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REVIEW

Modeling sarcomagenesis using multipotent mesenchymal stem cells

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Because of their unique properties, multipotent mesenchymal stem cells (MSCs) represent one of the most promising adult stem cells being used worldwide in a wide array of clinical applications. Overall, compelling evidence supports the long-term safety of *ex vivo* expanded human MSCs, which do not seem to transform spontaneously. However, experimental data reveal a link between MSCs and cancer, and MSCs have been reported to inhibit or promote tumor growth depending on yet undefined conditions. Interestingly, solid evidence based on transgenic mice and genetic intervention of MSCs has placed these cells as the most likely cell of origin for certain sarcomas. This research area is being increasingly explored to develop accurate MSC-based models of sarcomagenesis, which will be undoubtedly valuable in providing a better understanding about the etiology and pathogenesis of mesenchymal cancer, eventually leading to the development of more specific therapies directed against the sarcoma-initiating cell. Unfortunately, still little is known about the mechanisms underlying MSC transformation and further studies are required to develop bona fide sarcoma models based on human MSCs. Here, we comprehensively review the existing MSC-based models of sarcoma and discuss the most common mechanisms leading to tumoral transformation of MSCs and sarcomagenesis.

***Keywords*:** multipotent mesenchymal stem cells; adipose-derived stem cell; sarcoma; sarcoma models; fusion genes; cancer stem cell; sarcoma-initiating cell

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Introduction

Multipotent mesenchymal stem cells (MSCs), also called bone marrow (BM) stromal cells or skeletal stem cells, are multipotent cells that represent a rare subset of BM cells and constitute a source of progenitors for mesodermic tissues [1]. To date, the developmental origin and the histological localization of the more immature population of MSCs remain elusive and likewise, a reliable specific marker to define this population has not yet been identified. The International Society for Cellular Therapy proposed a minimal set of criteria to define the *ex vivo* MSC cultures: (i) the cells must be plastic adherent when maintained in standard culture conditions, (ii) they must express CD105, CD73 and CD90 and lack expression of CD45, CD34, CD14, CD11b, CD79b, CD19 and HLA-DR and (iii) they must be able to differentiate into osteoblasts, adipocytes and chondroblasts *in vitro* [2]. In addition to BM, cells fulfilling these properties are present in a variety of tissues during development and therefore can be isolated from several embryonic and adult tissues including adipose tissue, umbilical cord, liver or muscle [3-5]. The exact nature and localization of MSCs *in vivo* remain poorly understood but increasing evidence indicates that MSC precursors from different tissues could have a perivascular distribution [6]. Interestingly, *in vivo* transplantation has been proposed as a surrogate assay to address the multipotent differentiation ability of the stromal cells derived from different tissues [7]. In fact, this *in vivo* approach has already revealed differences in the differentiation potential of MSCs derived from BM and other tissues. While more accurate methods to derive

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and characterize MSC cultures are being developed, the prevailing consensus is that unfractionated populations of MSCs contain subpopulations spanning different stages of mesodermal development with distinct potency ranging from multilineage stem cells to unilineage precursors or even fully differentiated cells. Thus, cultures are heterogeneous in potency and it is likely that only a small MSC subset represents the *bona fide* multipotent stem cell population.

The potential of MSCs for cell-based therapies relies on several key properties: (i) capacity to differentiate into several cell lineages; (ii) lack of immunogenicity; (iii) immunomodulatory properties; (iv) robust *ex vivo* expansion potential; (v) ability to secrete factors, which regulate cell proliferation, differentiation and migration and; (vi) homing ability to damaged tissues and tumor sites [8]. Due to these properties, MSCs are being used worldwide in a variety of clinical applications including tissue repair, treatment of graft-versus-host disease and autoimmune diseases and are being used as vehicles to deliver anti-cancer therapies [8].

Nevertheless, recent evidence has revealed a link between MSCs and cancer. MSCs have been reported to inhibit or promote tumor growth depending on yet undefined conditions [9]. Likewise, the tumoral transformation of MSCs by different mechanisms gives rise to the formation of sarcomas *in vivo,* hence placing MSCs as the most likely cell of origin for certain sarcomas [10]. Sarcomas comprise a heterogenous group of malignant tumors of mesenchymal origin that were historically grouped according to the tumor location into two main types: soft tissue sarcoma (STS) and primary bone sarcoma [11]. An alternative genetic-based classification of sarcomas evolved upon the subsequent identification of molecular and genetic alterations associated with specific histological subtypes of sarcomas. According to this classification, sarcomas fall into two main categories. One group, including alveolar rhabdomyosarcoma (ARMS), myxoid liposarcoma (MLS), Ewing's sarcoma and synovial sarcoma, is characterized by the presence of tumor-specific translocations while the other group, represented by leiomyosarcoma, malignant fibrous histiocytoma (MFH) and osteosarcoma, is characterized by complex karyotypes indicative of severe genetic and chromosomal instability [12].

In the hierarchical model for cancer genesis it is hypothesized that different cells within the tumor have distinct potential to initiate and maintain a tumor, and that there exist a rare subset of so-called cancer stem cells (CSCs) capable of long-term tumor maintenance. These CSCs have been recently identified in several types of tumors and are thought to be the only cells within the bulk tumor with the ability to reinitiate and maintain tumor growth [13]. Likewise, there are cell types susceptible of acquiring early cancer-initiating mutation(s), which eventually result in *de novo* tumor formation. The cell-of-origin or tumor-initiating cell (TIC) is not necessarily the CSC since the TIC and CSC concepts refer to cancer-initiating cells and cancer-propagating cells, respectively [14]. In this regard, increasing evidence suggests that MSCs might be the TIC capable of initiating sarcomagenesis. Thus, several types of human sarcomas have been reproduced *in vivo* upon the overexpression of specific fusion oncoproteins or disruption of key signaling pathways in MSCs. Likewise, there are also studies supporting that sarcomas could represent good examples of the CSC model and that these sarcoma CSCs display MSC properties. Therefore, the development of human sarcoma models based on experimentally induced transformation of MSCs will constitute an unprecedented system in the search for target-specific therapies against sarcomas. Here, we review the existing models of sarcomas based on transformed MSCs.

Tumoral transformation of MSCs

It has been recently established that transformed MSCs may initiate sarcomagenesis *in vivo.* Many efforts have been directed to characterize the transformation process and also to prospectively generate specific models for different sarcomas. These studies include both spontaneous and induced transformation of MSCs mediated by specific alterations affecting key signaling pathways such as the cell cycle control.

Spontaneous transformation of MSCs

Mouse MSCs Mouse MSCs (mMSCs) are especially predisposed to acquiring transformation events after long-term *in vitro* culture favoring clonal selection of transformed cells [15-19] (Table 1). Upon inoculation into immunodeficient mice, spontaneously transformed mMSCs promote the formation of sarcomas resembling the histopathological properties of fibrosarcoma [15, 16] and osteosarcoma [17, 18]. In these reports, the transformation of mMSCs was associated with the accumulation of chromosome instability [16-19], *p53* mutations [15] or loss of *CDKN2A/p16* [17], highlighting the importance of a tight cell cycle control in MSC homeostasis.

Human MSCs More importantly, in the human setting, the *ex vivo* expansion of human MSCs (hMSCs) is a prerequisite for using these cells in some clinical applications. Consequently, the possibility that hMSCs may also undergo spontaneous transformation after long-term *in*

vitro culture became a concern, which has drawn especial attention in recent years. One group described the outgrowth of a cell population with a transformed phenotype derived from normal BM-hMSC cultures, although the authors could not rule out the presence of a rare $CD133^+$ non-stromal cell population in the starting material [20] (Table 1). Likewise, it is worth mentioning that two other comprehensive studies initially reporting spontaneous transformation of both BM-hMSCs and human adipose-derived mesenchymal stem cells (hASCs) after long-term *in vitro* culture have been recently retracted due to cross-contamination of the MSC cultures with cancer cell lines [21, 22]. On the other hand, many other authors have reported a lack of hMSC transformation after extensive *in vitro* culture [23-28]. These studies show that the life span of hMSCs is donor dependent and that cultures regularly become senescent after 15 to 25 passages. Likewise, no chromosomal abnormalities are normally detected by array-CGH and cytogenetic analysis [23], although in some preparations of clinical-grade hMSCs the occurrence of donor-associated aneuploidy without transformation was observed [28]. Altogether, these studies strongly suggest that *ex vivo* expanded hMSC cultures are genetically stable with no solid evidence of *in vitro* spontaneous transformation, thus supporting the safe use of hMSCs in clinical applications.

Cell cycle control in MSC-based sarcoma modeling

Cell cycle checkpoints play a crucial role in maintaining the cellular homeostasis and either gain- or loss-of-function mutations affecting cell cycle regulators are often associated with sarcoma development [12].

mMSCs In the mouse setting, the use of genetically targeted mMSCs has demonstrated how the deficiency of different cell cycle regulators, especially p53, triggers a transformation process in mMSCs resulting in the generation of sarcoma [29-31] (Table 2). For instance, in contrast to wild-type (wt) ASCs, p53-depleted mASCs were capable of originating leiomyosarcoma-like tumors after injection into immunodeficient mice [31]. This finding is further supported by a differentiation-based microRNA study, which has identified leiomyosarcoma as an MSC-related malignancy [32]. Another study has shown that the complete loss of *p53* expression in $p21^{-/-}p53^{+/-}$ mASCs after long-term culture induces cell growth, karyotypic instability and loss of $p16^{INK4A}$ which prevents senescence, resulting in the formation of fibrosarcoma-like tumors *in vivo* [30]. Similarly, alterations in other cell cycle regulators such as $p16^{INK4A}$ or $p19^{ARF}$ have also been detected during the process of MSC transformation [17, 30] whereas overexpression of c-MYC in $p16^{INK4A-/-}p19^{ARF-/-}$ BM-mMSCs results in osteosarcoma

Table 1 Studies reporting the occurrence (or the lack) of spontaneous transformation in MSC cultures

Cell type	Type of sarcomas[1]	Associated oncogenic events[2]	Reference
Spontaneous transformation			
BM-mMSCs	Fibrosarcoma	*p53* mutations	[15]
BM-mMSCs	Fibrosarcoma	Chromosomal instability + TERT and c-myc expression	[16]
BM-mMSCs	Osteosarcoma	Aneuploidy + *CDKN2A/p16* loss	[17]
BM-mMSCs	Osteosarcoma	Abnormal karyotype	[18]
BM-mMSCs	Undiff. soft tissue sarcomas	Aneuploidy + chromosomal translocations	[19]
BM-hMSCs[3]	Poorly differentiated tumors	Aneuploidy + chromosomal translocations	[20]
Absence of spontaneous transformation			
BM-mMSCs	NT	Spontaneous differentiation, loss of multipotent potential	[25]
BM-hMSCs	NT	No chromosomal abnormalities or hTERT detected, normal p53, p16 or Rb levels	[23]
BM-hMSCs	NT	No chromosomal abnormalities or hTERT detected, normal p53, p16 or Rb levels	[24]
BM-hMSCs	NT	Senescence observed at population doublings 33 to 55, chromosome 7 amplification in a sample.	[27]
BM-hMSCs	NT	Donor dependent aneuploidy	[28]
hASCs	NT	High level of genomic stability	[26]

[1]Type of tumors observed upon inoculation in immunodeficient mice. NT: no tumors formed upon inoculation in immunodeficient mice.
[2]Oncogenic events analyzed/reported in these studies.
[3]In this unique case of spontaneous transformation of hMSCs the presence of a rare population of $CD113^+$ non-stromal cells in the starting material is not ruled out.

development accompanied by loss of adipogenesis [33]. Importantly, the loss of other cell cycle regulators such as Rb does not transform mMSCs but its deficiency potentiates tumor development of p53-deficient mMSCs, generating more undifferentiated sarcomas [31].

Mouse models Several groups have also developed useful genetically engineered mouse models of sarcomagenesis based on the inactivation of *p53* and/or *Rb* specifically in the osteoblastic lineage [34, 35]. These studies not only confirm that sarcoma development is dependent on the loss of *p53* and potentiated by the loss of *Rb* but also that these tumors display many of the features of human osteosarcomas, linking the inactivation of *p53* and/or *Rb* in a committed mesenchymal lineage to osteosarcoma development. Similar results were shown in another mouse model in which *p53* and *Rb* were inactivated in early mesenchymal tissues of embryonic limb buds [36]. In this study, mice carrying a *p53* deletion developed different types of sarcomas, with osteosarcoma being the most common one. Interestingly, although *Rb*-deficient mice develop normally, *Rb* deficiency synergizes with *p53* deletion to accelerate sarcoma formation and increase the frequency of poorly differentiated sarcomas. In other mouse models where mutations are restricted to muscle or uterus by local delivery of the Cre recombinase, the expression of oncogenic *K-RAS* or the mutation of endogenous *K-RAS* is needed to efficiently induce sarcoma formation in *p53*-deficient tissues [37, 38]. Sarcomas developed in these models were characterized as pleomorphic rhabdomyosarcoma and high-grade sarcomas with myofibroblastic differentiation. Interestingly, deletion of the *INK4A-ARF* locus could substitute the *p53* mutation in this *K-RAS* mutation-based model of sarcoma development [37].

Human MSCs Fortunately, hMSCs do not undergo malignant transformation as easily as mMSCs. For instance, opposite to mMSCs the inactivation of *p53* or *p53* and *Rb* does not induce transformation in hMSCs, although *p53*-/*Rb*-deficient hMSCs display a higher growth rate *in vitro* coupled to an extended lifespan [39, 40]. In order to efficiently induce *in vivo* sarcomas from hMSCs, several non-physiological oncogenic events had to be combined [41, 42] (Table 2). Specifically, these oncogenic hits include the introduction of the catalytic subunit of the human telomerase (hTERT), HPV-16 E6 and E7 (which abrogate the functions of p53 and Rb family members), SV40 small T or large T antigens (which results in c-MYC stabilization and inactivates Rb and p53, respectively) and oncogenic H-RAS (which provides a constitutive mitogenic signal) [41, 42]. In one of these models, the process of transformation of hMSCs is associated with a gradual increase in genomic hypomethylation, although this phenomenon is not necessary for transformation [43]. Using an alternative approach, another group succeeded in transforming hMSCs through ectopic expression of hTERT, H-RAS and BMI-1, which inhibits the expression of genes controlled by polycomb response elements including *p16*^{INK4A} [44]. It was also reported that some hTERT-transduced hMSC lines lose contact inhibition, acquire anchorage-independent growth and form tumors in mice after long-term *in vitro* culture [45]. This transformation process was associated with the deletion of the Ink4a/ARF locus and with the acquisition of an activating mutation in *K-RAS*. Overall, *in vivo* tumors originated from most of these transformed hMSCs were classified as undifferentiated spindle cell sarcomas [41, 42, 44].

Other signaling pathways involved in MSC transformation

Besides the inactivation of cell cycle regulators, the transformation process of hMSCs has been linked to alterations in signaling pathways such as PI3K-AKT and WNT signaling (Table 2).

PI3K-AKT pathway The PI3K-AKT pathway is involved in cell survival and proliferation and is a downstream effector shared by different growth factor receptors abnormally activated in sarcomas, such as IGF1R, PDGFR or c-KIT receptor [12]. In this regard, it has been reported that the PI3K-AKT-mTOR signaling pathway plays a critical role in the development of leiomyosarcomas [46]. Thus, mice carrying a homozygous deletion of PTEN (a negative regulator of the PI3K-AKT pathway) in the smooth muscle lineage efficiently developed leiomyosarcoma [46]. The involvement of PTEN and PI3K-AKT in leiomyosarcoma is also implicated by the fact that these signaling pathways are dysregulated in leiomyosarcoma-forming *p53*-decifient mMSCs [40].

WNT pathway The WNT/β-catenin signaling pathway plays a central role in modulating the balance between self-renewal and differentiation in stem and progenitor cells [47]. In addition, WNT signaling also regulates the proliferation, differentiation and invasion capacity of hMSCs [48, 49]. While these functions exerted by the WNT pathway may be useful in tissue regeneration, an aberrant or inadequate activation of this pathway may deregulate the balance between proliferation, differentiation and apoptosis, leading to malignant transformation. Accordingly, a recent study supports a role for aberrant β-catenin stabilization in the promotion of MSC-derived

Table 2 Sarcoma models generated by targeting relevant pathways in MSCs or mesenchymal lineages

Sarcoma type[1]	Oncogenic events[2]		Cell/tissue of origin	Reference
	Inactivation	Expression/treatment		
Mouse MSCs				
Fibrosarcoma	*p21* + *p53*	-	mASCs	[30]
Leiomyosarcoma	*p53* or *p53* + *Rb*	-	mASCs	[31]
Osteosarcoma	*INK4A/ARF*	c-myc	BM-mMSCs	[33]
Mouse models[3]				
Osteosarcoma	*p53* or *p53* + *Rb*	-	Osteoblastic lineage	[34, 35]
Osteosarcoma	*p53*	-	Mesenchymal cells of limb buds	[36]
Undifferentiated sarcoma	*p53* + *Rb*	-	Mesenchymal cells of limb buds	[36]
High-grade sarcoma with myofibroblastic differentiation	*p53* or *INK4A/ARF*	K-RAS	Muscle, uterus	[37]
Pleomorphic rhabdomyosarcoma	*p53*	K-RAS	Muscle	[38]
Leiomyosarcoma	*PTEN*	-	Smooth muscle lineage	[46]
Aggressive fibromatosis	*APC*	-	MSC progenitors	[50]
Human MSCs				
NT	-	HPV16 E6/E7	BM-hMSCs	[39]
Undifferentiated spindle cell sarcoma	-	hTERT + HPV16 E6/ E7 + SV40-ST + H-RAS	BM-hMSCs	[41]
Undifferentiated spindle cell sarcoma	-	hTERT + SV40-LT + H-RAS	BM-hMSCs	[42]
Undifferentiated spindle cell sarcoma	-	hTERT + H-RAS + BMI-1	BM-hMSCs	[44]
Tumors with smooth muscle and bone properties	-	hTERT[4]	BM-hMSCs	[45]
Undifferentiated pleomorphic sarcomas	-	DKK1 (WNT signaling inhibition) + SV40-LT	BM-hMSCs	[51]

[1]Type of tumors observed upon inoculation in immunodeficient mice. NT: no tumors formed upon inoculation in immunodeficient mice.
[2]Transforming hits used to achieve tumoral transformation.
[3]In mouse models where distinct types of tumors were reported only the most represented one is denoted.
[4]The transformed phenotype of hTERT-BM-hMSCs in this study was observed after long-term culture and accompanied by the loss of the INK4a/ARF locus and DBCCR1 and the acquisition of K-RAS activating mutations.

tumorigenesis [50]. In this work, the development of a mouse model harboring a targeted mutation in the APC gene provided evidence for the link between aggressive fibromatosis, a mesenchymal neoplasm, and MSCs. More importantly, inactivation of WNT signaling upon treatment of previously SV40-immortalized hMSCs with the WNT inhibitor DKK1 led to full malignant transformation of these hMSCs and the consequent *in vivo* formation of MFH [51]. Conversely, restoration of WNT signaling in MFH cells allowed them to differentiate along different mesenchymal lineages [51]. Furthermore, it was reported that key components of the Wnt signaling pathway are downregulated in osteosarcoma as compared to normal hMSCs and hMSCs differentiated into osteoblasts [52]. Interestingly, the role of WNT signaling in sarcomas seems to differ from its role in carcinomas because hMSC transformation and sarcomagenesis are associated with WNT signaling inhibition while different models of carcinomas are linked to activating mutations in components of the WNT pathway [51].

The take-home messages from the aforementioned reports are as follows: (i) hMSCs do not seem to transform spontaneously during *ex vivo* expansion; (ii) mMSCs and mouse models highlight how the inactivation of key cell cycle regulators and/or alterations of other relevant signaling pathways induce the development of sarcomas from MSCs or their committed mesenchymal lineages; (iii) several cooperating oncogenic mutations have to work together in order to promote sarcoma development from difficult-to-transform hMSCs and; (iv) distinct cell populations at different developmental stages in the mesenchymal lineage hierarchy may serve as the cell of origin for different sarcoma subtypes.

Fusion gene-based models of sarcomas

The MSC origin of sarcomas characterized by the presence of tumor-specific fusion oncogenes as a result of chromosomal translocations has also been actively investigated and several types of tumors resembling human sarcomas have been reproduced *in vivo* upon the expression of sarcoma-specific fusion proteins in mMSCs and mouse models. Specifically, Ewing's sarcoma, MLS, ARMS and synovial sarcoma have been reproduced upon

Table 3 Models of sarcomas generated upon the expression of specific fusion proteins in MSCs or mesenchymal lineages

Sarcoma type[1]	Oncogenic events[2]		Cell/tissue of origin	Reference
	Fusion gene	2nd hit		
Mouse MSCs				
Ewing's sarcoma	EWS-FLI-1	*p53* loss	BM-mMSCs	[54]
Ewing's sarcoma	EWS-FLI-1	-	BM-mMSCs	[55]
Myxoid liposarcoma	FUS-CHOP	-	BM-mMSCs	[65]
Liposarcoma	FUS-CHOP	$p53^{-/-}$	mASCs	[40]
Alveolar rhabdomyosarcoma	PAX3/7-FKHR	*p53dn* (±H-RAS) or SV40-LT (±H-RAS)	BM-mMSCs	[74]
Mouse models[3]				
Ewing's sarcoma	EWS-FLI-1	$p53^{-/-}$	Mesenchymal cells of limb buds	[58]
Liposarcoma	FUS-CHOP	-	Ubiquitous	[66]
Liposarcoma	CHOP-FUS	-	Ubiquitous	[67]
Liposarcoma	FUS + CHOP	-	Ubiquitous	[68]
Alveolar rhabdomyosarcoma	PAX3-FKHR	$p53^{-/-}$ or $INK4A/ARF^{-/-}$	Differentiated muscle cells (MYF6-expressing cells)	[76]
Synovial sarcoma	SYT-SSX2	-	Immature myoblasts (MYF5-expressing cells)	[78]
Human MSCs				
NT	EWS-FLI-1	-	BM-hMSCs	[59]
NT	FUS-CHOP	*p53* depletion	hASCs	[40]
NT	SYT-SSX1	-	BM-hMSCs	[80]

[1]Type of tumors observed upon inoculation in immunodeficient mice. NT: no tumors formed upon inoculation in immunodeficient mice.
[2]Transforming hits used to achieve tumoral transformation.
[3]In mouse models where distinct types of tumors were reported only the most represented one is denoted.

expression of EWS-FLI-1, FUS-CHOP, PAX-FKHR and SYT-SSX, respectively (Table 3).

Ewing's sarcoma MSC models

Ewing's sarcoma is a poorly differentiated tumor of uncertain histogenesis and aggressive biologic behavior. Two decades ago, the understanding of the biology of Ewing's sarcoma took a leap forward with the identification of recurrent EWS fusions, which drive oncogenesis in this disease [53].

mMSCs Two different groups have reported that the expression of EWS-FLI-1 in BM-mMSCs resulted in cell transformation and sarcoma development when implanted into immunodeficient mice [54, 55]. These tumors shared some features with Ewing's sarcoma, including cell surface markers and cell morphology. Interestingly, the expression of EWS-FLI-1 was able to transform mMSCs on its own in one of the studies [55] while secondary hits acquired in culture (i.e. *p53* mutation) were required in another study [54]. Intriguingly, other Ewing's sarcoma-associated fusion genes such as EWS-ERG and FUS-ERG could not be stably expressed in mMSCs [56].

Mouse models Several knock-in and transgenic mouse models expressing EWS-FLI-1 or other Ewing's sarcoma-related fusion genes have been recently created [57]. The constitutive expression of EWS-FLI-1 has an embryonic lethal phenotype [58]. A tissue-specific cre-loxP-based strategy was thus used to achieve conditional expression of EWS-FLI-1 *in vivo*. Accordingly, mice with conditional expression of EWS-FLI-1 in primitive mesenchymal cells of the embryonic limb buds showed several developmental defects of the limbs but EWS-FLI-1 on its own was unable to induce formation of tumors in these mice [58]. However, when *p53* was simultaneously deleted, EWS-FLI-1 promoted sarcoma formation. Thus, as aforementioned, conditional deletion of *p53* in early mesenchymal tissues of embryonic limb buds predominantly gave rise to osteosarcomas [36], and the presence of EWS-FLI-1 shifted the tumor phenotype towards more undifferentiated sarcomas, similar to Ewing's sarcoma [58]. Overall, similar to that observed in EWS-FLI-1-expressing hMSCs, additional cooperating mutations seem to be required for transformation in Ewing's sarcoma mouse models.

Human MSCs In the human setting, EWS-FLI-1-expressing hMSCs failed to originate tumors when injected into immunodeficient mice, [59] although the expression of EWS-FLI-1 in hMSCs induced a transcriptional expression pattern similar to that observed in human Ewing's sarcoma and upregulated the expression of CD99, a specific marker for Ewing's sarcoma [59]. The lack of transformation in this model underpins the need of yet undefined secondary cooperating mutations to transform hMSCs. The MSC origin for Ewing's sarcoma has been strengthened further in other experiments based on hMSCs. For instance, treatment of Ewing's tumor cell lines with specific EWS-FLI1 short hairpin RNAs shifted their gene expression profile towards that of normal MSCs [60]. In addition, CSCs displaying MSC properties have been identified in Ewing's sarcoma [61]. In a follow-up study, these authors showed that human pediatric, but not adult, MSCs expressing EWS-FLI-1 display *in vitro* features of Ewing's sarcoma CSCs [62]. This phenotype was due to the EWS-FLI-1-mediated repression of the miR-145 promoter, which, in turn, leads to the upregulation of embryonic stem cell transcription factors SOX2, OCT4 and NANOG, thus linking development, cell ontogeny and cancer.

MLS models

Liposarcomas are the most common type of STS, representing ~20% of STS. Several types of liposarcoma are recognized, including well differentiated, dedifferentiated, myxoid, round cell and pleomorphic liposarcoma. MLS is characterized by the recurrent translocation t(12;16)(q13;p11), which fuses *FUS* to *CHOP* (also known as *DDIT3*) on chromosome 12. MLS has also been modeled by expressing of the FUS-CHOP fusion gene, which is found in > 90% of these tumors [63].

mMSCs Early *in vitro* approaches have shown the transforming effects of FUS-CHOP in NIH-3T3 fibroblasts [64], but not in 3T3-L1 pre-adipocytes, suggesting that the transformation activity of FUS-CHOP is influenced by the cellular environment. More importantly, the expression of FUS-CHOP in both BM-mMSCs and mASCs gave rise to MLS-like tumors [40, 65]. Nevertheless, in contrast to BM-mMSCs, secondary cooperating hits such as *p53* deficiency are required for liposarcoma development from mASCs, suggesting that BM-mMSCs are more susceptible to FUS-CHOP-induced transformation and sarcomagenesis than mASCs. In the absence of FUS-CHOP, *p53*$^{-/-}$ mASCs originated leiomyosarcoma [31], indicating that the expression of FUS-CHOP redirects the tumor genesis/phenotype [40]. These studies support the contention that the FUS-CHOP fusion is a critical event in MLS pathogenesis and that MSCs may represent the liposarcoma-initiating cell. Differential gene expression studies using these mMSCs have suggested potential genes and pathways specifically altered by FUS-CHOP

expression, which could be involved in liposarcoma development, including PDGF signaling, RXR signaling, and sphingolipid and fatty acid metabolism [40, 65].

Mouse models Transgenic mouse models have also provided clues about the transforming mechanisms and identity of the target cell where FUS-CHOP exerts its tumorigenic effect. Thus, transgenic mice expressing FUS-CHOP or CHOP-FUS transgenes under the control of the ubiquitous E1Fa promoter gave rise to similar liposarcomas that resemble their human counterparts [66, 67]. On the other hand, although the uncontrolled expression of CHOP after the chromosomal translocation seems to play a leading role in liposarcoma development [64], transgenic mice expressing CHOP alone do not develop any tumor [67] and the expression of FUS restores liposarcoma development in these CHOP-transgenic mice [68]. These results provide evidence that the FUS portion of FUS-CHOP also plays a specific and critical role in the pathogenesis of liposarcoma. Furthermore, the immature nature of liposarcoma cell progenitors was suggested by the generation of an aP2-FUS-CHOP transgenic model, where FUS-CHOP was not able to induce liposarcoma development when expressed in differentiated, aP2-expressing, adipocytes [69]. In addition, studies using embryonic fibroblasts from these mouse models have concluded that FUS-CHOP prevents the development of adipocytic precursors by repressing PPARγ and C/EBP as part of a mechanism of differentiation disruption that seems to be required for liposarcoma development [69, 70].

Human MSCs In the human setting, however, the expression of FUS-CHOP does not transform either wt or *p53*-deficient hASCs [40]. Nevertheless, the expression of either FUS-CHOP or CHOP in fibrosarcoma cell lines induces the formation of liposarcoma-like tumors [71], highlighting the need for further cooperating mutations in the FUS-CHOP-expressing hMSC model. A microarray gene expression profiling has revealed potential deregulated pathways (e.g., Wnt, PTEN or PI3K/AKT) in liposarcoma formation from *p53*-deficient FUS-CHOP-expressing mASCs, which might also be potential candidates for cooperating mutations in the transformation of hASCs [40].

ARMS models

The ARMS is a subtype of rhabdomyosarcoma characterized by an appearance similar to the alveoli of the lungs. There is also evidence that MSCs are the cells of origin for ARMS [72]. This tumor is characterized by the expression of either PAX3-FKHR or PAX7-FKHR fusion genes in MSCs, pushing MSC differentiation towards a myogenic lineage while inhibiting terminal differentiation.

mMSCs Initial studies showed the ability of PAX3-FKHR to transform mouse fibroblasts [73]. However, PAX3-FKHR and PAX7-FKHR fusions induced skeletal myogenesis but not transformation when introduced in BM-mMSCs [74]. Nevertheless, the expression of a dominant-negative form of p53 or SV40 large T antigen (which inactivates both p53 and Rb) elicits tumor formation in a proportion of the PAX-FKHR-expressing cells. Additional expression of the constitutively active H-RAS^{G12V} leads to tumor formation in all of the PAX-FKHR-expressing populations. These PAX-FKHR-expressing tumors display histological features and gene expression profiles similar to human ARMS [74].

Mouse models Similar to the aforementioned studies on primary MSCs, mouse models of PAX-FKHR fusions also several the need for additional secondary genetic events to develop overt ARMS. In one study using a *Pax3-Fkhr* knock-in approach, the heterozygous offspring of PAX3-FKHR chimeric mice showed developmental abnormalities although no signs of malignancy were observed [75]. In another model, a conditional *Pax3-Fkhr* knock-in allele was introduced specifically in MYF6-expressing skeletal muscle [76]. Although ARMS occurs at low frequency in these conditional mice, complementary disruption of *p53* or the *INK4A/ARF* locus substantially increases the frequency of ARMS [76].

Synovial sarcoma models

Synovial sarcomas (SS) often arise deep in the soft tissue near a joint in the extremity of young adult patients. Most SS are characterized by t(X;18)(p11.2;q11.2), resulting in a fusion between the *SS18* (*SYT*) gene on chromosome 18 and one of the *SSX* genes on the X chromosome, creating *SS18-SSX1*, *SS18-SSX2* or *SS18-SSX4* chimeric genes [77]. Although less studied than other *STS*, there are also several clues suggesting a potential MSC origin in synovial sarcoma.

Mouse models An interesting mouse model of SS based on the conditional expression of SYT-SSX2 in several skeletal muscle cell types has been reported [78]. Interestingly, SS tumor is reproduced when the SYT-SSX2 fusion is expressed in immature myoblasts (MYF5+) but not in more differentiated cells (MYF6+), highlighting how the same genetic alteration may lead to different outcomes/tumor phenotype in different cell populations down the lineage hierarchy.

Human MSCs In the human setting, silencing of the fusion gene expression with specific shRNA in primary SS cells induces the expression of mesenchymal markers and enhances their ability to differentiate into osteocytes, chondrocytes and adipocytes, suggesting that MSCs could be at the origin of this disease [79]. Similarly, the expression of SYT-SSX1 in hMSCs induces a transcriptional profile very similar to the SS expression signature [80].

We have reviewed mounting evidence suggesting that multipotent and long-lived MSCs may provide an ideal cellular target for the initiation of some sarcomas upon the expression of specific fusion genes. However, no fusion gene-based model of sarcoma has been developed so far using hMSCs. The fusion genes seem to primarily act to block differentiation towards a given pathway. According to the simplistic dogma of cancer where both differentiation and proliferation processes have to be impaired or deregulated for cancer initiation, the differentiation blockage is not sufficient for malignant transformation and secondary transforming hits would be needed to fully transform hMSCs. The identification of these relevant cooperating events will likely lead to the successful development of these currently non-existing sarcoma models (based on hMSCs). Interestingly, preliminary results from our lab suggest that it is possible to develop a human sarcoma model based on the enforced expression of a specific fusion gene in human MSCs harboring cooperating transforming hits (data not shown).

Sarcoma-initiating/stem cells

There is considerable evidence that sarcomas are hierarchically organized and sustained by a subpopulation of self-renewing cells that can generate the full repertoire of tumor cells. CSCs that display tumor re-initiating properties have been recently identified in osteosarcoma [81-83], chondrosarcoma [82], Ewing's sarcoma [61] and synovial sarcoma [79]. The identification of these sarcoma-initiating cells was based on both their ability to form spherical, clonal expanding colonies (called sarcospheres) in anchorage-independent and serum-starved conditions and the expression of stem cell markers [61, 79, 81-84]. All of these CSCs are characterized by the expression of the pluripotent stem cell markers OCT3/4, NANOG and SOX2 and are able to self-renew and to sustain the tumor in serial transplantation experiments. More importantly, many of these sarcoma-initiating cells express MSC markers [79, 81-83] and retain MSC *in vitro* differentiation properties, giving rise to adipogenic, chondrogenic and osteogenic lineages [61, 79, 82]. In addition, these MSC-like CSCs are associated with drug resistance and metastasis [81, 84] and therefore, they may be responsible for the frequent relapses observed in sarcomas [85].

It is worth mentioning that some of the factors involved in induced pluripotency are also involved in sarcomagenesis. For instance, SOX2 has been reported to play a key role in the development of Ewing's sarcoma [62], whereas BMI1, which has been previously shown to be involved in sarcomagenesis [44], has also very recently been shown to be relevant in induced pluripotency [86].

Although an alternative model in which stochastic genetic events determine the development of the tumor can not be excluded, the aforementioned data indicate that at least some sarcomas fulfill phenotypic and functional features reminiscent of the hierarchical model of cancer, suggesting a strong link between sarcomas and MSCs. Intriguingly, MSCs may not only be the TIC in sarcomas, but also, a population of altered MSCs could constitute the CSCs responsible for maintaining tumor growth, being able to initiate tumorigenesis upon serial transplantation.

MSCs and tumor growth

The role of MSCs in tumorigenesis could also be an indirect phenomenon [87, 88]. MSCs are frequently recruited to the site of tissue injury or tumor growth and sometimes, in the appropriate and permissive environment and under stress conditions, this could also represent a potential source of malignancy. Thus, MSCs within the tumor stroma facilitate breast cancer metastasis through the secretion of the chemokines CCL5 [89] and CCL2 [90]. Likewise, hMSCs target osteosarcoma and promote its growth and pulmonary metastasis through secretion of CCL5 [91]. Moreover, a recent study reported that MSCs protect breast cancer cells through the TGF-β1-mediated increase of regulatory T-cells [92].

The BM microenvironment plays a role in the pathogenesis of a variety of hematological malignancies including acute lymphoblastic leukemia (ALL), multiple myeloma or myelodysplastic syndrome [93-95]. The onset and progression of hematological malignancies are in many cases dependent on mutual interactions between the leukemic blasts/plasma cells and BM stroma/MSCs, which provide survival and growth-promoting signals [8, 95, 96]. Interestingly, the fusion MLL-AF4 was recently found expressed in both BM-MSCs and leukemic blasts in 100% of infants suffering from pro-B ALL highlighting an unrecognized role of the BM milieu in the pathogenesis of this dismal infant leukemia [97]. Importantly, this study revealed the absence of monoclonal

rearrangements in MLL-AF4+ BM-MSCs precluding the possibility of cellular plasticity or de-differentiation of B-ALL blasts and suggests that MLL-AF4 might arise in a population of mesodermal precursors. In addition, BM-MSCs are resistant to chemotherapy-induced apoptosis [98-100] and contribute to generating drug resistance in tumor cells [95, 101]. Likewise, several studies have evidenced that hMSCs are highly resistant to ionizing radiation [102]. Collectively, these data suggest important implications for cancer therapy as this chemo- and radio-resistance could lead to the accumulation of mutations, resulting in MSC transformation and eventual generation of refractory/secondary tumors [98].

Despite the reported ability of MSCs to contribute to tumor growth under certain circumstances, there are also solid studies claiming their potential to inhibit tumor growth. Thus, human and mMSCs can home to tumor sites and inhibit the growth of neoplastic cells as shown in models of gliomas [103], Kaposi's sarcomas [104] and hepatoma [105, 106]. Other study shows that hMSCs exhibit an antiproliferative activity on tumor cells, although in *in-vivo* experiments, the co-injection of MSCs caused an increase in tumor cell growth rate [107].

Intriguingly, experimental evidence suggests that MSCs may either favor or inhibit tumor growth depending on the genetic background of the tumor cells [108]. For instance, MSCs have been shown to accelerate tumoral growth and promote metastasis of estrogen receptor-alpha+ (ERα+) but not ERα– breast cancer cells [89]. Likewise, the capacity of MSCs to inhibit cellular growth in Kaposi's sarcoma depends on their ability to shut down AKT activity in the tumor cells [104]. These findings imply that if the interactions between cancer cells and MSCs in specific cancers can be elucidated, we could develop more effective anti-cancer strategies based on the use of wt or manipulated MSCs.

In addition to the ability of MSCs to promote tumor growth, other non-MSC cell types may acquire mesenchymal properties and exert a relevant function in cancer development. Thus, the epithelial to mesenchymal transition (EMT) plays crucial roles in the formation of the body plan and in the differentiation of multiple tissues and organs. EMT can also adversely cause organ fibrosis and promote carcinoma progression through a variety of mechanisms. EMT promotes the acquisition of a mesenchymal phenotype by tumoral epithelial cells, resulting in gain of migratory and invasive properties and induction of stem cell properties. Thus, the mesenchymal state is associated with the capacity of carcinoma (epithelial) cells to migrate to distant organs and maintain stemness, allowing their subsequent differentiation into multiple cell types and initiation of metastasis [109].

Worth mentioning, emerging evidence links mesenchymal-state metastasic cancer with infiltrating macrophages [110]. It has been proposed that many metastasic cancers may arise from myeloid/macrophages rather than from EMTs. In fact, numerous cancers exhibit multiple properties of macrophages, including phagocytosis. It is tempting to speculate that the macrophage properties expressed in metastasic cancers may arise from damage to an already existing mesenchymal cell. Although this is still a nascent area of investigation, the view of metastasis as a myeloid/macrophage disease might impact future cancer research and intervention [110].

In light of the roles of MSCs in tumorigenesis, it would be crucial to expand our understanding about the nature of MSCs in order to better utilize the immunosuppressive and regenerative properties of hMSCs without promoting tumor growth.

Open questions

Because of their unique properties, MSCs represent one of the most promising adult stem cells being used worldwide in many clinical applications. However, owing to some of their intrinsic aforementioned properties, MSCs may also become a double-edged sword since they may support tumor cell growth and are being explored as the target cell for the origin of sarcomas. At this point, it is important to stress that there is no solid evidence for hMSC transformation during *ex vivo* expansion, and therefore, hMSCs seem to be generally safe for clinical applications in terms of potential risk of transformation to sarcoma, based on early data. However, longer follow-up of patients is still highly demanded. In any case, the fact that hMSCs could be the target cell for transforming mutations giving rise to sarcoma formation should not necessarily hamper their clinical use, since the likelihood of transformation of the infused cells should not be higher than that of patient's own cells.

Here, we have reviewed the published literature suggesting a role of MSC populations as TIC and CSC for different types of sarcoma. Besides the intrinsic transforming ability of specific mutations, it is necessary that these mutations hit the appropriate target cell in order to induce sarcoma formation. A debate has emerged about the cell of origin that suffers these mutations responsible for sarcoma development. Two main models may be conceptualized to support the MSCs as a potential target cell for sarcomas. It is suggested that either (i) the different sarcomas come from MSCs at different stages of differentiation that suffer specific mutations resulting in a blockage of terminal differentiation which, in turn, determines the degree of tumor differentiation, or (ii) sar-

comas originate from a primitive MSC, which acquires relevant mutations that direct tumor genesis (Figure 1).

The first model is supported by studies showing that the gene expression signature is surprisingly similar between sarcomas in different stages of differentiation and normal MSCs in similar stages of differentiation. Such studies were reported for liposarcoma [111], chondrosarcoma [112], osteosarcoma [52] and leiomyosarcoma [32]. Likewise, osteosarcoma and ARMS have been modeled by the establishment of relevant oncogenic hits in nearly differentiated cells of the osteoblastic and myoblastic lineages, respectively [34, 35, 76]. The second model is supported by many studies reporting sarcoma formation from spontaneous or mutation-induced transformation of human or mouse primitive MSCs. Likewise, several types of CSCs presenting MSC properties have been reported [61, 79, 82] and moreover, sarcoma cells of certain subtypes can also differentiate into multiple mesenchymal lineages *in vitro* [30, 31, 42]. Existing sarcoma mouse models also provide arguments in favor of this theory. Thus, MLS development has been reported after ubiquitous expression of FUS-CHOP but not when the

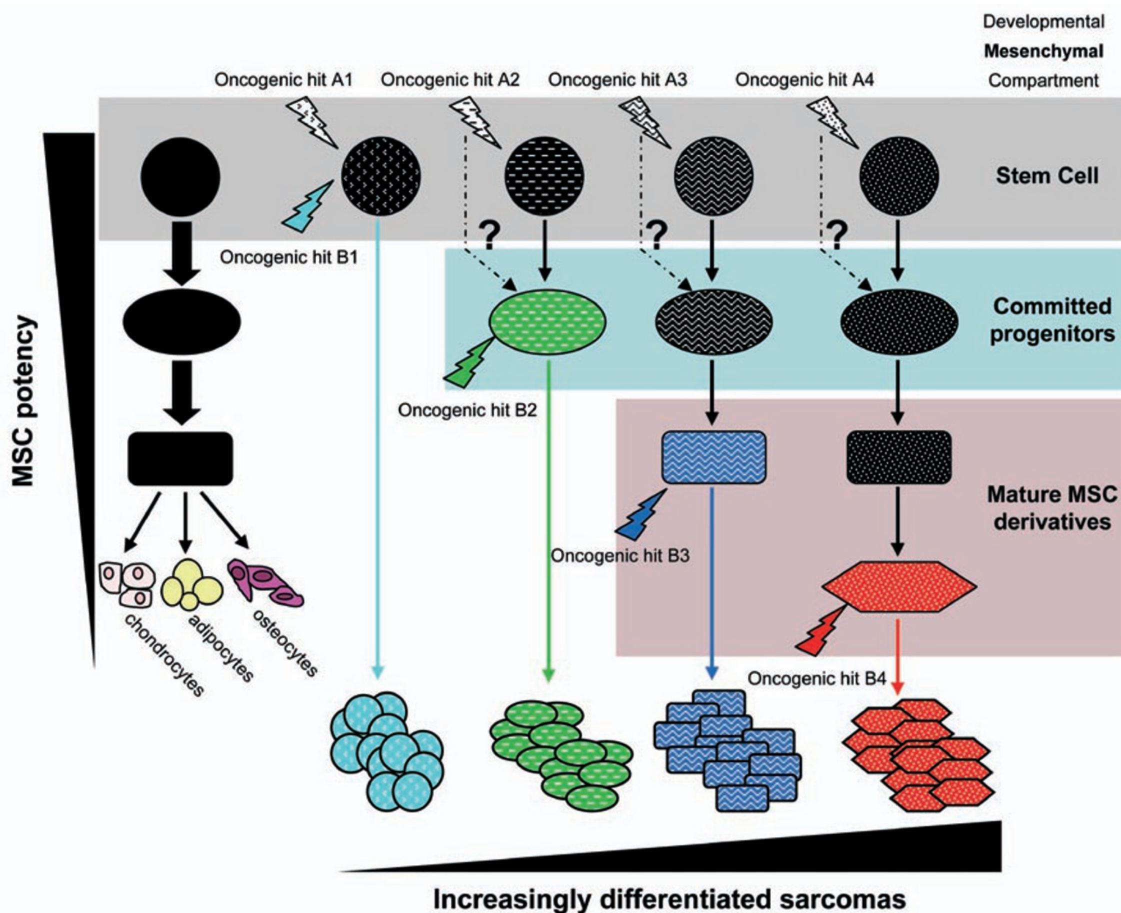

Figure 1 Schematic cartoon depicting how distinct sarcomas may result from a coordinated acquisition of cooperating oncogenic hits in the appropriate target cell throughout the mesenchymal hierarchy. In the absence of oncogenic hits the normal MSC (black circle; left) differentiates down the mesenchymal hierarchy eventually giving rise to mature and functional mesenchymal derivatives including adipocytes, chondrocytes and osteocytes. The "two-hit" model is thought to be necessary for explaining the eventual development of cancer: one hit is presumed to promote differentiation impairment while the second hit more frequently targets proliferation/apoptosis. Distinct early cancer-initiating hits (white-colored; hits A1-A4) are supposed to arise in long-lived MSCs, or perhaps, in early committed mesenchymal progenitors. It is very unlikely that a single early cancer-initiating hit induces sarcomagenesis on its own, and therefore sequential secondary cooperating hits (green, blue or red-coloured hits) are commonly required to achieve a malignant clonal expansion of mesenchymal derivatives. The more differentiated the stage (less MSC potency) along the mesenchymal hierarchy targeted by the secondary oncogenic mutation, the more differentiated the sarcoma appears. This model stresses both the importance of the intrinsic transforming ability of specific mutations and the need to target the appropriate cell type along the mesenchymal lineages.

fusion protein expression was restricted to aP2-expressing adipocytes suggesting that liposarcoma comes from a more immature cell type [66, 69]. Similarly, the SYT-SSX2 fusion gene is able to trigger SS formation when it is expressed in immature myoblasts but not in more differentiated cells [78].

Most likely, both models are not exclusive and could converge in a common model where sarcoma originates from MSCs that suffer sequential mutations targeting differentiation and proliferation pathways, resulting in sarcomas showing different degrees of differentiation depending on the potency of the cell along the MSC lineage that eventually gives rise to the tumor (Figure 1). The pathogenesis of Li-Fraumeni patients represents an interesting system to address whether these models could converge in a common model because these patients carry germline mutations of *p53*, which do not transform undifferentiated cells but might cooperate with subsequent mutations in more differentiated cells to induce sarcoma development. Further work aimed to better understand the nature of MSCs is needed to provide more specific markers, which will allow the identification of subpopulations with different differentiation capacities, which would facilitate the generation of both *in vitro* and *in vivo* sarcoma models based on the transformation of immature MSCs.

Worth mentioning, the tissue source of the MSCs may also represent an important factor influencing MSC transformation and the development of *bona fide* MSC-based models for sarcomagenesis. It should not be assumed that MSCs present in different tissues all have the same potential to differentiate along the different mesodermal lineages. Moreover, the environment and signaling stimuli that MSCs receive also vary among tissues. Therefore, future studies are expected to dissect the link between the tissue from which MSCs are sourced and the resulting sarcoma phenotype. For instance, *p53*-deficient mASCs originate leiomyosarcoma [31], while the loss of *p53* in mesenchymal cells of limb buds or in the osteogenic lineage gives rise to osteosarcoma development [34, 36]. Likewise, supporting a role for MSCs from tissues other than BM in the initiation of sarcomas, it has been recently reported that local resident MSCs and not BM-derived cells are the preferential target for initiation of undifferentiated pleomorphic sarcomas associated with *p53* and *Rb* deficiency [113].

Regarding the mechanisms underlying MSC transformation, mounting evidence suggests that the expression of sarcoma-related fusion genes in hMSCs does not suffice on its own for sarcoma initiation revealing the need for secondary oncogenic hits to achieve overt MSC transformation and *in vivo* sarcoma growth. Since fusion genes mostly disrupt differentiation, cooperating mutations are expected to target proliferation and apoptosis checkpoints. In this regard, the inactivation of p53 is sufficient to transform mMSCs [31] and generate sarcomas in mesenchymal tissues of mouse models [36]. Similarly, *p53* mutation has also been successfully used as a cooperating transforming hit in the development of several models of fusion gene-associated sarcomas from mMSCs [40, 54, 74] or in mouse models [58, 76] (Table 3). According to the available literature, the frequency of *p53* mutations in human sarcomas ranges from ~6% in well-differentiated/dedifferentiated liposacomas to 23% in osteosarcomas [114]. Nevertheless, the disruption of p53 signaling seems to be a key oncogenic event in many types of sarcomas [12]. For example, the expression of EWS-FLI-1 could silence p53 activity through the formation of an EWS-FLI-1/p53 complex [115] or inhibit NOTCH induced-p53 activation [116]. Moreover, the p53 inhibitor Mdm2 is often overexpressed in STS [117], and p53 and p16^{INK4A}/p14^{ARF} pathways are frequently disrupted in MLS [118]. Finally, other common abnormalities in sarcomas that could function as cooperating hits include defects in pathways controlled by Rb and different growth signaling factors, like those mediated by IGF1, PDGF or c-KIT [12].

In order to develop MSC-based models for sarcomagenesis, which closely reproduce the human disease it would be crucial to characterize more specific secondary cooperating mutations including point mutations, genomic losses and gains and copy number variations, etc, that are indispensable for sarcoma onset and progression. A desirable approach would be to apply cutting-edge whole-genome technologies such as deep sequencing to a cohort of different types of primary sarcomas in an attempt to identify specific mutations shared by a group of patients with the same tumor. The resulting data should then be functionally validated *in vitro* and *in vivo* in normal or fusion gene-harboring MSCs. Using this technology a recent study has identified frequently mutated genes in different subtypes of sarcomas that could constitute new targets for more specific therapies [119]. These frequently mutated genes include TP53 in pleomorphic liposarcomas, NF1 in myxofibrosarcomas and PIK3CA (the catalytic subunit of PI3K) in MLS.

Concluding remarks

Sarcomas are generally studied when the full transformation events have already occurred and therefore, the mechanisms of transformation and pathogenesis are not amenable to analysis with patient samples. Thus there exists the need to establish *bona fide* mouse and human-

based models to recapitulate sarcomagenesis *in vitro* and *in vivo*. Over recent years, mounting evidence indicates that MSCs from different sources (BM, adipose-tissue, etc) may represent the putative target cell of origin for a variety of human sarcomas, thus linking MSCs and cancer. Future research should be aimed at defining precisely the specific phenotype of the MSC populations at the origin of the different types of sarcomas as well as at dissecting the mechanisms governing MSC transformation. We envision that experimental research based on MSCs coupled to whole-genome sequencing of different types of primary sarcomas will advance our attempts to develop accurate MSC-based models of sarcomagenesis and to decipher the underlying mechanisms, provide a better understanding about the onset and progression of mesenchymal cancer, and lead to the eventual development of more specific therapies directed against the sarcoma-initiating cell.

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