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Review

Reprogramming of mesenchymal stem cells by oncogenes

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ABSTRACT

Mesenchymal stem cells (MSCs) originate from embryonic mesoderm and give rise to the multiple lineages of connective tissues. Transformed MSCs develop into aggressive sarcomas, some of which are initiated by specific chromosomal translocations that generate fusion proteins with potent oncogenic properties. The sarcoma oncogenes typically prime MSCs through aberrant reprogramming. They dictate commitment to a specific lineage but prevent mature differentiation, thus locking the cells in a state of proliferative precursors. Deregulated expression of lineage-specific transcription factors and controllers of chromatin structure play a central role in MSC reprogramming and sarcoma pathogenesis. This suggests that reversing the epigenetic aberrancies created by the sarcoma oncogenes with differentiation-related reagents holds great promise as a beneficial addition to sarcoma therapies.

1. Introduction

The mesenchymal stem cells (MSCs) originate from embryonic mesoderm and give rise to lineages of the connective tissue including adipose, bone, cartilage, tendon and ligament. These rare multipotent cells reside in multiple anatomical sites. In the bone marrow, they constitute a key component of the niches that support osteogenesis and hematopoiesis [1], and they are easily isolated from fat, umbilical cord Wharton's jelly, and amniotic fluid, mostly for applications in regenerative research. MSCs possess unique properties: they suppress pro-inflammatory responses by expressing a broad range of soluble factors and chemokines, and by activating regulatory T cells (Treg). This immunomodulatory function has made MSCs ideal candidates for cell-based treatment of autoimmune diseases such as diabetes, Crohn's disease, and for allogeneic tissue replacements [2–5]. Two distinguishing attributes, pro-angiogenesis and a remarkable capacity for homing to sites of injury including neoplasms, have made MSCs a very attractive tool for repairing damaged tissues such as post-infarction ischemic heart [6], and safe delivery vehicles for cancer gene therapies [7]. A distinctive feature of MSCs is their amazing plasticity that is harnessed in a variety of reprogramming approaches to generate tissues within and outside their classic mesenchymal lineages. Forced expression of defined transcription factors converts MSCs to pancreatic β-cells, cardiac muscle and neural cells [8], and grafted MSCs have shown potential for bone replacement in skeletal disorders such as osteogenesis imperfecta [9,10] and restoration of cardiac function [11,12]. Collectively, MSCs unparalleled characteristics of multiple tissue generation potential, directed migration, and immunosuppressive effects, coupled to easy extraction and *ex vivo* expansion, carry enormous potential for medicinal tissue therapies.

1.1. Indirect reprogramming of mesenchymal stem cells in cancer

In human cancer, neoplastic cells capitalize on MSC plasticity and tropism to assimilate the stem cells into their inflammatory stroma as tumor-associated fibroblasts, thereby reinforcing the neoplastic niche. There, the reprogrammed MSCs secrete a large repertory of chemokines and signaling mediators such as NOTCH and transforming growth factor beta (TGFβ) that promote neovascularization and epithelial–mesenchymal transition (EMT) of the cancer cells, leading to tumor dissemination [13–15]. MSCs recruited to prostate tumor sites through CXCL16 signaling were shown to promote metastasis [16]. Similarly, CCL5-mediated homing of MSCs enhanced the invasive capacity of breast cancer cells [17]. In stark contrast to these studies, a tumor inhibitory effect of MSCs was demonstrated in a diverse group of epithelial cancers, sarcoma, leukemia and lymphoma models, thus leading to a yet unresolved controversy regarding the role of MSCs as friends or enemies of neoplasia [18,19]. Clarification of the divergent results will require a more detailed knowledge of the cellular crosstalk in the tumor microenvironment.

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1.2. Direct reprogramming of mesenchymal stem cells in cancer

Despite the remarkable ease in shifting gene expression programs, MSCs possess a surprisingly stable genome. Compared to epithelial cancers, primary mesenchymal tumors commonly referred to as sarcomas rarely arise in adults. However, primary sarcomas constitute 10% of pediatric malignancies, and they are distinguished by aggressive behavior and poor response to chemotherapy [20]. Evidence gathered over the past decade supports MSCs as the likely cell of origin for certain sarcomas. In addition, tumor-initiating cells with stem-like features have been identified in several sarcoma types [21,22]. Sarcomas are divided in two broad groups based on their genetic aberrancies: tumors with complex karyotypes and those with specific chromosomal translocations. The latter are characterized by non-random reciprocal rearrangements that generate hybrid oncogenes. The translocated genes often code for transcription regulators and their fusion creates unique oncoproteins that disrupt global gene expression. These oncoproteins play a central role in sarcoma initiation, growth, and progression [22,23]. Perhaps owing to the unique biology of MSCs, functional studies have uncovered fascinating features pertaining to the sarcoma-associated translocations [21]. In the plastic MSC background, the sarcoma fusions appear to redirect developmental programs and disrupt normal differentiation as part of their transforming activities.

2. Mesenchymal stem cell reprogramming by oncogenes

Disruption of normal differentiation has been considered a hallmark of cellular transformation for over two decades [24]. Evidence for altered differentiation in mesenchymal stem cells by an oncogene, was first presented by Tzen and colleagues (1990) who showed that activated c-HA-ras in 3T3-T cells blocked adipogenesis and induced differentiation along the macrophage lineage [25]. Similarly the v-raf oncogene, a member of the ras pathway, converted Myc-transgenic B cells into macrophages [26]. MSCs transformed with the human polyomavirus JCV T-antigen lost their mesenchymal characteristics and acquired primitive neuroectodermal features [27]. An additional example of mesodermal progenitor reprogramming is illustrated by the c-fos oncoprotein whose forced expression induced mesenchymal cells to form cartilage or bone tumors, depending on the simultaneous expression of its co-activator Jun [28]. Together, these findings suggest that oncogenes control mesenchymal programs and their activated pathways determine cell fate.

The sarcoma-associated translocations have become a classic example of MSC reprogramming by oncogenes. Based on a number of recent studies, several sarcoma fusions drive oncogenesis by redirecting mesodermal progenitors toward specific lineages (Table 1). The data suggest that program specification is an intrinsic property of the fusions, which explains the osteogenic, myogenic, adipogenic, and neural features of sarcomas that arise in locations devoid of these lineages. In this report, we review the unique properties of a selected set of sarcoma oncoproteins and discuss their relevance to the understanding of sarcoma genesis and stem cell differentiation control.

2.1. EWSR1–FLI1

The t(11;22)(q24;q12) translocation is associated with Ewing's sarcoma, a pediatric malignancy of bone and soft tissues. Fusion of EWSR1, a member of the TET family of proteins involved in transcription and RNA processing, with FLI1, a member of the ETS family of transcription factors, generates an oncoprotein implicated in sarcoma initiation and progression [29]. The exact cell of origin for Ewing's sarcoma has long been debated, mainly due to lack of a definitive model. A neural crest origin was first suspected based on the observation that Ewing's tumors (ET) display a spectrum of primitive neuroectodermal markers and that ET lines undergo neural differentiation upon stimulation *in vitro* [30,31]. Ectopic expression of EWSR1–FLI caused differentiation arrest in murine bone marrow stromal cells and in C2C12 myoblasts [32,33]. However, when EWSR1–FLI1 switched the sympathetic neuronal program of neuroblastoma cells to a more primitive proliferative neuroectodermal phenotype [34], and redirected rhabdomyosarcoma cells toward a neuroectodermal program while blocking myogenesis [35], it became clear that EWSR1–FLI1 dictates a neural program regardless of cell context. A series of *in vitro* and *in vivo* models established by several groups suggested a mesodermal progenitor origin for ETs. EWSR1–FLI1 expression in bone marrow-derived MSCs (BM-MSCs) generated ET-like tumors in mice [36,37]. EWSR1–FLI1-depleted sarcoma cells exhibited MSC-like gene signatures and potential for mesenchymal multilineage differentiation [38]. Notably, a subset of sarcoma cells with cancer initiating properties was identified in ETs [39]. Altogether, these findings suggest that Ewing's tumor cells arise from MSCs diverted to the neural phenotype by EWSR1–FLI1, but are arrested before mature differentiation. Ewing's sarcoma is therefore believed to be a disease of aberrant differentiation elicited by the *EWSR1–FLI1* oncogene in mesenchymal precursors [40]. However, the results do not rule out an earlier mesodermal progenitor with dual mesenchymal and vascular potentials that would explain the blood lakes, vascular-like tubes, and the upregulated angiogenesis genes frequently associated with Ewing's tumors [38,41,42]. Alternatively, the vascular markers could be downstream targets EWSR1–FLI1, as wild-type FLI1 is a controller of early vasculogenesis [43].

Two important questions remain: (1) how does EWSR1–FLI1 control stem cell differentiation, and (2) what role does MSC reprogramming play in ET development? To this date, these problems are largely unsolved, but active investigations have yielded a number of key observations regarding the molecular functions of EWSR1–FLI1. The Lessnick group (2008) reported that EWSR1–FLI1 binds to the microsatellite repeat GGAA and regulates expression of the nuclear receptor NROB1, a necessary step for sarcoma genesis [44]. Cumulative work by several groups has shown that EWSR1–FLI1 interacts with myriad regulators of transcription, ranging from the transcriptional machinery and modulators of RNA splicing, to AP-1 factors, p300/CBP acetyltransferases, and nuclear receptors. These associations are believed responsible for mediating its oncogenic effects [45]. A later report showed EWSR1–FLI1 association with the osteogenesis controller RUNX2, thus providing a mechanism for osteogenic differentiation arrest by the oncogene [46].

Additional clues for the epigenetic effects of EWSR1–FLI1 were recently provided: in one study, ectopic expression of EWSR1–FLI1 in MSCs led to overexpression of the Polycomb Repressive Complex 2 (PRC2) methyltransferase EZH2 [47], and another study showed that EZH2 is a direct target of EWSR1–FLI1 and its overexpression is necessary for ET formation and metastasis. Notably, depletion of EZH2 or other members of the PRC2 complex rescued the neuroectodermal and endothelial differentiation potential of ET cells, thus confirming differentiation block by EWSR1–FLI1 through EZH2-mediated gene silencing [48]. Elevated EZH2 levels are frequently seen in human ETs [47]. Riggi and colleagues (2010) presented evidence for reprogramming of human pediatric mesenchymal stem cells (hpMSCs) by EWSR1–FLI1 toward a cancer stem cell phenotype as a priming event in sarcoma genesis. The data indicated that this was accomplished by a regulatory loop involving EWSR1–FLI1 and differential expression of the pluripotency regulators, miR-145 and SOX2 [49]. A recent study found that deregulation of the miR-145 pathway plays a role