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Human mesenchymal stromal cells do not promote recurrence of soft tissue sarcomas in mouse xenografts after radiation and surgery

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Abstract

Background. Mesenchymal stromal cells (MSCs) promote wound healing, including after radiotherapy (RT) and surgery. The use of MSCs in regenerative medicine in the context of malignancy, such as to enhance wound healing post-RT/surgery in patients with soft tissue sarcomas (STSs), requires safety validation. The aim of this study was to determine the effects of human MSCs on STS growth *in vitro* and local recurrence and metastasis *in vivo*. *Methods*. Human primary STS and HT-1080 fibrosarcoma lines were transduced to express luciferase/eGFP (enhanced green fluorescent protein). Sarcoma cells were co-cultured or co-injected with bone marrow–derived MSCs for growth studies. Xenograft tumor models were established with STS lines in NOD/SCID/γ_c^{null} mice. To emulate a clinical scenario, subcutaneous tumors were treated with RT/surgery prior to MSC injection into the tumor bed. Local and distant tumor recurrence was studied using histology and bioluminescence imaging. *Results*. MSCs did not promote STS proliferation upon co-culture *in vitro*, which was consistent among MSCs from different donors. Co-injection of MSCs with sarcoma cells in mice exhibited no significant tumor-stimulating effect, compared with control mice injected with sarcoma cells alone. MSC administration after RT/surgery had no effect on local recurrence or metastasis of STS. *Discussion*. These studies are important for the establishment of a safety profile for MSC administration in patients with STS. Our data suggest that MSCs are safe in STS management after standard of care RT/surgery, which can be further investigated in early-phase clinical trials to also determine the efficacy of MSCs in reducing morbidity and to mitigate wound complications in these patients.

Key Words: *human mesenchymal stromal cells, regenerative medicine, soft tissue sarcoma, tumor xenograft model, wound healing*

Introduction

Mesenchymal stromal cells (MSCs) are widely applied for tissue regeneration to restore normal structure and function of injured and defective tissues. In wound care, MSCs promote healing by accelerating wound closure, enhancing angiogenesis and remodeling extracellular matrix via a combination of mechanisms that mainly include modulation of immune responses, secretion of growth factors and recruitment/activation of local resident stem cells (1–4). Exploiting the beneficial role of MSCs in tissue repair, however, can be associated with, and limited by, safety concerns in the context of their tumor-supporting effects (5–9). This hampers therapeutic applications of MSCs in regenerative medicine in cases of an underlying or history of malignancy, in which MSCs may promote the growth of any residual microscopic cancer cells after surgical resection, for example. The inhibiting or promoting effects of MSCs on tumor growth are affected by several factors, including tumor type, model used and treatment dynamics (10,11), which warrants more directed investigations on the effect of MSCs on individual tumor types in clinically relevant scenarios.

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Soft tissue sarcomas (STSs) are rare, yet highly heterogeneous malignancies of mesenchymal origin. The current standard of care for most patients with STS includes surgical resection with pre- or post-operative radiation therapy (RT) (12–14). Pre-operative RT uses a lower total dose and substantially smaller treatment volume, thereby reducing the severity of subsequent complications, such as edema and fibrosis, and is associated with a better long-term functional outcome (15–17). However, the major disadvantage of pre-operative RT is a two-fold increase in the rate of post-operative wound complications compared with post-operative RT (18,19). Interventions that reduce the burden of wound complications after pre-operative RT, and thereby support its use in STS management, can further improve therapy outcome and the quality of life in these patients (20). We previously showed in a rat model of radiation-impaired incisional wound healing that injection of bone marrow MSCs significantly increased wound mechanics and improved the radiation-induced healing deficit (21). These studies suggest a potential therapeutic use of MSCs for STS patients with wound complications following RT/surgery. However, given the shared mesenchymal ontogeny, it is critically important to establish the safety profile of MSCs for clinical application in STS.

In this study, we sought to contribute to such a profile in the setting of wound management using MSCs after RT/surgery in patients with STS. More specifically, we aimed to determine the effects of MSCs on the growth of human STS cell lines *in vitro* and to evaluate the risk of sarcoma local recurrence and metastasis *in vivo* when human MSCs were injected into the tumor bed after pre-operative RT and subsequent tumor resection in xenograft models. We used established and patient-derived sarcoma cell lines and showed that MSCs had no significant effect on the growth and recurrence of STS tumors *in vitro* and *in vivo*. As the benefit of MSCs in overcoming radiation-impaired wound healing has previously been demonstrated (21), the establishment of a favorable safety profile with respect to tumor recurrence, as shown in these studies, is important for potential therapeutic application of MSCs in STS management.

Materials and Methods

Cell culture

Tumor cells. A human fibrosarcoma cell line (HT-1080) as well as primary tumor lines developed from patients' samples was used in this study. HT-1080 was obtained from American Type Culture Collection and cultured as recommended by the supplier. Medium was changed twice weekly and cells were passaged when cultures reached 80% confluence. Primary tissue samples were procured from patients after informed consent and following approval by the institutional Research Ethics Board (REB). Tumor samples were obtained under sterile conditions at the time of open surgical biopsy or definitive tumor resection from eligible patients who had not received pre-operative radiation. Samples were immediately transferred to Dulbecco's Modified Eagle's Medium (DMEM) for further processing. Four patient samples with histopathologic diagnosis of myxofibrosarcoma (one sample) and undifferentiated pleomorphic sarcoma (UPS; three samples) were used to develop primary STS lines. Each tumor tissue was mechanically dissociated using scalpels and incubated overnight (37°C; 5% CO_2) with collagenase (2 mg/mL; 1 mL/cm^3). Then, primary culture medium (DMEM/F12; Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin G and 100 μg/mL streptomycin, was added to the digested tissue and the cell suspension was centrifuged (1000*g*). Cells were collected and maintained at 37°C in a humidified incubator containing 5% CO_2. Culture medium was not changed during the first week and was replaced with fresh medium twice a week afterward. Cells were passaged when the cultures reached 80% confluence and kept under the same condition before being used for experiments.

MSCs. Approval for procurement of bone marrow was obtained from institutional REB. Bone marrow (BM) aspirates were obtained from healthy adult volunteers after informed consent, and mononuclear cells (MNCs) were isolated using Ficoll gradient. BM-MNCs collected from the interface were washed in phosphate-buffered saline (PBS) and resuspended in MSC culture medium containing DMEM/low glucose with 10% FBS and 1% antibiotic-antimycotic solution (Life Technologies). Cells were plated at 3×10^7 cells/175 cm^2 flask and maintained at 37°C in a humidified atmosphere with 5% CO_2. When cultures reached 80% confluence, cells were passaged and cultured at a density of 1×10^6 cells/175 cm^2 flask. Cells were passaged up to 3–4 times before use. Immunophenotype analyses were conducted to confirm that MSCs expressed the stromal markers CD44, CD49e, CD73, CD90 and CD105 and did not express CD11b, CD34 or CD45 (22).

eGFP/Fluc cell transduction

A recombinant lentiviral vector encoding enhanced green fluorescent protein (eGFP) and luciferase (eGFP/Fluc-LV) (304.pCCL.sin.cPPT.polyA.CTE.eGFP.minhCMV.hPGK.Fluc.Wpre)