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CCL5/CCR1 axis regulates multipotency of human adipose tissue derived stromal cells

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Abstract Several potential clinical applications of stem cells rely on their capacity to migrate into sites of inflammation where they contribute to tissue regeneration processes. Inflammatory signals are partially mediated by chemokines acting via their receptors expressed on the target cells. Data concerning the repertoire and biological activities of chemokine receptors in human adipose tissue derived stromal cells (ADSCs) are limited. Here we show that CCR1 is one of the few chemokine receptors expressed in ADSCs at a high level. *CCR1* expression varies in ADSCs derived from different donors. It sharply decreases in the early phase of ADSCs in vitro propagation, but further demonstrates relative stability. Expression of *CCR1* positively correlates with expression of *SOX2*, *OCT4* and *NANOG*, transcription factors responsible for maintenance of the stemness properties of the cells. We demonstrate that signaling via CCL5/CCR1 axis triggers migration of ADSCs, activates ERK and AKT kinases, stimulates NFκB transcriptional activity and culminates in increased proliferation of $CCR1^+$ cells accompanied with up-regulation of *SOX2*, *OCT4* and *NANOG* expression. Our data suggest that chemokine signaling via CCR1 may be involved in regulation of stemness of ADSCs.

Introduction

Adherent, fibroblastic cell populations have been isolated from many connective tissues, and some have been found to contain a subset of local stem/progenitor cells. Some of these cell populations are suggested to be nonimmunogenic, and may have the ability to migrate to injured or inflamed sites. Those features support their potential clinical applications including treatment of immune, cardiovascular and degenerative diseases (osteoarthritis and osteoporosis), and their use as drug delivery vehicles (Caplan, 2007; Gimble et al., 2007).

It has been suggested that similarly to embryonic stem cells (ESCs), stemness-related properties of some stromal stem/progenitor cells are supported by regulatory networks of transcription factors OCT4, SOX2 and NANOG (Greco et al., 2007; Ng and Surani, 2011; Park et al., 2012; Riekstina et al., 2009). When over-expressed, these factors reprogram differentiated cells to an embryonic-like state designated as

Abbreviations: ADSCs, adipose tissue derived stromal cells; BCs, peripheral blood mononuclear cells; BMSCs, bone marrow derived stromal cells; ESCs, embryonic stem cells; GPCR, G-protein coupled receptor; p, passage

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pluripotent stem cells. These reprogrammed cells exhibit the morphology and growth characteristics of ESCs and express ESC marker genes (Takahashi et al., 2007).

Stromal cells with stem/progenitor-like properties can be found in a diverse range of tissues and organs (Parekkadan and Milwid, 2010). White adipose tissue is considered as a rich source of stromal cells due to its simple harvesting methods and high numbers of isolated cells (Gimble et al., 2007). Growth kinetics, immunogenic characteristics, in vitro differentiation potential, senescence ratio and angiogenic activity of adipose tissue derived stromal cells (ADSCs) (also known as adipose-derived "mesenchymal stem cells") are comparable with stromal cells obtained from other sources; however, several differences have been reported (De Ugarte et al., 2003; Izadpanah et al., 2006; Noel et al., 2008; Wagner et al., 2005; Winter et al., 2003).

Purification of homogenous functionally relevant stem/progenitor cells from adipose tissue is still a challenge. Widely used methods of ADSC isolation include separation of adipose tissue stromal vascular fraction, followed by sorting according to the immunophenotype guidelines suggested by the International Society for Cellular Therapy, and selection of a plastic adherent population (Dominici et al., 2006). It has become clear that this method yields highly heterogeneous populations with only a fraction of cells with stem/progenitor characteristics (Gimble et al., 2010; Madonna et al., 2009; Tallone et al., 2011). Heterogeneity of initial populations may result in unpredictable effects and non-reproducible results, and therefore it remains one of the key problems in the field.

Numerous clinical applications of stromal cell populations isolated from different tissues rely on the migration and homing of therapeutic cells in the site of injury or inflammation (Chamberlain et al., 2007). Also, it has been suggested that inflammatory conditions result in activation and directional movement of endogenous cells to the sites of injury where they participate in the processes of tissue regeneration. Signals of inflammation are partly mediated by chemokines. These signaling molecules act via binding to their seven-transmembrane receptors belonging to the G-protein coupled receptor (GPCR) family. Chemokines and their receptors play essential roles in the immune system by regulating mobilization and migration of several cell types including neutrophils and B- and T-lymphocytes (Bendall, 2005). Also, chemokines are associated with a variety of other cellular functions including proliferation, differentiation and establishment of cellular polarity. Several data also indicate that chemokines have cell type and concentration dependent anti- and pro-apoptotic effects (Vlahakis et al., 2002). Twenty chemokine receptors and approximately 50 chemokines are identified in humans.

Bone marrow derived stromal cells (BMSCs) express several chemokine receptors (CCR1, CCR4, CCR6, CCR7, CCR9, CCR10, CXCR4, CXCR5, CXCR6, CX3CR1), and respective chemokines induce migration of BMSCs in vitro (Fox et al., 2007; Honczarenko et al., 2006; Ruster et al., 2006; Sordi et al., 2005; Von Luttichau et al., 2005; Wynn et al., 2004). Data concerning chemokine system in ADSCs are very limited. It has been demonstrated that ADSCs are chemotactic in vitro, but, compared to BMSCs, ADSCs express a smaller subset of chemokine receptors (CCR1, CCR7, CXCR4, CXCR5 and CXCR6) (Baek et al., 2011).

Here we analyze the entire repertoire of chemokine receptors in human ADSCs, peripheral blood mononuclear cells and dermal fibroblasts, and show that *CCR1* is one of the few chemokine receptors highly expressed in ADSCs. Interestingly, expression of *CCR1* in different pools of ADSCs positively correlates with the expression levels of the stem cell marker genes *SOX2*, *OCT4* and *NANOG*, whereas exposure of ADSCs to CCL5, a ligand for CCR1, stimulates proliferation of $CCR1^+$ cells accompanied with increased expression of *SOX2*, *OCT4* and *NANOG*. Our results also show that CCR1 is an active receptor in ADSCs, and signaling via the CCL5/CCR1 axis triggers not only migration of cells but also activation of ERK and AKT kinases as well as NFκB signaling pathway.

Materials and methods

Cell culture

ADSCs were isolated from human subcutaneous adipose tissue as described (Lin et al., 2007) with some modifications. Minced adipose tissue was digested with 0.1% collagenase (Gibco, Invitrogen, Carlsbad, CA, USA) in serum-free low glucose Dulbecco's modified Eagle's medium (DMEM-LG) (Gibco, Invitrogen) at 37 °C for 1.5 h, followed by neutralization with normal growth medium (DMEM-LG) supplemented with 10% fetal bovine serum (HI-FBS) (PAA, Pasching, Austria) heat-inactivated at 56 °C for 30 min and 1% penicillin–streptomycin (PEST) (Invitrogen). After centrifugation at 1000 rpm for 5 min, the cell pellet was incubated for 15 min at room temperature (RT) in NH_4Cl, passed through a 100-mm nylon mesh (BD Biosciences Pharmingen, San Jose, CA, USA), re-suspended in normal growth medium and plated at density of 10,000 cells/cm^2 (passage 0). Cells were propagated at 37 °C and 5% CO_2 for 24 h, and non-adherent cells were removed by changing the medium. Each splitting of the confluent cells was considered as the next passage. Immunophenotype of the isolated ADSCs was assessed as $CD73^+/CD90^+/CD105^+/CD45^-/CD34^-$ (Supplementary Fig. 1). Human peripheral blood mononuclear cells were isolated using a Histopaque-1077 (Sigma-Aldrich, Steinheim, Germany) density gradient centrifuge. Primary fibroblast cultures were established by migration from skin explants placed onto Primaria dish containing normal growth medium (Takashima, 2001). The donors of adipose tissue, skin fibroblasts and blood mononuclear cells are described in the Supplementary Table 1.

For kinase activation assay, ADSCs were pre-incubated overnight in serum-free DMEM-LG, treated with recombinant CCL5 (PeproTech, Rock Hill, NJ, USA) for 10, 15 or 60 min, washed with phosphate buffered saline (PBS) and lysed directly in Laemmli sample buffer (60 mM Tris–Cl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.01% bromophenol blue). Prolonged treatments of ADSCs were carried out in Light medium (DMEM-LG containing 3% HI-FBS and 1% PEST) supplemented with 50 ng/ml of CCL5 for 8, 24, 48 or 72 h. Treatments were stopped by washing the cells with PBS, trypsinization (trypsin–EDTA) (PAA), fractionation for total RNA and protein isolations and immediate lysis in the respective lysis buffer. Viability of ADSCs was tested using ViaLight™ plus kit (Lonza, Basel, Switzerland) according to the manufacturer's instructions.