Blocking tumor-educated MSC paracrine activity halts osteosarcoma progression.


Author information

Abstract

PURPOSE:
Human osteosarcoma is a genetically heterogeneous bone malignancy with poor prognosis despite the employment of aggressive chemotherapy regimens. Because druggable driver mutations have not been established, dissecting the interactions between osteosarcoma cells and supporting stroma may provide insights into novel therapeutic targets.

EXPERIMENTAL DESIGN:
By using a bioluminescent orthotopic xenograft mouse model of osteosarcoma we evaluated the effect of tumor extracellular vesicle (EV)-educated mesenchymal stem cells (TEMSCs) on osteosarcoma progression. Characterization and functional studies were designed to assess the mechanisms underlying MSC education. Independent series of tissue specimens were analyzed to corroborate the preclinical findings, and the composition of patient serum EVs was analyzed after isolation with size-exclusion chromatography.

RESULTS:
We show that EVs secreted by highly malignant osteosarcoma cells selectively incorporate a membrane-associated form of TGFB, which induces pro-inflammatory IL-6 production by MSCs. TEMSCs promote tumor growth, accompanied with intratumor STAT3 activation and lung metastasis formation, which was not observed with control MSCs. Importantly, intravenous administration of the anti-IL-6 receptor antibody tocilizumab abrogated the tumor-promoting effects of TEMSCs. RNA-seq analysis of human osteosarcoma tissues revealed a distinct TGFB-induced pro-metastatic gene signature. Tissue microarray immunostaining indicated active STAT3 signaling in human osteosarcoma, consistent with the observations in TEMSC-treated mice. Finally, we isolated pure populations of EVs from serum and demonstrated that circulating levels of EV-associated TGFB are increased in osteosarcoma patients.

CONCLUSION:
Collectively, our findings suggest that TEMSCs promote osteosarcoma progression and provide the basis for testing IL-6 and TGFB blocking agents as new therapeutic strategies for osteosarcoma patients.
Oxidative status predicts quality in human mesenchymal stem cells.

Bertolo A¹, Capossela S¹, Fränkl G¹, Baur M²³, Pötzel T², Stoyanov J⁴⁵.

Abstract

BACKGROUND:
Human bone marrow-derived mesenchymal stem cells (MSC) are adult progenitor cells with great potential for application in cell-based therapies. From a cell-based therapy perspective, there are two limitations to MSC use: (1) these therapies require large numbers of cells, and long-term expansion of MSC in vitro promotes replicative senescence; and (2) patient variability is a challenge for defining MSC quality standards for transplantation. This study aimed to determine whether low or high oxidative status of MSC correlate with changes in cell expansion and differentiation potentials.

METHODS:
We investigated functional aspects of mitochondria, such as cell metabolic activity indicators and expression of antioxidant enzymes. Furthermore, we tested if senescence-induced changes in oxidative status of MSC could be counteracted by methylene blue (MB), an alternative mitochondrial electron transfer known to enhance cell bioenergetics.

RESULTS:
MSC isolated from donors of the same age showed distinctive behavior in culture and were grouped as weak (low colony-forming units (CFU) and a short life in vitro) and vigorous MSC (high CFU and a long life in vitro). In comparison to weak MSC, vigorous MSC had oxidative status characterized by lower mitochondrial membrane potential, lower mitochondrial activity, and fewer reactive oxygen species production, as well as reduced mitochondrial biogenesis. Vigorous MSC had a significantly higher expansion potential compared to weak MSC, while no differences were observed during differentiation. MB treatment significantly improved expansion and differentiation potential, however only in vigorous MSC.

CONCLUSIONS:
Together, these results demonstrate the importance of mitochondrial function in MSC in vitro, and that cells with low oxidative status levels are better candidates for cell-based therapies.
by xenogeneic nutrients for their culture. Human derivatives or recombinant materials are the first choice candidates to reduce these reactions. Therefore, culture supplements and materials of autologous origin represent the best nutrients and the safest products. Here, we describe a new protocol for the isolation and culture of bone marrow hMSCs in autologous conditions - namely, patient-derived serum as a supplement for the culture medium and fibrin as a scaffold for hMSC administration. Indeed, hMSC/fibrin clot constructs could be extremely useful for several clinical applications. In particular, we focus on their use in orthopedic surgery, where the fibrin clot derived from the donor’s own blood allowed effective cell delivery and nutrient/waste exchanges. To ensure optimal safety conditions, it is of the utmost importance to avoid the risks of hMSC transformation and tissue overgrowth. For these reasons, the approach described in this paper also indicates a minimally ex vivo hMSC expansion, to reduce cell senescence and morphologic changes, and short-term osteo-differentiation before implantation, to induce osteogenic lineage specification, thus decreasing the risk of subsequent uncontrolled proliferation.