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Mohseny, A.B.; Hogendoorn, P.C.W.

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Concise Review: Mesenchymal Tumors: When Stem Cells Go Mad

ALEXANDER B. MOHSENY, PANCRAS C. W. HOGENDOORN

Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands

Key Words. MSC • Bone marrow • Cancer stem cell • Transplantation • Multipotential differentiation • Stem cell therapy

ABSTRACT

Sarcomas are nonepithelial, nonhematopoietic malignant tumors that arise from the embryonic mesoderm. Despite their rarity, less than 10% of all cancers, sarcomas are accountable for relatively high morbidity and mortality especially in children and adolescents. Although there are some hereditary conditions predisposing sarcoma, such as the Li-Fraumeni and Retinoblastoma syndrome, the vast majority of these tumors are sporadic. Based on their histological morphology, sarcomas have been divided into a broad spectrum of subtypes recognized in the 2002 WHO classification of tumors. This wide lineage range suggests that sarcomas originate from either many committed different cell types or from a multipotent cell, subsequently driven into a certain lineage. Mesenchymal stem cells (MSCs) are able to differentiate into many cell types needed to create mature structures like vessels, muscle, and bone. These multipotent cells can be isolated from several adult human tissues and massively expanded in culture, making them both of use for research as well as potential beneficial therapeutical agents. For this reason MSCs are being extensively studied, however, concerns have raised about whether they are the putative originating cells of sarcoma and their questionable role in cancer progression. Recent accomplishments in the field have broadened our knowledge of MSCs in relation to sarcoma origin, sarcoma treatment and the safety of MSCs usage in therapeutic settings. STEM CELLS *2011;29:397–403*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

It was only half a century ago when McCulloch and coworkers revealed the existence and the clonal nature of marrow stem cells [1]. Yet nowadays these so-called mesenchymal stem cells or marrow/multipotent stromal cells (MSCs) have been extensively subjected to a wide range of biomedical studies. This growing interest in MSCs is explained by the relative ease with which these cells can be isolated from several adult human tissues [2], expanded just on plastic, and the MSCs' multipotential differentiation capacity into many cell types [3].

Together with all the success stories, also concerns about the possible negative or harmful effects, when brought into a potential therapeutic area, of the cells have been raised. The role of MSCs as originating cells of several sarcomas has been debated in literature. Both in translocation-driven and in genomically unstable tumors, MSCs have been proven or proposed to be the cells of origin. Most recently, a shift of attention for research into MSCs' possible pathogenesis and potentiality as therapeutical agents was evident. Here, we aim to illuminate the current state of the art of MSCs in relation to sarcoma (-genesis) by highlighting the latest achievements concerning:

(a). Sarcoma origin: multiple versus multipotent cells of origin. The two currently debated assumptions explaining the origin of sarcoma are described and the possibility of joining these two into one comprehensive hypothesis, proposing impaired differentiation of a "vulnerable" MSC, is discussed.
(b). MSCs versus cancer stem cells (CSCs). Based on recent identification of so-called CSCs in sarcoma, we draw a parallel between CSCs and transformed MSCs.
(c). Cause versus cure. Although very new, very controversial and not shown in most exemplary sarcoma types, MSCs have been reported to be involved in inhibiting and promoting sarcoma progression. In this section, we briefly mention possible roles of MSCs in sarcoma treatment.
(d). The other side of the coin. Beneficial aspects of MSCs in therapeutical settings are mentioned together with the possible hidden dangers indicating the safety issues.

Investigating a possible relationship between MSCs and sarcoma is not only logical because of their shared mesenchymal origin but also fundamental for a better understanding of sarcoma biology. Knowing the exact cell of origin of a complex malignancy offers a more accurate target to hit. Moreover, it provides better opportunities to model the tumor which in turn allows for discovering and assaying novel treatment strategies.

SARCOMA ORIGIN: MULTIPLE VERSUS MULTIPOTENT CELLS OF ORIGIN

Sarcoma is the collective name for a relatively rare, yet heterogeneous group of cancers, most probably derived from

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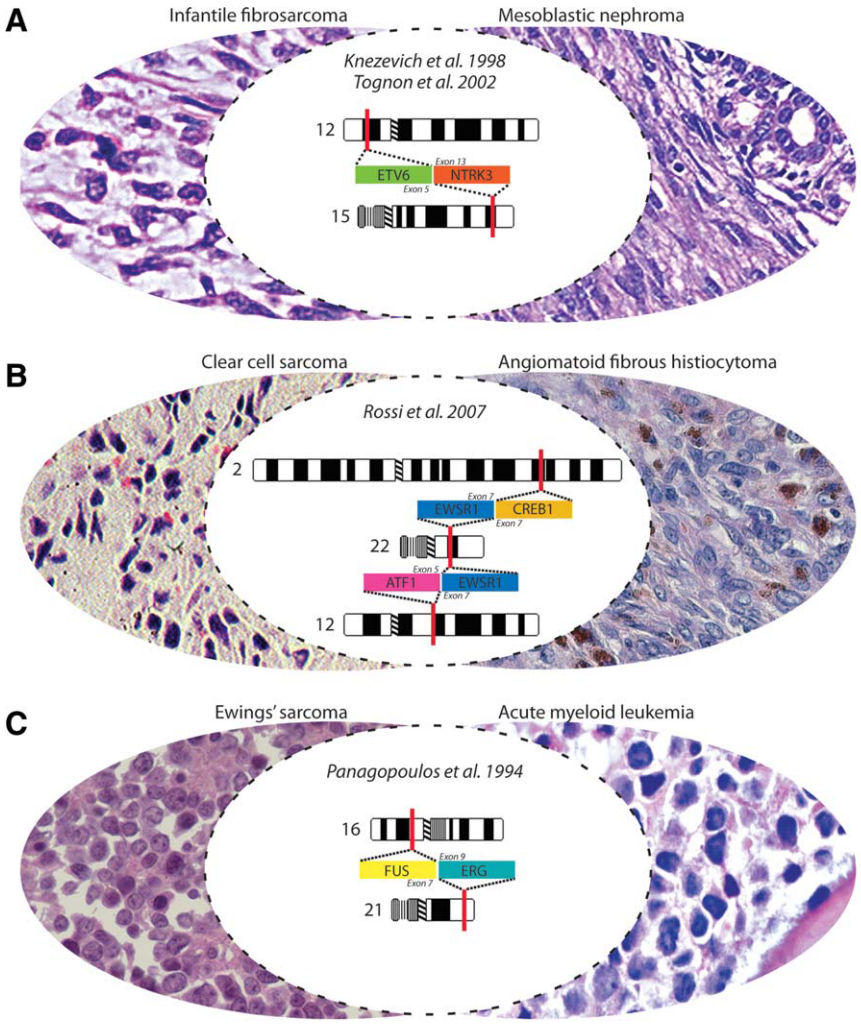
Correspondence: Pancras C. W. Hogendoorn, M.D., Ph.D., Professor of Pathology, Leiden University Medical Center, P.O. box 9600, L1-Q, Leiden, The Netherlands. Telephone: +31-71-526-6639; Fax: 31-71-526-6952; e-mail: p.c.w.hogendoorn@lumc.nl Received September 22, 2010; accepted for publication December 23, 2010; first published online in STEM CELLS *EXPRESS* January 7, 2011. 1066-5099/2009/$30.00/0 doi: 10.1002/stem.596



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Name	Primary location	Common symptoms	Nature
Infantile fibrosarcoma	Distal extremities	Swelling	Intermediate
Mesoblastic nephroma	Kidney	None	Benign
Clear cell sarcoma	Extremities	Swelling	Malignant
Angiomatoid fibrous histiocytoma	Extremities/trunk	Subcutaneous swelling	Benign
Ewings' sarcoma	Long bones	Bone pain	Malignant
Acute myeloid leukemia	Blood	Fatigue, weight loss, pain	Malignant

Figure 1. Molecular genetic background of distinct cancer subtypes shows communal origin, despite their different clinical and morphological characteristics. **(A):** Infantile fibrosarcoma, a tumor of fibroblasts and cellular mesoblastic nephroma, a spindle cell tumor of the kidney, are found at different locations and show each a distinct clinical course. In both a translocation (12;15) associated *ETV6-NTRK3* gene fusion is found [5]. A third malignancy, that is, secretory breast carcinoma, can be added to this group sharing the same translocation [6]. Here the translocation related phenotype is not restricted to lineage being both mesenchymal as well as epithelial. **(B):** The presence of either *EWSR1-CREB1* (2;22) or *EWSR1-ATF1* (12;22) gene fusions has been identified in both phenotypically as well as clinically very distinct clear cell sarcoma, a high-grade soft tissue tumor with melanocytic differentiation, and angiomatoid fibrious histiocytoma, a low-grade mesenchymal neoplasm from as yet undefined lineage [7]. **(C):** *ERG* is a member of the *ETS* gene family of transcription factors and is fused with *FUS* in a subset of Ewing's sarcoma, a highly malignant round cell tumor of bone and soft tissue. The same fusion has also been shown to be present in acute myeloid leukemia, a progressive hematopoietic malignancy, with (16;21) translocations [8]. **(D):** The main clinical characteristics of the genetically described tumors in **(A–C)** are depicted here. Please note that infantile fibrosarcoma is rarely metastasizing making it of "intermediate" grade and that mesoblastic nephroma is asymptomatic because it is usually found before birth. All microscopic images shown here represent ×400 magnified snapshots of the tumors. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mesenchymal tissues differentiating into bone, muscle, fat, and cartilage [4]. Sarcomas are divided into many subtypes based on the histological appearance and the anatomical site of the tumors, indicating either distinct cells of origin for every subtype or a multipotent cell of origin responsible for the development of all subtypes. In favor of the latter possibility, distinct anatomical and/or histological sarcoma, and even some epithelial nonsarcoma, types with simple karyotypic defects have proven to share a molecular pathogenesis and origin. This has been shown in infantile (congenital) fibrosarcoma, mesoblastic nephroma, and secretory breast carcinoma sharing the *ETV6-NTRK3* fusion gene (Fig. 1A), in clear cell sarcoma and angiomatoid fibrious histiocytoma sharing both the *EWSR1-CREB1* and the *EWSR1-ATF1* fusion genes (Fig.

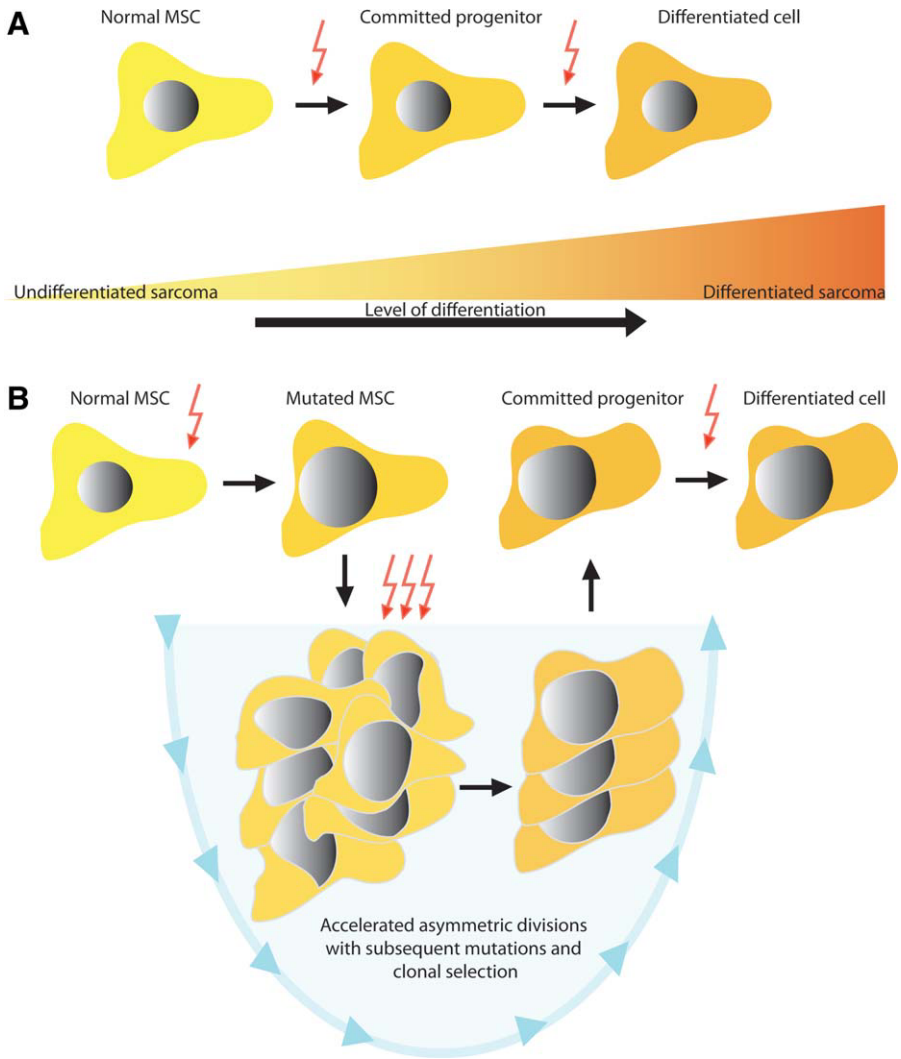

Figure 2. Differentiation and sarcoma genesis. **(A):** Impaired differentiation caused by mutations at every stage from a naïve stem cell to a fully differentiated daughter cell. The stage where the mutation happens might explain the degree of differentiation of the sarcoma, however, it does not explain the initiation of malignant outgrowth and sarcoma formation. **(B):** Impaired differentiation like in **(A)**, however, now starting from a mutated "vulnerable" stem cell instead of a normal MSC. Because of this early mutation, the stem cell undergoes asymmetric divisions giving rise to a mixture of cells with all additional mutations. Subsequently, immortalized clones with partially or fully impaired differentiation capacity (based on the mutations) generate sarcoma with a certain degree of differentiation. Abbreviation: MSC, mesenchymal stem cell. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

1B) as well as in Ewing's sarcoma and acute myeloid leukemia both having the *FUS-ERG* fusion. Moreover, even within a subtype of sarcoma, like osteosarcoma, many histological subgroups are described again highlighting the multipotency of tumor cells [9]. Although the exact cell of origin for these mostly sporadic malignancies has not been conclusively identified yet, one way or the other, the MSCs seem to be pivotal. In general, there are two potential theories about a sarcoma cell of origin (Fig. 2).

The first theory presumes that sarcoma is a differentiation disease, caused by mutations hampering terminal differentiation of MSCs. Depending on the lineage and the stage of differentiation at the time of the mutation, sarcomas with variable phenotype and histological grade could be initiated. This is, for example, suggested to explain the different stages of differentiation seen in osteosarcoma [10], the most prevalent bone tumor of nonhematopoietic origin. In addition, most evidence supporting this theory is based on studies where researchers compare gene expression signatures of sarcoma with the signature of tissue-specific differentiation stages of MSCs indicating overlap of the signatures of tumor and normal tissues according to their lineage of differentiation. Accordingly, differentiated chondrosarcoma, a malignant cartilage-forming bone tumor, was shown to share similarities with fully differentiated chondrocytes, whereas less differentiated chondrosarcoma showed overlap with prechondrogenic stages of MSCs [11, 12]. Similar studies proposed defective differentiation underlying leiomyosarcoma, an aggressive malignancy of smooth muscle tissues [13], pleomorphic/dedifferentiated liposarcoma, a malignant tumor mainly consisting of anaplastic fat cells [14], osteosarcoma [15], and sarcomas in general [16].

Although this hypothesis is based on the idea that differentiation is a tightly regulated process in which any mistake could be catastrophic, and it provides plausible explanations for the wide variety of histological subtypes of sarcoma, it might oversimplify the real situation. A caveat lies within the experimental designs comparing tumor tissues with in vitro

differentiated cells. First, the gene expression profiles of tumors for a substantial part might represent the stroma instead of the tumor cells themselves [17] and therefore not represent the cell of origin. Second, the in vitro culturing conditions possibly will influence cells' gene expression, not to mention that these cells are being pushed toward differentiation by adding several growth factors, which strengthens this bias even more. So comparing these two states might not tell us more than what already could be concluded from the morphology and the histology of the tumor. A differentiated sarcoma is more similar to a differentiated MSC than an undifferentiated one on the gene expression level; however, it does not provide solid evidence for the tumor's origin. Another argument against this theory is that sarcoma cells of a certain subtype often can differentiate into multiple lineages in vitro [18, 19]. This was shown even more clearly when we observed that transformed murine MSCs that formed differentiated osteoblastic osteosarcoma in vivo easily could be pushed toward not only osteoblasts but also adipocytes and chondrocytes in vitro [19–21]. So, the tumor environment seems to be very important for the final tumor phenotype next to its cell of origin. Moreover, looking at the genetic hallmarks of a fully developed sarcoma might not represent its state at origin. But the biggest disagreement with this hypothesis comes when one compares the biology of MSCs to that of differentiated daughter cells. Unlike differentiated cells, MSCs have the ability of replicating as undifferentiated cells, suppressing immune response, homing, and the plasticity to change phenotype [18, 22], as seen during the process of mesenchymal to epithelial transition (MET) [23], all characteristics necessary for malignant outgrowth and metastasis.

It is exactly for these reasons that sarcoma researchers have formulated the second theory, which argues that sarcoma is more likely to originate form a primitive MSC than a differentiated one [18]. Also, here, comparisons of sarcoma gene expression profiles have been made to those of normal cells and tissues [15, 24] obviously with the same caveats regarding expression profile comparisons mentioned earlier. Stronger evidence supporting this hypothesis comes from many laboratories world-wide observing spontaneous malignant transformation of murine [20, 21] and human [25] MSCs after which the cells produced sarcomas on grafting. Others have shown that in a more controlled situation transformation of MSCs indeed could be achieved by deletion or expression of certain genes initiating sarcoma [18, 26–29]. This has been studied in depth for Ewing's sarcoma, a malignant bone tumor with relatively simple karyotypic defects [30] containing known chromosomal translocations, where the expression of *EWS-FLI-1* chimeric gene in human MSCs was shown to induce Ewing's sarcoma formation [31]. Similarly, deleting *p53* in human MSCs induced again transformation of the cell; however, this time leiomyosarcoma formation was initiated [26]. Other studies have indicated likewise MSCs transformation resulting into other subtypes of sarcoma. So, spontaneous or induced MSCs tend to transform and initiate sarcoma formation. Our own more recent effort was to take advantage of this transformation by studying it in a stepwise manner to gain knowledge of early steps in osteosarcoma genesis [20] and the parallels with human osteosarcoma such as the *CDKN2A* deletions [32]. Another finding supporting a MSC origin of sarcoma is the expression of embryonal markers like *Oct-4* in sarcoma [33] and in aged MSCs, which indicates the stemness of these tumors.

The difficulty of these studies is that they do not provide a direct understanding for the final phenotype of the sarcoma, in other words; how can we explain the formation of different sarcoma subtypes from the same transformed MSCs just at different laboratories? Explanations for this phenomenon could be hidden in MSCs characteristics as well as the influence of the microenvironment. One possibility is that the mutation at the base of the malignant transformation of the MSC simultaneously could interfere with its path of differentiation toward a certain lineage resulting into a subtype of sarcoma. Alternatively, subsequent mutations can drive the transformed MSC to undergo differentiation and when this does not occur, an undifferentiated sarcoma could develop like high-grade undifferentiated pleomorphic sarcoma not otherwise specified (sarcoma NOS) [27]. This might imply a more balanced genomics for these tumors or the genomic alterations might be that severe that all differentiation paths are blocked, in agreement with the often complex karyotypes described in sarcoma NOS [34, 35]. Recently, this group of sarcomas that previously contained all unclassifiable tumors has been revisited [36] resulting in a diagnosis of exclusion approach that might lead to a better defined group of sarcoma, genetically to be studied. Even with the same mutation in the same originating cell, the time of the mutation and the location of the mutated cell might result in different subtypes of sarcoma [30]. One good example is osteosarcoma, which clinically represents with a broad spectrum of histological subtypes [9]. This suggests that the second theory, implying a communal cell of origin, can not exclude the first one, indicating impaired differentiation of the cell of origin. Indeed, we can not deny an important role of the differentiation pathways in sarcoma development as interfering with this pathways has been shown to induce sarcoma [37] and impaired/overactivated developmental pathways, such as Wnt and Hedgehog pathways, that are important for MSC's differentiation, have been found in sarcoma [15, 19]. So, it might be more sensible to join these two models into one; sarcoma originates from a mutated MSC, which is vulnerable for subsequent mutations. Depending on the impact of the initial mutation and/or subsequent additional mutations and/or environmental factors, as shown for myxofibrosarcoma [38], differentiation pathways are deregulated resulting into a specific subtype of sarcoma (Fig. 2B). This might provide an explanation for phenotypically altered recurrence of certain sarcoma subtypes as occasionally reported. Sequential accumulation of carcinogenic hits toward malignancy, as often seen in carcinomas by chronological transformations of precursor lesions, is rare in sarcomas. This indicates that several mutational factors need to meet each other in the same MSC for its malignant outgrowth, which might explain the rarity of sarcoma compared with carcinoma and the full-blown character of some sarcomas at diagnosis [30]. Accordingly in conditions where these predisposing factors are partly congenitally present, like the Li-Fraumeni syndrome, the incidence of sarcoma indeed increases tremendously.

MSC Versus CSC

Contradicting the historical clonal, or stochastic, model of cancer expansion, CSCs were identified at first in acute myeloid leukemia. Subsequently, stem-like cells were reported in brain tumors, breast tumors, and sarcomas [33, 39, 40]. The CSCs are described as a small subpopulation of cells within a tumor that have the potential of self-renewal and asymmetrical division, producing both stem-like cells and more differentiated cells that divide further and form the majority of the tumor. Indeed, when CSCs are selectively isolated from a tumor and xenografted, they show a much higher tumorigenic capacity as compared with the other tumor cells.

The essence of identifying CSCs in sarcoma lies within its clinical implications. When the CSCs theory holds in sarcoma, meaning that the tumors are heterogeneous and not all cells are identical, it indicates that only a 100% removal of the tumor bulk will cure the patient. Even if only a small percentage of the tumor, presumably the stem cells because of their higher resistance to therapy, remains in the patient, local and distant recurrences are to be expected [41]. This is in line with the often high insensitivity of sarcomas to systemic therapies which fail to eradicate all tumor cells and which can only be overcome by combining these with surgery to achieve complete resection. Accordingly, in recent reports tumor-initiating cells were found [33, 39, 42] and shown to be associated with metastasis and drug resistance [42, 43] in sarcoma. Taken together, there is not only growing evidence for the existence of CSCs in sarcoma, their existence also explains the clinical behavior of these tumors. The question that remains is what these stem cells exactly are. Considering the best known features of these cells until now, that is, their self-renewal, resistance to systemic therapy, and high migratory ability, they seem to be most reminiscent of normal adult stem cells such as the MSCs. In agreement with the theory that tumorigenesis could be seen as aberrant organogenesis [44], mutated MSCs have all it needs to source and maintain sarcoma [45] as recently exemplified by the effects of *HMGA2* alteration in mesenchymal stem-like cells [46]. Especially children's osteosarcomas are often found at locations with a high rate of tissue turnover (growth plate area) indicating that MSCs are rapidly proliferating and differentiating, making them potentially more prone to undergo mutations and transform into CSCs. Interestingly, this transformation is not exclusive for MSCs toward sarcoma as parallels are shown in neural stem cells and pediatric brain tumors [47, 48], suggesting that these mechanisms might be applicable to all childhood malignancies if not to cancer as a whole.

Cause Versus Cure

Although still in infancy and very controversial, recent work indicates that MSCs could have therapeutical implications in sarcoma. Survival advantage was shown in Ewing's sarcoma patients treated with autologous stem cell transplantation [49]. In a mouse model using Ewing's sarcoma, the beneficial effect of MSCs was related to their ability to locate and migrate to the tumors and deliver interleukin-12 [50]. Furthermore, human MSCs were reported to exert antitumorigenic effects in a model of Kaposi's sarcoma [51]. On the other hand, also protumorigenic activities of MSCs should be mentioned as they were described to promote growth and pulmonary metastasis of osteosarcoma [52] and provide a niche for cancer metastasis in breast cancer [53].

These studies, favoring stem cell therapy for sarcoma, are very recent and many underlying mechanisms have yet to be identified and investigated. For example, the exact role of the MSCs within a stem cell transplant needs to be explored and whether, in xenotransplantation models of sarcoma, MSCs really migrate to the tumors or are just attracted to the site of injury needs to be addressed. Especially in Ewing's sarcoma, for which a MSC origin is better established than other sarcomas [31], this might seem counterintuitive. Although it is hard to speculate without further research, possible antitumorigenic effects of MSCs again indicate the plasticity of these cells and their multifaceted behavior under different circumstances. So, if MSCs could cure sarcoma, they neither exclude the possibility that they are the cell of origin of sarcoma nor it does prove that they could cause sarcoma.

The Other Side of the Coin

MSCs have fascinated sarcoma researchers because of the putative origin of sarcoma, their possible antitumorigenic and protumorigenic abilities, their differentiation capacities, and last but not least because of their relatively straightforward availability for research. Moreover, the possibility to isolate MSCs from several human tissues [2] and the ability to expand these cells in vitro have caused MSCs usage to become broader and increased interest in clinical settings. MSCs are advantageous for modulation of the immune response (hematopoietic engraftment, graft-versus-host disease, and autoimmune diseases), for reparative/regenerative cell therapy (osteogenesis imperfecta, leukodystrophy, Hurler syndrome, and tissue engineering) and to deliver therapy for malignancies. Even in more prevalent skeletal diseases, such as osteoporosis and rheumatoid arthritis, MSCs might be instrumental therapeutics as described in a recent review [22].

At the same time, the safety issues using MSCs in clinical settings worry medical doctors and researchers [30]. This is mainly based on repeatedly reported transformation of MSCs in vitro and more importantly creation of sarcoma in vivo by these cells originating from mouse or human [20, 21, 25]. Occasionally, sarcoma formation in bone marrow recipients treated for unrelated diseases has been reported [54] and recently highly unexpected osteosarcoma recurrence was related to an autologous fat graft [55]. Does this mean we should give up transplanting patients with MSCs despite all shown and expected advantages?

The safety issues again remind us of the plasticity and complexity of MSCs. If these are the same cells, cells that originate sarcoma, cells that target sarcoma, cells that promote sarcoma's growth and metastasis, and cells that have tremendous advantages in almost every other human disease, there is only one conclusion to make, that is, MSCs are deceiving cells. From recent work in our laboratory, we observed that slight changes in culturing conditions of fresh human MSCs can change their phenotype from completely MSC-like to fibroblastic to neuro-like and back and that is before adding any differentiation supplements. Consequently, if these cells change their phenotype that easily, we must not be too confident about the phenotypical markers to identify them; and moreover, it stresses their flexibility regarding the functional activities in a much more complex system as the human body. Moreover, cross-contamination of human MSCs with established cancer cell lines was very recently reported [56], indicating not only the importance of highly strict regular quality checks for characterization of MSCs before and after any type of experiment but also another possible danger of MSC treatment. Without any doubt there are many possibilities in MSC-based therapies, however, great dangers might be there as well and until we get to know these cells better, extensive caution should precede any MSC transplantation in patients.

Future Aspects

In future studies, it is crucial to characterize MSCs in a comprehensive way. A better understanding is needed about the markers, these cells express in a wide range of in vitro and in vivo settings and their behavioral changes in response to these

settings. Once there is more knowledge about this, we might be able to understand better the versatility of MSCs. Only after this, we could draw conclusions whether sarcoma is originating from MSCs or not and prove the hypothesis by generating transgenic mice for the specific sarcoma subtypes.

Conclusion

In conclusion, a clearer understanding of the MSCs might be helpful to set up better and faster screening methods to distinguish between "the good" and "the bad" MSCs. This would allow us to help patients one way or the other by directly using the good MSCs for treatment or alternatively by using the bad MSCs to model the disease.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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