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Migrational changes of mesenchymal stem cells in response to cytokines, growth factors, hypoxia, and aging

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ABSTRACT

Mesenchymal stem cells (MSCs) are non-immunogenic, multipotent cells with at least trilineage differentiation potential. They promote wound healing, improve regeneration of injured tissue, and mediate numerous other health effects. MSCs migrate to sites of injury and stimulate repair either through direct differentiation or indirectly through the stimulation of endogenous repair mechanisms. Using the *in vitro* scratch assay, we show that the inflammatory cytokines, chemokines, and growth factors TNF-α, SDF-1, PDGF, and bFGF enhance migration of rat MSCs under normoxic conditions, while TNF-α, IFN-γ, PDGF, and bFGF promote MSC migration under hypoxic conditions. This indicates that the oxygen concentration affects how MSCs will migrate in response to specific factors and, consistent with this, differential expression of cytokines was observed under hypoxic versus normoxic conditions. Using the transwell migration assay, we find that TNF-α, IFN-γ, bFGF, IGF-1, PDGF, and SDF-1 significantly increase transmigration of rat MSCs compared to unstimulated medium. MSCs derived from aged rats exhibited comparable migration to MSCs derived from young rats under hypoxic and normoxic conditions, even after application with specific factors. Similarly, migration in MSCs from aged, human donors did not statistically differ compared to migration in MSCs derived from human umbilical cord tissue or younger donors.

1. Introduction

Mesenchymal stem cells (MSCs) are multipotent, non-hematopoietic cells which can differentiate into three distinct lineages: adipocytes, chondrocytes, and osteocytes [1,2]. Although MSCs were first identified in bone marrow [1,2], subsequent work has identified MSCs in peripheral blood, adipose tissue, heart, lung, and other adult tissues [3]. MSCs are also present in a plethora of birth-associated tissues, including placenta, umbilical cord, and cord blood [3]. Unlike other cell types, MSCs lack unique markers and are characterized by a unique cell surface antigen profile as well as by their adhesive nature [3,4]. MSCs can be expanded in culture, retaining their growth and multilineage potential for several passages. They slowly age *in vitro*, reaching maximum population doublings of 30–40 [5]. Unlike induced pluripotent stem cells, which show distinct differences compared to endogenous stem cells and require genetic reprogramming to generate [6], MSCs are native stem cells that are easy to procure.

Because of their multipotency and ease of obtainment, MSCs have received considerable interest with regard to therapeutics. They have immunosuppressive properties [7] and are regarded as non-immunogenic [8], two qualities which are highly desirable for transplantation into an allogeneic host. MSCs have been widely used in bone marrow transplantation [9], tissue engineering [10], and cell therapy [11]. They have been shown to enhance wound healing [12,13], improve post-ischemic myocardial regeneration [14], reduce hypertension and right ventricular overload [15], and mediate other health effects [16]. MSCs are recruited to wounded skin to contribute to repair [12] and, through the production of various growth factors and cytokines, are believed to stimulate endogenous repair mechanisms [4]. Numerous clinical trials are currently underway investigating the ability of MSCs to treat a wide array of diseases (http://www.clinicaltrials.gov/).

Since MSCs need to arrive at a site of injury to promote repair either directly by differentiation or indirectly through the

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stimulation of endogenous mechanisms, their ability to migrate to a damaged site is of critical importance. This migratory process has been thoroughly investigated in recent years [4]. Labeled, bone-marrow derived MSCs that were delivered to rats intravenously were found to distribute in lung, liver, spleen, and bone marrow [17]. Similarly, MSCs were shown to travel to the lung in response to pulmonary insult in mice [18]. More recent studies have shown that MSCs journey to sites of pulmonary fibrosis [19], ocular damage [20,21], injured liver [22], infarcted myocardium [23], ischemic brain [24], and other sites of tissue injury [4,25]. Thus, the movement of MSCs to damaged areas appears to play an important role in the regenerative response.

Unfortunately, a large portion of systematically delivered MSCs will fail to migrate to target sites and will either accumulate in specific organs or passively arrest and fail to migrate to damaged tissues [25]. Aging may also be a relevant factor, as problems in MSC therapies have been reported using aged donors and aging is known to detrimentally effect the functionality of MSCs [26,27]. Various strategies have been employed to improve the migratory ability of MSCs [4,25]. Treatment with bFGF [28] or inhibition with Rho [29], for example, have both been shown to enhance MSC migration. Engineering or preconditioning MSCs before infusion might also be an effective strategy [30,31]. Culturing MSCs in a hypoxic environment [32,33] or with insulin-like growth factor has been reported to promote the migration of MSCs [34].

To gain further insight into strategies aimed at optimizing MSC migration, we evaluated the migrational ability of both rat and human MSCs in response to age, cytokines, growth factors, and hypoxic conditioning. We identify numerous cytokines and growth factors which enhance migration and transmigration of MSCs. We also find that hypoxic versus normoxic conditions alter how MSCs will migrate in response to specific factors. Similarly, we show that MSCs exhibit a differential expression of cytokines under hypoxic versus normoxic conditions. Finally, we show that MSCs from aged rats and humans exhibit comparable migration to MSCs from young rats and humans *in vitro*, suggesting that changes in migration do not contribute to problems in cell therapies using aged donors.

2. Materials and methods

2.1. Chemicals

Unless stated otherwise, all chemicals were obtained from Sigma-Aldrich.

2.2. Isolation of rat bone marrow stem cells

Sprague Darley rats were purchased from the local animal house and kept on site according to regulations. Bone marrow cells were obtained centrifugally from tibiae and femurae according to the method of Dobson et al. [35]. MSCs were isolated similarly to before in our previous work [36] and according to the method of Sekiya et al. [37].

2.3. Isolation of MSCs from adipose tissue

Human subcutaneous adipose tissue was obtained from different donors undergoing surgery (Berufsgenossenschaftliche Kliniken Bergmannstrost Halle, Germany). Approval to perform the study was obtained by the Ethical Commission of Halle University and written consent forms were obtained by all donors.

The adipose tissues were cut into 1–2 mm pieces under the laminar hood and transferred into a 15 ml tube and digested in equal volumes of collagenase I (200 U/ml) and dispase (30 U/ml, 37 °C). The digested tissues were mixed with equal volume of media and centrifuged at 1000 rpm for 5 min. As a result of the digestion three layers are visible after centrifugation. The first layer contains adipose tissue, the second layer contains enzymes, and the last layer includes the stromal vascular fraction (SVF), which consists of stem cells and endothelial cells. The first and second layers were carefully removed with a pipette and 10 ml of culture media was added to the layer containing the SVF. The SVF was sieved using a 40 μm nylon mesh in a 50 ml tube. The filtered supernatant, which contains adipose-derived mesenchymal stem cells (ADSCs), was centrifuged at 1000 rpm for 5 min. The supernatant was removed and 10 ml of growth media was added. Then, cells were transferred into a T75 flask. ADSCs were cultivated in Dulbecco's modified Eagle's medium (1 g/L D-Glucose; Invitrogen) containing 10% fetal bovine serum (Hyclone) and 1% penicillin/streptomycin (Invitrogen). Culture media was changed every 3 days until cell proliferation was visible. ADSCs were trypsinized at 80% confluency. For experiments, ADSCs at passages 3–5 were used.

2.4. Isolation of MSCs from umbilical cord tissue

Umbilical cord-derived mesenchymal stem cells (UC-MSCs) were isolated according to previously described protocols [38,39]. UC-MSCs were cultivated in Dulbecco's modified Eagle's medium (1 g/L D-Glucose; Invitrogen) containing 10% fetal bovine serum (Hyclone) and 1% penicillin/streptomycin (Invitrogen) until 80% confluency was reached. UC-MSCs were used at passages 1–3 for the scratch experiments.

2.5. Characterization of MSCs

Cells were harvested by trypsinization and stained with antibodies against specific antigens. CD44, CD73, and CD105 were used as positive markers and CD11b was used as a negative marker (1:100; 4 °C; 30 min; Serotec). Cells were then analyzed using the personal flow cytometry system (Beckman Coulter). In addition to immunophenotyping, the ability to differentiate into bone, fat, and/or cartilage was assessed for rat and human MSCs. Scratch closure tests were also performed.

2.6. Scratch assay

MSCs were grown to confluence in 24-well plates and mechanically scratched to remove a fixed area of cells. This scratching was done using a standard P200 Gilson pipette tip. Tested substances were added to the media during the assay. This technique has been previously described and has been shown to be suitable for studies on cell migration [40].

2.7. Transwell assay

2×10^5 MSCs were seeded into the upper chamber of Costar, 24-well Transwell plates with 8 μm pore filters (Corning). Medium containing different chemicals was placed into the lower chamber. MSCs were incubated for 24 h and the cells were counted using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.

2.8. MTT assay

The MTT assay was used similarly to before in our previous work [36]. 30,000 MSCs were seeded onto 24-well plates and incubated overnight. Prior to the addition of MTT, the medium was changed to phenol-free medium. Absorbance was measured using a microplate reader (Tecan Infinity Pro 200 series) at 550 nm.

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