, osteoid osteoma, enchondroma, osteochondroma, chondrosarcoma, myeloma, lymphoma, chordoma and adamantinoma. Our findings show that there is differential SMA expression in primary bone tumours and that identifying the presence or absence of SMA is useful in the differential diagnosis of these lesions. The nature of SMA-expressing cells in bone tumours is uncertain but they are negative for desmin and caldesmon and could represent either myofibroblasts or perivascular cells, such as pericytes.

F. Hemingway · T. G. Kashima · G. Mahendra · A. Dhongre · N. A. Athanasou (✉)
Department of Pathology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences,
University of Oxford,
Oxford OX3 7LD, UK
e-mail: nick.athanasou@noc.anglox.nhs.uk

P. C. W. Hogendoorn
Department of Pathology, Leiden University Medical Centre,
Leiden, The Netherlands

F. Mertens
Department of Clinical Genetics, University and Regional Laboratories, Skane University Hospital, Lund University,
Lund, Sweden

Keywords Bone tumours · Alpha smooth muscle actin · Giant cells

Abbreviations

SMA	Alpha-smooth muscle actin
GCTB	Giant cell tumour of bone
CB	Chondroblastoma
ABC	Aneurysmal bone cyst
MSC	Mononuclear stromal cells
OS	Osteosarcoma
CMF	Chondromyxoid fibroma
GCRG	Giant cell reparative granuloma
SBC	Simple bone cyst
NOF	Non-ossifying fibroma
LCH	Langerhans cell histiocytosis
FD	Fibrous dysplasia

Introduction

Actin is a 42-kDa cytoskeletal microfilament protein that is ubiquitously expressed in all cell types. Actin interacts with a variety of membrane and cytoplasmic cytoskeletal proteins; it plays a role in cell contraction, adhesion and motility and is particularly abundant in muscle cells where

it is bundled into myofilaments. There are several homologous actin isoforms and these have specificities for different cell types [1, 2]. Alpha actin is found in muscle cells and there are specific alpha actins for smooth, skeletal and cardiac muscle. Beta and gamma actin are found in muscle and non-muscle cells. The alpha isoform of smooth muscle actin (SMA) is known to be expressed in smooth muscle cells, myoepithelial cells, pericytes and myofibroblasts [3, 4]. Immunophenotypically, myofibroblasts are distinguished from smooth muscle cells by the absence of expression of desmin and caldesmon, and from pericytes by variable expression of SMA, desmin and other markers [5–7].

Specific monoclonal antibodies which recognise SMA are used to characterise soft tissue tumours of smooth muscle, myoepithelial and myofibroblast origin [3]. SMA has also been identified in a number of primary bone tumours, including leiomyosarcoma, malignant fibrous histiocytoma (MFH), osteosarcoma (OS), giant cell tumour of bone (GCTB) and chondromyxoid fibroma [8–13]. SMA expression has also been noted in leukaemia, myeloma and some secondary tumours of bone [14–16]; the nature of cells expressing SMA in these tumours has not been established. In normal bone, SMA expression has been reported in marrow stromal cells, osteoblasts and perivascular cells [14–16].

In this study, our aim has been to identify whether SMA expression is useful in the differential diagnosis of primary bone tumours and tumour-like lesions and to provide information on the nature of SMA-expressing cells in normal and

Table 1 Percentage of MSC in primary bone tumours showing SMA expression

Diagnosis	SMA staining			
	− (%)	+ (%)	++ (%)	+++ (%)
Osteogenic tumours				
Osteoblastoma (*N*=5)	100	0	0	0
Osteoid osteoma (*N*=5)	100	0	0	0
Osteosarcoma (*N*=17)	29	35	24	12
Telangiectatic osteosarcoma (*N*=11)	36	36	18	9
Giant cell-rich osteosarcoma (*N*=1)	0	100	0	0
Cartilage tumours				
Enchondroma (*N*=5)	100	0	0	0
Osteochondroma (*N*=5)	100	0	0	0
Chondroblastoma (*N*=20)	35	30	10	25
Chondromyxoid fibroma (*N*=11)	45	27	9	18
Chondrosarcoma (*N*=5)	100	0	0	0
Mesenchymal Chondrosarcoma (*N*=5)	80	20	0	0
Dedifferentiated Chondrosarcoma (*N*=5)	0	20	40	40
Giant cell-rich tumours/lesions				
Giant cell tumour (*N*=11)	0	0	36	64
Giant cell tumour recurrent/metastatic (*N*=8)	0	0	88	13
Giant cell granuloma of jaw (*N*=4)	0	25	75	0
Aneurysmal bone cyst (*N*=15)	0	40	60	0
Brown Tumour (*N*=3)	0	0	33	67
Non-ossifying fibroma (*N*=15)	0	0	27	73
Other benign tumours/lesions				
Fibrous dysplasia (*N*=16)	0	0	25	75
Simple bone cyst (*N*=5)	0	100	0	0
Langerhans cell histiocytosis (*N*=11)	91	9	0	18
Other malignant tumours				
Adamantinoma (*N*=5)	100	0	0	0
Chordoma (*N*=5)	100	0	0	0
Ewing sarcoma (*N*=5)	100	0	0	0
Malignant fibrous histiocytoma (*N*=12)	25	25	33	17
Myeloma (*N*=5)	100	0	0	0
Lymphoma (*N*=5)	100	0	0	0

SMA expression in (extravascular) mononuclear stromal cells (MSC) was scored semiquantitatively as follows: − no staining, + <10 % cells stained, ++ 10–50 % cells stained, +++ >50 % cells stained

N number of cases

pathological bone tissue. We have carried out a survey of the expression of SMA and other smooth muscle antigens in normal bone as well as in a wide range of tumour and tumour-like lesions of bone. To determine the identity of SMA-expressing cells we have also cultured mononuclear stromal cells (MSC) from several giant cell-rich lesions of bone, including GCTB, and examined whether these cells express SMA and other antigens.

Materials and methods

Specimen and tissue processing

Archival specimens of bone lesions were retrieved from the files of the Department of Pathology, Nuffield Orthopaedic Centre, Oxford, UK and the Department of Pathology Leiden University Medical Center, the Netherlands. The number and nature of lesions examined in this study are shown in Table 1. Bone specimens were decalcified in 5 % nitric or formic acid for 24 h prior to processing and embedding in paraffin wax. Sections (5 μm) of decalcified, formalin-fixed, paraffin-embedded bone specimens were cut onto silane-coated slides (Surgipath, Peterborough, UK) and incubated at 37°C for at least 24 h to improve tissue adhesion before immunohistochemistry was carried out using an indirect immunoperoxidase technique (ChemMate Envision, Dako UK). Tissue sections were dewaxed and rehydrated by successive immersion in xylene, graded ethanol and water. The monoclonal antibodies employed in this study are shown in Table 2. Antigen retrieval was not required for SMA and muscle-specific actin antibody staining but was required for other antibodies; this was carried out by microwave treatment (700 W, 2×4 min) in Target Retrieval Solution (Dako). Endogenous peroxidase was blocked in a 0.2 % (v/v) hydrogen peroxide solution in 80 % ethanol, and a protein serum block. Antigen expression was visualised by incubation with labelled polymer and diamenobenzidene. The sections were then counterstained with haematoxylin, dehydrated, cleared, and mounted.

SMA expression in (extravascular) MSC was scored semiquantitatively as follows: − no staining; + <10 % cells stained; ++ 10–50 % cells stained; +++ >50 % cells stained. Distinction of SMA staining of smooth muscle cells in the wall of blood vessels from MSC was carried out by staining serial sections with antibodies directed against endothelial cell markers (Factor VIII, CD31 and CD 34). All assays were scored by three independent observers (TGK, GM and NAA) who closely agreed on the findings. Cases with equivocal MSC staining or discordant scoring were reviewed by the pathologist together after immunohistochemical studies were repeated.

Analysis of SMA expression in cultured MSC from GCTB and other giant cell-rich lesions

MSC were isolated from 14 cases of GCTB, 13 cases of aneurysmal bone cyst (ABC) and two cases of chondroblastoma (CB) as previously described [17]. A small piece of tumour tissue was washed in sterile phosphate buffered saline, placed in a tissue culture flask and covered with α minimal essential medium (MEM; Lonza, Wokingham, UK) supplemented with 10 % heat-inactivated fetal bovine serum 50 IU/ml Penicillin, 50 μg/ml Streptomycin (Invitrogen, Paisley, UK) and 2 mM L-glutamine (Lonza, UK). Cultures were maintained at 37°C in 5 % CO_2, and the medium was replenished every 7 days. When the cells were 80 % confluent, the medium was removed and the cells were washed with α-MEM. The cells were incubated with trypsin for 5 min at 37°C and then α-MEM was added to inactivate the trypsin. The cells were pelleted and seeded onto 15 glass coverslips within a 6-cm Petri dish at 1×10^5 cells/dish. When the cells on the coverslips were 90 % confluent, they were fixed in acetone, allowed to dry and immunohistochemically stained for SMA

Table 2 Details of monoclonal antibodies employed in this study

Antigen	Antibody	Specificity	Dilution
Smooth muscle actin (SMA)	1A4	Smooth muscle cells Myofibroblasts Myoepithelial cells	1/50
Muscle specific actin	HHF 35	skeletal, cardiac and smooth muscle cells	1/50
Desmin	D33 and DE-R11	Smooth striated and muscle cells	1/100
Caldesmon	h-CD	H-caldesmon (smooth muscle cells)	1/100
Calponin	CALP	Calponin binding protein in smooth muscle cells and myofibroblasts	1/50
CD31	JC70	Endothelial cells	1/200
CD34	QBEnd 10	Endothelial cells	1/20
CD45	PD7/26	Leucocyte common antigen	1/50
CD68	KP-1	Pan-macrophage; osteoclast	1/50

and other smooth muscle and vascular markers as well as for CD45 and CD68 with the monoclonal antibodies PD7/26 and KP1, respectively (both obtained from Dako, UK).

In order to determine more precisely the nature of SMA-expressing MSC, the presence (and frequency) of telomere associations in short-term MSC cultures from six GCTBs was also performed as previously described [18].

Results

SMA expression in normal mature bone

In normal compact and cancellous bone, SMA was confined to the wall of blood vessels, some of which had an obvious smooth muscle wall which contained several layers of SMA+cells. Numerous small, thin-walled vascular channels were seen in fatty and haematopoietic marrow and immediately beneath bone-lining cells and osteoblasts covering the surface of bone trabeculae (Fig. 1). Although in some areas it appeared that there were small collections of SMA-positive cells in the stroma of fatty and haematopoietic marrow, serial sections revealed that these were related to thin-walled vascular channels. The fibrous periosteum did not contain cells expressing SMA or other muscle markers outside blood vessels; the cambium layer of periosteal cells covering bone also did not express SMA. Muscle-specific actin showed a similar pattern of expression to SMA. Desmin, calponin and caldesmon expression was also only noted in the smooth muscle wall of large- and medium-sized vessels and, unlike SMA, rarely stained small vessels. Articular cartilage was negative for SMA.

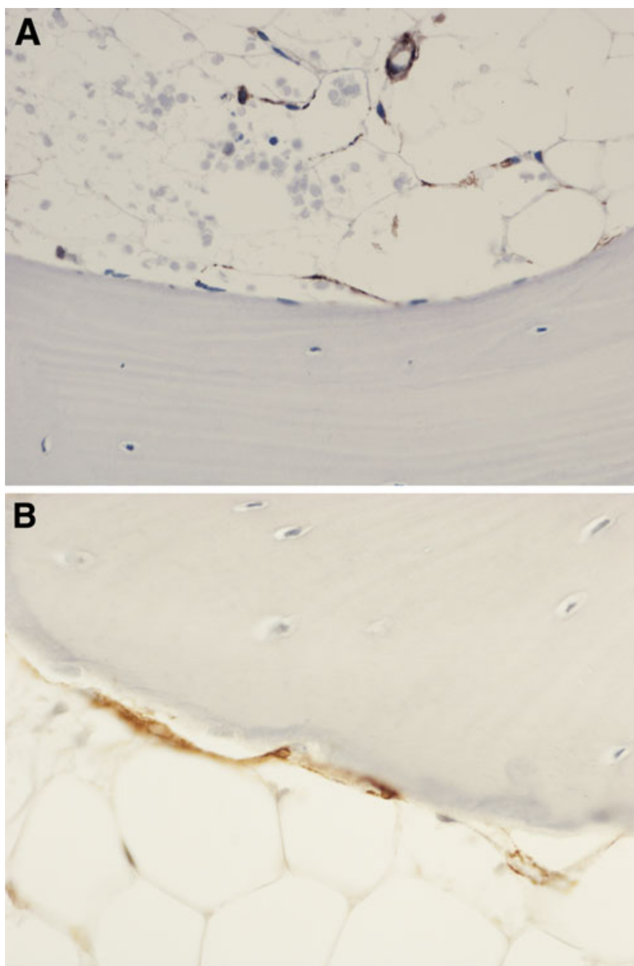

Fig. 1 **a** Low and **b** high-power view of normal bone showing SMA expression in the lining of vascular spaces, including those covering cancellous bone; bone lining cells are unstained

Analysis of SMA expression in tumours and tumour-like lesions of bone

SMA-expressing MSC (unrelated to the bone vasculature) were identified in a wide range of tumours and tumour-like lesions of bone (Table 1). These cells also expressed muscle-specific actin but were negative for desmin, calponin and caldesmon. We identified three patterns of SMA expression in the lesions analysed. A few tumours (non-ossifying fibroma [NOF], “brown tumour”, fibrous dysplasia [FD]) consistently showed strong diffuse (++/+++) expression of SMA by MSC. The second group of tumours showed highly variable expression of SMA which was not uniformly distributed throughout the tumour. The third (and largest) group of tumours showed no obvious expression of SMA outside blood vessels. These three patterns were useful in distinguishing specific types and subtypes of bone tumours (Fig. 2).

Among giant cell-rich lesions of bone, diffuse strong (+++) expression of SMA was noted in NOF and the brown tumour of hyperparathyroid bone disease (Fig. 3). SMA was more variably expressed in GCTB (Fig. 4a), giant cell reparative granuloma (GCRG) and ABC (Fig. 4b); these tumours were well-vascularised as shown by staining for endothelial markers. SMA was expressed by MSC and not multinucleated osteoclast-like giant cells in all the giant cell-rich lesions of bone analysed. Most cases of chondromyxoid fibroma (CMF) and CB showed little (+) expression of SMA by MSC, but a few cases showed more pronounced staining (++) (Fig. 4c). SMA expression was not identified in Langerhans cell histiocytosis (LCH) or simple bone cyst (SBC) (Fig. 5), but was strongly and diffusely expressed in fibrous dysplasia (Fig. 3c).

No obvious difference in SMA expression was noted between primary and recurrent GCTBs. In some cases of GCTB, SMA expression was quite prominent at the advancing edge of the tumour, but in these areas the tumour was often highly vascular; SMA expression was also not particularly prominent in fibrotic areas of the lesion where

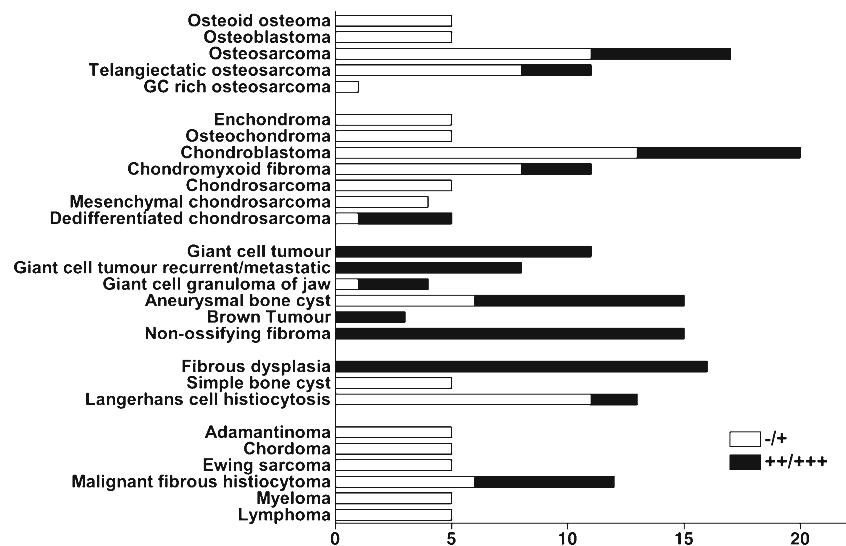

Fig. 2 Differential SMA expression in primary bone tumours. *empty rectangles* −/+, *filled rectangles* ++/+++ scored semiquantitatively

there were relatively few giant cells and there was proliferation of MSC.

MSC cultured from GCTB, ABC and CB expressed SMA and muscle-specific actin but not desmin (Fig. 6), calponin, caldesmon, the vascular marker CD31 and CD45 (leukocyte common antigen) or CD68, a macrophage marker. Cytogenetic analysis showed that cultured SMA + MSC from all the GCTBs contained telomere associations (mean: 25 % of cultured cells).

Fibroblast-like MSC and pleomorphic tumour cells expressed SMA in MFH and OS (Fig. 7a). OS showed a variable degree of SMA expression with most cases being +/++; a few cases, however, were entirely negative for SMA and some diffusely and strongly positive (+++). In contrast, SMA was not expressed outside blood vessels in the benign bone-forming tumours osteoid osteoma and osteoblastoma (Fig. 7b); plump osteoblasts and stromal MSC in these tumours did not express SMA. In Ewing's sarcoma, lymphoma, myeloma, adamantinoma and chordoma, SMA expression was also confined to the wall of blood vessels and neither tumour cells nor MSC expressed SMA. Chondromas, osteochondromas, conventional, mesenchymal and clear cell chondrosarcomas did not express SMA outside blood vessels. Dedifferentiated chondrosarcomas showing dedifferentiation towards OS or MFH variably (+/++) expressed SMA in the dedifferentiated but not the low-grade chondrosarcoma component (Fig. 7c).

Discussion

This study has shown that immunohistochemical expression of SMA is confined to blood vessels in normal adult cortical and cancellous bone and that it is not seen in marrow stromal cells, osteoblasts, osteocytes or bone lining cells. It has also found that SMA is differentially expressed by MSC in primary tumours and tumour-like lesions of bone. Diffuse strong expression of SMA was noted in several lesions including NOF, brown tumour of hyperparathyroidism and FD. In contrast to most giant cell-rich and cystic lesions of bone, little or no SMA expression was noted in LCH and SBC. Many primary benign and malignant bone tumours, including benign bone-forming tumours were negative for SMA, but MFH, OS and dedifferentiated chondrosarcoma variably expressed this marker.

Previous studies have shown that a variable number of actin-expressing cells are present in giant cell-rich containing lesions of bone [13, 19–22]. SMA expression has also been noted in giant cell tumours of soft tissue, skin and other extraskeletal sites [23–25]. In contrast to previous studies [13, 22], we did not identify actin expression by giant cells in GCTB or other giant cell-rich lesions of bone, although we did note that the giant cells in these lesions were often surrounded by thin-walled vessels or MSC expressing SMA and muscle-specific actin. SMA expression in GCTB was seen in both primary and recurrent GCTBs as well as in some tumours that produced lung nodules, a feature noted in previous studies [20, 26]; this suggests that SMA expression is not related to tumour invasion or an indicator of GCTB behaviour.

The differential diagnosis of giant cell-rich and cystic lesions of bone requires correlation of clinical, radiological and pathological findings. Interpretation of the histopathology of these lesions can be challenging and there are relatively few immunohistochemical markers which can usefully be employed to categorise these lesions. We found that SMA was strongly and diffusely expressed by MSC in NOF and brown tumour and that it was absent in LCH and SBC. Other giant cell-rich lesions of bone such

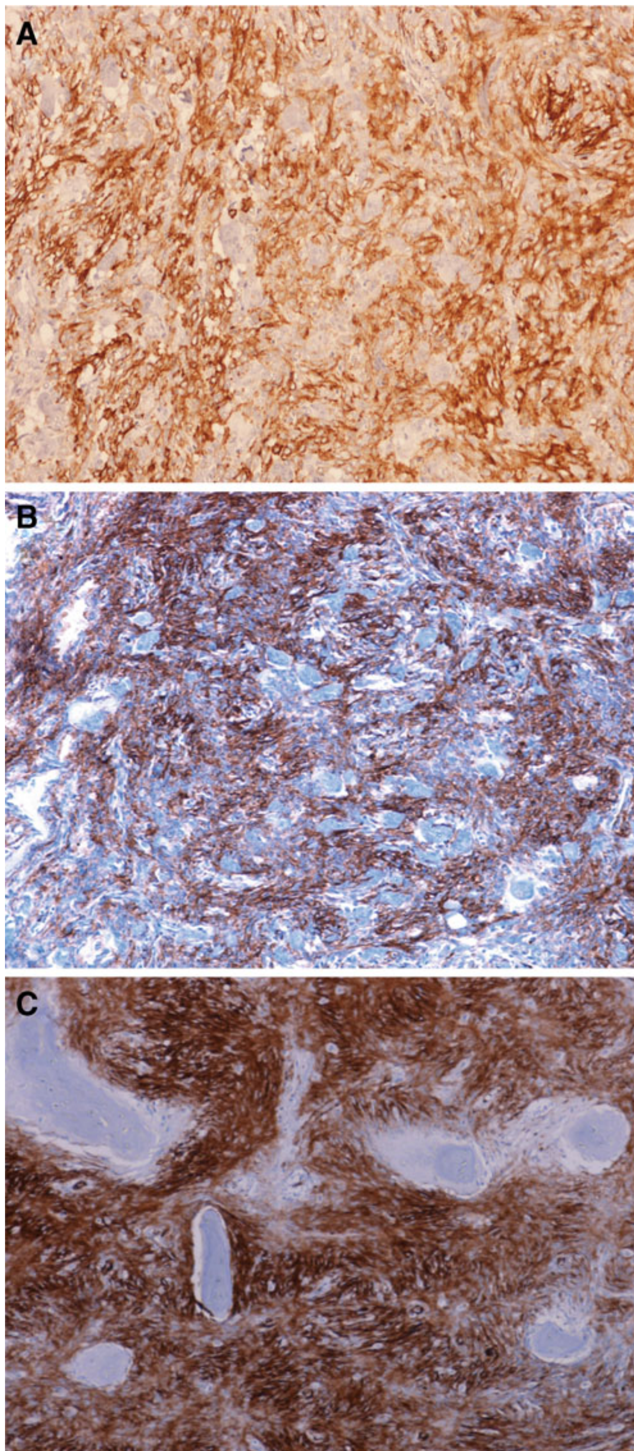

Fig. 3 Strong diffuse expression of SMA by MSC in: **a** non-ossifying fibroma, **b** "brown tumour" of hyperparathyroidism, **c** fibrous dysplasia

as GCTB, GCRG, ABC, CB, CMF and telangiectatic OS variably expressed SMA. Thus, with regard to cystic lesions of bone, expression of SMA may be useful in distinguishing ABC from SBC and, with regard to giant cell-rich lesions, absence of SMA may favour a diagnosis of LCH.

FD, a lesion which can occasionally contain focal giant cell-rich areas, was also distinguished by strong diffuse expression of SMA by intertrabecular MSC. FD enters

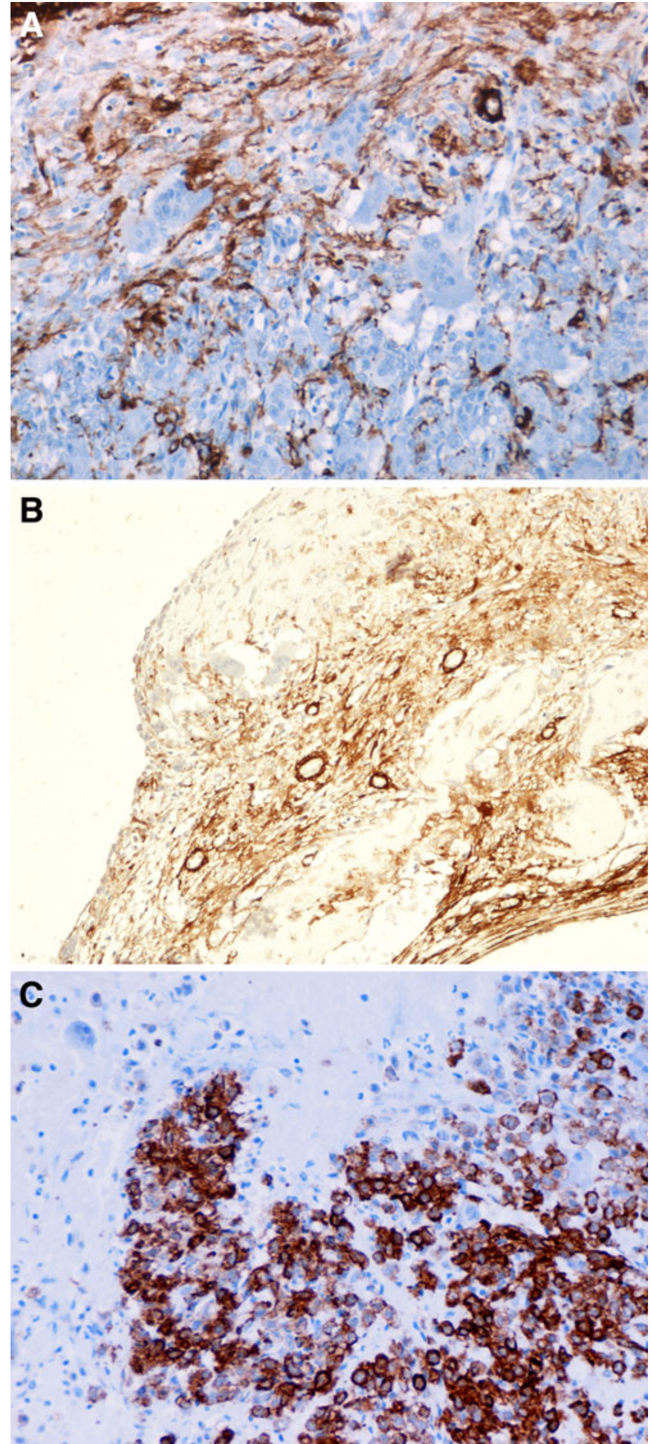

Fig. 4 SMA expression by MSC in: **a** giant cell tumour of bone, **b** aneurysmal bone cyst, **c** chondroblastoma

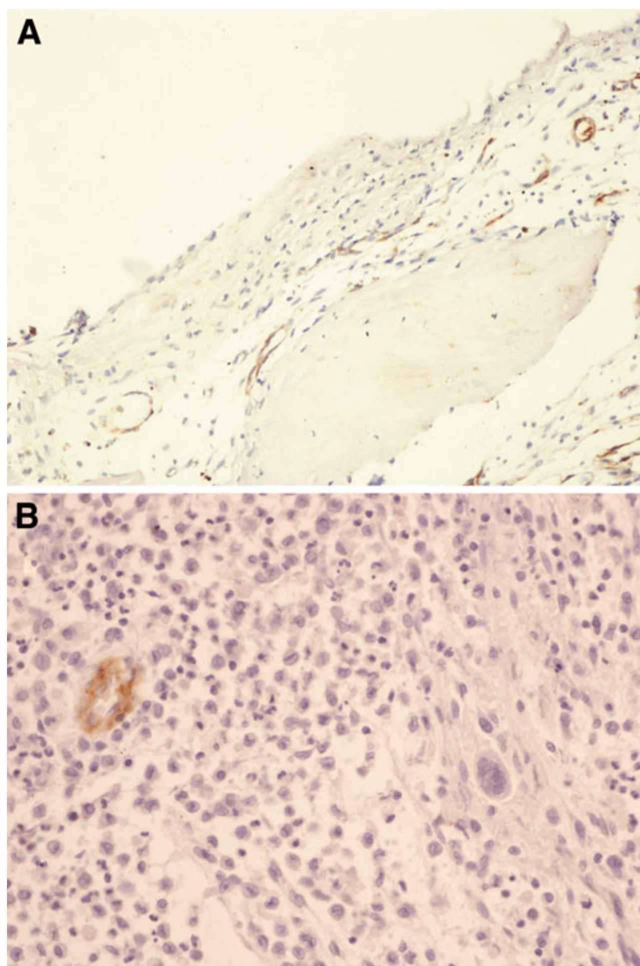

Fig. 5 Absence of (extravascular) SMA expression in **a** simple bone cyst, **b** Langerhans cell histiocytosis

into the differential diagnosis of many tumours, particularly fibro-osseous tumours of bone, such as MFH and OS. As noted in a previous study [12], we found that MFH showed relatively frequent but variable expression of SMA. We also noted SMA in most OS, including giant cell-rich and telangiectatic subtypes. In most cases of OS, SMA expression was restricted to a minority of MSC and tumour cells (+), but in some cases there was increased (++ or +++) expression. In contrast, osteoid osteoma and osteoblastoma did not show expression of SMA outside blood vessels. SMA expression may thus be useful in distinguishing benign bone-forming tumours from OS. Previous ultrastructural studies have shown that myofibroblast-like cells with a well-developed rough endoplasmic reticulum are present in FD and OS, particularly low-grade forms of OS [27]. The human SMA gene is a transcriptional target for the p53 tumour suppressor gene; expression of contractile proteins has been reported to be more common in low-grade OS and it has been suggested that SMA expression may have prognostic significance [10]. In the present study, we noted SMA expression in both high- and low-grade OS.

Many primary benign and malignant bone tumours showed no expression of SMA outside blood vessels. Osteochondroma, enchondroma as well as conventional, clear cell and mesenchymal chondrosarcoma did not express SMA; absence of SMA and calponin in conventional chondrosarcomas was similarly noted in a previous study [28]. Unsurprisingly, dedifferentiated chondrosarcomas showed SMA expression in the high-grade component exhibiting dedifferentiation towards MFH or OS. Other primary malignant bone tumours, including Ewing's sarcoma, myeloma, lymphoma, adamantinoma and chordoma, showed no expression of SMA outside blood vessels. Many of these tumours need to be considered in the differential diagnosis of round cell, spindle cell and pleomorphic sarcomas of bone. The status of SMA expression, taken with other immunohistochemical findings, may thus be useful in distinguishing these tumours.

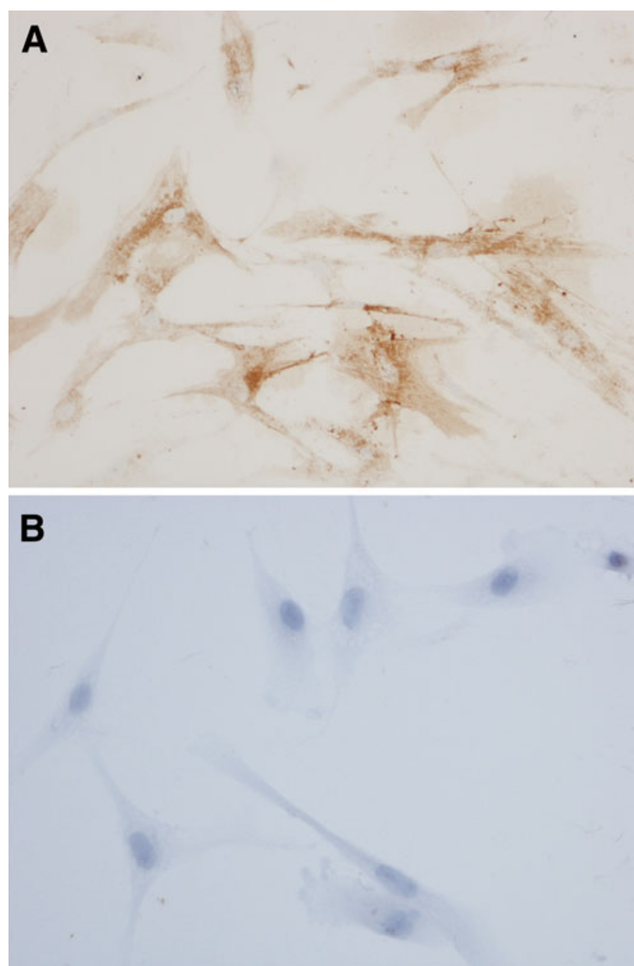

Fig. 6 MSC cultured from GCTB showing expression of **a** SMA but not **b** desmin

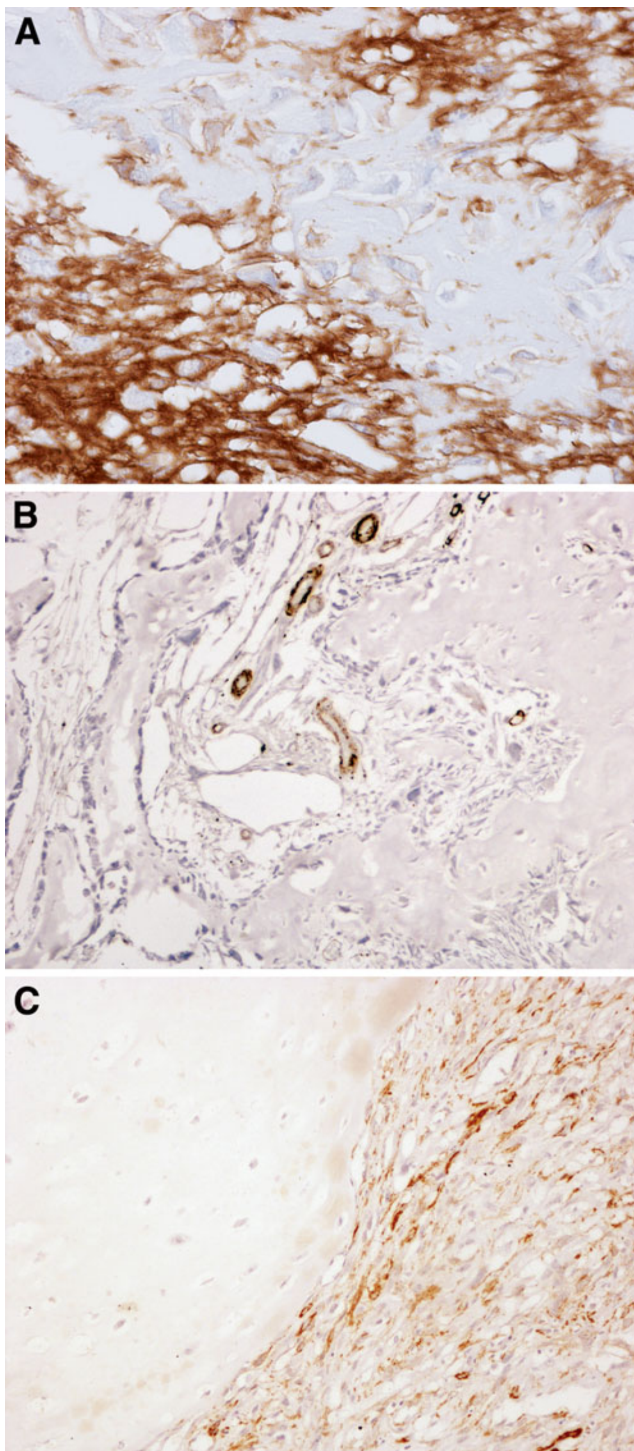

Fig. 7 SMA expression by tumour cells in **a** osteosarcoma but not **b** osteoblastoma or **c** low-grade chondrosarcoma component of a dedifferentiated chondrosarcoma; the dedifferentiated (MFH-like) component is positive for SMA

There are several reports of expression of SMA in normal adult bone marrow and in osteoblasts and bone lining cells [16, 29, 30]. One of the features which became apparent in the course of this study was the highly vascular nature of normal and pathological bone tissues; a dense meshwork of thin walled vessels is found in bone tumours and in normal bone marrow where a thin-walled vascular net is present over bone lining cells. This makes the assessment of extravascular SMA expression difficult to establish with certainty by immunohistochemistry and may account for the differences noted in SMA expression between the present and previous studies [14]. To verify that SMA was expressed by MSC in the extravascular component of the specimens analysed, we carried out staining with endothelial cell markers in serial sections. We also confirmed in cell culture studies of GCTB, ABC and CB that lesional SMA + mononuclear cells did not express endothelial, leucocyte or other smooth muscle cell markers. This was best seen in cell cultures of MSC derived from GCTB where cytogenetic analysis showed that these cells had telomere associations, a common feature of MSC in GCTB. SMA expression did not, however, obviously correlate with tumour vascularity, strong staining being noted, for example, in less-vascularised, fibrotic and well-vascularised cellular areas of GCTB.

We noted that SMA + MSC in many primary bone tumours also expressed muscle-specific actin but not desmin, calponin or H-caldesmon. This immunophenotypic profile has been noted in previous studies and believed to indicate that the SMA-expressing MSC in these lesions are reactive myofibroblasts [13, 22, 31]. Myofibroblast-like cells have been identified in several bone tumours in ultrastructural studies [27, 32–36]. We did not identify co-expression of calponin, which is typically expressed by myofibroblasts, on SMA-expressing cells. It remains possible, however, that the SMA-expressing cells in these lesions could represent this cell type as expression of smooth muscle markers by myofibroblast-like cells may depend on specific growth factor (e.g., transforming growth factor-β) stimulation [7, 21, 37]. Another cell type which expresses SMA (and more variably other smooth muscle-related antigens) is the pericyte [6]. Pericytes are perivascular cells which have been shown to be present in the bone marrow and other tissues; these cells are considered to represent a mesenchymal stromal cell precursor population which has the capacity for widely divergent differentiation into several mesenchymal cell types (including fibroblasts/myofibroblasts, chondroblasts and osteoblasts) [6, 38, 39]. Pericyte progenitor cells have been identified in the bone marrow, particularly in tumours [40]; these cells, like pericytes and myofibroblasts, can exhibit variable antigen expression. The precise nature and significance in terms of pathobiology of SMA-expressing MSC in bone tumours remains uncertain, but our findings suggest that identification of these cells may be useful in the differential diagnosis of primary bone tumours.

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Conflict of interest We declare that we have no conflict of interest.

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