Review Article

Stem Cell INVESTIGATION

The role of mesenchymal stem/progenitor cells in sarcoma: update and dispute

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Abstract: Sarcoma is the collective name for a relatively rare, yet heterogeneous group of cancers, most probably derived from mesenchymal tissues. There are currently over 50 sarcoma subtypes described underscoring the clinical and biologic diversity of this group of malignant cancers. This wide lineage range might suggest that sarcomas originate from either many committed different cell types or from a multipotent cell. Mesenchymal stem/progenitor cells (MSCs) are able to differentiate into many cell types and these multipotent cells have been isolated from several adult human tumors, making them available for research as well as potential beneficial therapeutical agents. Recent accomplishments in the field have broadened our knowledge of MSCs in relation to sarcoma origin and sarcoma treatment in therapeutic settings. However, numerous concerns and disputes have been raised about whether they are the putative originating cells of sarcoma and their questionable role in sarcomagenesis and progression. We summarize the update and dispute about MSC investigations in sarcomas including the definition, cell origin hypothesis, functional and descriptive assays, roles in sarcomagenesis and targeted therapy, with the purpose to give a comprehensive view of the role of MSCs in sarcomas.

Keywords: Sarcoma; mesenchymal stem cells; tumorigenesis; targeted therapy

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Introduction

Sarcomas are biologically and clinically heterogeneous malignant connective tissue tumors arising from mesenchymal or ectodermal tissues. They often harbor relatively specific genetic aberrations, the recognition of which can be used as a diagnostic tool as well as a potential prognostic/predictive marker (1,2). For instance, the fusion of *PAX3-FOXO1* in ARMS, *EWS-FLI1* in Ewing sarcoma, *COL1A1-PDGFB* fusion in dermatofibrosarcoma protuberans (DFSP) and *SYT-SSX* in synovial sarcoma can be used as diagnostic makers (3). Mutations in key genes and signaling pathways such as *C-KIT*, *PDGFRA* and *BRAF* have been targeted by specific drugs such as imatinib and vemurafenib (1,2). Co-amplified oncogenes cyclin-dependent kinase 4 (*CDK4*) and *MDM2* can serve as confirmatory diagnostic markers and as potential pharmacological targets in well-differentiated and dedifferentiated liposarcomas (4).

Based on the multiple histological morphology and genetic characteristics, sarcomas have been divided into a broad spectrum of subtypes recognized in the 2013 WHO classification of tumors (3). Despite conventional multi-modality treatment approaches (surgery, chemotherapy, and radiation therapy), sarcoma patients have disproportionally higher rates of morbidity and mortality than those with other cancers. Investigations into the biology of sarcoma resistance to therapy and sarcoma relapses have resulted in the development of the mesenchymal stem/progenitor cell (MSC) hypothesis (5). Investigating the possible

relationship between the MSCs and sarcoma will gain a better understanding of sarcoma biology. Moreover, these studies might provide better opportunities to discover novel treatment strategies.

Correlation of MSCs, cancer stem cell (CSCs) and tumor-initiating cells (TICs)

Increasing evidence suggests that MSCs might be the TICs capable of initiating sarcomagenesis (6-9), although there is still great controversy about the nature and relation of MSCs, CSCs and TICs. There are also studies supporting that sarcomas could represent good examples of the CSC model and these sarcoma CSCs display MSCs properties (10,11). CSCs that display tumor re-initiating properties have been recently identified in chondrosarcoma (9), osteosarcoma (8), Ewing's sarcoma (12) and synovial sarcoma (13). These CSCs are characterized by expression of markers OCT3/4, NANOG and SOX2 (5,8,9,14). These CSCs are also able to self-renew and able to sustain the tumor in the serial transplantation experiments (5,8,9,14). More importantly, many of these CSCs express MSC markers and retain MSC differentiation properties *in vitro* (13). In addition, these MSC-like CSCs are associated with drug resistance and metastasis, which might be responsible for the relapse of sarcomas (8,15-17). Therefore, MSCs may not only be the TICs in sarcomas, but also a population of altered CSCs which are responsible for maintaining tumor growth and initiating tumorigenesis upon serial transplantation.

Important factors involved in the MSC transformation

Transformation of MSCs has been achieved by several methods including knockout of tumor suppressor genes, overexpression of oncogenes and drug administration to affect signaling pathways (6).

During the transformation process there are remarkable deregulations of the tumor suppressor genes and signaling pathways. For instance, in mouse adipose derived MSCs (ADSC), the loss of tumor suppressor P21 and TP53 could induce *in vitro* transformation and so-called fibrosarcoma formation *in vivo* after transplantation (18). In another study, both $Tp53^{-/-}$ $Rb^{-/-}$ and $Tp53^{-/-}$ mouse ADSCs were generated and leiomyosarcoma-like tumors were developed in the *in vivo* tumorigenicity assays of these two types of mouse ADSCs (19). Furthermore, the combination of *Cdkn2a* loss and *C-myc* overexpression in mouse MSCs induced osteosarcomas accompanied by the loss of adipogenic differentiation capacity (20).

In addition to directly targeting *in vitro* cultured MSCs, the genetically engineered mouse models have been used to explore the roles of such genes in the MSC transformation. In P53-deficient mice, many types of sarcomas occurred in the mesenchymal cells of limb buds, which osteosarcoma was the most common type (21). These induced transformation studies established the importance of the P53 and P53 pathway in preventing mouse MSC transformation (22,23).

Additionally, upregulated oncogenic pathways also can induce or potentiate mouse MSC transformation. For instance, the *Fos* overexpression transgenic mice resulted in the development of bone tumors, with chondrosarcomas as the main type (24). In mice, overexpression of *K-ras* in addition to P53 loss induced sarcoma formation more efficiently than with P53 loss alone (25). These studies evaluate the roles of different oncogenic pathways in mouse MSC transformation.

Similarly, most studies of human MSC transformation are also based on genetic methods to knock out important tumor suppressor genes and overexpress certain oncogenes, such as the exogenous expression of *hTERT* (11,26-28). Consistent with MSC studies in mice, the disruption of P53 and RB pathways are also important for human MSC transformation. For instance, the introduction of *SV40-LT*, which perturbs both P53 and RB proteins, potently promoted human MSC transformation (26). Furthermore, the overexpression of some oncogenes such as *H-RAS* has also been shown to contribute to the transformation (26). In all, the deregulation of some important signal pathways including P53 pathway, RB pathway, PI3K-AKT pathway, WNT/β-catenin signaling pathway and MAPK pathway might be involved in the MSC transformation and sarcoma formation (29).

It is worth noting that overexpression of sarcoma-specific fusion proteins in mMSCs and mouse models could reproduce several sarcomas (13,14,19,30-36). Ewing's sarcoma, MLS, ARMS and synovial sarcoma have been reproduced upon expression of EWS-FLI-1, FUS-CHOP, PAX-FKHR and SYT-SSX, respectively (13,14,19,30-36). For instance in mouse MSCs the exogenous expression of the fusion gene *EWS-FLI1* alone could transform these MSCs, which are characterized by *in vitro* immortalization and *in vivo* sarcomatous tumor formation, even a secondary genetic alteration was

needed (33,34). Similarly, human MSCs with exogenous *EWS-FLI1* expression were transformed and expressed neuroectodermal markers (35). On the contrary, the knockdown of *EWS-FLI1* expression in Ewing sarcoma cell lines restored the *in vitro* trilineage differentiation ability of the cells (12). In a transgenic mouse model, by expressing *EWS-FLI1* gene specifically in the mesoderm-originated tissues in limbs and simultaneous *Tp53* knockout, sarcomas with similar characteristics as Ewing sarcoma occurred (36).

Functional and descriptive assays of MSCs

The stem cell assays including functional and descriptive assays are used to characterize MSCs. The functional assays include the colony-forming assay, tumorigenesis, dye efflux, chemoresistance and differentiation. The descriptive assays include the expression of stem cell genes, aldehyde dehydrogenase activity (ALDH), and side population (SP) (5,7,10,11,17,37,38). Approaches used to identify both normal stem cells and MSC populations include cell surface markers, ALDH, SPs etc. (7,37,38).

MSCs are believed to have an increased ability to form colonies from a single cell and the colony-forming assays are the most commonly used methods although there are several issues about this method (39). The general "gold standard" characteristic of MSCs is the ability to grow serially transplantable tumors in immunodeficient mice (40). ATP-binding cassette (ABC) transporter efflux of DNA-binding dyes is also generally used as an indicator of stem cell properties (41). Somewhat related to drug efflux, chemotherapy resistance is also considered as a hallmark of MSCs (17). Differentiation into mesenchymal cell types such as osteoblasts, adipocytes, and chondrocytes is typically evaluated, based on the theory that a MSC has undergone malignant transformation (42).

Expression of so-called "stem cell genes" such as CD20, CD24, CD34, CD44, CD90, CD117, CD133, OCT4, SOX2, and NANOG is used as markers of MSCs (43,44). High activity of ADLH may play a role in early differentiation of stem cells through oxidizing retinol to retinoic acid and can confer resistance to chemotherapeutic agents such as cyclophosphamide (45,46). Cells termed the "SP" due to its position in the flow cytometry plot were found to have many of the characteristics of stem cells (47). Several groups have used SP as a sign of "stemness" to support the idea that the marker they were studying identified stem cells (38,41).

The MSC origin of sarcomas

Osteosarcoma

Different studies have supported the MSC origin of osteosarcoma (20,22). Tsuchida and colleagues firstly reported that SP cells isolated from the osteosarcoma cell line by exposure of the HOS osteosarcoma cell line to cisplatin showed stem cell-like properties (48). Tirino *et al*. evaluated the utility of CD133 expression to identify and isolate a subpopulation of cells with stem cell properties in osteosarcoma cell lines. Examination of osteosarcoma cell lines identified a subpopulation of $CD133^+$ cells which exhibited self-renewal properties, higher proliferative rates, spherical colony formation, and expression of the stem cell-associated gene *OCT3/4* (49,50). Wang *et al*. demonstrated a subpopulation with high ALDH activity in the osteosarcoma cell line OS99-1 (51). These cells were able to grow xenografts in NOD/SCID mice, and showed characteristic CSC features including self-renewal, ability to produce differentiated progeny, and increased expression of the stem cell genes OCT3/4A, NANOG, and SOX-2 (51). Most importantly, Adhikari and colleagues discovered that mouse and human osteosarcoma cell lines positive for both CD117 and Stro-1 (two MSC markers) demonstrated typical stem cell characteristics (8). Validation of the stem cell-like properties would further strengthen the utility of CD117 and Stro-1 marker expression as specific markers for the identification and isolation of MSC population in osteosarcoma.

Ewing sarcoma

Suva and colleagues were the first to report on the isolation of $CD133^+$ cells derived from primary Ewing sarcoma tumors. They showed that these $CD133^+$ cells demonstrated abilities of initiating and forming tumors in NOD/SCID mice and recapitulating the parental tumor phenotype (5,52). The $CD133^+$ Ewing's sarcoma also expressed significantly higher levels of the stem cell genes OCT4, SOX2, and NANOG. Jiang and colleagues investigated the expression of CD133 in 48 primary Ewing's tumors and cell lines and found that most of them had very low or absent expression of the $CD133^-$ encoding gene PROM1 while 4 cases had overexpression of PROM1. Of these four cases with overexpression of PROM1, two were found to have quickly developed a chemoresistant tumor while the other two were long-term survivors after receiving chemotherapy (53). These results suggest that heterogeneity

of CD133 expression in Ewing's sarcomas and a variable prognostic impact of the level of CD133 expression.

The utility of SP cells to identify MSCs in Ewing sarcoma has also been explored. Yang and colleagues isolated the SP fraction from the Ewing sarcoma cell line SK-ES-1 and showed that the SP cells exhibited increased stem cell features such as clonogenicity, invasive behavior, and cytotoxic drug resistance (54). Isolation of cells with high ALDH activity has also shown promise in identifying cell populations in Ewing sarcoma enriched for MSCs. Awad and colleagues examined Ewing sarcoma cell lines and patient-derived Ewing sarcoma xenograft tumors and found that the cells from the $ALDH^{High}$ sub-population demonstrated stem cell properties including highly tumorigenic and more resistant to the chemotherapy (55).

Rhabdomyosarcoma

Similar to Ewing sarcoma, the PAX3/FOXO1and *PAX7/FOXO1* have classically characterized a more clinically aggressive subset of RMS referred to as the alveolar variant. Komuro and colleagues showed that RMS possessed SP cells based on Hoechst 33342 dye exclusion (56). More recently, Walter and colleagues demonstrated that some RMS cell lines possessed stem cell properties including elevated expression of stem cell markers (*OCT-4*, *NANOG*, *c-MYC*, *SOX2*, and *PAX3*) and formed tumors at lower cell densities compared to adherent cells (57). The investigators further sorted the cells and isolated a $CD133^{+}$ cell fraction which showed typical stem cell features. Furthermore, this study also suggested that CD133 expression in RMS may not only be useful in enriching for candidate sarcoma stem cells, but may also have clinical utility in predicting survival outcomes (53,57).

Synovial sarcoma

In synovial sarcoma, exogenous expression of *SYT-SSX2* fusion gene in the skeletal-muscle-specific Myf5 expressing lineage induced the formation of synovial sarcomas *in vivo* (58). Additionally, in primary synovial sarcoma cells the fusion gene silencing restored both the trilineage differentiation capacity and the MSC marker expression, which strongly suggests the cells of MSC lineage was the origin of synovial sarcoma (13). Terry and Nielsen have shown subpopulations of CD133 expressing cells in primary synovial sarcomas and synovial sarcoma cell lines (59).

Other sarcomas

In chondrosarcoma, a study found that less differentiated tumors were shown to have more similarity with MSCs of pre-chondrogenic stages, while more differentiated tumors share more similarity with fully differentiated chondrocytes (60). This suggests that chondrosarcoma progression probably parallels deregulated chondrocytes differentiation process of MSCs (60,61). Additional, chondrosarcoma cells derived from primary tumors and enriched for CD133, which showed potent stem cell potential (50).

Liposarcoma is also found in MSC origin. In a mouse model of liposarcoma, where FUS-*CHOP* was able to induce liposarcoma genesis in MSCs, whereas no liposarcoma was formed when FUS-CHOP gene was manipulated to be only expressed in differentiated adipocytes. This study also evaluated the exact cell status as a crucial factor in sarcomagenesis (62,63). Stratford and colleagues have identified candidate sarcoma stem cells in a liposarcoma cell line (SW872) after double enrichment for CD133 and $ALDH^{High}$ activity ($ALDH^{High}$) and these cells were more clonogenic and tumorigenic (64). Clear cell sarcoma and other subtype sarcomas were also characterized with MSC origin (65,66).

Therapeutic role of MSCs in sarcomas

Although still in infancy and very controversial, recent work indicates that MSCs could have therapeutical implications in sarcoma. The potential of MSCs for cell-based therapies relies on several key properties such as capacity to differentiate into several cell lineages, lack of immunogenicity, immunomodulatory properties, robust *ex vivo* expansion potential, ability to secrete factors and homing ability to damaged tissues and tumor sites (67,68). Furthermore, MSCs are resistant to chemotherapy-induced apoptosis, and contribute to generating drug resistance and resistant to ionizing radiation in tumor cells (69-72). Due to these properties, MSCs have considerable therapeutic potential in a variety of clinical applications in several disease processes, including cardiovascular disease, as well as in human malignancies (67-69,73).

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 (74). Survival advantage was shown in Ewing's sarcoma patients treated with autologous stem cell transplantation (75). Furthermore human MSCs were reported to exert anti-

tumorigenic effects in a model of Kaposi's sarcoma (73). This effect is mediated by direct cell contact leading to the inhibition of Akt activation within KS cells (73). The result suggests that in contrast to other stem cells or normal stromal cells, MSCs possess intrinsic antineoplastic properties and that this stem cell population might be of particular utility for treating those human malignancies characterized by dysregulated Akt (73).

At the same time, the safety issues using MSCs in clinical settings should be paid with more attention (76). This is mainly because MSCs were reported to promote growth and pulmonary metastasis of osteosarcoma and also provide a niche for cancer metastasis in breast cancer (77,78). Also sarcoma formation in bone marrow recipients treated for unrelated diseases has been also reported (79) and highly unexpected osteosarcoma recurrence was related to an autologous fat graft (80). Moreover, cross-contamination of human MSCs with established cancer cell lines was very recently reported (81). All these issues indicate that there is a long way to go.

Debates about the origin of sarcoma tumor cell

The exact cell of origin for sarcomas has not been conclusively identified yet. By now two main models have been conceptualized to support the MSCs as the cell of origin for sarcomas. The first theory presumes that sarcoma is a differentiation disease, caused by mutations hampering terminal differentiation of MSCs (82). The second theory argues that sarcoma is more likely to originate from a primitive MSC than a differentiated one, and the primitive MSC acquires relevant mutations, which directs sarcoma genesis (26).

As for the first theory, different sarcomas could be initiated depending on the mutations according to different lineage and different stage of differentiation (7). This hypothesis is suggested to explain the variable subtypes of osteosarcoma (83). In addition, most powerful evidence supporting this theory is based on studies which show overlap of the gene expression signatures of differentiation stages of MSCs from sarcomas and normal tissues. For instance, differentiated chondrosarcoma was shown to share similarities with fully differentiated chondrocytes while less differentiated chondrosarcoma showed overlap with prechondrogenic stages of MSCs (7,60,84). Similar studies were found in leiomyosarcoma, pleomorphic/dedifferentiated liposarcoma (7,85).

However, this hypothesis is debated because the overlap of the signature might be due to the similar stroma of the different tissues (26,86). Therefore, another hypothesis has been suggested, which considers that sarcoma is more likely to originate from a primitive MSC rather than a differentiated MSC (26). Evidences supporting this hypothesis come from the experimental spontaneous malignant transformation of human and murine MSCs (87,88). Others also have shown that targeting deletion or expression of certain genes in MSCs indeed induce sarcoma formation (76,89). This hypothesis has been confirmed in depth in Ewing's sarcoma where the expression of EWS-FLI-1 chimeric gene in human MSCs induces Ewing's sarcoma formation (31). Similarly, transformation of MSCs and leiomyosarcoma formation were initiated by deleting p53 in certain human MSCs (22). Other studies have also indicated MSCs transformation resulting into other subtypes of sarcoma (9,22).

All these studies do not provide a direct evidence for the transformation of different sarcoma subtypes from MSCs. It is more acceptable that some sarcomas originate from mutated MSCs which are vulnerable for subsequent mutations. Depending on the impact of the initial and/or subsequent additional mutations and/or different environmental factors, the MSCs may differentiate into different sarcoma subtypes (90).

Summary

Osteosarcoma, ewing sarcoma, rhabdomyosarcoma, synovial sarcoma, chondrosarcoma, liposarcoma and other sarcomas are found shown MSC origin. It is more acceptable that sarcomas originate from mutated MSCs which are vulnerable for subsequent mutations. Transformation of MSCs can be achieved by several methods including knockout of tumor suppressor genes, overexpression of oncogenes and drug administration to affect signaling pathways. The potential of MSCs for cell-based therapies relies on several key properties such as capacity to differentiate into several cell lineages, lack of immunogenicity, immunomodulatory properties, robust *ex vivo* expansion potential, ability to secrete factors and homing ability to damaged tissues and tumor sites. Due to these properties, MSCs have considerable therapeutic potential in a variety of clinical applications in human malignancies. These recent accomplishments have broadened our knowledge of the role of MSCs in sarcomas.

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Footnote

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