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Mesenchymal stem cells as a gene therapy carrier for treatment of fibrosarcoma

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Background aims

Cell-based gene therapy is an alternative to viral and non-viral gene therapy. Emerging evidence suggests that mesenchymal stem cells (MSC) are able to migrate to sites of tissue injury and have immunosuppressive properties that may be useful in targeted gene therapy for sustained specific tissue engraftment.

Methods

In this study, we injected intravenously (i.v.) 1×10^6 MSC, isolated from green fluorescent protein (GFP) transgenic rats, into Rif-1 fibrosarcoma-bearing C3H/HeN mice. The MSC had been infected using a lentiviral vector to express stably the luciferase reporter gene (MSC-GFP-luci). An in vivo *imaging system (IVIS 200) and Western blotting techniques were used to detect the distribution of MSC-GFP-luci in tumor-bearing animals.*

Results

We observed that xenogenic MSC selectively migrated to the tumor site, proliferated and expressed the exogenous gene in subcutaneous fibrosarcoma transplants. No MSC distribution was detected in other organs, such as the liver, spleen, colon and kidney. We further showed that the FGF2/FGFR pathways may play a role in the directional movement of MSC to the Rif-1 fibrosarcoma. We performed in vitro *co-culture and* in vivo *tumor growth analysis, showing that MSC did not affect the proliferation of Rif-1 cells and fibrosarcoma growth compared with an untreated control group. Finally, we demonstrated that the xenogenic MSC stably expressing inducible nitric oxide synthase (iNOS) protein transferred by a lentivirus-based system had a significant inhibitory effect on the growth of Rif-1 tumors compared with MSC alone and the non-treatment control group.*

Conclusions

iNOS delivered by genetically modified iNOS-MSC showed a significant anti-tumor effect both in vitro *and* in vivo. *MSC may be used as a target gene delivery vehicle for the treatment of fibrosarcoma and other tumors.*

Keywords

Fibrosarcoma, gene therapy, inducible nitric oxide synthase, mesenchymal stem cells.

Introduction

Solid tumors comprise two distinct but interdependent compartments: neoplastic cells and the stroma that the neoplastic cells induce and in which they are dispersed. Stem cells are mainly referred to as tumor-supporting fibroblasts and they may derive from resident fibroblasts in the organ/tissue [1] or circulating mesenchymal progenitor cells [2–5].

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Bone marrow (BM) is a major source of mesenchymal stem cells (MSC), a well-characterized population of adult stem cells. MSC can differentiate into cells of connective tissue lineages, including bone, fat, cartilage and muscle, and other types of tissue, such as hepatic, renal, cardiac and neural [6]. MSC preferentially home to bone, lung and spleen when injected intravenously (i.v.), as they favor adhering to matrix components. MSC have been used in regenerative medicine and tissue-engineering applications, as they are readily collected and mobilized and can differentiate into various tissue cells in response to wound signals [7–9].

A number of studies have demonstrated that MSC selectively home to sites of injury [10,11]. During the wound-healing process, many factors, including growth factors, hormones, cytokines and extracellular matrices, may regulate the recruitment and homing of MSC to sites of injury. Similar to the repair responses, inflammatory cytokines, growth factors and extracellular matrices also play important roles in cancer/tumor development and progression. The stroma of malignant tumors closely resembles the granulation tissue of a healing wound [11]; solid tumors generate a wound-like environment on their boundary, causing the physical and chemical stresses associated with their unrestrained growth. Tumors can, therefore, be regarded as sites of tissue damage or wounds that never heal.

Given the limitations in the efficiency and safety of most current cancer therapy, cell-based gene therapy could be the alternative to the use of currently available vectors. Developing a systemic delivery system would allow this approach to be used clinically for tumors inaccessible to direct transgene injection. The injury-homing characteristic of MSC is very attractive for this application. The direct targeting of anti-tumor agents into the tumor microenvironment might increase anti-cancer treatment efficacy and reduce the side-effects to other organs. The tumor microenvironment has been demonstrated to promote preferentially the engraftment of MSC compared with other tissues [2–4,11,12]. Therefore, the application of MSC to cancer therapy is of growing interest to medical researchers [2,3,12,13].

Genetically modified MSC producing anti-cancer molecules such as interferon (INF)-β and nitric oxide (NO*) may be used for anti-cancer treatment. Inducible nitric oxide synthase (iNOS) gene therapy, leading to targeted generation of high levels of NO*, has been identified as an anti-tumor strategy in pre-clinical models, leading to extensive apoptosis [14]. iNOS gene therapy has also been shown to cause dilation of the tumor-associated vasculature and increased tumor blood flow, which may sensitize the radiotherapy and chemotherapy of cancer [15–17]. The aim of the current study was to determine whether xenogenic MSC can be used as a delivery vehicle for iNOS gene therapy in a fibrosarcoma cell culture system and animal model.

Methods

Mononuclear cell culture and experimental animals

Green fluorescent protein (GFP)-transgenic rats were kindly provided by Professor M Okabe (Osaka University, Osaka, Japan). Rat MSC were obtained from BM aspirates of healthy young GFP-transgenic rats. The isolation and culture of MSC were performed using previously described methods [18,19]. Murine fibrosarcoma (Rif-1) cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum at 37°C under 5% CO_2 and 95% air.

Syngeneic transplanted fibrosarcomas were set up by intradermally injecting 4×10^5 Rif-1 cells into the rear dorsum of 10-week-old female C3H/HeN mice (Harlan, Huntington, UK). The animals were examined every day after the injection and treated in different groups when the tumor masses reached a volume of 100 mm^3. All animal experiments were carried out in accordance with the Animal (Scientific Procedure) Act 1986 and conformed to the current UKCCCR guidelines.

Lentiviral vectors and MSC transfection

The lentiviruses were created using the ViraPower™ Lentiviral Expression System (Invitrogen, Paisley, UK). The coding sequence of luciferase or iNOS was subcloned into pLenti6/V5-D-TOPO (Invitrogen). pLenti6/V5-D-TOPO/luciferase or pLenti6/V5-D-TOPO/iNOS vector and the ViraPower™ Packaging Mix (Invitrogen) were co-transfected using a gene carrier kit (Epoch-Biolabs, Missouri City, TX, USA.) into the 293T cell line to produce a lentiviral stock. Forty-eight hours post-transfection, virus-containing supernatant was harvested by collecting the medium. Viral particles were purified by ultracentrifugation through a 20% sucrose cushion. For infecting MSC, cells were cultured in 24-well plates and, when the culture reached 80% confluence, the concentrated lentivirus was