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Investigation of bone marrow mesenchymal stem cells (BM MSCs) involvement in idiopathic pulmonary fibrosis (IPF)

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KEYWORDS

Mesenchymal stem cells;
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Summary

Background: Experimental data have provided evidence that progenitor cells of bone marrow (BM) origin may play a role in the fibrogenic process of the lung.

Objective: To probe the possible involvement of BM mesenchymal stem cells (MSCs) in the pathophysiology of Idiopathic Pulmonary Fibrosis (IPF) by investigating the molecular profile of these cells.

Design: BM MSCs were studied in 10 IPF patients and 10 healthy controls. MSCs were identified by their immunophenotypic characteristics and their potential to differentiate towards adipocytes/osteocytes/chondrocytes. We evaluated the mRNA expression of genes involved in the lung injury of IPF, namely the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor beta-1 (TGF- β 1) and the axis stromal-cell-derived factor-1 (SDF-1)/CXCR4 in BM MSCs using quantitative RT-PCR.

Results: The BM MSCs of IPF patients displayed normal immunophenotypic characteristics and differentiation potential. No statistically significant difference was found between patients and controls in VEGF and FGF mRNA expression. TGF- β 1 was not expressed in either patients or controls. A significant increase in SDF-1-TR1 and CXCR4 mRNA expression was detected in IPF patients ($1.6 \times 10^{25} \pm 1.2 \times 10^{25}$ and $3.1 \times 10^7 \pm 3.1 \times 10^7$, respectively) compared to

Abbreviation List: IPF, idiopathic pulmonary fibrosis; BM, bone marrow; MSCs, bone marrow mesenchymal stem cells; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; TGF- β 1, transforming growth factor beta-1; SDF-1, stromal-cell-derived factor-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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controls ($0.32 \times 10^{25} \pm 0.07 \times 10^{25}$ and $1.67 \times 10^7 \pm 0.30 \times 10^7$, respectively) ($p = 0.001$ and $p = 0.001$, respectively) whereas SDF-1 levels in MSC supernatants were similar in patients and controls.

Conclusions: The increased CXCR4 expression by patient MSCs suggests that the BM is probably implicated in the pathophysiology of IPF by mobilizing MSCs in response to or preceding lung injury. The potential role of BM MSCs in IPF is another interesting field for further investigation. © 2010 Elsevier Ltd. All rights reserved.

Introduction

Idiopathic Pulmonary Fibrosis (IPF), the most common and devastating form of pulmonary fibrosis, is distinguished from other interstitial lung diseases by important prognostic implications. IPF does not respond to current medical therapies and its clinical course is marked by inexorable deterioration, with a mortality rate of approximately 70% five years after diagnosis.^{1,2} Fibroblasts, especially in their activated differentiated state named myofibroblasts, are considered to be the key elements in the pathogenesis of fibrosis.³ The hallmark lesions are the fibroblast foci representing focal areas of active fibrogenesis featuring vigorous fibroblast replication and exuberant extracellular matrix deposition, which may lead to obliteration of the distal air space.^{1,2}

Understanding the source of lung fibroblasts and myofibroblasts and the mechanism of recruitment are critical issues in the pathogenesis of fibrotic lung diseases. While these cells were classically thought to derive exclusively from resident lung fibroblasts, recent studies indicate that they may originate from pulmonary epithelial cells⁴ or even from extrapulmonary cellular sources.⁵ In this context, a profibrotic role of bone marrow (BM) derived circulating fibroblast like cells, has been reported.^{5–10} In other studies, however, a protective rather than a profibrotic effect of BM derived mesenchymal stem cells (MSCs) has been indicated.¹¹ These contradictory data suggest that the role of BM in the pathogenesis of fibrotic lung diseases remains unclear.

The BM MSCs are multipotent cells of non-hematopoietic origin that may differentiate into several cell lineages of mesenchymal tissues.^{12–15} These cells have been demonstrated to participate in tissue homeostasis and repair under the influence of appropriate signals such as the chemokine CXCL12, also known as stromal-cell-derived factor (SDF)-1.⁸ Interestingly, chronic ischemic heart disease in experimental mice models has been associated with reduced migratory response of BM MSCs to local SDF-1 and vascular endothelial growth factor (VEGF) stimuli.¹⁶ The role of these cells in the repair of liver injury has also been reported.¹⁷

The aim of the current study is to investigate the possible involvement of BM MSCs in the pathophysiology of IPF by evaluating the mRNA expression of genes involved in the lung injury/repair process, namely the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor beta-1 (TGF- β 1) and the CXCL12/CXCR4 ligand/receptor dyad.¹⁸ To our knowledge, this is one of the few studies investigating the role of BM MSCs in IPF in humans.

Methods

Patients

We have studied 10 patients with IPF and 10 healthy individuals (from a previous study of the University of Crete,¹⁹ age- and sex-matched with the patients (Table 1)). Patients were recruited from the Interstitial Lung Disease Unit (ILDU) at the Department of Thoracic Medicine of Heraklion.

The diagnosis of IPF was made in 3 cases by surgical biopsy (in the correct clinical context, detailed below) and the histologic diagnosis of Usual Interstitial Pneumonia (UIP) was obtained. In the remaining 7 cases the diagnosis was made on the basis of clinical and high-resolution computed tomography (HRCT) criteria: (1) bilateral basal or widespread crackles; (2) restrictive ventilatory defect or isolated depression of DL_{CO}; (3) computed tomography (CT) appearances indicative of IPF with predominantly basal and subpleural microcystic or macrocystic honeycombing, with variably extensive ground-glass and reticular abnormalities but no consolidation, nodular abnormalities, or other parenchymal abnormalities (apart from centrilobular emphysema); and (4) no environmental exposure to a fibrogenic agent or connective tissue disease.¹ According to the aforementioned criteria a known cause of pulmonary fibrosis, such as a connective tissue disorder, has been excluded by both immunologic screening and rheumatologic clinical evaluation.

Ethical Committee of the University of Crete has approved the study,¹⁹ and all participants (patients and

Table 1 Demographic and spirometric characteristics of IPF patients and control subjects.

Characteristics	Control subjects	IPF patients
Number	10	10
Sex: Male/Female	5/5	7/3
Age, median (yr)	59 (32–65)	65 (40–75)
Smokers/non smokers	6/4	8/2
FVC, (% pred)	103 \pm 14	77.3 \pm 13.0*
TLC, (% pred)	101 \pm 19	67.4 \pm 14.2*
TL _{CO} , (% pred)	96 \pm 6	60.3 \pm 17.8*
P α O ₂ , (mmHg)	—	80.3 \pm 10.0

Values are expressed as mean \pm SD and age as median (range). *Statistically significance difference between IPF patients and healthy controls ($p < 0.05$).

Abbreviations: FVC, Forced Vital Capacity; TLC, Total Lung Capacity; TL_{CO}, Diffusing Capacity for Carbon Monoxide; P α O₂, Arterial Partial Pressure of Oxygen.

control subjects) were informed of the scope of the study and gave their written informed consent.

MSC culture and identification

BM MSCs were grown from posterior iliac crest aspirates, as previously described.^{19,20} In brief, BM mononuclear cells (BMMCs) isolated with Histopaque-1077 (Sigma, Saint Louis MO) were cultured in Dulbecco's Modified Eagle Medium-Low Glucose (DMEM-LG; Gibco Invitrogen, Paisley Scotland)/10% fetal calf serum (FCS; Hyclone, Logan, Utah, USA)/100 IU/ml penicillin–streptomycin (PS, Gibco) (MSC medium) at a concentration of 2×10^5 cells/cm² in 25 cm² culture flasks in 37 °C/5%CO₂ humidified atmosphere. One to three days after seeding, floating cells were removed and the medium was replaced by fresh MSC medium. Thereafter, attached cells were fed with fresh medium every 3–4 days. Cells were passaged when 70–90% confluence was reached, using 0.25% trypsin-1 mM EDTA (Gibco).

MSCs were identified by their morphologic and immunophenotypic characteristics and their potential to differentiate towards three different pathways, namely adipocytes, osteocytes and chondrocytes.

MSC quantification in the BMMC fraction

A colony forming unit fibroblastic assay (CFU-F) was used to evaluate MSC frequency within BMMCs. Briefly, day-0 BMMCs were seeded at four different concentrations, expanded for 14 days and CFU-F number was estimated using linear regression analysis.

Immunophenotypic characteristics of MSCs

Trypsinized MSCs from passage-2 (P2) were immunophenotypically characterized by flow cytometry using anti-human monoclonal antibodies against CD29 (4B4; Cyto-Stat/Beckman-Coulter, Florida, USA), anti-CD44 (J173; Immunotech/Coulter, Marseille, France), anti-CD73 (AD2; Becton Dickinson-Pharmingen, San Diego, CA), anti-CDw90 (F15.42; Immunotech/Coulter), anti-CD105 (SN6; Caltag, Burlingame, CA), anti-CD146 (P1H12; Becton Dickinson-Pharmingen), anti-CD45 (IMMU19.2; Immunotech/Coulter) and anti-CD34 (QBend10; Beckman-Coulter). Data were processed in an Epics Elite flow cytometer (Coulter, Miami, FL) (Fig. 1A).

Differentiation potential of MSCs at P2

Adipogenic differentiation was induced using MSC medium supplemented with 10%FCS/0.5 mM 1-methyl-3-butylisoxanthine/1 μM dexamethasone/0.2 μM indomethacin/10 μg/ml insulin. Differentiation was assessed by Oil Red O stain and adipose fatty acid-binding protein (aP2) and peroxisome proliferator activated receptor-γ (PPARG) mRNA expression (Fig. 1B,C).^{19–21} Osteogenic differentiation was induced using MSC medium supplemented with 0.1 μM dexamethasone/0.15 mM ascorbate-2-phosphate/3 mM NaH₂PO₄. Differentiation was assessed by alkaline phosphatase (ALP)/Von Kossa stain and ALP and runt-related transcription factor 2 (RUNX2) mRNA expression (Fig. 1D,E).^{19–21} For chondrogenic induction, cells were pelleted in 15 ml tubes and cultured in DMEM-High Glucose (Gibco), supplemented with 6.25 μg/ml insulin/6.25 μg/ml transferrin/1.33 μg/ml linoleic acid/1.25 mg/ml bovine

serum albumin/1 mM sodium pyruvate/0.17 mM ascorbate-2-phosphate/0.1 μM dexamethasone/0.35 mM L-proline/6.25 ng/ml selenous acid/0.01 μg/ml TGF-β3 (R&D Systems). Differentiation was identified with Alcian blue stain and collagen type II (COL2A1) and aggrecan (AGC1) mRNA expression (Fig. 1F,G).^{19–21} RT-PCR conditions and primer sequences for differentiation-specific gene identification have been reported elsewhere.^{19,21}

Real-time reverse transcriptase-polymerase chain reaction assay

MSCs at P2 were homogenized in the TRIzol[®] reagent (Invitrogen, Carlsband, CA), total RNA was extracted and cDNA synthesized by reverse transcription (RT) with the ThermoScript[™] RT kit (Invitrogen). Peptide growth factors mRNA expression was measured using a real-time RT-PCR assay with SYBR-Green I. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control, in order to normalize VEGF, TGF-β1, FGF, SDF-1 (TR1 and TR2) and CXCR4 expression levels.^{22,23} The mRNA-specific primers used are listed in Table 2.

ELISA for SDF-1

SDF-1 levels in MSC culture supernatants at P2 were evaluated by means of ELISA (Quantikine; R&D Systems, Minn., MN). The sensitivity of the assays is below 47 pg/mL.

Statistical analysis

Data were analyzed using the nonparametric Mann–Whitney test (GraphPad Software; San Diego, CA). Values are expressed as means ± 1 standard error of the mean (SEM) and a value of $p < 0.05$ was considered significant.

Results

MSC frequency in the BMMC fraction

The estimated frequency of MSCs in the BMMC fraction did not differ significantly between patients ($3.33 \pm 1.44/10^5$ BMMCs) and controls ($6.64 \pm 2.94/10^5$ BMMCs) ($p = 0.0667$) by CFU-F analysis, suggesting normal numbers of MSCs in IPF patients.

MSC immunophenotype and differentiation potential

Immunophenotypic analysis of MSCs from IPF 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 (Fig. 1A). P2 MSCs from both IPF patients and controls were able to differentiate towards the adipogenic, osteogenic and chondrogenic lineages as shown by the respective cytochemical staining and differentiation-specific gene mRNA expression (Fig. 1B–G).

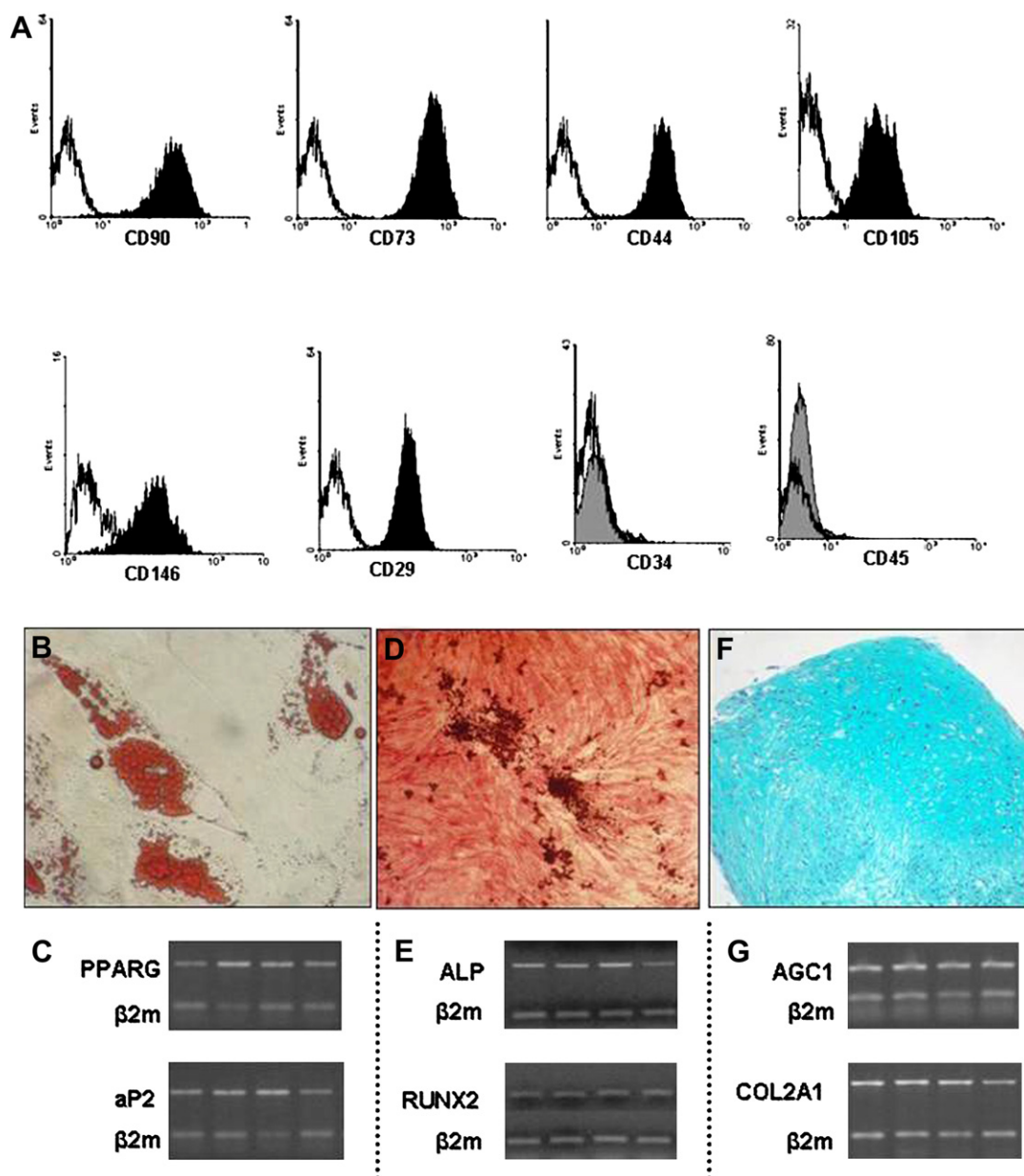


Figure 1 Immunophenotypic characteristics and differentiation potential of BM MSCs in IPF. Representative plots from flow cytometric analysis of BM MSCs from an IPF patient at P2 stained with surface monoclonal antibodies. Black filled histograms show the positive markers and gray filled histograms depict the negative markers in comparison to the isotype-matched controls (open histograms) (A). Differentiated BM MSCs from P2 of the same patient towards the adipogenic (B, C), osteogenic (D, E) and chondrogenic (F, G) lineages. Adipogenic differentiation was identified by the Oil red O stain (B) and PPAR γ and aP2 mRNA expression (C), osteogenic differentiation with ALP/Von Kossa staining (D) and ALP and RUNX2 mRNA expression (E) and chondrogenic differentiation with Alcian blue (F) staining and AGC1 and COL2A1 mRNA expression (G).

mRNA expression of IPF-related genes

We first evaluated the expression of genes implicated in the recruitment of MSCs at sites of injury. Results from the SDF-1 and CXCR4 mRNA expression in BM MSCs are shown in Figs. 2 and 3, respectively. A statistically significant increase was detected in both SDF-1-TR1 and CXCR4 mRNA expression in IPF patients ($1.6 \times 10^{25} \pm 1.2 \times 10^{25}$ and $3.1 \times 10^7 \pm 3.1 \times 10^7$, respectively) compared to controls ($0.32 \times 10^{25} \pm 0.07 \times 10^{25}$ and $1.67 \times 10^7 \pm 0.30 \times 10^7$,

respectively) ($p = 0.001$ and $p = 0.001$, respectively). In contrast, no statistically significant difference was documented in SDF-1-TR2 mRNA expression between patients and controls (1502.6 ± 1477.9 and 36.9 ± 11.4 , respectively).

Regarding the BM MSC expression of genes implicated in the pathogenesis of lung injury in IPF, no statistically significant difference was documented in FGF and VEGF mRNA expression between patients (3662.0 ± 395.3 and 1242.1 ± 12.8 , respectively) and healthy controls

Table 2 Primer sequences used for quantitative Real-time RT-PCR.

Growth factor or cytokine	Primer pair Sequence (5'–3')	Annealing temperature (°C)	Product size (bp)
VEGF	ATGACGAGGGCCTGGAGTGTG CCTATGTGCTGGCCTTGGTGAG	60	91
TGF-β1	AAGGACCTCGGCTGGAAGTG CCCGGGTTATGCTGGTTGTA		137
FGF	CTGGCTATGAAGGAAGATGGA TGCCCAAGTTCGTTTCAGTG	55	149
SDF-1-T1	TGAGAGCTCGCTTTGAGTGA CACCAGGACCTTCTGTGGAT	55	233
SDF-1-T2	CTAGTCAAGTGCCTCCACGA GGACACACCACAGCACAAAC	55	221
CXCR4	GGTGGTCTATGTTGGCGTCT TGGAGTGTGACAGCTTGGAG	55	229
GAPDH	GGAAGGTGAAGTCCGAGTCA GTCATTGATGGCAACAATATCCACT	60	101

Abbreviations: VEGF, Vascular Endothelial Growth Factor; FGF2, Fibroblast Growth Factor 2; TGF-β1, Transforming growth factor beta-1; SDF-1, Stromal-cell-Derived Factor-1; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

(4162.8 ± 1903.6 and 1045.8 ± 41.8 , respectively), whereas TGF-β1 was not expressed in either patients or controls.

SDF-1 in MSC culture supernatants

As we found a statistically significant increase in SDF-1-TR1 but not SDF-1-TR2 mRNA expression of IPF patients compared to controls, we measured the SDF-1 total protein levels in MSC culture supernatants at P2 by means of ELISA. No statistically significant difference was found between patient and controls (3031.56 ± 461.96 pg/mL and 4236.36 ± 582.27 pg/mL, respectively, $p = 0.1$) in SDF-1 supernatant levels. In addition, the fibrocyte attractant chemokine CXCL12 has been found increased in plasma of IPF patients in comparison with healthy controls (3021.0 ± 69.2 pg/mL and 2636.36 ± 98.57 pg/mL, respectively, $p = 0.01$).

Discussion

IPF is a devastating disease leading to progressive lung destruction and scarring. Previous mechanistic research has

been mainly focused on the local fibroproliferative process. However, experimental data have provided evidence that progenitor cells of BM origin may play a crucial role in the fibrogenetic process. The aim of the current study was to investigate the possible involvement of BM MSCs in the pathogenesis of IPF in humans.

We found that the frequency of MSCs within BMSCs did not differ between patients and controls, although the lack of statistical significance may result from lack of statistical power. Our findings suggest that patients with IPF have rather normal BM MSCs reserves, in agreement with previous report from our institute.²⁰ In detail, recent data from a larger study population showed that MSCs from RA patients and age-/sex-matched healthy individuals were similar in frequency, differentiation potential, survival, immunophenotypic characteristics and protein profile.

It has been reported that MSC can produce a variety of cytokines and chemokines that play a role in the regulation of cell migratory properties.^{24–26} In this respect, MSCs have been shown to express a restricted pattern of chemokine receptors, including CXCR4, allowing them to migrate to tissues upon specific chemotactic triggers.^{27–30} These

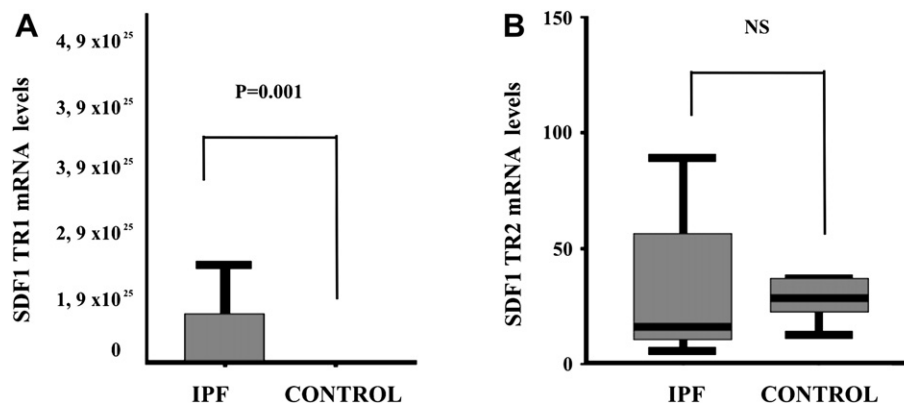


Figure 2 SDF-1-TR1 and SDF-1-TR2 mRNA expression in IPF ($N = 10$) and control subjects ($N = 10$). (A). SDF-1-TR1 mRNA levels were higher in IPF patients compared to controls ($P = 0.001$) (B). No difference in SDF-1-TR2 mRNA levels was observed in IPF patients and controls ($P > 0.05$).

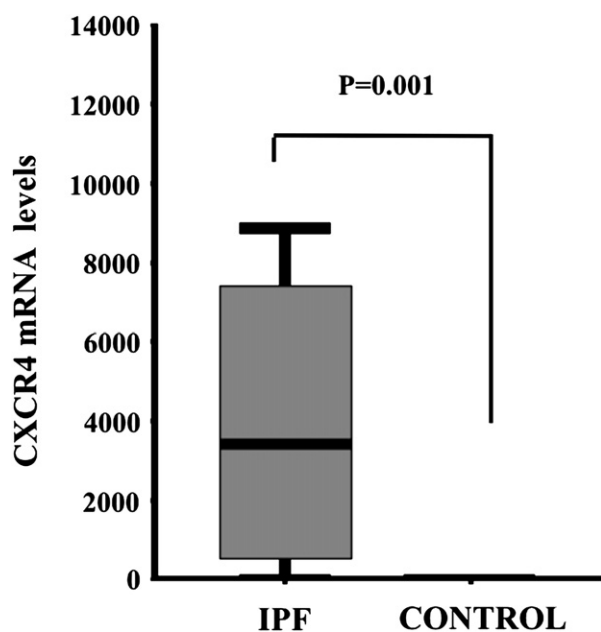


Figure 3 CXCR4 mRNA expression in IPF ($N = 10$) and control subjects ($N = 10$). Significantly higher CXCR4 mRNA levels were observed in IPF patients compared to controls ($P = 0.001$).

receptors represent the basis for MSC homing to multiple organs where they undergo a program of tissue-specific differentiation.³¹ In our study we found that CXCR4 mRNA was overexpressed in BM MSCs of IPF patients compared to controls suggesting probably upregulation of this chemokine receptor in an attempt of patients' BM to respond to the lung injury by MSC mobilization. We also found increased levels of one SDF-1 mRNA transcript in BM MSCs of IPF patients. However, this finding was not confirmed at the protein level, as SDF-1 concentration in MSC supernatants did not differ significantly between patients and controls.

The current paper has different limitations. Indeed, the fact that our findings could be interpreted in more than one way could be articulated as the major limitation of the study. We therefore hypothesize a mobilization process of CXCR4 overexpressing MSCs from the BM to peripheral blood, and thence to the injured lung in response to a SDF-1 concentration gradient.^{32,33} However, the increased expression of CXCR4 could also precede lung injury, at least in part, and contributed to it in some way. Findings in the current study do not appear to discriminate between these two models. In addition, we have not excluded the possibility that increased mobility of bone marrow cells is a key early event in IPF. Therefore, changes in the expression of other cytokines or their contribution to alternative key mechanisms may occur once circulating bone marrow cells reach the lungs.

The small number of cases constitutes a further limitation of this study. The lack of statistical significance could be due to lack of power in different occasions. Firstly, the average number of cells is 50% lower among the patients compared to controls, however does not reach statistical significance. Secondly, but not less importantly, the age of the controls may not be statistically different from the IPF patients, however they are younger with fewer males. It has been previously shown that aging may affect the

proliferative potential of MSCs.^{34,35} An age-related defect in the clonogenic and proliferative capacity of patient MSCs cannot be excluded in the current study. However, it has been showed that RA and IPF patients' MSCs displayed age-inappropriate relative telomere loss,^{19,36} suggesting that the replicative capacity of cells speculates that the defective growth potential of patient MSCs is due to inappropriate telomere loss.¹⁹ Furthermore, lower proportion of smokers among the controls is a potential bias of the current study, as it has been demonstrated that oxidative stress is accelerating telomere length.³⁷ Finally, there aren't data regarding sex influence on MSCs population from ours or other studies.

The early nature of the IPF, compared to other populations, could also be articulated as a potential limitation, which is, may be, a strength. This is important because in more advanced disease, the non-specific effects of hypoxia might be expected to confound attempts to compare bone marrow parameters with those in normal subjects.

A number of studies are in accordance with our hypothesis. Specifically, in an experimental mouse model of bleomycin-induced lung injury it was shown that BM derived progenitor cells may participate in the fibrotic process in response to locally produced chemokine signals as neutralization of CXCL12 resulted in reduction of cell recruitment and amelioration of lung fibrosis.⁹ Increased expression of CXCL12 has been found in the lungs of patients with IPF substantiating further the significance of the CXCR4/CXCL12 axis in the pathogenesis of the fibrotic process.^{32,33,38–40} It has also been reported that SDF-1 levels in BAL and peripheral blood specimens increase late after bleomycin injury and this increase is accompanied by an increase in CXCR4 expression in the lungs with a peak at the second week after injury.¹⁰ These data are consistent with the hypothesis that lungs with bleomycin-induced injury stimulate a late increase in the expression of SDF-1, which can be implicated in the mobilization of CXCR4⁺ expressing BM MSCs.

However, verification in human IPF is rather limited.^{38–40} In the fibrogenic environment of the injured lung, CXCR4⁺ cells may acquire a fibroblast phenotype finally contributing to the fibrogenetic process.³⁸ A histological study demonstrated the presence of cells that coexpressed mesenchymal and leukocyte markers in IPF lung.³⁹ Finally, a recent study suggests that the percentage of fibrocytes in circulation may serve as a biomarker for the presence of fibrosis and a potential biomarker for acute exacerbations.⁴⁰ Furthermore, SDF-1/CXCL12 was increased in plasma from IPF patients. A similar increase of this chemokine was also recently reported, in agreement with the latter finding.^{38,39}

A number of studies have investigated the possible involvement of BM MSCs in the pathophysiology of inflammatory, degenerative, vascular and autoimmune disease.⁴¹ For example, it has been suggested that the BM may contribute in pathophysiology of rheumatoid arthritis by providing MSCs with altered properties in the affected joints.^{42,43} However, recent data regarding the potential use of MSCs for cartilage and bone repair in rheumatoid arthritis showed that these patients have normal BM MSC reserves and that culture expanded MSCs from RA patients display normal differentiation capacity and proteomic profile.²⁰ In contrast, the cartilage and bone damage

associated with osteoarthritis has been attributed to the impaired chondrogenic and adipogenic differentiation potential of BM derived MSCs.⁴⁴ In patients with systemic sclerosis, the BM MSCs have not been shown to contribute to the sclerosing process as they exhibit the normal phenotypic, proliferative, differentiation potential and immunosuppressive properties.⁴⁵

To investigate the hypothesis that BM MSCs from IPF patients may be primarily involved in the pathogenesis of IPF, we evaluated the expression of cytokines previously reported to be upregulated in the lungs of the affected subjects. Our patient MSCs displayed normal VEGF, FGF, and TGF- β 1 mRNA expression compared to healthy controls. This finding suggests that the BM MSC population seems unlikely to be primarily involved in lung injury but represents rather a tissue repair cellular source. In favour of this hypothesis is a recent study indicating that myelosuppression may increase the lung susceptibility to bleomycin and transfer of BM derived MSCs may display a protective action.¹¹ In addition, intratracheal or systemic administration of MSCs immediately after intratracheal bleomycin administration decreased subsequent lung collagen accumulation, fibrosis and levels of matrix metalloproteinases.⁴⁶ Furthermore, systemic MSC administration also decreased lung inflammation after endotoxin administration in mice.^{47–49} Based on their anti-inflammatory and immunoregulatory properties, BM MSCs are considered as potential therapeutic modalities for autologous and allogeneic usage in immune-mediated diseases including graft versus host disease.^{36,50}

The complete absence of any difference between IPF and normals in cytokines upregulated in the lung can be set against the striking increase in a cytokine linked to mobilization of bone marrow cells. However, we cannot be certain that bone marrow cells are not contributing at some level to lung injury, as we have selected a panel of cytokine markers implicated in IPF. In addition, we have not excluded the possibility that bone marrow cells without cytokine upregulation in the marrow might, nonetheless, play a key pathogenetic role in injury, simply by virtue of enhanced mobilization.

In conclusion, these findings establish that the mobilization of bone marrow cells could reasonably be interpolated into a pathogenetic model for IPF, at one level or another. In summary, the current study evaluates the molecular characteristics of BM MSCs in patients with IPF. The increased CXCR4 expression by patient MSCs suggests that the BM is probably implicated in the pathophysiology of IPF by mobilizing MSCs in response to or preceding lung injury. The normal VEGF, FGF, and TGF- β 1 mRNA expression indicates that the BM derived MSCs is rather unlikely to be the cellular population promoting the tissue injury in IPF, at least by their upregulation. The potential role of the abnormal lung microenvironment in the functional characteristics of the recruited MSCs in IPF is another interesting field for further investigation.

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Author contributions: Dr. Antoniou had full access to all of the data in the study and takes responsibility for the integrity and accuracy of the data.

Study concept and design: Antoniou, Siafakas, Papadaki. Acquisition of data: Kastrinaki, Koutala, Damianaki, Soufla.

Analysis and interpretation of data: Antoniou, Papadaki. Drafting of the manuscript: Antoniou, Papadaki, Siafakas.

Critical revision of the manuscript: Papadaki, Spandidos, Siafakas.

Statistical analysis: Soufla, Kastrinaki.

Administrative, technical, or material support: Antoniou.

Study supervision: Siafakas, Papadaki, Antoniou.

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