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Expression of SDF-1/ CXCR4 axis in bone marrow mesenchymal stem cells derived from rheumatoid arthritis-usual interstitial pneumonia

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ABSTRACT

Objective. *To explore the SDF-1/ CXCR4 axis as driving mechanism of bone marrow mesenchymal stem-cells to the injured lung in patients with rheumatoid arthritis associated usual interstitial pneumonia (RA-UIP).*

Methods. *We evaluated the m-RNA expression of SDF-1 and CXCR4 with real-time PCR in bone marrow mesenchymal stem cells of 7 RA-UIP and 10 RA patients without lung involvement.*

Results. *The axis was not expressed in RA whereas both SDF-1 and CXCR4 were expressed in RA-UIP [1.93 (1.32, 2.00) and 0.008 (0, 0.01)] respectively.*

Conclusion. *The development of pulmonary fibrosis in RA may be considered as the key event for the migration of stem cells to the injured lung through the SDF-1/CXCR4 axis.*

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disorder that typically involves the diarthrodial joints. However, pulmonary complications are not uncommon and account for the death of 10–20% of the patients (1). Moreover, it was recently observed that clinically significant interstitial lung disease associated with RA (RA-ILD) occurs in 10% of the patients and is associated with shorter survival and more severe underlying disease (2). Interestingly, the subgroup of patients with RA associated usual interstitial pneumonia (RA-UIP,) which is also the pathologic counterpart of idiopathic pulmonary fibrosis (IPF), tends to have the same dismal outcome with IPF (3). It is now believed that pulmonary fibrosis is the result of repeated epithelial lung injuries followed by aberrant wound healing. However, despite recent advances (4) pathogenesis of pulmonary fibrosis is not fully understood and thus far there is no effective treatment available.

Mesenchymal stem-cells (MSCs) are one of the most intriguing novel therapeutic approaches in the field of chronic diseases (5) because of the ability to repair injured tissues. Inhibition of adipogenesis of MSCs in RA leads to the development of bone oedema, a characteristic lesion of the disease (6). In animal models of bleomycin (BLM) induced fibrosis it was shown that bone marrow (BM)-MSCs administered systematically can differentiate in type II epithelial cells and suppress the expression of proinflammatory and profibrotic genes (5). We have recently shown that BM-MCSs from patients with IPF and RA-UIP present relative telomere length which did not differ compared to healthy controls and thus could be used for cell replacement treatment (7). Moreover, they express, although weakly, both genes of telomerase at the same level with healthy controls suggesting a plausible mechanism for the maintenance of telomere length. But how do these cells travel in the lung?

Again, in animal models it was shown that (BM)-MSCs express several chemokine receptors such as CXCR4 which ligand, stromal cell derived actor 1 (SDF-1) a CXC chemokine with angiogenic activity, is induced in murine lungs (8-10) suggesting that bone marrow stem cells could be recruited and mobilised to the injured lung through a CXCR4 dependent mechanism. Recently we have shown that CXCR4 is overexpressed in BM-MSCs of patients with IPF (11) suggesting that this gradient could be responsible for the recruitment of BM-MSCs also in humans.

With this in mind, we aimed to evaluate the axis SDF-1/CXCR4 in BM-MSCs of patients with RA-UIP and RA without lung involvement. We hypothesise that the axis could be activated in patients with RA-UIP and could be a possible driving mechanism for the mobilisation of BM-MSCs and that differences in the expression between the two groups could explain different behaviour of these cells.

Materials and methods

We have studied prospectively 7 patients with RA-UIP and 10 patients with RA but without ILD. Patients were recruited from the Interstitial Lung Disease Unit (ILDU) at the Department of Thoracic Medicine of Heraklion. Ethics Committee of the University of Crete has approved the study and all participants were informed on the scope of the study and gave their written informed consent.

BM mononuclear cells (BMMCs) were obtained from posterior iliac crest aspirates. *In vitro* expansion and differentiation methodology have been described previously (11). Immunophenotypic analysis of MSCs from all groups of patients and healthy controls at the end of P2 demonstrated that cultures constituted of a homogenous cell population positive for CD73, CD90, CD146, CD105, CD29, CD44 and negative for CD45 and CD34 surface antigens. P2 MSCs were able to differentiate towards the adipogenic, osteogenic and chondrogenic lineages in healthy individuals, as well as in all groups of patients.

MSCs at P2 were homogenised in the TRIzol® reagent (Invitrogen, Carlsband, CA), total RNA was extracted and cDNA synthesised by reverse transcription (RT) with the Thermoscript™ RT kit (Invitrogen). Genes mRNA expression was measured using a real-time RT-PCR assay with SYBR-Green I. Beta-actin was used as the internal control.

The data are presented as median (interquartile ranges).

Results

The axis SDF-1/CXCR4 was not expressed in patients with RA without ILD. Conversely, all patients with RA-UIP have expressed both genes of the axis [(SDF-1: 1.93 (1.32, 2.00) and CXCR4: 0.008 (0, 0.01), respectively].

Discussion

BM-MSCs may represent an intriguing therapeutic option for epithelial lung injury because of their ability for cell differentiation and repair. Interestingly, we have previously observed that BM-MSCs from patients with IPF and RA-UIP are able to maintain their telomere length and thus their reparative ability unlike what has been observed in other cell types in familial and sporadic cases of IPF. (12-15). In this study the most important finding is that the SDF-1/CXCR4 axis is not expressed in RA patients without ILD whereas it is expressed in patients with RA-UIP. We believe that the lung injury and the development of lung fibrosis may be the key event for the expression of the axis and for the mobilisation of BM-MSCs to the site of injury in order to begin the reparative process. We have previously shown that both components of the axis are expressed in the bronchoalveolar lavage fluid (BAL) of patients with RA-ILD (16) suggesting that can be produced by the injured lung and attract BM-MSCs in the injured lung. Clearly we acknowledge that the small number of patients represents a limitation of our research and that further studies with larger cohorts are needed in order to confirm our findings.

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