h-Caldesmon as a Specific Marker for Smooth Muscle Tumors

Comparison With Other Smooth Muscle Markers in Bone Tumors

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Abstract

Caldesmon is a protein widely distributed in smooth and non–smooth muscle cells and is thought to regulate cellular contraction. Its isoform, high-molecular-weight caldesmon (h-CD), was demonstrated to be specific for smooth muscle cells and smooth muscle tumors of the soft tissue and to never be expressed in myofibroblasts. We performed an immunohistochemical study to examine h-CD expression in the following bone tumors: conventional and nonconventional osteosarcoma, 13; malignant fibrous histiocytoma of bone, 5; giant cell tumors of bone, 5; chondroblastoma, 3; metastatic leiomyosarcoma, 2; and rhabdomyosarcoma, 1. Frequent immunoreactivity for muscle actin (alpha-smooth muscle actin or muscle-specific actin) was seen in 11 of 13 osteosarcomas and all other tumors, whereas h-CD was expressed intensely only in 2 leiomyosarcomas. h-CD is considered a specific and useful marker to distinguish smooth muscle tumor from bone tumors with myoid differentiation.

It is well known that a considerable number of soft tissue mesenchymal tumors, in addition to leiomyoma and leiomyosarcoma, exhibit myoid or myofibroblastic differentiation immunohistochemically or ultrastructurally.[1-7] They include glomus tumor,[4] perivascular myoma,[8] inflammatory myofibroblastic tumor,[6,9,10] benign and malignant fibrous histiocytoma (MFH),[2,3,7,11] desmoid and other fibromatoses,[2,5] gastrointestinal stromal tumor,[12-14] angiomyolipoma,[15,16] and hemangiopericytoma.[4] Furthermore, it has been reported that various bone tumors such as osteosarcoma (OS),[17-20] chondroma, chondrosarcoma,[21] chondroblastoma (CB),[21,22] and giant cell tumor of the bone (GCT)[23] are also immunoreactive for actin or desmin to various degrees. Although some investigators think that they show myofibroblastic or myoid differentiation, their cellular nature has not been elucidated fully.[17,21,22]

Caldesmon is a protein widely distributed in smooth and non–smooth muscle cells.[24,25] It combines with calmodulin, tropomyosin, and actin and is thought to regulate cellular contraction.[25] It was demonstrated that its isoform, high-molecular-weight caldesmon (h-CD), is specific for smooth muscle cells and soft tissue smooth muscle tumors and is never expressed in myofibroblasts and pericytes, in contrast to other muscle markers.[26] We studied h-CD expression in various bone tumors, and we report the usefulness of h-CD for distinguishing leiomyosarcomas (LMS) from other bone tumors. We also briefly discuss the relation between bone tumors and myofibroblasts.

Materials and Methods

Clinical and pathologic details of the tumors examined are summarized in **Table 1**. The tumors were as follows:

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Table 1
Clinical Data and Results of Immunohistochemical Studies of Tumor Tissues

Histology/Subtype	Sex/Age (y)	Location	h-CD	Desmin	aSMA	MSA
Metastatic bone tumor						
Leiomyosarcoma (thigh)	F/43	Ilium	+++	+++	+++	+++
Leiomyosarcoma (uterine)	F/63	Humerus	+++	+++	+++	+++
Rhabdomyosarcoma (uterine)	F/48	Vertebra	–	+	–	++
Osteosarcoma						
Osteoblastic	M/10	Femur	–	–	+	+++
	M/10	Femur	–	–	+++	+++
	F/12	Humerus	–	–	–	–
	F/13	Humerus	–	–	+	+
	F/25	Tibia	–	–	–	+
Chondroblastic	F/11	Sacrum	–	–	++	++
	M/16	Femur	–	–	+	+
Fibroblastic	F/57	Scapula	–	–	++	+
MFH-like	M/47	Pubis	–	–	++	++
	F/61	Fibula	–	–	+++	+++
	F/70	Humerus	–	–	–	–
GCT-like	F/37	Femur	–	–	+	++
Telangiectatic	M/10	Femur	–	–	+	+
MFH of bone	M/13	Femur	–	–	–	++
	M/21	Femur	–	–	+	++
	M/60	Tibia	–	–	+++	+++
	F/63	Femur	–	–	–	+++
	F/66	Sacrum	–	–	+	+
GCT of bone	F/29	Tibia	–	–	++	+++
	F/32	Tibia	–	–	–	++
	M/42	Femur	–	–	++	++
	M/43	Ilium	–	–	++	+++
	M/47	Rib	–	–	++	++
Chondroblastoma	M/13	Femur	–	–	+	+++
	M/14	Tibia	–	–	++	+++
	F/15	Femur	–	–	+++	+++

aSMA, alpha-smooth muscle actin; GCT, giant cell tumor; h-CD, high-molecular-weight caldesmon; MFH, malignant fibrous histiocytoma; MSA, muscle-specific actin (HHF35, Enzo Diagnostics, New York, NY); –, negative; +, <25% positive cells; ++, 25%-75% positive cells; +++, >75% positive cells.

OS, 13, including 5 osteoblastic, 3 MFH-like, 2 chondroblastic, and 1 each fibroblastic, GCT-like, and telangiectatic; MFH of the bone, 5; GCT, 5; and CB, 3. Three of 5 cases of GCT were included in a previous report.[23] In addition, 2 metastatic LMSs, arising in the uterus and soft tissue of the thigh, and 1 metastatic rhabdomyosarcoma (RMS), pleomorphic type, arising in the uterus also were examined immunohistochemically.

Specimens were fixed in 20% formalin and embedded in paraffin. Sections 3- to 4-μm thick were cut and stained with H&E. Immunohistochemical studies using the streptavidin-biotin peroxidase complex method were performed on the paraffin-embedded sections. The primary antibodies and their final dilutions were h-caldesmon (h-CD, 1:50, Dako, Kyoto, Japan), desmin (D33, 1:50, Dako), alpha-smooth muscle actin (aSMA, 1:200, Dako), and muscle-specific actin (MSA; HHF35, 1:8,000, Enzo Diagnostics, New York, NY). Microwave pretreatment was performed for 15 minutes for h-CD and desmin. The sections were deparaffinized and then immersed in methanol containing 1% hydrogen peroxide to block endogenous peroxidase activity. The sections were incubated in primary antibodies for 2 hours and then with anti-mouse immunoglobulin conjugated with biotin (SAB kit, Dako) and streptavidin-peroxidase complex (SAB kit, Dako), respectively, for 30 minutes at room temperature. The immunoreactive products were visualized after immersion in a solution containing 20 mg of 3,3¢-diaminobenzidine (Dojin Laboratories, Kumamoto, Japan) in 100 mL of a 0.01-mol/L concentration of phosphate-buffered saline. Finally, the slides were counterstained for nuclei with hematoxylin.

Results

Histologic Features

Osteoblastic OSs were composed of anaplastic round or polygonal cells with various degrees of neoplastic osteoid formation. Chondroblastic OSs contained large amounts of neoplastic cartilaginous tissue in addition to osteoblastic tumor cells and osteoids. In contrast, fibroblastic OS was composed chiefly of fibroblastic spindle tumor cells with

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much osteoid tissue. MFH-like OSs were composed of spindle or polygonal tumor cells with various degrees of pleomorphism, occasionally arranged in fascicular and storiform patterns. In part, small amounts of osteoid tissue were present in all tumors. GCT-like OS was constituted of polygonal or short spindle tumor cells and osteoclast-like giant cells with conspicuous osteoid formation. The histologic features of telangiectatic OS were characterized by cyst-like spaces filled with blood. The pleomorphic polygonal tumors associated with osteoclast-like giant cells were present between the cyst-like spaces. Osteoid formation was also conspicuous.

The histologic features of MFH primarily arising in the bone were analogous to those of storiform-pleomorphic MFH of the soft tissue. Coagulation necrosis and hyalinous degeneration were seen in parts of each tumor. All 5 cases of GCT of the bone had typical histologic features and were composed of numerous osteoclast-like giant cells and mononuclear stromal cells. In addition, all 3 CBs were constituted of characteristic polygonal eosinophilic cells with immature cartilaginous matrices and numerous osteoclast-like giant cells

In the metastatic bone tumors, 2 LMSs were composed of conventional eosinophilic spindle cells arranged in interlacing fascicles. In part, small storiform arrangements of pleomorphic tumor cells mimicking MFH were seen. Although metastatic RMS, originally pleomorphic RMS of the uterus, was composed of pleomorphic spindle or round cells and showed no structural differentiation, large eosinophilic cells reminiscent of rhabdomyoblasts were scattered in the tumor.

Immunohistochemistry

Immunohistochemical results are detailed in Table 1. Two cases of metastatic LMS **Image 1A** intensely stained for h-CD **Image 1B**, desmin **Image 1C**, aSMA, and MSA (HHF35) in the pleomorphic tumor cells in addition to mature spindle cells. One metastatic RMS was positive only for desmin and MSA in part of the tumor.

Eleven of 13 cases of OS were positive for aSMA, MSA, or both, and 6 of them exhibited intense positivity. Three of the 6 osteoblastic subtypes showed intense immunoreactivity for aSMA **Image 2A** and MSA. In the chondroblastic type, osteoblastic tumor cells stained for aSMA and MSA, whereas tumor cells within the cartilaginous matrices did not show reactivity. Both fibroblastic spindle cells and osteoblastic polygonal cells in fibroblastic OS were positive for aSMA. Two of the 3 MFH-like OSs showed intense reactivity for aSMA and MSA, whereas 1 OS was negative. Polygonal mononuclear tumor cells in GCT-like and telangiectatic OS exhibited positive reactivity for aSMA and MSA. Although osteoclast-like giant cells were negative for aSMA, their cytoplasmic periphery occasionally stained for MSA. All cases of MFH **Image 2B**, GCT **Image 2C**, and CB were intensely positive for MSA, and most of them also for aSMA. In GCT, fibroblastic spindle cells and monocytoid round cells showed reactivity for aSMA and MSA. In addition, the cytoplasmic periphery of osteoclast-like giant cells stained for MSA (Image 2C). In contrast, h-CD and desmin were completely negative in all OSs, MFHs, GCTs, and CBs.

Discussion

Although actin and desmin have been used as superior smooth muscle cell markers in diagnostic pathology,[1-3] it has come to be recognized that many kinds of mesenchymal tumors other than conventional leiomyoma and LMS express these markers in various degrees. Among these, tumors and tumor-like lesions with myofibroblastic cells, including inflammatory myofibroblastic tumor,[5,6,9,10] MFH,[3,7,11] desmoid, and fibromatosis,[2,5] frequently exhibit intense immunoreactivity for the muscle markers, and, therefore, some of them occasionally may be confused with leiomyoma or LMS. In addition, it was demonstrated by electron microscopy that storiform-pleomorphic type MFH, which is one of the most common soft tissue sarcomas, frequently exhibits various degrees of myogenic differentiation.[7] On the basis of these observations, storiform-pleomorphic type MFH is now thought to include pleomorphic RMS, LMS, myofibroblastic tumor, and other unspecified undifferentiated sarcomas.[7,11] However, it is difficult, if not impossible, to strictly differentiate pleomorphic RMS, LMS, and myofibroblastic tumor by light microscopy, even when using immunohistochemistry, since they occasionally express common muscle markers, including actin and desmin.[1-3,7]

Caldesmon is a protein widely distributed in smooth and non–smooth muscle cells.[24,25] Its isoform, h-CD, was identified to be specific for smooth muscle cells and soft tissue smooth muscle tumors and was not to be expressed in myofibroblastic tumor.[26] In addition, recently Miettinen et al[27] also reported that among various soft tissue tumors, h-CD is immunoreactive only in the smooth muscle tumors. Thus, h-CD is thought to be an extremely useful marker of smooth muscle tumors, especially for the differentiation of soft tissue tumors with various myofibroblastic characteristics, but the specificity of h-CD has not been examined in bone tumors. Therefore, we attempted to define h-CD expression in OS and MFH of the bone and in CB and GCT, in which frequent or occasional actin expression has been reported,[17-20,22,23] in addition to metastatic LMS and RMS.

We found that h-CD was expressed intensely only in 2 metastatic LMSs and never in the other tumors examined,

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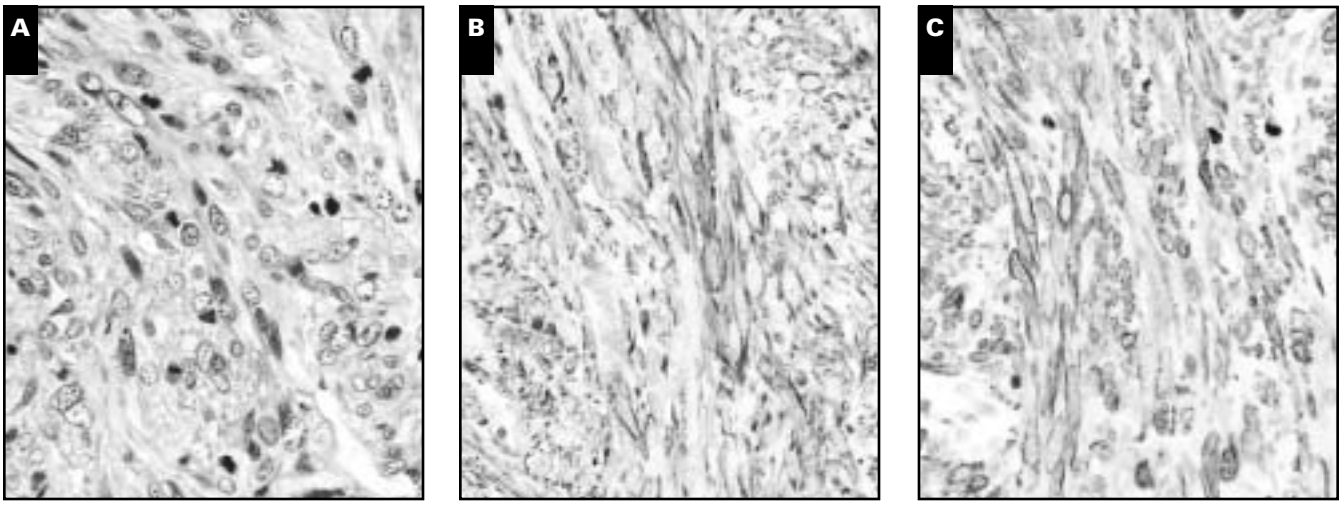

Image 1 Metastatic leiomyosarcoma (A, H&E, ·250). Almost all tumor cells exhibit immunoreactivity (B, h-caldesmon, ·250; C, desmin, ·250).

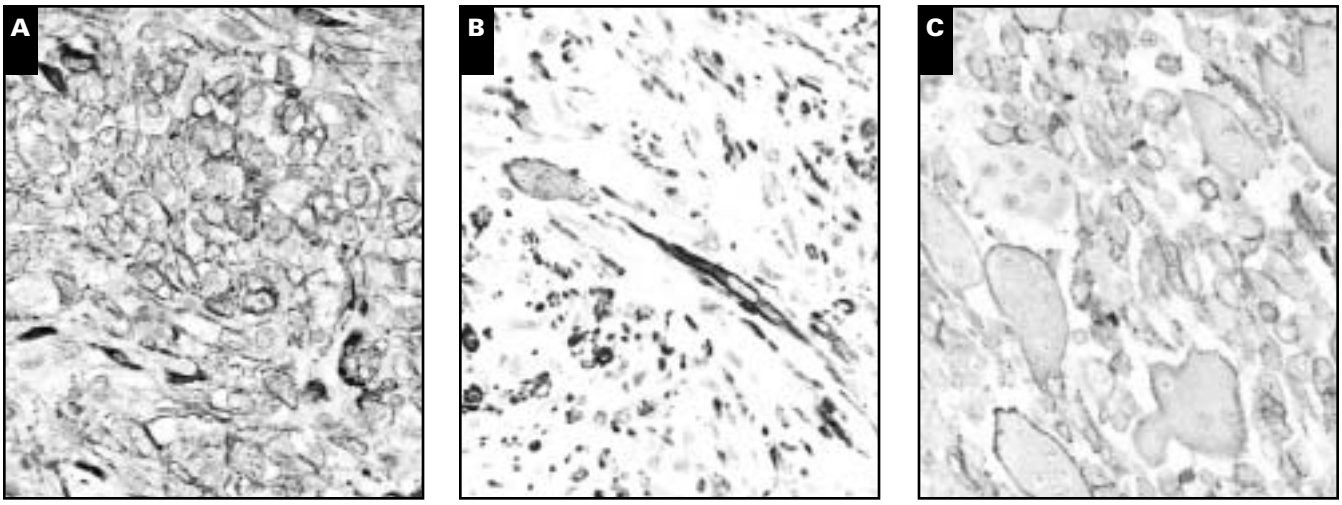

Image 2 A, Immunoreactivity in osteoblastic osteosarcoma (alpha-smooth muscle actin [aSMA; Dako, Kyoto, Japan], ·250). B, Malignant fibrous histiocytoma of the bone also shows intense immunoreactivity (aSMA, ·125). C, Giant cell tumor of the bone. Round or short spindle mononuclear cells immunostained for muscle-specific actin (HHF35, Enzo Diagnostics, New York, NY). In addition, the cellular periphery of osteoclast-like giant cells was frequently immunolabeled (muscle-specific actin, ·250).

in contrast with aSMA, MSA (HHF35), and desmin. It was shown that h-CD may be a specific and sensitive marker for smooth muscle tumors in the bone in addition to soft tissue tumors. In addition, the fact that h-CD is not expressed in myofibroblasts and other actin-rich cells may indicate their different origins compared with smooth muscle cells.

Actin expression in various bone tumors, confirmed in the present study by immunoreactivity for aSMA and MSA, has been well established previously.[17-20,22,23] It is likely that the positivity for aSMA and MSA of bony MFH is due to its myofibroblastic differentiation, as well as MFH of the soft part. In addition, the immunoreactivity of OS is thought to be due to myofibroblastic differentiation of the tumor cells.[17-20] Myofibroblasts are distinctive cells with spindle-shaped fibrillary cytoplasm, often with stellate and long cytoplasmic extensions. They also are characterized ultrastructurally by well-developed actin filaments with dense bodies and fibronexus, well-developed rough endoplasmic reticulum, and intermediate and gap cellular junctions.[28-31] Although the irregular polygonal shape of osteoblastic cells usually is not seen in myofibroblasts, the actin-like thin filaments with dense patches adjacent to the plasma membrane, numerous rough endoplasmic reticula, and rudimentary cellular junctions are observed in osteoblastic tumors cells.[32-36] These common ultrastructural features suggest that osteoblastic cells and myofibroblasts, if not identical, may originate from common mesenchymal cells.

Finally, the phenomenon of frequent and intense immunoreactivity for MSA and aSMA in GCT of the bone

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and CB is an issue of controversy.[22,23,37] Although actin expression in the fibroblast-like tumor cells of GCT can be explained by the electron microscopic observation that these tumor cells occasionally contain abundant microfilaments in their cytoplasm,[38,39] its significance is unclear. Myofibroblastic differentiation of these tumor cells is unlikely because of their electron microscopic features. Their lack of the dense patch, basal lamina, and fibronexus seems to be considerably different from myofibroblasts, except for the existence of thin filaments. Ultrastructurally, CB cells also contain abundant thin filaments, sometimes with condensations. However, it seems unlikely that CB cells belong directly to myofibroblasts or smooth muscle cells, since they are well-established cells characterized by cartilage formation and frequent S-100 protein expression.[22] Although the meaning of actin expression in CB has not been elucidated, the designations of myochondroblast and myofibroblast were proposed for such cells with peculiar differentiation.[22,40]

Expression of h-CD was studied immunohistochemically in OS, MFH of the bone, GCT, CB, and metastatic LMS and RMS. As a result, h-CD was expressed distinctively in LMS and never in other tumors in which actin and desmin were present in various degrees. We think h-CD is a specific and sensitive marker of smooth muscle tumors and is useful for differentiating bone tumors with myoid features.

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