Ⅳ. 研究成果の刊行物

Chapter 2

Cancer Stem Cells of Sarcoma

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Introduction

Sarcomas are malignant mesenchymal tumors. Mesenchymal tissue is defined as a complex of nonepithelial structures of the body exclusive of reproductive tissue, glia, and hematopoietic and lymphoid tissue. Embryonically, these nonepithelial structures are derived from the mesoderm and, to a lesser degree, from the neuroectoderm and are grouped into fibrous tissue, adipose tissue, skeletal muscle, blood and lymph vessels, and peripheral nervous tissue (Aogi et al. 2000). The word "sarcoma" is derived from the Greek word *sarkoma* meaning "fleshy outgrowth"; sarcomas can present as either a bone sarcoma or a soft tissue sarcoma (Misra et al. 2009).

Bone Sarcoma

Bone tissue is composed of many kinds of cells, such as osteoblasts, osteocytes, chondrocytes, and periosteal cells, and, in bone marrow, hematopoietic cells, adipocytes, neurocytes, fibroblasts, and vascular

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smooth myocytes. Since the origin of sarcoma has not been identified, bone sarcoma classification is based on morphological findings, such as cell type, architecture, and matrix production. The morphological features of benign and malignant as well as non-neoplastic conditions may overlap. The classification of the World Health Organization (WHO) is presented in Table 1 (Fletcher et al. 2002).

Table 1. WHO classification of bone sarcoma.
(adapted from Fletcher, C.D.M., K.K. Unni., and F. Mertens. 2002.
Pathology and genetics of tumors of soft tissue and bone. p. 226. IARC Press. Lyon)

CARTILAGE TUMORS	EWING SARCOMA/PRIMITIVE
Osteochondroma	NEUROECTODERMAL TUMOR
Chondroma	Ewing sarcoma
Enchondroma	HEMATOPOIETIC TUMORS
Periosteal chondroma	Plasma cell myeloma
Multiple chondromatosis	Malignant lymphoma, NOS
Chondroblastoma	GIANT CELL TUMOR
Chondromyxoid fibroma	Giant cell tumor
Chondrosarcoma	Malignancy in giant cell tumor
Central, primary, and secondary	NOTOCHORDAL TUMORS
Peripheral	Chordoma
Dedifferentiated	VASCULAR TUMORS
Mesenchymal	Hemangioma
Clear cell	Angiosarcoma
OSTEOGENIC TUMORS	SMOOTH MUSCLE TUMORS
Osteoid osteoma	Leiomyoma
Osteoblastoma	Leiomyosarcoma
Osteosarcoma	LIPOGENIC TUMORS
Conventional	Lipoma

Table 1. contd....

Table 1. contd....

OSTEOGENIC TUMORS	LIPOGENIC TUMORS
chondroblastic	Liposarcoma
fibroblastic	NEURAL TUMORS
osteoblastic	Neurilemmoma
Telangiectatic	MISCELLANEOUS TUMORS
Small cell	Adamantinoma
Low grade central	Metastatic malignancy
Secondary	MISCELLANEOUS LESIONS
Parosteal	Aneurysmal bone cyst
Periosteal	Simple cyst
High grade surface	Fibrous dysplasia
FIBROGENIC TUMORS	Osteofibrous dysplasia
Desmoplastic fibroma	Langerhans cell histiocytosis
Fibrosarcoma	Erdheim-Chester disease
FIBROHISTIOCYTIC TUMORS	Chest wall hamartoma
Benign fibrous histiocytoma	JOINT LESIONS
Malignant fibrous histiocytoma	Synovial chondromatosis

Excluding myeloma and lymphoma, malignant primary bone sarcomas constitute 0.2% of all malignancies in adults and approximately 5% of childhood malignancies, for which data were obtained in one large series (Surveillance, Epidemiology, and End-Results (SEER) study). In North America and Europe, the incidence rate for bone sarcomas in males is approximately 0.8 new cases per 100,000 population a year (Fletcher et al. 2002). Cancer registry data with histological stratification indicate that osteosarcoma is the most common primary malignant tumor of bone, accounting for approximately 35% of cases, followed by chondrosarcoma for 25%, Ewing sarcoma for 16%, and chordoma for 8% (Table 3) (Dorfman and Czerniak 1995).

Soft tissue sarcoma

Soft tissue is defined as the supportive tissue of various organs and the nonepithelial, extraskeletal structures exclusive of lymphohematopoietic tissues. It includes fibrous connective tissue, adipose tissue, skeletal muscle, blood/lymph vessels, and the peripheral nervous system. Embryologically, most of it is derived from the mesoderm, with a neuroectodermal contribution in the case of peripheral nerves. Soft tissue sarcomas are malignant mesenchymal neoplasms that share a common embryological and connective tissue origin. Since their origin has not been clarified, the classification system commonly used in soft tissue sarcoma is also based on histopathology. The generally accepted basis for soft tissue tumor classification is also the WHO system (Table 2) (Fletcher et al. 2002).

According to the SEER study, which included 26,758 cases for 1978–2001, leiomyosarcoma was the most common sarcoma, accounting for 23% of cases. Other major histological types included malignant fibrous histiocytoma (MFH; 17%), liposarcoma (11%), dermatofibrosarcoma (10%), and rhabdomyosarcoma (4%). Together, these 6 histological types accounted for 67% of all cases (Table 3) (Toro et al. 2006). In this report, MFH was the second most common soft tissue sarcoma in their series. However, it is accepted that MHF does not show true histiocytic differentiation and its morphological pattern is shared by a variety of poorly differentiated malignancies. As a result, the diagnostic term MFH is now reserved for pleomorphic sarcomas without defined differentiation. Therefore, the decline in MFH incidence rates identified since 1990 is probably due to changes in diagnostic criteria that parallel changes in the understanding of MFH.

Problems with the current treatments

According to the histological type, treatment options for most sarcoma patients include surgical resection followed by limb or trunk reconstruction, pre-operative (neoadjuvant) and/or post-operative (adjuvant) chemotherapy, and radiotherapy. Although surgical resection is the mainstay of treatment for musculoskeletal sarcomas, chemotherapy also has a proven role in the primary therapy of certain types of bone sarcomas and a potential role for some patients with soft tissue sarcomas (Wesolowski and Budd 2010). In osteosarcoma cases, for example, recruitment of chemotherapy in the 1970s drastically improved the prognosis of patients (Ferrari et al. 2009). More recent randomized trials have shown that treatment of osteosarcoma patients with modern multiagent chemotherapy regimens, which include doxorubicin (DOX), cisplatin (CDDP), methotrexate (MTX), and ifosfamide (IFO), results in a 5-year survival rate of approximately 60–80%. Furthermore, response to neoadjuvant (preoperative) treatment has

Table 2. WHO classification of soft tissue sarcoma.
(adapted from Fletcher, C.D.M., K.K. Unni., and F. Mertens. 2002. Pathology and genetics of tumors of soft tissue and bone. p. 10-11. IARC Press. Lyon)

ADIPOCYTIC TUMORS	SO-CALLED FIBROHISTIOCYTIC TUMORS	CHONDRO-OSSEOUS TUMORS
Benign	Benign	Soft tissue chondroma
Lipoma	Giant cell tumor of tendon sheath	Mesenchymal chondrosarcoma
Lipomatosis	Diffuse-type giant cell tumor	Extraskeletal osteosarcoma
Lipomatosis of nerve	Deep benign fibrous histiocytoma	
Lipoblastoma / Lipoblastomatosis	Intermediate (rarely metastasizing)	TUMORS OF UNCERTAIN DIFFERENTIATION
Angiolipoma	Plexiform fibrohistiocytic tumor	Benign
Myolipoma	Giant cell tumor of soft tissues	Intramuscular myxoma
Chondroid lipoma	Malignant	(incl. cellular variant)
Extrarenal angiomyolipoma	Pleomorphic 'MFH' / Undifferentiated	Juxta-articular myxoma
Extra-adrenal myelolipoma	pleomorphic sarcoma	Deep ('aggressive') angiomyxoma
Spindle cell	Giant cell 'MFH' / Undifferentiated	Pleomorphic hyalinizing
Pleomorphic lipoma	pleomorphic sarcoma	angiectatic tumor
Hibernoma	with giant cells	Ectopic hamartomatous thymoma
Intermediate (locally aggressive)	Inflammatory 'MFH' / Undifferentiated	Intermediate (rarely metastasizing)

Table 2. contd....

Table 2. contd....

ADIPOCYTIC TUMORS	SO-CALLED FIBROHISTIOCYTIC TUMORS	TUMORS OF UNCERTAIN DIFFERENTIATION
Atypical lipomatous tumor/	pleomorphic sarcoma with	Angiomatoid fibrous histiocytoma
Well differentiated liposarcoma	prominent inflammation	Ossifying fibromyxoid tumor
Malignant		(incl. atypical / malignant)
Dedifferentiated liposarcoma	SMOOTH MUSCLE TUMORS	Mixed tumor/
Myxoid liposarcoma	Angioleiomyoma	Myoepithelioma/
Round cell liposarcoma	Deep leiomyoma	Parachordoma
Pleomorphic liposarcoma	Genital leiomyoma	Malignant
Mixed-type liposarcoma	Leiomyosarcoma (excluding skin)	Synovial sarcoma
Liposarcoma, not otherwise specified		Epithelioid sarcoma
	PERICYTIC (PERIVASCULAR) TUMORS	Alveolar soft part sarcoma
FIBROBLASTIC / MYOFIBROBLASTIC	Glomus tumor (and variants)	Clear cell sarcoma of soft tissue
TUMORS	malignant glomus tumor	Extraskeletal myxoid chondrosarcoma
Benign	Myopericytoma	("chordoid" type)
Nodular fasciitis		PNET / Extraskeletal Ewing tumor
Proliferative fasciitis	SKELETAL MUSCLE TUMORS	pPNET

Proliferative myositis

Myositis ossificans

fibro-osseous pseudotumor of digits

Iischemic fasciitis

Elastofibroma

Fibrous hamartoma of infancy

Myofibroma / Myofibromatosis

Fibromatosis colli

Juvenile hyaline fibromatosis

Inclusion body fibromatosis

Fibroma of tendon sheath

Desmoplastic fibroblastoma

Mammary-type myofibroblastoma

Calcifying aponeurotic fibroma

Angiomyofibroblastoma

Cellular angiofibroma

Nuchal-type fibroma

Benign

Rhabdomyoma

adult type

fetal type

genital type

Malignant

Embryonal rhabdomyosarcoma

(incl. spindle cell,

botryoid, anaplastic)

Alveolar rhabdomyosarcoma

(incl. solid, anaplastic)

Pleomorphic rhabdomyosarcoma

VASCULAR TUMORS

Benign

Hemangiomas of

subcut/deep soft tissue

extraskeletal Ewing tumor

Desmoplastic small round cell tumor

Extra-renal rhabdoid tumor

Malignant mesenchymoma

Neoplasms with perivascular epithelioid

cell differentiation (PEComa)

clear cell myomelanocytic tumor

Intimal sarcoma

Table 2. contd....

Table 2. contd....

FIBROBLASTIC/MYOFIBROBLASTIC TUMORS	VASCULAR TUMORS
Gardner fibroma	capillary
Calcifying fibrous tumor	cavernous
Giant cell angiofibroma	arteriovenous
Intermediate (locally aggressive)	venous
Superficial fibromatoses (palmar / plantar)	intramuscular
Desmoid-type fibromatosis	synovial
Lipofibromatosis	Epithelioid hemangioma
Intermediate (rarely metastasizing)	Angiomatosis
Solitary fibrous tumor	Lymphangioma
and hemangiopericytoma	Intermediate (locally aggressive)
(incl. lipomatous hemangiopericytoma)	Kaposiform hemangioendothelioma
Inflammatory myofibroblastic tumor	Intermediate (rarely metastasizing)
Low grade myofibroblastic sarcoma	Retiform hemangioendothelioma
Myxoinflammatory	Papillary intralymphatic angioendothelioma
fibroblastic sarcoma	

Infantile fibrosarcoma

Malignant

Adult fibrosarcoma

Myxofibrosarcoma

Low grade fibromyxoid sarcoma

hyalinizing spindle cell tumor

Sclerosing epithelioid fibrosarcoma

Composite hemangioendothelioma

Kaposi sarcoma

Malignant

Epithelioid hemangioendothelioma

Angiosarcoma of soft tissue

Table 3. Incidence of bone and soft tissue sarcomas.

(A) Relative Frequencies of Bone Sarcomas by Histologic Type: SEER Data 1973–1987

	Cases	Percent
Osteosarcoma	922	35.1
Chondrosarcoma	677	25.8
Ewing sarcoma	420	16.0
Chordoma	221	8.4
Malignant fibrous histiocytoma (MFH)	149	5.7
Angiosarcoma	36	1.4
Unspecified	32	1.2
Other	170	6.4
Total	*2627*	*100.0*

(adapted from Dorfman, H.D. and B. Czerniak. 1995. Bone cancers. Cancer. 75: 203–210)

(B) Soft tissue sarcomas* diagnosed during 1978–2001 in the 9 SEER program areas by histologic type

	Cases	Percent
Leiomyosarcoma	6393	23.9
Malignant fibrous histiocytoma (MFH)	4577	17.1
Liposarcoma	3086	11.5
Dermatofibrosarcoma	2810	10.5
Rhabdomyosarcoma	1218	4.6
Angiosarcoma	1092	4.1
Nerve sheath tumor/MPNST	1061	4.0
Fibrosarcoma	964	3.6
Sarcoma, NOS	3424	12.8
Other specified soft tissue sarcomas Synovial sarcoma	2133 615	8.0 2.3
Total	*26758*	*100.0*

MPNST, malignant peripheral nerve sheath tumor, NOS, not otherwise specified.

*Excluding sarcomas of bones and joints and Kaposi sarcoma.

(adapted from Toro, J.R. and L.B. Travis, H.J. Wu, K. Zhu, C.D. Fletcher, and S.S. Devesa. 2006. Incidence patterns of soft tissue sarcomas, regardless of primary site, in the surveillance, epidemiology and end results program, 1978–2001: An analysis of 26,758 cases. Int J Cancer 15; 119: 2922–2930)

become the most important predictor of outcome, as the median survival of osteosarcoma patients who have greater than 90% necrosis in the resected specimen following neoadjuvant chemotherapy is about 90% at 5 years.

However, one of the current problems is that the prognosis of osteosarcoma patients began to plateau about 20 years ago and many patients develop resistance in the standard therapies and tumor recurrence. The prognosis is much worse for patients with metastases (Longhi et al.

2006, Ta et al. 2009), most of which are lung metastases. Targeting molecules associated with sarcomagenesis, "targeted therapy," has been an exciting development in sarcoma treatment in the past 10 years. However, such therapies are currently limited for many kinds of sarcoma. Furthermore, there are no fewer cases involving metastases long after initial treatments (Halldorsson et al. 2009). Many problems remain to be solved before the prognosis of sarcoma patients improves.

Considering the characteristics and heterogeneity of sarcoma described below, it is possible that a subset of sarcoma cells might resist various stresses producing recurrences or metastases; this is known as the "cancer stem cell hypothesis." Therefore, there is a great need to identify, characterize, and target sarcoma cancer stem cells for the improvement of sarcoma treatments.

Origin of Sarcoma

Sarcomas constitute a large and heterogeneous group of neoplasms in terms of pathophysiology and molecular oncology. Considering the heterogeneity of sarcomas, a possible speculation is that pluripotent cells, such as tissue stem cells, could be involved in sarcomagenesis (Fig. 1A).

Tissue stem cells

Mesenchymal stem cell (MSC). Speculation had been that the pluripotent cells exist in bone morrow. Friedenstein et al. were the first to demonstrate this idea in an experimental approach (Friedenstein et al. 1976). Currently, MSCs from not only bone marrow stroma but also alternative sources, such as adipose tissue, have provided exciting prospects for cell-based tissue engineering and regeneration (Tuan et al. 2003, Banas et al. 2007). However, in most studies, it remains to be determined whether true stem cells are present or whether the population is instead a diverse mixture of lineage-specific progenitors (Toguchida 2009). Inconsistency in the published reports of the growth characteristics and differentiation potential of MSCs underscores the need for a functional definition of these cells. At present, one of the criteria that the International Society for Cellular Therapy (ISCT) proposed to define an MSC population is that the cells must be "greater than or equal to 95% positive for CD73 (ecto-50-nucleotidase), CD90 (Thy-1), and CD105 (endoglin), and no more than 2% of the cells may express CD34 (hematopoietic progenitor and endothelial cell marker), CD45 (pan-leukocyte marker), CD11b or CD14 (monocyte and macrophage markers), CD19 or CD79α (B cell markers), and HLA-DR (marker of stimulated MSCs)" (Kuhn and Tuan 2010). Other proposed MSC markers include CD44, CD49a, STRO-1, CD200, CD271, and CD146 (Kuhn and Tuan 2010).

It is assumed that MSC or MSC-like cells are localized not only in bone marrow but also in other tissues, such as adipose tissue, for example, and can be isolated (Fibbe 2002).

Neural crest stem cell (NCSC). Other multipotent stem cells that differentiate into bone and cartilage include neural crest stem cells. Originally, in the vertebrate embryo, neuroectodermal neural crest cells (NCC) have remarkably broad potencies, giving rise, after a migratory phase, to neurons and glial cells in the peripheral nervous system and to skin melanocytes, all being designated here as "neural" derivatives (Le Douarin et al. 2008). NC-derived cells also include non-neural, "mesenchymal" cell types, such as chondrocytes and bone cells, myofibroblasts and adipocytes, which largely contribute to the head structures in amniotes. A multipotent progenitor cells isolated from neural crest have the capacity to self-renew and to generate neurons, glia, and smooth muscle and have therefore been termed neural crest stem cells (NCSCs) (Morrison et al. 1999, Nagoshi et al. 2008). NCSCs are highly migratory and invasive and, during embryogenesis, travel to numerous tissues throughout the body (von Levetzow et al. 2011). As they are also identified in bone marrow (Nagoshi et al. 2009), the relationship between MSC and NCSC has been a matter of interest.

Clinical implications

There are some clinical features supporting the hypothesis that sarcomas originate from these tissue stem cells (Toguchida 2009).

Sarcomas containing different mesenchymal components

Three types of sarcomas, described below, represent a mixture of distinct histological subtypes in one sarcoma tissue.

Malignant mesenchymoma. Malignant mesenchymomas are rare soft tissue tumors that contain two or more distinct histological subtypes of sarcoma within the same tumor. They are generally considered high-grade neoplasms and are associated with a poor prognosis (Brady et al. 1996).

Malignant Triton tumor. Malignant triton tumors (Fig. 1Ba) are malignant peripheral nerve sheath tumors with rhabdomyosarcomatous differentiation (James et al. 2008). This entity was originally described by Masson and Martin in 1938, who suggested that the neural elements induced skeletal muscle differentiation, as was believed to occur in skeletal muscle of the Triton salamander.

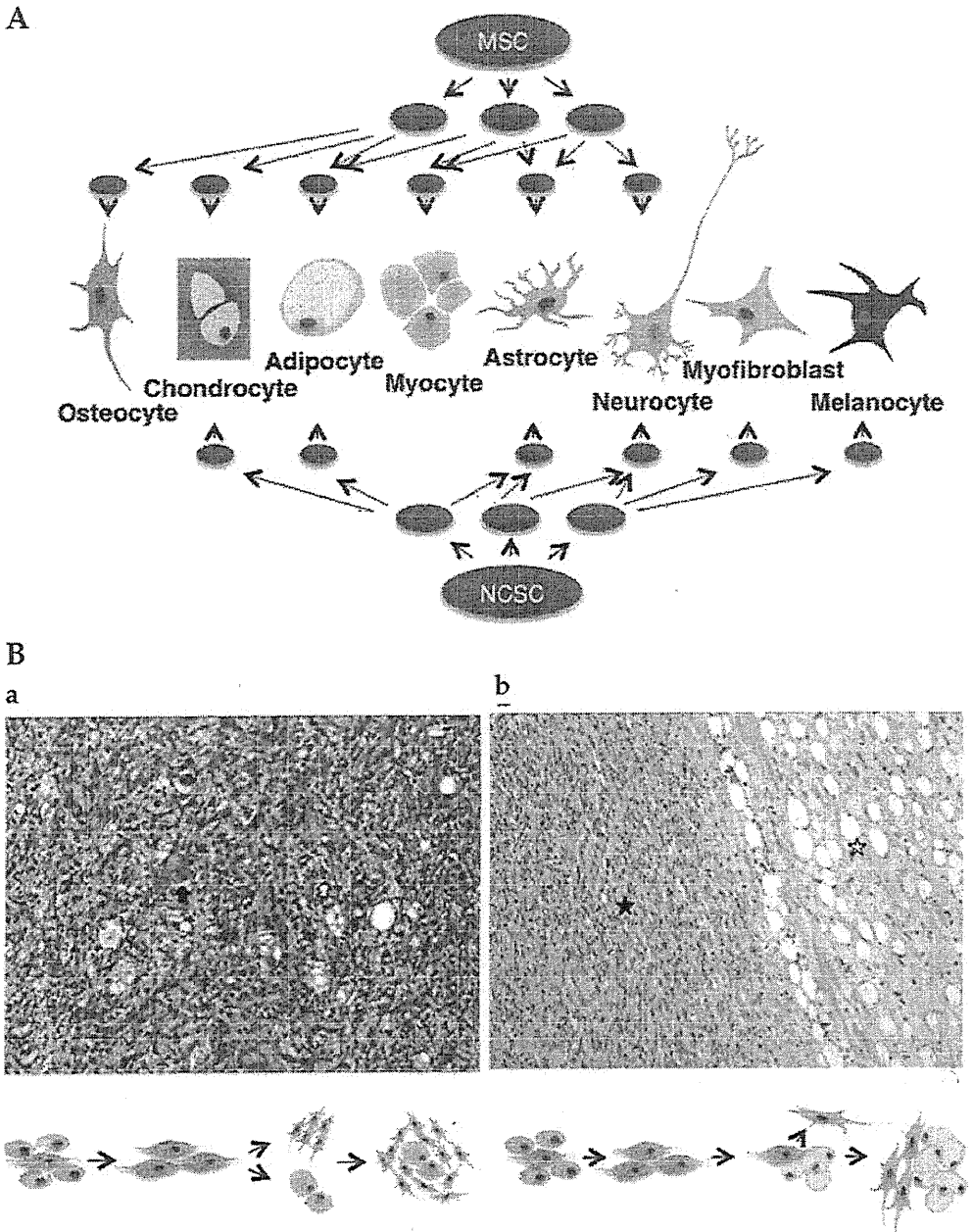

Fig. 1. Considerations for the origin of sarcoma. (A) Multi-differentiating potential of the mesenchymal stem cell (MSC) and neural crest stem cell (NCSC) (adapted from Toguchida J. Sarcoma-initiating cells and tissue stem cells. pp. 65–71. *In:* H. Esumi, N. Takakura, K. Miyazono, M. Mori [eds.] 2009. Experimental Medicine. 27. Yodosha, Tokyo, Japan). (B) Schemes of sarcomagenesis suggesting that tissue stem cells might be the origin of sarcomas. a. Malignant Triton tumor. Microscopically, the tumor cells exhibited pleomorphic (Schwannian) spindle tumor cells (white arrow) with focal rhabdomyosarcomatous differentiation (black arrow). b. Dedifferentiated liposarcoma. An abrupt transition from conventional well-differentiated liposarcoma (☆) to dedifferentiated liposarcoma composed of pleomorphic spindle cells (★).

Color image of this figure appears in the color plate section at the end of the book.

Ectomesenchymoma. Ectomesenchymoma is a rare malignant neoplasm usually consisting of rhabdomyosarcoma (RMS) with a neural component (Kawamoto et al. 1987).

These tumors are speculated to be composed of a subpopulation of cells that have two or more specific lineages of differentiation.

Dedifferentiated sarcoma

Dedifferentiation is the progression of cells toward a less differentiated state in which the original line of differentiation is no longer evident (Katoh et al. 2004). The concept of dedifferentiation was first proposed by Dahlin and Beabout in 1971, when they described dedifferentiated chondrosarcoma as a distinct clinicopathological entity characterized by a low-grade chondrosarcoma juxtaposed to a histologically different high-grade sarcoma (Dahlin and Beabout 1971).

Dedifferentiated liposarcoma. Dedifferentiated liposarcoma (DDLS) (Fig. 1Bb) is a term that was first introduced by Evans in 1979 to describe liposarcomas containing a mixture of atypical lipomatous tumor (ALT)/ well-differentiated liposarcoma (WDLS) and a high-grade nonlipogenic sarcomatous component, usually with an abrupt transition between the two components (Cha 2011). Dedifferentiated areas exhibit a wide morphological spectrum. Most cases have areas of high-grade poorly differentiated sarcoma resembling pleomorphic malignant fibrous histiocytoma, fibrosarcoma, malignant hemangiopericytoma, or high-grade myxofibrosarcoma.

Dedifferentiated chondrosarcoma. Dedifferentiated chondrosarcoma (DCS) is a high-grade, aggressive anaplastic sarcoma that progresses from a previous low-grade chondrosarcoma. DCSs have been described as "osteosarcomatous" or "fibrosarcomatous" transformations of low-grade chondrogenic neoplasms (Wick et al. 1987).

These sarcomas are speculated to include a subpopulation of the pluripotent cells with acquired novel genetic alterations that transform into completely different lineages.

Experimental implications

Some experimental results also indicate that tissue stem cells are the origins of sarcomas. If the hypothesis is right, cell lines originating from sarcoma tissues might have a potential of multi-directional differentiation (Toguchida 2009).

Multi-differentiation potential of osteosarcoma. Osteosarcoma is defined as a malignant tumor composed of neoplastic mesenchymal cells synthesizing osteoid or immature bone. On the other hand, the histological findings can be extremely variable. Osteosarcoma can be subdivided into several histological subtypes; osteoblastic, chondroblastic, and fibroblastic osteosarcoma. Aoyama et al. demonstrated that the cell line established from chondroblastic osteosarcoma expressed not only osteoblastic but also chondroblastic genetic markers and represented both histological types under a differentiation environment *in vitro* and *in vivo* (Aoyama et al. 2004). Furthermore, this cell line could differentiate into adipose, neural, muscular, and vascular lineages, suggesting ability similar to that of MSCs.

Multi-differentiation potential of synovial sarcoma. Synovial sarcoma (SS) is a mesenchymal spindle cell tumor that displays variable epithelial differentiation, including glandular formation, and has a specific chromosomal translocation t(X; 18) (p11; q11). The name "synovial" comes from the morphological similarity with joint synovium; however, it does not arise from or differentiate toward synovium, which lacks epithelial differentiation and has different histochemistry. No origin from or continuity with pre-existing epithelium has been identified. Nagayama et al. examined the genome-wide gene expression profiles of 13 SS cases and 34 other spindle cell sarcoma cases by cDNA microarray consisting of 23,040 genes (Nagayama et al. 2002). A hierarchical clustering analysis grouped SS and malignant peripheral nerve sheath tumor (MPNST) into the same category, and these two types of tumors shared expression patterns of numerous genes relating to neural differentiation. Several genes were up-regulated in almost all SS cases, and the presumed functions of known genes among them were related to migration or differentiation of neural crest cells, suggesting the possibility of the neuroectodermal origin of SS. On the other hand, Naka et al. demonstrated that SS cells, on SS18-SSX silencing with siRNAs, exhibited morphological transition from spherical growth in suspension to adherent growth in the monolayer, additional expression of later mesenchymal and hematopoietic lineage genes, and broader differentiation potentials into osteocytes, chondrocytes, adipocytes, and macrophages in appropriate differentiation environments. These data suggest that SS is a stem cell malignancy (Naka et al. 2010).

Sarcomagenesis of MSC

Several publications have questioned the ability of MSCs to undergo malignant transformation (Shima et al. 2007, Mohseny et al. 2009). Shima et al. reported the spontaneous transformation of bone marrow-derived human MSC (hMSC), isolated and expanded independently in two

laboratories. They tried to immortalize hMSC by inactivating the $p16^{INK4A}$ gene using the *Bmi1* gene, established immortalized human MSC (ihMSC), which retained the potential for the multi-directional differentiation of the original cells, and tested the feasibility of using ihMSC as presarcomatous cells. The transformation of ihMSC by the *H-ras* gene showed the phenotype of fully transformed cells and retained the adipogenic and chondrogenic, but not osteogenic, potential (Shima et al. 2007).

Cancer Stem Cells of Sarcoma

The cancer stem cell hypothesis proposes that, within a heterogeneous tumor, there is a small subpopulation of cells called "cancer stem cells (CSCs)" that are responsible for forming the bulk of the tumor (Clarke et al. 2006, Visvader and Lindeman 2008). These cells are considered to be similar to stem cells and may arise from the transformation of stem cells or the dedifferentiation of non-stem cells (Visvader 2011). The common consensus is that they are capable of both self-renewal and differentiation into all of the cells within a tumor (Clarke et al. 2006).

The first evidence of the existence of CSCs came from studies of hematological malignancies (Clevers 2011). Initial attempts to characterize CSCs were accomplished using cell surface molecules in acute myeloid leukemia. Several groups demonstrated that CSCs capable of initiating leukemia were found in the $CD34^+CD38^-$ fraction (Lapidot et al. 1994, Warner et al. 2004). Recently, CSCs have been isolated from several human solid tumors that have markers for putative normal stem cells, including breast cancer ($CD44^+CD24^-ESA^+$) (Al-Hajj et al. 2003), brain cancer ($CD133^+$) (Singh et al. 2004), prostate cancer ($CD44^+/\alpha2\beta1^{high}/CD133^+$) (Collins et al. 2005), hepatocellular carcinoma ($CD133^+$) (Yin et al. 2007), pancreatic cancer ($CD44^+CD24^-ESA^+$, $CD133^+CXCR4^+$) (Hermann et al. 2007, Li et al. 2007) and colon cancer ($CD133^+$) (Ricci-Vitiani et al. 2007).

Gibbs et al. were the first to demonstrate CSC in sarcomas in 2005. They demonstrated that spheres from osteosarcoma cell line possessed the CSC phenotype as described below. To date, many reports have been published as to CSCs or TICs in both bone and soft tissue sarcomas using various methods as follows:

1) Sphere formation
2) Side population (SP)
3) Cell surface markers
4) Self-renewal marker genes

Regardless of these methods, the common phenotype has been strong tumorigenicity *in vivo*. This might be regarded as a common consensus of CSCs. The implications of sarcoma CSCs have been reported most frequently in osteosarcoma, followed by Ewing sarcoma. These reports are summarized in Tables 4 and 5.

Bone sarcoma

Osteosarcoma-Sarcosphere, SP, ALDH, CD133, CD117, Stro-1, Oct4, and Sox2. Osteosarcoma is a primary mesenchymal tumor that is characterized histologically by the production of osteoid by malignant cells. It is the most common primary malignancy of bone, with approximately 900 new cases reported in the United States annually (Geller 2010). It represents less than 1% of cancers reported within the United States, with a peak incidence of 4.4 cases per million annually in the adolescent and young adult population. The most common primary sites are the distal femur, proximal tibia, and proximal humerus, with approximately 50% of cases originating in the vicinity of the knee.

The WHO classification recognizes additional histological variants in addition to the conventional osteosarcoma (osteoblastic, chondroblastic, and fibroblastic types): telangiectatic osteosarcoma, small cell osteosarcoma, low-grade central osteosarcoma, secondary osteosarcoma, parosteal osteosarcoma, and periosteal osteosarcoma according to the dominant histological feature (Fletcher et al. 2002).

The standard treatment of patients with conventional osteosarcoma consists of neoadjuvant chemotherapy, resection, and adjuvant chemotherapy (Marina et al. 2004). In the past, treatment of the primary tumor was amputation, whereas a high percentage of patients are currently being treated by limb salvage surgery (Bacci et al. 2000, Bielack et al. 1999, Weis 1999, Lindner et al. 1999). With combined treatment (neoadjuvant chemotherapy, surgery, and adjuvant chemotherapy), the 5-year survival for patients with no metastatic disease at diagnosis has been 60% to 80% (Provisor et al. 1997, Bacci et al. 2000, Rytting et al. 2000, Meyers et al. 2008). However, for patients who present with metastatic disease, outcomes are far worse at <30% survival (Ferguson and Goorin 2001). Pulmonary metastasis is the predominant site of osteosarcoma recurrence and the most common cause of death. The survival rate has not improved for 20 years despite multiple clinical trials with increased intensity; therefore, new therapeutic targets and approaches must be sought to suppress pulmonary metastasis of osteosarcoma for better prognosis.

Table 4. Multiple phenotypes of sarcoma CSCs in biopsies and cell lines of human/mouse bone sarcoma according to the CSC markers.

Sarcoma type	CSC marker	Cell line (No.)	Frequency, %[a]	*In vitro*				*In vivo*		Year	Refs (et al.)
				Asymmetric division	Stem cell marker	Chemo-resistance	Invasion	Tumorigenicity	Metastasis		
Osteosarcoma	Sarcosphere	Biopsy (5) Human (3) Mouse (3)	0.1–1 0.025–0.25 0.1–1.5	✓ ✓	✓ ✓ ✓	 ✓				2005 2009 2008	Gibbs Wang, Fujii Wilson
	SP	Biopsy (5) Human (2)	NA 0.17–0.31		 ✓	 ✓	 ✓	✓ ✓		2007 2008, 2009	Wu Tsuchida, Murase
	CD133	Biopsy (2, 18) Human (3)	5–7.8 0.92–10.86	✓	✓ ✓	 ✓		✓		2009, 2011 2008, 2009	Veselska, Tirino Tirino, Kelly
	CD117/ Stro-1	Biopsy (1) Human (2) Mouse (3)	NA 1 1–3	 ✓	 ✓	 ✓		✓ ✓ ✓	 ✓	2010	Adhikari
	ALDH	Human (4)	0.59–45.07	✓	✓	✓		✓		2010	Wang, Honoki
	Oct3/4	Biopsy (1)	NA	✓	✓			✓	✓	2009	Levings
	Sox2	Biopsy (18) Human (7) Mouse (4)	>50 (IHC) NA 45–74.5[b]	 ✓	✓ ✓ ✓	 	 ✓	 ✓		2011	Basu-Roy
Ewing sarcoma	Sarcosphere	Human (1)	0.78		✓	✓				2009	Fujii
	CD133	Biopsy (4, 48) Human (1)	0–99[c]		✓	✓		✓		2009, 2010	Suva, Jiang
	ALDH	Human (5)	2	✓		✓		✓		2010	Awad

Chondrosarcoma	Sarcosphere	Biopsy (4)	0.01–0.1							2005	Gibbs
	SP	Biopsy (4)	NA							2007	Wu
	CD133	Biopsy (6)	0.21–3.5					✓		2011	Tirino
Chordoma	SP	Biopsy (1)	NA							2007	Wu
	CD133	Biopsy (1)	0.8							2011	Tirino

SP, side population. NA, not available. [a]analyzed by flow cytometry. [b]Sca-$1^{high}$$Sox2^{high}$ population. [c]analyzed by immunohistochemistry.