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Adipose mesenchymal stem cells protect chondrocytes from degeneration associated with osteoarthritis

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Abstract Our work aimed at evaluating the role of adipose stem cells (ASC) on chondrocytes from osteoarthritic (OA) patients and identifying the mediators involved. We used primary chondrocytes, ASCs from different sources and bone marrow mesenchymal stromal cells (MSC) from OA donors. ASCs or MSCs were co-cultured with chondrocytes in a minimal medium and using cell culture inserts. Under these conditions, ASCs did not affect the proliferation of chondrocytes but significantly decreased camptothecin-induced apoptosis. Both MSCs and ASCs from different sources allowed chondrocytes in the cocultures maintaining a stable expression of markers specific for a mature phenotype, while expression of hypertrophic and fibrotic markers was decreased. A number of factors known to regulate the chondrocyte phenotype (IL-1β, IL-1RA, TNF-α) and matrix remodeling (TIMP-1 and -2, MMP-1 and -9, TSP-1) were not affected. However, a significant decrease of TGF-β1 secretion by chondrocytes and induction of HGF secretion by ASCs was observed. Addition of a neutralizing anti-HGF antibody reversed the anti-fibrotic effect of ASCs whereas hypertrophic markers were not modulated. In summary, ASCs are an interesting source of stem cells for efficiently reducing hypertrophy and dedifferentiation of chondrocytes, at least partly via the secretion of HGF. This supports the interest of using these cells in therapies for osteo-articular diseases.

Introduction

Osteoarthritis (OA) is the most frequent rheumatic disease, characterized by a degeneration of articular cartilage, mainly due to changes in the activity of chondrocytes in favor of

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catabolic activity as well as reduced cartilage cellularity (Bijlsma et al., 2011; Loeser et al., 2012). The capacity of adult articular chondrocytes to regenerate the normal cartilage matrix architecture declines with aging, due to cell death (apoptosis) and abnormal responsiveness to anabolic stimuli. OA chondrocytes lose their capacity to secrete the specific components of the extracellular matrix, such as collagen type IIB or aggregan, and adopt a hypertrophic or fibroblastic phenotype. Currently, no treatment capable of markedly altering the progression of OA exists and therapeutic options are essentially pain management and surgical intervention (Hunter, 2011). Indeed, new innovative therapeutic strategies for cartilage protection/repair are currently being evaluated mainly based on stem cell-mediated approaches.

Mesenchymal stem cells or stromal cells which reside in bone marrow (MSCs) or in adipose tissue (adipose-derived stromal cells or ASC) are capable of self-renew and are characterized by their differentiation capabilities toward three lineages namely, chondrocytes, osteoblasts and adipocytes (Dominici et al., 2006). Beside this property, MSCs produce a number of secreted factors, such as cytokines, chemokines or growth factors, which mediate diverse functions (Doorn et al., 2012; Meirelles Lda et al., 2009; Salgado et al., 2010). Indeed, the use of MSCs for stem cell-based therapy of degenerative diseases such as OA may rely on two modes of action: MSC differentiation into chondrocytes, or prevention of cartilage degeneration through secretion of bioactive factors. Previous studies have demonstrated the potent role of MSCs combined with biomaterials and chondrogenesis-inducing factors for cartilage engineering (Vinatier et al., 2009). Moreover, the co-culture of human MSCs with OA chondrocytes allowed the differentiation of MSCs towards chondrocytes in the absence of growth factors and prevented their hypertrophic differentiation frequently occurring during in vitro growth factor-induced chondrogenesis (Aung et al., 2011). The possibility that MSCs, through their trophic role, may prevent cartilage degradation or stimulate cartilage formation would be of interest for the treatment of OA. Recent works have shown that trophic factors secreted by MSCs regulate the chondrocyte phenotype when the two cells were mixed in pellet- or alginate-based co-cultures (Mo et al., 2009; Wu et al., 2011, 2012). Altogether, these studies have highlighted the role of the cross-talk between MSCs and chondrocytes with an improved chondrogenic differentiation of MSCs by factors secreted by chondrocytes and the positive role of MSC trophic mediators on chondrocyte phenotype. Most of these studies were conducted using bone marrow-derived MSCs and less data are available on ASCs. These last cells have been intensively tested in various pre-clinical models since their initial characterization and are considered as a more accessible source of cells than MSCs with comparable properties (Pittenger et al., 1999; Zuk et al., 2001). However, neither the effect of ASC or MSC secretome when these cells are cultured with chondrocytes in the absence of direct contact, nor the identification of the proteins responsible for the paracrine effects, has been investigated.

In the present study, we therefore evaluated in vitro the effects of factors secreted by ASCs on the proliferation, apoptosis and phenotype of OA chondrocytes in a co-culture assay where chondrocytes were physically separated from ASCs by a porous membrane and cultured in a minimal medium. Our main findings show that ASCs reduce the expression of both hypertrophic and fibroblastic markers on OA chondrocytes and that HGF is one key mediator involved in the anti-fibrotic effect of ASCs.

Materials and methods

Tissue samples

Samples for ASC isolation were obtained from patients undergoing plastic surgery or total joint replacement surgery (hip or knee hoffa). Subcutaneous abdominal fat (SC-ASC) was obtained from 17 patients (15 women and 2 men; mean age: 44.9 ± 4.3 years). Infrapatellar Hoffa (Hoffa-ASC) and hip fat (Hip-ASC) were also obtained from OA patients. Chondrocytes and MSCs were isolated from patients with OA undergoing total knee replacement surgery. Articular cartilage was harvested from the femoral condyles of 12 patients (10 women and 2 men; mean age: 68.9 ± 2.3 years; body max index (BMI): 26.6 ± 1.4). MSCs were from 4 patients (3 women and 1 man; mean age: 68 ± 3.7 years). Consent of donors was approved by the French Ministry of Research and Innovation (approvals DC2009-1052 and DC-2010-1185).

Cell isolation

For ASC isolation, adipose tissue was digested with 250 U/mL collagenase type II for 1 h at 37 °C and centrifuged (300 *g* for 10 min) using routine laboratory practices. The stroma vascular fraction was collected and cells filtered successively through a 100 μm, 70 μm and 40 μm porous membrane (Cell Strainer, BD-Biosciences, Le-Pont-de-Claix, France). Single cells were seeded at the initial density of 4000 cell/cm^2 in αMEM supplemented with 100 U/mL penicillin/streptomycin (PS), and 2 mmol/mL glutamine (Glu) and 10% fetal calf serum (FCS) was from PAA laboratories (Mureaux, France). After 24 h, cultures were washed twice with PBS. After 1 week, cells were trypsinized and expanded at 2000 cells/cm^2 till day 14 (end of passage 1), where ASC preparations were used. Clinical grade ASCs were isolated and expanded in platelet-enriched plasma (PLP) as described (Bourin et al., 2011). Typically, the doubling time of ASCs was evaluated to be 1.25 ± 0.05 days.

For MSC isolation, trabecular bone pieces were flushed out and cultured as previously described (Bouffi et al., 2010b). For chondrocyte isolation, knee cartilage slices were incubated in 2.5 mg/mL pronase (Sigma-Aldrich, Saint-Quentin-Fallavier, France) for 1 h at 37 °C followed by 2 mg/mL collagenase type II (Sigma), overnight at 37 °C. Digested pieces were filtrated through a 70 μm cell strainer and the cell suspension was cultured in DMEM/PS/Glu/10% FCS (proliferative medium) at the density of 25,000 cell/cm^2 till the end of passage 1.

Flow cytometry analysis

ASCs (1 × 10^5) in PBS containing 0.2% bovine serum albumin (BSA) were incubated with different antibodies (BD-Biosciences): fluorescein isothiocyanate (FITC)-conjugated CD31 or CD106 antibodies, peridinin chlorophyll protein (PerCP)-Cy5.5-conjugated CD34 antibody, phycoerythrin (PE)-conjugated CD73 or CD105 antibodies, Allophycocyanin (APC)-conjugated