Shared Cell Surface Marker Expression in Mesenchymal Stem Cells and Adult Sarcomas

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**ABSTRACT**
Advanced adult soft-tissue sarcomas (STSs) are rare tumors with a dismal prognosis and limited systemic treatment options. STSs may originate from mesenchymal stem cells (MSCs); the latter have mainly been isolated from adult bone marrow as plastic-adherent cells with differentiation capacity into mesenchymal tissues. Recently, a panel of antibodies has been established that allows for the prospective isolation of primary MSCs with high selectivity. Similar to cancer stem cells in other malignancies, sarcoma stem cells may bear immunophenotypic similarity with the corresponding precursor, that is, MSCs. We therefore set out to establish the expression pattern of MSC markers in sarcoma cell lines and primary tumor samples by flow cytometry. In addition, fibroblasts from different sources were examined. The results document a significant amount of MSC markers shared by sarcoma cells. The expression pattern includes uniformly expressed markers, as well as MSC markers that only stained subpopulations of sarcoma cells. Expression of W5C5, TNAP, CD344, and CD271 marked subpopulations of MSC markers in sarcoma cell lines and primary tumor samples by flow cytometry. In addition, fibroblasts from different sources were examined. The results document a significant amount of MSC markers shared by sarcoma cells. The expression pattern includes uniformly expressed markers, as well as MSC markers that only stained subpopulations of sarcoma cells. Expression of W5C5, TNAP, CD344, and CD271 marked subpopulations displaying increased proliferation potential. Moreover, CD271+ cells displayed in vitro doxorubicin resistance and an increased capacity to form spheres under serum-free conditions. Interestingly, another set of antigens, including the bona fide progenitor cell markers CD117 and CD133, were not expressed. Comparative expression patterns of novel MSC markers in sarcoma cells, as well as fibroblasts and MSCs, are presented. Our data suggest a hierarchical cytoarchitecture of the most common adult type sarcomas and introduce W5C5, TNAP, CD344, and CD271 as potential sarcoma progenitor cell markers.

**INTRODUCTION**

Soft-tissue sarcomas (STSs) include different histologic subtypes, which reflect a spectrum of biologically and prognostically different diseases. Patients with STS typically present at young age, display a good performance status that does not necessarily correspond to tumor burden, but do not respond well to systemic treatment and therefore still have a poor prognosis, with every second patient ultimately dying from the disease [1]. Indeed, despite the rarity of STSs, they range among the five leading causes of death in adolescents and young adults in the U.S. [2].

The histopathological classification of STSs is based on the morphological similarity to the tumor’s corresponding normal tissue. In other words, morphological diagnosis is determined by malignant cell differentiation [3]. Immunohistochemistry allows discernment of STS subentities based on residual differentiation patterns from not otherwise specified, undifferentiated pleomorphic STSs, formerly known as malignant fibrous histiocytoma [3–5].

Success in the treatment of rare STS subtypes such as gastrointestinal stromal tumors (GISTs) and dermatofibrosarcoma protuberans has shown that a better understanding of molecular mechanisms of STS biology may help us find therapeutic targets and improve the prognosis of patients with STS [6, 7]. However, the absolute majority of adult STSs belong to a group of sarcomas with complex karyotypes and a lack of consistently identified molecular changes such as pleomorphic sarcoma, leiomyosarcoma, pleomorphic lipo- and rhabdomyosarcoma, angiosarcoma, osteosarcoma, myxofibrosarcoma, and myofibroblastic sarcoma [8].

The mesenchymal origin of STS appears obvious, because tumors frequently display adipose, smooth or striated muscular, or cartilage-like differentiation. Analogous to hematopoietic stem cells as the origin of malignancy in leukemia (leukemia stem cells [LSCs]) [9] and epithelial stem cells in carcinomas (cancer stem cells [CSCs]) [10–12], mesenchymal stem cells are hypothesized to be the cell of origin of sarcoma [13–18]. Therefore, sarcoma stem cells may
recapitulate the development of their normal tissue equivalents [19] and bear immunophenotypic similarity to MSCs. Recently, a novel panel of mouse anti-human antibodies was developed that are not only suitable for phenotypic characterization of cultured MSCs but also for the prospective isolation of bone marrow- and/or placenta-derived MSC populations [20].

Following the above-mentioned analogy to the development of leukemias [21], we sought to establish the expression profile of sarcomas using a novel MSC marker panel together with selected markers of hematopoietic and tissue-specific stem cells including CD34, CD117, and CD133. To this end, we analyzed both cell lines and primary tumor samples of the most common histologic features by flow cytometry. Both cell types showed frequent expression of mesenchymal, but not of hematopoietic or epithelial progenitor cell markers. When fibroblasts derived from foreskin or embryonic tissues were monitored, another set of markers shared with MSCs was discovered, establishing a high degree of relationship between fibroblasts, MSCs, and STSs.

However, we also identified markers that allow the distinction between benign and malignant progenitor cells on the one hand and differentiated fibroblasts on the other hand. The same set of markers including W5CS, WB82 (tissue nonspecific alkaline phosphatase [TNAP]), CD344 (frizzled-4), and CD271 (low-affinity nerve growth factor receptor) stained tumor subpopulations with increased proliferative potential and increased sarcomphere formation capacity and doxorubicin resistance as compared with unsorted cells. Our results not only reveal cellular hierarchies within the most common adult STS entities but also open up the door for the study of potential sarcoma progenitor cell subpopulations including the identification of novel therapeutic targets.

**Materials and Methods**

**Primary Tumor Samples and STS Cell Lines**

Patients from the University of Tuebingen Center for Soft Tissue Sarcomas, GIST, and Bone Tumors who underwent surgical resection of primary or metastatic tumors were eligible. Fresh tumor samples were obtained from surgical specimens immediately after surgical resection. All patients had given their written informed consent to the scientific analysis of tissue samples, the study of which was approved by the ethics committee of the University of Tuebingen.

Primary tumor samples were processed as follows: tumor samples obtained directly after surgical removal of primary tumor or metastases were mechanically dissociated, digested in 0.05% trypsin (Biochrom AG, Berlin, Germany, http://www.biochrom.de) or Liberase DL and DH (Roche, Indianapolis, IN, http://www.roche.com). After several washing steps, cells either underwent direct flow cytometric analysis or were incubated in Dulbecco’s modified Eagle’s medium (DMEM), Eagle’s minimum essential medium (EMEM), and RPMI medium (Invitrogen, Carlsbad, CA, http://www.invitrogen.com) containing 20% fetal calf serum (FCS; Biochrom) and 1% penicillin/streptomycin (Biochrom) at 37°C for up to 1 week prior to flow cytometric analysis. Human STS cell lines HT1080, SK-LMS1, SW872, and SW982 were obtained from the American Type Culture Collection (Manassas, VA, http://www.atcc.org).

**Antibodies**

The antibodies used in this study are summarized in supplemental online Table 1. The generation and characterization of all proprietary antibodies have been published. The references are listed in supplemental online Table 1.

**Proliferation Assay**

Sarcoma cells were transferred or flow-sorted into white flat-bottomed 96-well plates at limiting dilutions, and proliferation was analyzed after 8 days of growth with the luciferase-based CellTiter-Glo luminescent-cell viability assay (Promega, Mannheim, Germany, http://www.promega.com) according to the manufacturer’s instructions.

**Flow Cytometry**

The cells were transferred to round-bottomed 96-well plates in phosphate-buffered saline (PBS) with 1% FCS and 0.1 mg/ml human IgG1 at a concentration of at least 1 × 10^5 cells per well, incubated with culture supernatants of the antibodies for 15 minutes at 4°C, washed, and incubated again for 15 minutes at 4°C with a fluorescein isothiocyanate-conjugated horse anti-mouse secondary antibody (1:100 in PBS/FCS/human IgG1; Vector Laboratories, Eching, Germany, http://www.vectorlabs.com). After washing, free binding sites were blocked with mouse IgG1 (1:100 in PBS/FCS/human IgG1, 15 minutes 4°C; BD Biosciences, Heidelberg, Germany, http://www.bdbiosciences.com), washed, stained with CD271-APC (1:10 in PBS/FCS/human IgG1; Miltenyi Biotec, Bergisch Gladbach, Germany, http://www.miltenyibiotec.com), and finally analyzed in fluorescence-activated cell sorting buffer (PBS/FCS/human IgG1) containing 1 μg/ml propidium iodide (PI). Pl-positive cells were excluded from the flow cytometric analysis. Where indicated, specific fluorescence indices were calculated by dividing the median fluorescence obtained with specific mAb by median fluorescence obtained with isotype control. The cells were analyzed on a FACSCanto II flow cytometer (BD Biosciences). The data were analyzed using FlowJo 7.4 software (Tree Star, Ashland, OR, http://www.treestar.com).

**Isolation of MSCs From Adult Human Bone Marrow**

MSCs were isolated and propagated in culture as described before [22] and confirmed to represent multilineage differentiation potential toward chondrocytes, adipocytes, and osteocytes (data not shown).

**In Vitro Differentiation of MSCs and Sarcoma Cells**

Adipogenic, osteogenic, and chondrogenic differentiation of mesenchymal stem cells and primary sarcoma cells was obtained with the StemPro osteo-, adipo-, and chondrogenes differentiation kit from Invitrogen, which was used according to the manufacturer’s instructions. The cells were cultured in chamber slides (Falcon; BD Deutschland, Heidelberg, Germany, http://www.bd.com/de/). Adipocytes were distinguished with HCS LipidTOX Green (Invitrogen) after 10 days of adipogenic differentiation. The cells were fixed with 4% paraformaldehyde for 30 minutes and stained with HCS LipidTOX Green (Invitrogen) for an additional 30 minutes. The HCS LipidTOX neutral stain was diluted 1:200 in PBS. The slides were coverslipped with VectaShield HardSet mounting medium with 4’,6-diamidino-2-phenylindole. Calcium accumulations in osteocytes were detected after 21 days of osteogenic differentiation and fixation with 4% paraformaldehyde for 30 minutes. The cells were subsequently stained for 3 minutes in 2% alizarin red S (Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com) at room temperature.

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temperature. After 21 days of chondrogenic differentiation incubation, the medium was removed, and the cells were fixed with 4% paraformaldehyde for 30 minutes and either stained for 30 minutes with 1% Alcian blue (Sigma-Aldrich) at room temperature or first cut into 5-μm sections before staining.

Sarcosphere Initiating Capacity
An ascending number of cells (8, 16, 31, 63, 125, 250, 500, and 1,000 cells) were flow-sorted in 96-well flat-bottomed ultra-low attachment plates (Corning, New York, NY, http://www.corning.com) and cultured in adequate medium (DMEM, EMEM, or DMEM/F-12; Invitrogen) with 1% penicillin/streptomycin (Invitrogen), 2% B27 supplement (Invitrogen), 0.1% epidermal growth factor (Sigma-Aldrich), 0.1% basic fibroblast growth factor (R&D Systems Inc., Minneapolis, MN, http://www.rndsystems.com), and 0.014% heparin (Braun, Melsungen, Germany, http://www.bbraun.de). After 10 days of growing, the formed sarcospheres were counted.

Immunohistochemistry
Primary tissues were cut into 5-μm sections and fixed with acetone. The slides were washed with PBS, and endogenous peroxidase was blocked with 3% H2O2 for 10 minutes followed by using tone. The slides were washed with PBS, and endogenous peroxidase (Merck & Co., Whitehouse Station, NY, http://www.merck.com) for 7 minutes and subsequently counterstained with hematoxylin (Merck & Co., Whitehouse Station, NY, http://www.merck.com).

RESULTS

Screening of Sarcoma Cell Lines Identifies Several Surface-Expressed Epitopes
As a first step, sarcoma cell lines HT1080 (fibrosarcoma), SK-LMS (leiomyosarcoma), SW872 (liposarcoma), and SW982 (synovial sarcoma) were analyzed by flow cytometry for expression of MSC antigens (supplemental online Table 1). Interestingly and unexpectedly, all cell lines showed coexpression of several MSC antigens. CD105, 2E4B4, S8B1, W5C4, and CD109 were uniformly expressed by all cell lines at high density (supplemental online Fig. 1A), whereas CD340, CD164, W1C3, W4A5, W7C6, and CD271 stained only subpopulations in each cell line (supplemental online Fig. 1B). Other markers were differentially expressed by the cell lines (CD140b, CD56 [clone 39D5], NPC, CD10, CD318, CD344, F9–3C2F1, HEK3D6, CD172a, CD349, W3D5A9, W5C5, and TNAP; supplemental online Fig. 1C). Another set of antigens, including the bona fide progenitor cell markers CD117 and CD133, as well as CD326, CD34, CD324, and W3C3, was not expressed by any of the cell lines tested (supplemental online Fig. 4). Because sarcoma cell lines have passed through serial passages and therefore through clonal evolution and may harbor many transforming mutations, they may not compare well with the situation in primary tumors. We therefore next analyzed primary sarcoma samples from our hospital that were freshly obtained directly after resection to undergo flow cytometric analysis.

Primary Sarcoma Samples Display Similar Expression Patterns as Compared With STS Cell Lines
In order to establish the in vivo relevance of above-mentioned expression profiles, freshly resected primary tumor samples were carefully converted to single cell suspensions and processed in the same way. Tumor samples of the most common entities (supplemental online Table 2) were examined, and again each sample showed surface marker expression of at least several MSC markers (supplemental online Fig. 2). Interestingly, an extensive overlap of expression patterns was observed when the immunophenotypic analysis of primary cells with their corresponding cell lines was compared: again, CD105, 2E4B4, S8B1, W5C4, and CD109 were uniformly expressed by all cell lines at high density with the exception of CD109, which was expressed by only 10% of cells in a primary leiomyosarcoma sample (supplemental online Fig. 2A). Subpopulations of differing sizes expressed CD340, CD164, W1C3, W4A5, W7C6, and CD271 (supplemental online Fig. 2B), whereas the same markers as above displayed differential expression by the primary tumor cells in comparison (CD140b, CD56 [clone 39D5], NPC, CD10, CD318, CD344, F9–3C2F1, HEK3D6, CD172a, CD349, W3D5A9, W5C5, and TNAP; supplemental online Fig. 2C). As with STS cell lines, established hematopoietic and epithelial stem/progenitor cell markers CD326, CD34, CD324, CD117, CD133, and W3C3 were not expressed by any of the primary tumor samples (supplemental online Fig. 4).

High Level of Marker Coexpression in MSCs and Fibroblasts
A complete expression analysis of MSCs for all selected markers of this study has not been published. Therefore, we obtained bone marrow-derived MSCs as previously described by harvesting adherent bone marrow mononuclear cells after culture [23] and performed flow cytometric analysis using the same panels as above (supplemental online Fig. 3). In addition, fibroblasts derived from the embryonic fibroblast cell line MRC5 (American Type Culture Collection) and from human foreskin were examined. Similar to sarcoma cell lines and primary sarcoma samples, CD105, 2E4B4, S8B1, W5C4, and CD109 were uniformly expressed by more than 80% of MSCs and fibroblasts (supplemental online Fig. 3A). Indeed, when the remaining markers were screened on MSCs in comparison with fibroblasts, a remarkable immunophenotypic overlap was found, especially between MSCs and foreskin fibroblasts (supplemental online Fig. 3B, 3C). Exceptions to this observation are CD271+ and TNAP+ cells: 33% of MSCs versus less than 1% of fibroblasts expressed CD271 (supplemental online Fig. 3B), and 11% of MSCs versus 0.4% and 1% of fibroblasts expressed TNAP (supplemental online Fig. 3C). We conclude that MSC markers may be expressed on bona fide fibroblasts, as well as on STS cells, which may impede their diagnostic utility. On the other hand, these findings point to the close biological relation of the respective cell types [24].

Comparative Analysis of “Tissue-Specific” Marker Expression
In order to facilitate comparative expression profile analyses of the above-mentioned markers (CD271 and TNAP), as well as
markers of high-proliferative potential sarcoma cells (W5C5 and CD344; see below), figures were created that allow for a side-by-side comparison between sarcoma cell lines and their corresponding morphological primary sarcoma equivalent, as well as with MSCs and fibroblasts.

**Fibroblast Morphology**

The sarcoma group with fibroblast morphology consisted of the human fibrosarcoma cell line HT1080, one case of myxofibrosarcoma (MFS), and two cases of not otherwise specified pleomorphic sarcoma (formerly malignant fibrous histiocytoma; supplemental online Table 2). MFS and pleomorphic sarcomas overlapped in their expression profiles; data derived from analysis of MFS are shown (Fig. 1). W5C5, CD271, and TNAP were expressed by subpopulations of sarcoma cells, as well as MSCs, with varying coexpression in embryonic fibroblast cell line MRC5 and foreskin fibroblasts (Fig. 1). CD344 identified only minute populations in HT1080 cells, as well as foreskin fibroblasts.

**Smooth and Striated Muscle Morphology**

The human leiomyosarcoma (LMS) cell line SK-LMS1 was directly compared with primary LMS and rhabdomyosarcoma. All primary sarcoma samples coexpressed W5C5, CD344, TNAP, and CD271. In contrast, SK-LMS1 contained less than 1.5% W5C5+, CD344+, or TNAP+ cells. CD271 was expressed in less than 5% of SK-LMS1 cells (Fig. 2).

**Synovial Cell Morphology**

One case of primary biphasic synovial sarcoma was analyzed and compared with the human synovial sarcoma cell line SW982. Again, CD271 and TNAP turned out to be more specific markers of MSCs coexpressed on malignant cells. CD344 was expressed by almost 50% of SW982 cells but on less than 1% of primary synovial sarcoma cells (Fig. 3; supplemental online Figs. 1C, 2C). W5C5 was expressed in 16% of SW982 cells (Fig. 3; supplemental online Fig. 1C) and approximately 35% of primary synovial sarcoma cells (Fig. 3; supplemental online Fig. 2C).

**Adipocyte Morphology**

The human liposarcoma cell line SW872 was compared with a single case of primary liposarcoma. SW872 strongly expressed CD271, W5C5, and CD344 but very little TNAP (<0.5% of cells; Fig. 4; supplemental online Fig. 1C). In contrast, primary liposarcoma cells strongly expressed TNAP but little CD344. Expression of CD271 and W5C5 were comparable between cell line and primary sarcoma cells (Fig. 4).

**Immunohistochemistry Reveals Specific Expression of CD271 and TNAP in Human Leiomyosarcoma, Rhabdomyosarcoma, and Liposarcoma**

In order to validate our results in a larger number of sarcoma specimens, cryosections of freshly frozen soft-tissue sarcoma samples were analyzed immunohistochemically. Our results show that both CD271 and TNAP can be detected as cytoplasmic staining in LMS (n = 4), rhabdomyosarcoma (n = 4), and liposarcoma (n = 4) cells.

Supplemental online Figure 5 shows representative results, and an overview of all staining results is given in supplemental online Table 3.
STS Subpopulations With Increased Proliferative Activity and In Vitro Characteristics of Cancer Stem Cells

Prospectively isolated HT1080 subpopulations positive for W5C5, W8B2 (TNAP), or CD344 (frizzled-4) displayed a strongly increased in vitro proliferation potential (Fig. 5A). Regardless of their histology, all primary STS samples contained subpopulations expressing CD271 (low-affinity nerve growth factor receptor). CD271 has previously been described to be a suitable marker of primary human MSCs [25]. When proliferative activity of CD271+/H11001 versus CD271+/H11002 tumor cells were compared both within the tested cell lines and the primary STS samples, there was a significant difference with the CD271-expressing cells displaying increased proliferative activity (Fig. 5B) as compared with the CD271-negative subpopulations. CD271+/H11001 cells underwent further in vitro testing for CSC characteristics, namely their capacity to form sarcospheres under serum-free conditions in limiting dilutions and doxorubicin resistance [26]. Indeed, the CD271+ population of all tested cells both showed increased resistance to doxorubicin and retained sarcosphere initiating capacity down to a number of only 63 cells seeded per well, whereas CD271− cells formed sarcospheres out of a minimum number of 250 cells (Fig. 6). We conclude that CD271+ sarcoma cells may represent a population enriched for sarcoma stem/progenitor cells, displaying increased proliferative potential, doxorubicin resistance, and sarcosphere initiating capacity.

Soft-Tissue Sarcoma Cell Lines Display MSC-Like Differentiation Capacity

MSCs are defined by their proliferative and differentiation capacity. Under appropriate culture conditions, MSCs are able to differentiate into adipose, cartilage, muscle, and bone tissue. To test the hypothesis of a close relationship between MSCs and soft-tissue sarcoma cells, we sought to differentiate STS cells into the respective tissues. Compared with MSCs, all of the tested STS cell lines were able to differentiate into tissue types different from their original histology type. Rhabdomyosarcoma formed adipocyte-like cells, and liposarcoma differentiated into cartilage- and bone-like tissue. An overview of differentiation capacities is given in Figure 7. These findings may mean that sarcoma stem and progenitor cells may retain their differentiation capacity or that both STS cell lines and primary tumors contain MSCs such as was described for osteosarcoma [27].

DISCUSSION

Similar to leukemias, which represent rare diseases but whose study has helped us comprehend tumor biology including the prediction of clinical acuity and response to therapy by immunophenotyping, an understanding of mesenchymal tissue differentiation marker expression pattern may enable us to provide better clinical care for patients with adult-type STS [21].

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hypothesis also pertains to the cancer stem cell hypothesis, according to which malignant tumors harbor a subset of self-sustaining cells that are exclusively able to contribute to tumor maintenance, whereas the bulk of cancer cells within the tumor has lost its self-renewal capacity [28]. Analogous to LSCs, CSCs may resemble their normal tissue-specific stem cell counterparts. Recent evidence strongly suggests that sarcomas originate from mesenchymal stem cells [13–16], which represent a plastic-adherent spectrum of different bone marrow-derived cells until recently, when expression analysis using a set of novel antibodies made their prospective isolation possible [20]. Herein, we present the results of a systematic phenotypic analysis of STS cells for the expression of markers with known reactivity against MSCs, as well as hematopoietic or tissue-specific stem cell counterparts. It is important to note, however, that our flow data reflect analyses of single-patient specimens. The validity of these data, however, was proven by immunohistochemistry of a total of \( n = 12 \) fresh frozen leiomyosarcoma, rhabdomyosarcoma, and liposarcoma samples. Immunohistochemistry showed high interindividual variability of the percentage of tumor cells stained, which underscores the hypothesis-generating nature of our results.

Herein, a library of antibodies raised against MSCs with frequent reactivity against sarcoma cell lines and against the most common subtypes of primary adult STS is presented. Several markers discriminated against subpopulations within sarcoma cell lines, suggesting a hierarchical structure even after serial passages. Moreover, MSC marker-positive subpopulations showed different growth kinetics as compared with their marker-negative counterparts. Nonetheless, the flow cytometric analysis of MSCs, as well as fibroblasts derived from both embryonic and adult tissue, revealed a significant overlap of MSC marker expression among all of the tested cell types, reminding us of the important issue of whether all tested cells belong to an extended fibroblast family [24]. Nonetheless, several markers shared by sarcoma cells and MSCs may be more specific for malignant cells as compared with fibroblasts: TNAP, CD271, CD344, and W5C5.

MSCA-1, which was recently identified to be identical to TNAP [30], was highly expressed on MSCs but not on fibroblasts. TNAP was expressed by subpopulations of STS cells of histology-specific sizes, with myogenic sarcomas containing the largest TNAP-positive populations. We suggest that TNAP-positive...
vated cell sorting technology, and 2 CD271 (C): served down to a number of 63 CD271 -cells. Mean values of quadruplicate experiments are shown. (C): CD271 + and CD271 - cells were separated using magnetic activated cell sorting technology, and 2 x 10^5 cells, respectively, were seeded in each well of white flat-bottomed 96-well plates. After 3 days of doxorubicin treatment, the plates were analyzed with the CellTiter-Glo luminescent cell viability assay (Promega GmbH) according to the manufacturer’s instructions. Abbreviations: neg, negative; pos, positive.

HT1080 cells displaying an increased proliferation potential may represent sarcoma progenitor cells.

Among the tested markers, only CD271 (low-affinity nerve growth factor receptor, tumor necrosis factor receptor superfamily 16, and p75NTR) was specifically expressed in all primary tumor samples and all cell lines as well as in MSCs, but not in fibroblasts. CD271 is the most commonly used MSC marker, and bone marrow-derived clonogenic MSCs have been found to reside exclusively in the CD271 bright cell fraction [20]. In addition, CD271 has been successfully used as a marker for the prospective isolation of multipotent adipose tissue-derived stem cells [31], as well as human melanoma-initiating cells [32]. CD271 belongs to the tumor necrosis factor receptor superfamily and serves as a receptor for the neurotrophin nerve growth factor, brain-derived neural factor, and neurotrophin-3 and -4 [33].

Herein, we show that in absolute concordance to MSCs, dense expression of CD271 belongs to the typical characteristics of sarcoma cells. Moreover, we show that CD271 + cells of sarcoma cell lines and in primary tumor samples, expression of CD271 correlated with a strongly increased proliferation potential, as well as an increased capacity for sarcomatous formation and doxorubicin resistance, potentially suggesting a hierarchically prominent position of CD271-positive cells in both normal and malignant mesenchymal tissues. The functional importance of this finding remains to be established.

W5C5 has been introduced as a marker suitable for the prospective isolation of highly enriched bone marrow-derived MSCs [20]. In HT1080, W5C5 marks a small subpopulation of cells with increased proliferative potential as compared with the W5C5 population and is expressed by large fractions of primary synovial sarcoma, rhabdomyosarcoma, and liposarcoma cells, as well as their corresponding cell lines (Figs. 1–4). Up to 15.3% of foreskin fibroblasts coexpressed W5C5 (supplemental online Fig. 3C).
interpretation; H.-J.B.: conception and design, data analysis and interpretation; H.-G.K.: financial support, conception and design, data analysis and interpretation, manuscript writing.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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