

Table 5. Multiple phenotypes of sarcoma CSCs in biopsies and cell lines of human/mouse soft tissue sarcoma according to the CSC markers.

Sarcoma type	CSC marker	Cells (No.)	Frequency, % ^a	<i>In vitro</i>				<i>In vivo</i>		Year	Refs (et al.)
				Asymmetric division	Stem cell marker	Chemo-resistance	Invasion	Tumorigenicity	Metastasis		
Leiomyosarcoma	SP	Biopsy (1)	3							2007	Wu
	CD133	Biopsy (1)	0.9							2011	Tirino
MFH	SP	Biopsy (4)	NA	✓	✓			✓		2011	Tirino
		Human (1)	5.28								
Liposarcoma	SP	Biopsy (2)	NA							2007	Wu
	CD133	Human (3)	0.31–5.2							2011	Tirino
	ALDH/CD133	Human (1)	NA	✓				✓		2011	Stratford
Rhabdomyosarcoma	SP	Human (1)	1.5–2			✓	✓	✓		2008	Tsuchida
	CD133	Human (3)	20	✓		✓		✓		2011	Walter
	FGFR-4	Human (3)	1.6–2.6	✓	✓			✓		2009	Hirotsu
Synovial sarcoma	SP	Biopsy (3)	NA					✓		2007	Wu
	CD133	Biopsy (8) Human (2)	0.84–7.23 1.6–20.5 ^b 0.1–1.7							2010, 2011	Jefferson, Tirino

Fibrosarcoma	Sarcosphere	Human (1)	0.25	✓	✓					2009	Fujii
	CD133	Biopsy (4) Human (1)	0.42-1.23		✓			✓		2011	Tirino
	ALDH	Human (1)	9							2010	Honoki

SP, side population. NA, not available. ^aanalyzed by flow cytometry. ^banalyzed by immunohistochemistry.

Sarcosphere. Reynolds and Weiss first cultured cells that exhibit stem cell properties, such as free-floating spheres, called neurospheres, from the adult brain (Reynolds and Weiss 1992). They dissected striatal tissue to single cells and plated them in nonadherent conditions in serum-free medium in the presence of an epidermal growth factor (EGF). Spheres from sarcoma cell lines with the similar procedures, called "sarcospheres," have been reported to possess CSC phenotypes (Gibbs et al. 2005, Wilson et al. 2008).

Gibbs et al. reported that all of the several cell lines established from patient biopsies and the MG63 cell line formed spheres at a frequency of 0.1–1% under an anchorage-independent environment with a serum-free N2 medium with growth factors, human EGF, and human FGF. The marker genes of pluripotent embryonic stem cells, Oct3/4 and Nanog, were greater in sarcospheres than in adherent cells. These spheres could form secondary spheres after dissociated single cells, suggesting that they contained a small subpopulation that had self-renewing ability.

Similarities between human and canine osteosarcoma cell lines were identified in view of the CSC phenotype of the sarcosphere (Wilson et al. 2008). Sarcosphere formation was identified in all of 3 canine cell lines, D-17, UWOS-1, and UWOS-2, and 1 human cell line, MG63, expressing Nanog, Oct4, and STAT3 at a frequency of 0.1–1.5%. Their ability to reproduce consistently after multiple passages was also confirmed. Wang et al. also identified sarcosphere formation from human cell lines OS99-1, MG63, HuO9, and SaOS2 and confirmed the expression of Oct3/4 A, Oct3/4 B, and Nanog by RT-PCR, immunohistochemistry, and flow cytometry (Wang et al. 2009).

Fujii et al. investigated drug resistance between sarcosphere and adherent cells (Fujii et al. 2009). MG63 spheres showed strong resistance to DOX and CDDP and increased expression of DNA repair enzyme genes MLH1 and MSH2 compared to adherent cells, indicating that a DNA repair inhibitor had the potential to enhance the efficacy of chemotherapeutics.

Side population (SP). In the analysis of hematopoietic stem cells, a subpopulation that effluxes the DNA-binding dye Hoechst 33342 out of the cell membrane through an ATP-binding cassette (ABC) transporter was recognized as a stem cell population (Goodell et al. 1996, Zhou et al. 2001, Ibrahim et al. 2007). This cell population expressing the ABC transporter is defined as side population (SP) cells, which are distinguished from cells of the other population (main population; MP) (Murase et al. 2009). SP cells showed CSC phenotypes characterized by asymmetric cell division, drug resistance, and tumorigenicity (Huber et al. 2005, Chiba et al. 2008). However, the following problems have been reported regarding SP cells as

a CSC fraction (Wu and Alman 2008). First, cells resistant to Hoechst 33342 dye do not necessarily show tumorigenicity and metastatic ability, as CSCs do (Patrawala et al. 2005). Second, cytometry gating strategies used to isolate SP cells lack the consistency of gating strategies used in marker staining (Chiba et al. 2008). These problems might lead to cross-contamination of the SP and the non-SP cells, resulting in controversial data.

Wu et al. demonstrated that SP cells were identified in 5 surgically excised osteosarcoma samples; the frequencies were not proven (Wu et al. 2007). The SP cells from one sample were analyzed for tumorigenicity in NOD/SCID mice, showing higher tumorigenicity than MP cells. Murase et al. screened the frequency of SP cells in 7 osteosarcoma cell lines (OS2000, KIKU, NY, HuO9, HOS, U2OS, and SaOS2). As a result, only NY included SP cells, at a small percentage (0.38%; Murase et al. 2009). No further study was done to confirm the phenotype of SP cells of NY. Tsuchida et al. have shown that CDDP treatment could transform a non-tumorigenic osteosarcoma SP fraction to a highly tumorigenic phenotype, which is described later in this chapter.

The enrichment of the CSC fraction as a result of chemotherapeutics have described. Di Fiore et al. reported that 3AB-OS, i.e., MG-63 treated with 3-aminobenzamide (3AB) for 100 days, possessed much greater capacity to form spheres, stronger self-renewal ability, and higher expression of genes associated with cell cycle (*ppRb*, *E2F2*, *cyclin A*, *B1*, *D1*, *E*), stemness (*Oct3/4*, *hTERT*, *nucleostemin*, *Nanog*), and inhibition of apoptosis (*HIF-1 α* , *FLIP-L*, *Bcl-2*, *XIAP*, *IAPs*, *survivin*) than parental MG-63 (Di Fiore et al. 2009). They were also characterized by high expression of ABCG2 and CD133, indicating that CSCs were enriched by chemotherapeutics. Tang et al. found that MTX-resistant U2OS/MTX300 cells, cultured in DMEM with 300ng/ml MTX, showed CSC phenotypes (Tang et al. 2011). They were larger in size and showed a higher potential to form spheres *in vitro* and tumors *in vivo*. Flow cytometric analysis revealed that the percentage of SP cells was 0% for U2OS and 0.11% for U2OS/MTX300, whereas the percentage of CD117⁺/Stro-1⁺ cells was 0% for U2OS and 0.6% for U2OS/MTX300, concluding that chemotherapy enrichment was a feasible and practical way to enrich osteosarcoma CSCs.

CD133. Recent studies have demonstrated that CD133 (prominin-1) is a specific marker of CSCs in a wide spectrum of malignant tumors (Singh et al. 2003, Ricci-Vitiani et al. 2007, Maeda et al. 2008). CD133 was the first identified member of the prominin family of the five-transmembrane glycoprotein with an extracellular N-terminus, a cytoplasmic C-terminus, and two large extracellular loops with eight consensus sites for N-linked glycosylation (Mizrak et al. 2008, Chen et al. 2009). The characteristic feature

of CD133 is its rapid downregulation during cell differentiation (Lee et al. 2005). This feature makes CD133 a unique cell surface marker for the identification and isolation of stem cells and progenitor cells in several tissues (Chen et al. 2009, Takahashi et al. 2011).

Tirino et al. were the first to identify that CD133⁺ cells possessed many features of CSCs (Tirino et al. 2008). SaOS2, MG-63, and U2OS cell lines contained a small fraction of CD133⁺ cells ranging from 3% to 5% and showed the following phenotype: high proliferation rate, cell cycle detection in a G2/M phase, positive for Ki-67, formation of spheres, and inclusion of a small subset of SP cells (0.97%). Their further analysis revealed that all of the 21 primary human sarcoma samples including osteosarcoma contained a small population of CD133⁺ cells by flow cytometric analysis (Tirino et al. 2011). CD133⁺ cells of 2 stabilized cell lines from osteosarcoma samples, 7.8% and 5.0%, respectively, also showed self-renewal, formation of spheres, adipogenic and osteogenic differentiation, high expression of stemness genes, and tumorigenicity *in vivo*, reinforcing the hypothesis that CD133 is a reliable marker. Veselska et al. identified the cells expressing nestin in all 18 of the osteosarcoma primary samples (Veselska et al. 2009). In 4 of the 18 stabilized cell lines, 3 contained nestin⁺/CD133⁺ cells at a frequency of 11–100% by immunohistochemistry.

The cell growth of CD133⁺ cells increased using the Hydro Focusing Bioreactor (HFB), simulating aspects of hypogravity (Kelly et al. 2010). Interestingly, Kelly et al., using the SaOS2 cell line containing about 11% of CD133⁺ cells, found that the hypogravity environment of the HFB greatly sensitized the CD133⁺ cancer cells, which are normally resistant to chemotherapy, to become susceptible to various chemotherapeutic agents.

CD117 and Stro-1. The CD117/c-kit is a 145-kDa transmembrane glycoprotein and is expressed in both hematopoietic stem cells (HSCs) and MSCs (Adhikari et al. 2010). CD117 has been suggested as a prognostic marker for osteosarcoma because its higher expression is associated with a poorer outcome in patients, metastasis, and local recurrence (Entz-Werle et al. 2005, Sulzbacher et al. 2007, Wei et al. 2008). Stro-1, originally identified as an antigen expressed by stromal elements in human bone marrow, is exclusively expressed in MSCs (Gronthos et al. 1994).

CD117⁺Stro-1⁺ cells of osteosarcoma showed CSC phenotypes with high invasiveness and chemoresistance (Adhikari et al. 2010). Adhikari et al. found that spheres from murine osteosarcoma cell lines (318-1, P932, and K7M2) showed higher expression of *ABCG2*, *CXCR4*, *CD117*, and *Stro-1*, and higher tumorigenicity *in vivo* than those of the monolayer (Mo) cells. Furthermore, CD117⁺Stro-1⁺ (DP) cells had differentiation ability, enriched for *CXCR4* and *ABCG2*, and showed higher resistance to DOX and higher

tumorigenicity and metastatic ability than CD117⁺Stro-1⁻ (DN) cells and Mo cells. These properties of CD117⁺Stro-1⁺ (DP) cells were validated on human osteosarcoma cell lines, KHOS and MNNG/HOS, and a primary cell line, BCOS. Thus, both mouse and human CD117⁺Stro-1⁺ cells of osteosarcoma were shown to possess CSC properties, including self-renewal, drug resistance, tumorigenicity, and high metastatic potential. However, the reliability of CD117⁺Stro-1⁺ in clinical samples remains controversial. Tirino et al. reported that all 21 samples in their analysis were negative for CD117 (Tirino et al. 2011). Further investigation and discussion including clinical samples are necessary to confirm the possibility that they are appropriate markers of CSCs.

ALDH. ALDH is a detoxifying enzyme responsible for the oxidation of intracellular aldehydes (Sladek 2003). ALDH has been reported to play a role in the early differentiation of stem cells in the oxidation of retinol to retinoic acid (Ambroziak et al. 1999, Duester et al. 2003). High ALDH activity has been observed in murine and human hematopoietic and neural stem and progenitor cells (Armstrong et al. 2004, Hess et al. 2004, Matsui et al. 2004). ALDH activity may provide a common marker for both normal and malignant stem and progenitor cells. For example, an increase in ALDH activity has been found in stem cell populations in multiple myeloma and AML (Matsui et al. 2004, Pearce et al. 2005). The measurement of ALDH activity has been a useful approach in the identification and isolation of CSCs in several tumors (Awad et al. 2010, Charafe-Jauffret et al. 2010, Carpentino et al. 2009).

Wang et al. found that OS99-1, derived from a highly aggressive primary human osteosarcoma, contained cells with high ALDH activity (ALDH^{br} cells) at a frequency of 45.07%, while 1.84% in HuO9, 1.56% in SaOS2, and 0.59% in MG63 (Wang et al. 2010). No statistically significant difference in tumor formation was found between ALDH^{br} and ALDH^{lo} cells sorted from the OS99-1 cell line. Interestingly, the percentage of ALDH^{br} cells in the OS99-1 xenograft was decreased to 3.13%, which was around a 15-fold reduction, suggesting that ALDH activity might differ according to the environment of the tumor. ALDH^{br} cells from the xenograft showed a higher proliferation rate, clone formation, and expression of *Oct3/4A*, *Nanog*, and *Sox2* than ALDH^{lo} cells from the xenograft. In an additional animal experiment, ALDH^{br} cells from tumors generated from a xenograft with as few as 100 cells maintained the phenotype after serial transplantation, whereas ALDH^{lo} cells from the xenograft formed no tumors with at least 1x10⁴ cells. Thus, it seemed to be more reliable to use an *in vivo* model that provides a physiologic environment to develop the CSCs that can be isolated in human osteosarcoma (Wang et al. 2010). On the other hand, Honoki et al. demonstrated that MG63 contained 11% ALDH1 positive cells with the

ability to form spheres, self-renew with secondary sphere formation, and increase expression level of *Oct3/4A*, *Nanog*, *Sox2*, and *Stat3*. Furthermore, these spheres had a higher level of chemoresistance to DOX and CDDP than monolayer adherent cells (Honoki et al. 2010).

Oct3/4. The transcription factor Oct3/4 (also known as POU5F1 and Oct-4) is regarded as one of the key regulators of pluripotency (Jong and Looijenga 2006). Expression in nonmalignant cells is restricted to the pluripotent cells in the embryo and the primordial germ cells that will pass pluripotency to future generations via the gametes. Aberrant expression of Oct3/4 has also been suggested to fulfill an oncogenic role in tumorigenesis (Gidekel et al. 2003, Tai et al. 2005, Monk and Holding 2001).

Levings et al. found that osteosarcoma cells derived from biopsies contained a small population of self-renewed spherical clones in an anchorage-independent, serum-starved environment in which the spheres showed a significant enhancement of *Oct-4* and *Nanog* (Levings et al. 2009). A tumorigenic osteosarcoma cell line from biopsy, OS521, was engineered to activate an Oct-4 promoter/GFP reporter (OS521Oct4-p). GFP⁺ cells made up 23% of the culture and 67% of the xenograft. The mesenchymal stem cell-associated surface antigens, CD105 and ICAM-1, were positive in GFP⁺ cells from the xenograft tumor. The GFP⁺ cells were at least 100-fold more tumorigenic, capable of forming tumors at less than 300 cells, and formed metastases in the lung. On the other hand, in the GFP-depleted group, only 1 of 8 mice developed a tumor at 3,000 cells, and none of the mice developed tumors at 300 cells. Finally, OS521Oct4-pGFP⁺ cells were capable of self-renewal in several passages, forming heterogeneous tumors for Oct-4/GFP expression.

Sox2. Sox2 is a transcription factor of the high mobility group (HMG) domain family that plays a critical role in embryonic development and in maintaining pluripotency and self renewal in embryonic stem cells (Yuan et al. 1995, Avilion et al. 2003, Masui 2007) and several cell lineages, such as neural (Pevny and Nicolis 2010) and tracheal cells (Que et al. 2009).

Basu-Roy et al. found that Sox2 mRNA and protein were highly expressed in 7 human osteosarcoma cell lines (MG63, HOS, U2OS, OS187, SJSA, OS-99-1, SaOS2, and SaOS2-LM7) and 4 murine osteosarcoma cell lines (mOS-202M, mOS-379, mOS-482, and mOS-648). All 18 osteosarcoma tissues were also positive for Sox2 at variably high levels (Basu-Roy et al. 2011). Sox2 depletion by shRNA decreased colony formation in soft agar, migration, invasion, and tumorigenicity *in vivo*. Spheres of osteosarcoma were enriched for Sox2 and Sca-1, a stem cell antigen of the hematopoietic system. The Sca-1^{hi}Sox2^{hi} fraction was identified at frequencies of 45–74.5% in 3 murine cell lines and showed asymmetric division and adipogenic differentiation. Interestingly, high Sox2 expression was accompanied by

reduced Wnt signaling, while activation of the Wnt signaling resulted in low Sox2 expression, suggesting that activation of Wnt signaling led to antagonize the effect of Sox2 in maintaining osteosarcoma cells.

Ewing sarcoma-Sarcosphere, CD133, and ALDH. Since its first description by James Ewing in 1921, Ewing sarcoma has been a cryptic malignancy (Jedlicka 2010). Ewing sarcoma is relatively uncommon: it accounts for 6–8% of primary malignant bone tumors and is less common than osteosarcoma and chondrosarcoma (Fletcher et al. 2002). The most common bony sites are the long bones of the extremities, pelvis, chest wall, and spine. Lesions of long bones typically involve the diaphysis. Biologically, Ewing sarcoma is a classic example of a malignancy driven by a fusion oncogene. Detection of EWS-/FLI-1 gene fusion transcripts by RT-PCR is widely used as a tool in the diagnosis of tumors of Ewing sarcoma (Downing et al. 1995). Since Ewing's sarcoma is relatively sensitive to chemotherapy and radiotherapy, the combination with surgery, chemotherapy, and radiotherapy is the standard treatment. Multi-disciplinary care incorporating advances in diagnosis, surgery, chemotherapy, and radiation has substantially improved the survival rate of patients with localized Ewing sarcoma to nearly 70% (Subbiah et al. 2009).

Fujii et al. demonstrated that the HTB166 cell line formed spheres at a frequency of 1/128 (466 colonies/60,000 cells), which expressed a higher level of Oct3/4, Nanog, STAT3, Sox2, Sox10, and EWS-FLI1 than the adherent cells (Fujii et al. 2009). The spheres also showed higher chemoresistance to DOX and CDDP and increased expression of DNA repair enzyme genes *MLH1* and *MSH2*, similarly to osteosarcoma cells.

A subpopulation of ESFT cells expressing CD133 was demonstrated to show CSC phenotypes (Suva et al. 2009). Suva et al. isolated CD133⁺ cells from 3 ESFT surgical samples using an autoMACS device and revealed that 4% to 8% of bulk ESFT cell populations expressed CD133. Both CD133⁺ and CD133⁻ cells were injected beneath the renal capsule of NOD-SCID mice, which displayed higher tumorigenicity in CD133⁺ cells than in CD133⁻ cells. Moreover, CD133⁺ cells displayed spherogenic potential, differentiation along adipogenic, osteogenic, and chondrogenic lineages, and a high expression level of *Oct4* and *Nanog*. Jiang et al. also focused on CD133 as a CSC marker of ESFT (Jiang et al. 2010). Their analysis demonstrated heterogeneity in CD133 expression in ESFT tumors and cell lines. CD133 expression in 48 primary ESFT samples was absent or extremely low by qRT-PCR: < 1% in 37, 1–2% in 5, 2–3% in 2, > 3% in 4 tumors. Of the last 4 patients with CD133⁺, two cases were drug-resistant, but the others were long-term, event-free survivors. Moreover, the frequency of CD133⁺ cells ranged from 2–99%, and, with one exception, no differences were detected in the chemoresistance or tumorigenicity between CD133⁺ and CD133⁻ cells.

Briefly, only a few cases showed CSC phenotypes. Therefore, their study suggests that CD133 expression alone will be insufficient to isolate drug-resistant CSCs in ESFT.

Awad et al. investigated whether ALDH^{high} cells of ESFT possessed CSC phenotypes (Awad et al. 2010). They isolated the cells with the highest and lowest 2% of ALDH activity from 5 ESFT cell lines (TC-71, MHH-ES, SK-ES-1, A4573, RD-ES). ALDH^{high} cells were enriched for clonogenicity, sphere formation, expression of *Oct4*, *Bmi-1*, and *Nanog*, chemoresistance against DOX, and tumorigenicity *in vivo*. Interestingly, ALDH^{high} cells were resistant to DOX but sensitive to YK-4-279, a small molecule of the inhibitor of EWS-FLI1.

Chondrosarcoma-Sarcosphere, SP, CD133, and ALDH. Chondrosarcomas are a heterogeneous group of malignant tumors showing hyaline cartilage differentiation. Most cases of chondrosarcomas are slow-growing and rarely metastasize (Gelderblom et al. 2008). They are primarily bone tumors and are predominantly found in adults, among whom they are the third most common malignant bone tumor after myeloma and osteosarcoma (Fletcher et al. 2002). Prognosis depends largely on the histological grade. Conventional chondrosarcomas account for the vast majority, and approximately 90% of them are low- to intermediate-grade tumors characterized by indolent clinical behavior and low metastatic potential. Most of the others are high-grade lesions, which commonly metastasize. They are generally unresponsive to chemotherapy and radiotherapy. Treatment is usually limited to surgical resection; however, patient survival with high-grade chondrosarcoma is poor, even with wide surgical resection (Ozaki et al. 1997, Jamil et al. 2010).

Gibbs et al. found that chondrosarcoma cells that originated from patient biopsies formed spherical colonies in a serum-starved, anchorage-independent environment. These spheres, as well as tissue specimens, expressed activated *STAT3*, *Oct3/4*, and *Nanog* more than adherent cells. They also displayed *Stro-1*, *CD105*, and *CD44* as well as the marker genes of mesodermal, ectodermal, and endodermal differentiation (Gibbs et al. 2005). Wu et al. identified SP cells in 2 of 4 primary tissue samples by flow cytometry (Wu et al. 2007); however, the frequencies were not proven. Tirino et al. identified CD133⁺ cells in all 6 clinical samples at a frequency of 0.39–3.5% by flow cytometry. Of 6 samples, 2 could be used to establish cell lines, which formed spheres and showed higher tumorigenicity in NOD-SCID mice than adherent cells (Tirino et al. 2011). To date, no functional analysis according to the markers has been conducted in chondrosarcoma.

Chordoma-CD133, and SP. Chordoma is a rare, low-grade, primary malignant bone tumor arising from primitive notochord remnants of the axial skeleton. The sacrum represents a more common anatomical site of origin accounting

for 50–60% of all cases followed by the skull base region (spheno-occipital/nasal) (25–35% of cases), the cervical vertebrae (approximately 10% of cases), and the thoracolumbar vertebrae (approximately 5% of cases). Although it is considered to be of low metastatic potential, up to 40–60% of patients are reported to develop distant metastases over the course of their disease (Higinbotham et al. 1967, Chambers et al. 1979, Catton et al. 1996). Adequate wide surgery still remains the cornerstone of treatment for chordomas even though safe margins are often hard to obtain because of their anatomical sites of origin. Sensitivity to chemotherapy is very low and generally reported in a small subgroup of patients with high-grade dedifferentiated chordomas and agents active in high-grade sarcomas (Fleming et al. 1993). Proton radiotherapy may succeed in offering better tumor control and fewer side effect even if it is still not as readily available in comparison to external-beam radiotherapy. The 5- and 10-year survival rates of the entire series of patients were 76 and 59%, respectively (Ferraresi et al. 2010).

Only two studies have been reported on the markers of CSC in chordoma. Tirino et al. found that chordoma biopsy cells from the sacrum of a 39-year-old female contained CD133⁺ cells at a frequency of 0.8%. The cells were positive for CD90, CD44, and CD117 at 13.98%, 14.56%, and 0.01%, respectively (Tirino et al. 2011). Wu et al. reported that SP cells were found in a primary chordoma sample; however, the amount was not indicated (Wu et al. 2007). The functional analysis of CSC phenotypes would be the next step for chordoma CSC research.

Soft tissue sarcoma

Synovial sarcoma: CD133 and SP. Synovial sarcomas account for up to 10 percent of soft-tissue sarcomas and include two major histological subtypes, biphasic and monophasic, defined respectively by the presence and absence of glandular epithelial differentiation in a background of spindle tumor cells (Kawai et al. 1998). They can occur anywhere in the body and feature local invasiveness and a propensity to metastasize (Ferrari et al. 2008). A characteristic SYT–SSX fusion gene resulting from the chromosomal translocation t(X;18) (p11;q11) is detectable in almost all synovial sarcomas. Although treatment is predicated on surgery, adjuvant radiation and/or chemotherapy, such as DOX and IFO, may be beneficial, particularly in high-risk patients. Eilber et al. demonstrated that the 4-year disease-free survival of patients treated with ifosfamide was 88% compared with 67% for the patients who did not undergo chemotherapy (Eilber et al. 2007).

Terry et al. investigated 5 primary synovial sarcoma and 3 synovial sarcoma cell lines for the expression of CD133 by flow cytometry and immunohistochemistry. Subsequently, the Fuji, SYO-1, and KU-SS-1 cell lines contained 2.6–16.8% of CD133⁺ cells, and all 5 of the primary samples

showed the presence of CD133 at a frequency of 1.5–20.5%. CD133⁺ of three cell lines were dispersed and appeared to have dendritic processes (Terry et al. 2010). Tirino et al. also identified CD133⁺ cells from the biopsies of 3 patients at a frequency of 0.84–7.23%. All samples were negative for CD117 (Tirino et al. 2011). No functional analysis on CSC phenotypes was conducted. Wu et al. found SP cells in 2/3 of cases, although the frequencies were not shown. One sample was sorted into both SP and non-SP cells, and SP cells formed tumors at a higher frequency than non-SP cells (Wu et al. 2007).

Leiomyosarcoma: SP and CD133. Leiomyosarcoma is a malignant tumor composed of cells showing distinct smooth muscle features. Soft-tissue leiomyosarcoma usually occurs in middle-aged or older individuals, although it may develop in young adults and even children. It arises in retroperitoneal lesions (40–45%), extremities (30–35%), skin (15–20%), and larger blood vessels (5%). Surgical wide resection is the most reliable treatment. Although the effectiveness of chemotherapy and radiotherapy is uncertain, a clear survival benefit is evident if surgical margins are narrow or not clear of tumor cells. The prognosis is poor, with survival rates among the lowest of all soft tissue sarcomas (Mankin et al. 2004). For patients with leiomyosarcomas in the extremities, Farshid et al. reported 10–25% as local recurrence rate, 64% as 5-year survival rate (Farshid et al. 2002).

SP cells were detected in 2 primary leiomyosarcoma samples, which were positive at about 3% in one sample (Wu et al. 2007). The other sample was grade 2 and did not contain SP cells. Tirino et al. reported that CD133⁺ cells were present at 0.9% in a biopsy sample from the scapulohumeral region in a 39-year-old male (Tirino et al. 2011). The cells were positive at 78.45% for CD90 and 15.0% for CD44 and negative for CD117. Neither study included an *in vitro* and *in vivo* functional analysis, which should be considered in any further research.

Malignant fibrous histiocytoma (MFH): SP. In 2002, the World Health Organization (WHO) declassified MFH as a formal diagnostic entity and renamed it as an undifferentiated pleomorphic sarcoma not otherwise specified (NOS) (Fletcher et al. 2002). While it remains unclear how to most accurately organize these tumors, the term malignant fibrous histiocytoma represents the diagnosis for thousands of patients and is still commonly used by both patients and physicians. MFH of soft tissue typically presents in a patient that is approximately 50 to 70 years of age, although it can appear at any age. It can arise in any part of the body but most commonly in the lower extremities, especially the thigh. Since MFH tends to be resistant to chemotherapy and radiotherapy, surgical resection is the most reliable treatment. Chemotherapy has often been administered to patients who already have metastatic disease or who are at the highest risk for metastasis.

The overall 5-year survival rate of undifferentiated pleomorphic sarcomas/NOS of the extremities has been reported to be 60–80% (Lehnhardt et al. 2009).

Murase et al. demonstrated that the MFH2003 cell line contained 5.28% of SP cells. These cells showed a higher capacity for the formation of spherical colonies, self-renewal, differentiation into non-SP cells, and greater tumorigenic potential than non-SP cells *in vivo* (Murase et al. 2009). Wu et al. investigated SP cells in four primary leiomyosarcoma samples, three of which were positive. One sample of grade 3 contained SP cells at a frequency of 10%. Compared with other grade 1 and 2 samples, higher-grade tumors included a higher proportion of SP cells. SP cells could form tumors at 100 cells, whereas non-SP cells could not (Wu et al. 2008):

Liposarcoma: ALDH/CD133 and SP. Liposarcomas (LSs) are subdivided into four major types: atypical lipoma (ATL)/well-differentiated liposarcoma (WDLS), myxoid liposarcoma (MLS), pleomorphic liposarcoma (PLS), and mixed liposarcoma. Dedifferentiated liposarcoma (DDLs) is defined as a WDLS that shows abrupt transition to a non-lipogenic sarcoma at least several millimeters in diameter. In addition to distinctive morphology, each of the subgroups has a different prognosis and treatment strategy. Surgical resection is the mainstay of curative treatment, however, large, high-grade liposarcomas may benefit from multimodality treatment with chemotherapy and radiation. MLS is relatively chemosensitive in comparison to the other types (Jones et al. 2005). Although the prognosis of WDLS is good, the 5-year survival of DDLs is about 30% and that of liposarcoma of the retroperitoneum is much worse.

Stratford et al. demonstrated that ALDH^{high}CD133^{high} cells of liposarcoma have CSC characteristics (Stratford et al. 2011). Immunohistochemical analyses of ALDH1 expression in liposarcoma patient samples including a range of subtypes of WDLS, DDLs, MLS, and PLS confirmed that all 10 samples expressed ALDH1. Furthermore, 8 of 10 samples expressed ALDH1 in more than 50% of the tumor cells. To determine whether ALDH^{high} cells show CSC activity, they used a xenograft model of the SW872 cell line from a surgical specimen with histopathology of undifferentiated malignant liposarcoma. In their screening of xenograft derived cells for the presence of MSC markers and CSC markers, ALDH^{high}CD133^{high} cells were observed in a small population (0.1–1.7%) in a stem cell medium but not a RPMI medium. These cells have an enhanced ability to form spheroids, to differentiate into adipocytes, and to form tumors more efficiently *in vivo* than ALDH⁺CD133⁻, ALDH⁻CD133⁺, and ALDH⁻CD133⁻ cells. Wu et al. investigated SP cells in one MLS and one PLS, both of which were positive for SP fractions, although the frequencies were not reported (Wu et al. 2008). Tirino et al. found CD133⁺ cells in 3 patient biopsies at 0.31–5.20%.

All samples were negative for CD117 (Tirino et al. 2011). Further analysis of CSC phenotypes would be expected.

Rhabdomyosarcoma: SP, CD133 and FGFR3. Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood, representing 5 to 8% of all pediatric malignancies (De Giovanni et al. 2009). Histopathologically, RMS is classified into three types; embryonal (eRMS), alveolar (aRMS), and pleomorphic types. It occurs in most parts of the body but more frequently in spaces surrounding the brain, the trunk, and genitourinary tract (McDowell 2003). It has been suggested that mesenchymal stem cells (MSCs) might be the origin of rhabdomyosarcomas (Hirotsu et al. 2009, Merlino and Khanna 2007), whereas some reports indicate that also neuronal cells can transform into malignant myogenic cells after activation and a large number of neuronal genes are expressed in RMS. Hence, the origin of potential RMS stem cells remains to be determined (Galli et al. 2000, Wachtel et al. 2006). All cases with RMS are treated with chemotherapy. Depending upon the size and location of the primary tumor and how much of it can be surgically removed, most cases will also receive some combination of radiation therapy and surgery. Adult patients who had a complete response to chemotherapy had a 5-year survival rate of 57% compared with a rate of only 7% for poor responders (Esnaola et al. 2001).

Tsuchida et al. found an SP fraction in RH-4, a drug-resistant rhabdomyosarcoma cell line, at a frequency of 1.5–2.0% (Tsuchida et al. 2008). SP cells were resistant to CDDP treatment, and tumors formed after injection of 2.5×10^5 cells, whereas tumors did not form with non-SP cells. CDDP increased the SP fraction and enhanced the clonogenic activity and stemness gene expression of SP cells. “Rhabdospheres” formed from eRMS cells, which are enriched in a CD133⁺ CSC population (Walter et al. 2011). Three eRMS cell lines (RD, Rh36, and Ruch2) formed spheres in a stem cell medium, which is more tumorigenic *in vivo* than their adherent cells. Stem cell genes, such as *Oct4*, *Nanog*, *c-Myc*, *Pax3*, and *Sox2*, are significantly upregulated in spheres that were differentiated into adipocytes, myocytes, and neuronal cells. Furthermore, CD133 was upregulated in rhabdospheres both on mRNA and protein levels. RD CD133⁺ sorted cells were subsequently shown to be more tumorigenic and more resistant to cisplatin and chlorambucil. Finally, a tissue microarray of eRMS patients was performed and found that high expression of CD133 correlated with poor overall survival.

Hirotsu et al. screened many markers, including SP, CD9, CD10, CD13, CD29, CD31, CD34, CD44, CD117, CD113, FLT3, LNGFR, and FGFR3, for KYM-1, RD, and A204 cell lines. These markers contained a small population of FGFR3⁺ cells at a frequency of 1.6–2.6%. Surprisingly, 2 of 6

mice inoculated with only single FGFR3⁺ KYM-1 cells formed tumors *in vivo*, whereas those inoculated with FGFR3⁻ KYM-1 cells did not. FGFR3⁺ KYM-1 cells showed upregulation of undifferentiated markers, such as *CD34*, *PAX3*, *PAX7*, *MYF5*, *NANOG*, *OCT3*, and *SOX2*, and downregulation of differentiated muscle markers, such as *MYH1*, *desmin*, *myogenin*, and *dystrophin*. The expression of FGFR3 was validated in eRMS patient biopsy specimens to be positive or upregulated by immunohistochemistry and RT-PCR (Hirotsu et al. 2009).

Fibrosarcoma: Sarcosphere, CD133, and ALDH. Fibrosarcoma, in which histologically the predominant cells are fibroblasts that divide excessively without cellular control, can arise from both bone and soft tissue. Fibrosarcoma of bone can occur in patients of any age but are most common between the second and sixth decades of life. Fibrosarcoma of soft tissue is classified into infantile fibrosarcoma and adult fibrosarcoma. Surgical resection is the standard treatment, and some patients are candidates for chemotherapy, although the efficacy has not been proven. The 5-year-survival rate of soft tissue fibrosarcoma is reported to be 58% in grade 1 and 2, 34% in grade 3, and 21% in grade 4 cases (Scott et al. 1989).

The HT1080 cell line was found to form spheres at a frequency of 1/180 (553 colonies/100,000 cells), which showed an increased level of *Oct3/4*, *Nanog*, *STAT3*, *Sox2*, *Sox10*, *MLH1*, and *MSH2* compared with adherent cells (Fujii et al. 2009). Honoki et al. found a population of cells expressing high ALDH activity with frequencies of 9% in an HT1080 cell line. ALDH1⁺ cells showed high frequency of sphere formation in an anchorage-independent environment, in contrast to ALDH1⁻ cells (Honoki et al. 2010). A small subset of CD133⁺ cells were identified in 4 fibrosarcoma biopsies, ranging from 0.42% to 1.23% (Tirino et al. 2011). The HT1080 cell line expressed 3% of CD133⁺ by flow cytometry. Spheres of HT1080 CD133⁺ cells, showing increased expression of *Oct3/4*, *Nanog*, *Sox2*, and *nestin*, formed colonies in soft agar more efficiently and showed 2500-fold greater tumorigenicity than adherent culture cells.

Others: Hiwi, hTERT, Survivin, LGR5 and the other markers. Several reports have focused on the stem cell genes and cell surface markers of MSC and NCSC in various histological soft tissue sarcomas.

Because of difficulty in the detection and isolation of CSCs and controversy as to the presence of CSC, Taubert et al. approached this subject by analysis of stem cell-associated genes for stem cell identification (Taubert et al. 2007). The expression of three genes, *Hiwi*, *hTERT*, and *survivin*, was evaluated, which had previously been shown to be overexpressed in both stem cells and cancer cells. *Hiwi* belongs to the Piwi-domain proteins, which are components of ribonucleoprotein complexes. Taubert et al. had already reported an association between altered expression of *Hiwi*

mRNA and a poor prognosis for soft tissue sarcoma patients (Qiao et al. 2002, Liu et al. 2006, Taubert et al. 2007). hTERT is the catalytic subunit of telomerase, which functions in the synthesis and extension of telomeric DNA. High telomerase activity is observed in more than 85% of human cancer cells, strongly suggesting a key role during tumorigenesis (Pendino et al. 2006). Survivin is a member of a family of apoptosis inhibitors (Altieri 2003) and is considered to be an interface molecule between the regulation of apoptosis and the control of cell proliferation (Altieri 2003). A cohort of 104 primary STS, including liposarcoma, MFH, neurogenic sarcoma, rhabdomyosarcoma, leiomyosarcoma, and other STS, was investigated by quantitative real-time PCR for their RNA coexpression of the three genes. Upon analysis of a single gene, only an increased expression of *hTERT* was associated with a poor outcome. Moreover, coexpression of all three genes showed a 74.6-fold increased risk of tumor-related death compared with patients with an intermediate level of *Hiwi*, low level of *survivin* and low level of *hTERT* mRNA expression. Additional analysis was performed with regard to the association among the expression of these three genes and the tumor stage and prognosis, and the findings show that the risk of tumor-related death was based on the coexpression of stem cell-associated genes rather than on tumor stage (Taubert et al. 2007).

The prognostic impact of the stem cell marker, cancer-associated gene, and Wnt/Tcf4 target gene LGR5/GPR49 in 77 frozen tumor samples from liposarcoma, MFH, neurogenic sarcoma, RMS, and the other STS was investigated by qRT-PCR (Rot et al. 2011). LGR5/GPR49 had been identified as a novel stem cell marker in intestinal epithelia, stomach, and hair follicles (Barker et al. 2010). Their analysis included a transcript variant of *LGR5/GPR49* lacking exon 5 (*LGR5/GPR49Δ5*). As a result, a low mRNA expression level of *GPR49Δ5*, a transcript variant of *LGR5/GPR49* lacking exon 5, but not of wild type *LGR5/GPR49*, was significantly correlated with a poor prognosis for the disease-associated survival of STS patients and associated with a shorter recurrence-free survival. Thus, transcript variant *GPR49Δ5* was a negative prognostic marker for disease-associated and recurrence-free survival in STS patients (Rot et al. 2011).

Sadikovic et al. screened Ewing sarcoma, eRMS, aRMS, SS, MPNST, and the other undifferentiated soft tissue sarcomas (USTSs) for the presence of 3 mesenchymal stem cell markers (CD44, CD105, and CD166) and 5 neural stem cell markers (CD15, CD29, CD56, CD133, and nestin) by immunohistochemistry. Subsequently, CD56 could distinguish ES and USTS/eRMS, aRMS, MPNST, and SS, and CD166 could distinguish between ES and USTS by cluster analysis (Sadikovic et al. 2011). However, no discussion was presented with regard to the relationship with CSCs, which is a matter of interest.

Targeting CSCs in Sarcoma—Molecular Mechanisms Underlying the CSC Phenotype

Self-renewal pathways

For the principles of stem-cell biology to apply to tumorigenesis, cancers would have to be organized hierarchically into clonally derived populations of cells with different proliferative potentials, just like cells within normal tissues (Pardal et al. 2003). Considering the self-renewability of CSCs, there may be some kinds of self-renewal pathway in their biology. There are some reports on CSCs in carcinomas, but they are rare in sarcomas, which is the current matter of interest. Indeed, little is known about molecular mechanisms underlying the CSC phenotype of sarcomas. Multiple pathways including self-renewal pathway within sarcomas or MSCs might be clues to the mystery.

Wnt pathway. The Wnt signal transduction pathway coordinates myriad activities, from development and differentiation to proliferation and tumorigenesis (Matushansky et al. 2008). Aberrant Wnt signaling has been reported in tumors from patients with hepatocellular carcinoma, hepatoblastoma, colorectal cancer, AML, CML, multiple myeloma, gastric cancer, and Wilms tumor (reviewed in Takebe et al. 2011). In addition, deregulated Wnt signaling has been associated with CSC activity; specifically, cutaneous CSCs require β -catenin signaling to maintain their tumorigenic phenotype (Malanchi et al. 2008).

In striking contrast to carcinomas as described above, rarely has Wnt/ β -catenin nuclear activity been noted in sarcomas (Matushansky et al. 2008). Ng et al. examined 549 cases of a diverse group of sarcomas. No high-level nuclear β -catenin expression was observed in any of 381 cases of fibrohistocytic, muscular, adipocytic, chondroid, or osseous tumors representing 42 diagnostic categories. However, there were several other categories that did show high levels of nuclear β -catenin, including desmoid-type fibromatosis (71% of cases), solitary fibrous tumors (40%), and synovial sarcoma (28%) (Ng et al. 2005). These data suggest that Wnt signaling may have a very different role in sarcomagenesis in comparison to the development of other malignancies (Matushansky et al. 2008). Matushansky et al. showed that the Wnt inhibitor Dickkopf-1 (Dkk1) is overexpressed in MFH and that it mediates the transformation of MSCs into MFH-like tumors *in vivo*. They also showed that activating Wnt signaling in MFH allows for their controlled differentiation toward mature connective tissue lineages (Matushansky et al. 2006).

Wnt signaling has also been well studied in MSCs. Gregory et al. demonstrated that Dkk1 promotes MSC self-renewal (Gregory et al. 2003). More importantly, Dkk1 expanded MSCs and maintained their

pluripotentiality. In contrast to this work, Baksh et al. showed that Wnt3a (and resultant β -catenin activation) can similarly expand MSCs capable of maintaining their pluripotentiality (Baksh et al. 2007). Whether β -catenin promotes or inhibits MSC self-renewal remains unclear.

One study on regulating CSCs of sarcoma by inhibition of Wnt/ β -catenin signaling was reported. Tang et al. investigated whether salinomycin acts against osteosarcoma stem cells (Tang et al. 2011). Salinomycin treatment was reported to overcome ABC transporter-mediated multi-drug resistance and regarded as a P-glycoprotein inhibitor. It was also recognized as a selective inhibitor of breast CSCs and leukemia stem cells (Gupta et al. 2009, Fuchs et al. 2010). Salinomycin was found to inhibit the sarcosphere formation of these cells and sensitize spheres to DOX *in vitro* and *in vivo*. Subsequently, salinomycin was shown to downregulate Wnt/ β -catenin signaling by decreased β -catenin and cyclin D1 by immunohistochemistry and decreased protein level of phosphor-GSK3 β and cyclin D1 by Western blot analysis (Tang et al. 2011).

Notch signaling. Notch signaling plays a key role in the normal development of many tissues and cell types through diverse effects on cell fate decision, stem cell renewal, differentiation, survival, and proliferation (Artavanis-Tsakonas et al. 1999). Notch itself is a transmembrane receptor. When Notch interacts with membrane-bound ligand Delta or Jagged on the surface of neighboring cells, the Notch intracellular domain (NICD) is proteolytically cleaved from the membrane by γ -secretase and translocates to the nucleus to complex with and to activate the transcription factor CSL (human CBF1, fly Suppressor of Hairless, worm Lag-1). CSL then recruits the co-activator Mastermind-like (MAML) and initiates transcription of target genes such as Hes and Hey (Bolós et al. 2007, Watt et al. 2008).

Notch signaling seems to function as an oncogene or a tumor suppressor, depending on the cellular context (Zhang et al. 2008). In addition to the reports on carcinomas and blood malignancies, the roles of Notch signaling in osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma are demonstrated. Through pharmacological and direct retroviral modulation of the Notch pathway, Zhang et al. found that Notch signaling induces invasiveness and metastasis of osteosarcoma *in vitro* and *in vivo* but does not affect cell proliferation, survival, or tumorigenesis (Zhang et al. 2008). The Notch target gene *HES1* was sufficient to induce an invasive and metastatic phenotype in osteosarcoma. In ESFT, Bennani-Baiti et al. reported that Notch signaling is switched off despite the expression of Notch receptors and *HES1* being uncoupled from Notch in ESFT (Bennani-Baiti et al. 2011). Furthermore, activated Notch proved to block ESFT cell proliferation and reduce the ESFT clonogenic potential, suggesting that Notch exerts tumor suppressor-like functions in ESFT. In rhabdomyosarcoma, the Notch target

gene HES1 is upregulated and prevents tumor cell differentiation in a Notch-dependent manner (Raimondi et al. 2011). Raimondi et al. demonstrated that inhibition of Notch3 signaling induces rhabdomyosarcoma cell differentiation promoting p38 phosphorylation and p21Cip1 expression and hampers tumor cell growth *in vitro* and *in vivo*. However, the role of Notch signaling in sarcoma CSCs is unknown.

Notch signaling is also important for MSC differentiation into osteoblasts. Notch signaling improves osteoblastogenesis but does not necessarily enhance bone formation (Lin and Hankenson 2011). The osteoblast-specific gain of the Notch function in the Notch intracellular domain (NICD)-overexpressing transgenic mouse results in abnormally dense or osteosclerotic bone, whereas loss of Notch signaling via γ -secretase mutations leads to late-onset, age-related osteoporosis (Engin et al. 2008). The osteosclerotic phenotype is caused by enhanced proliferation of immature osteoblasts and not due to decreased osteoclastic activity. Conversely, the osteoporotic phenotype in loss-of-Notch-function mice displayed increased osteoclast numbers as a result of decreased Notch signaling in osteoblasts (Engin et al. 2008). Notch signaling also influences Runx2 function, a transcription factor required for osteoblastogenesis. Runx2 transcriptional activity is physically antagonized by the protein encoded by the Notch target gene *Hey1* (Zamurovic et al. 2004). Therefore, this signaling might have some role with regard to the CSC phenotype; however, none is known.

Hedgehog signaling. The hedgehog (Hh) pathway, which sets off a chain of events in target cells leading to the activation and repression of target genes by transcription factors in the GLI family, is implicated in the maintenance of stem cells in a variety of cancers, including multiple myeloma, myeloid leukemia, and colorectal cancer, therefore representing another potentially druggable target for cancer stem cell elimination (Alison et al. 2010). The Hh receptor Patched 1 (PTC1) inhibits signaling by the membrane protein Smoothed (Smo), and this inhibition is relieved by binding Sonic Hedgehog to PTC. Unrestricted Smo signaling can affect target gene transcription via the GLI family of transcription factors (GLI1, GLI2, and GLI3).

Reduction of human embryonal rhabdomyosarcoma tumor growth by inhibition of the Hh signaling pathway was reported (Tostar et al. 2010). Tostar et al. showed that Hh signaling is critical for the maintenance/survival of human eRMS tumor cell lines. In addition, inhibition of GLI1 by small molecule antagonists, such as GANT61, could be an effective therapeutic option in pediatric eRMS. On the other hand, Nagao et al. showed that GLI2 was aberrantly overexpressed in human osteosarcoma biopsy specimens. Inhibition of GLI2 prevented osteosarcoma growth *in vivo*

and overexpression of GLI2 promoted MSC proliferation and accelerated their cell cycle progression (Nagao et al. 2011). No reports, however, has been reported on the role of the Hh pathway in CSCs of sarcomas.

Plaisant et al. showed that hMSC was endowed with a basal level of Hh signaling that decreased after differentiation of these cells (Plaisant et al. 2009). Inhibition of Hh signaling, using the classical inhibitor cyclopamine, or a siRNA directed against GLI2, leads to a decrease in hMSC proliferation (Plaisant et al. 2011). There seems to be some potential for regulating CSCs in sarcomas by controlling the Hh pathway.

Alternative molecular pathways

MAPK pathway. In addition to Wnt, Notch, and Hedgehog pathways playing a large role in many kinds of sarcomas and CSCs, the mitogen-activated protein kinase (MAPK) pathway has also been observed to play an important role in the pathogenesis of sarcoma. The MAPK signaling pathway occurs downstream of RAS, which is an intracellular central signal transduction mediator also activated by growth factors, such as the platelet-derived growth factor (PDGF) or the vascular endothelial growth factor (VEGF). This pathway consists of three kinases, including RAF1 (MAP kinase-kinase-kinase), MEK (MAP kinase-kinase), and ERK (extracellular signal-related kinase). This kinase cascade is closely related to tumor proliferation and metastasis in several types of cancer (Young et al. 2009, Wang et al. 2009, Ding et al. 2008).

Sasaki et al. demonstrated the role of the MAPK pathway in bone and soft tissue tumors. RAF1 and MEK 1/2 mRNA was detected in 6 human sarcoma cell lines (osteosarcoma and MFH) and all of the 7 surgically obtained OS specimens. Treatment with MEK inhibitor, U0126 [1,4-diamino-2,3-dicyano-1,4-bis (2-aminophenyltio) butadiene], resulted in dose- and time-dependent inhibition of cell proliferation and suppression of p-ERK expression, opposite to the promotion of p-MEK. In view of CSC regulation, Tsuchida et al. demonstrated that U0126 downregulated CDDP-induced VEGF and Flt1, whereas CDDP treatment could upregulate VEGF and Flt1 expression, leading to the survival and expansion of SP cells, as formerly described (Table 6).

miRNAs. MicroRNAs (miRNAs) have been focused on as a novel approach for regulating the phenotypes of CSCs. miRNAs are small regulatory RNA molecules that modulate the expression of their target genes and play important roles in a variety of physiological and pathological processes, such as development, differentiation, cell proliferation, apoptosis, and stress responses (Bartel 2004, Kosaka et al. 2010). Over the past several years, many miRNAs have been investigated in various human cancers (Croce

Table 6. Experimental trials for targeting CSCs or regulating the phenotypes of sarcoma CSCs.

Sarcoma	Agents	Target CSC	Mechanism	Year	Refs. (et al.)
Osteosarcoma	Salinomycin	Sarcosphere	Impairing of Wnt/ β -catenin signaling by degradation of β -catenin	2011	Tang
Osteosarcoma	U0126 (MEK inhibitor)	Side population cells	Downregulation of CDDP-induced VEGF and Flt1 expression through MAPK/ERK1,2 signaling	2008	Tsuchida
Ewing sarcoma	miR-145	EWS-FLI-1-mediated hpMSC	Making a feedback loop with EWS-FLI-1 in reprogramming hpMSC into ESFT CSCs through repression of SOX2, a direct and an indirect EWS-FLI-1 target gene	2010	Riggi
Ewing sarcoma	YK-4-209 (EWS-FLI1 inhibitor)	ALDH ^{high} cells	Blocking RNA helicase A (RHA) binding to EWS-FLI1 (Not understood about the correlations with ALDH)	2010	Awad
MFH	Tc4C-6 (CTL clone)	Side population cells	Specific cytotoxicity against human leukocyte antigen (HLA) Class 1 molecules on the cell surface of the SP cells	2011	Tsukahara