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Hematopoietic Derived Fibrocytes: Emerging Effector Cells in Fibrotic Disorders

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1. Introduction

Fibrocytes constitute a unique population of mesenchymal progenitor cells from hematopoietic origin. They display a unique spectrum of immune and molecular characteristics such as the simultaneous expression of mesenchymal (collagen types I and III, fibronectin), leukocyte (CD45), monocyte (CD14), and hematopoietic stem cell (CD34) markers. Fibrocytes were initially described in the context of wound repair and since their original description in 1994, our understanding and knowledge of this novel cell population has grown considerably. They have the potential to differentiate into fibroblasts and myofibroblasts among other mesenchymal cells such adipocytes, osteoblasts, and chondrocytes. Fibrocytes are a rich source of inflammatory cytokines, growth factors, and chemokines that provide important intercellular signals within the local tissue microenvironment. Moreover, fibrocytes possess the immunological features typical of an antigen-presenting cell (APC), and they have the capacity for the presentation of antigens to naïve T-cells.

The aim of this chapter is to present a comprehensive overview over the history and recent findings on the biology of fibrocytes as well as their putative participation in fibrotic disorders.

2. History

After an injury occurs, a number of extracellular and intercellular responses are initiated and coordinated in order to restore the tissue integrity and homeostasis. Wound healing is a dynamic, interactive process in which cellular components of the immune system, the blood coagulation cascade and the inflammatory pathways are activated. The cells involved including neutrophils, monocytes, lymphocytes, dendritic cells, endothelial cells, keratinocytes and fibroblasts undergo marked changes in gene expression and phenotype, leading to cell proliferation, differentiation and migration (Singer & Clark 1999; Arabi et al., 2007; Gurtner et al., 2008)

Tissue fibroblasts play a key role not only in normal reparative processes, but also in pathological fibrotic processes. In the past decade it has been established that fibroblasts/myofibroblasts, which participate in repair and fibrosis have their origin not only in the fibroblasts already present in the injured tissues, but also may derive from other sources such as mesenchymal and hematopoietic stem cells (Hinz et al., 2007). The notion of a

monocytic fibroblast precursor was first proposed more than a hundred years ago by James Paget, and probably represents the first observations of cells with the molecular features of circulating fibrocytes (Herzog & Bucala 2010). Afterward in the early 1960´s the hypothesis of the blood borne origin of fibroblast appeared again in the literature; of particular significance are the observations of Petrakis and co-workers who reported the in vivo differentiation of human leukocytes into fibroblasts, histiocytes and adipocytes in subcutaneous diffusion chambers (Petrakis et al., 1961). More recently, it was demonstrated that bone-marrow (BM) contributes to the expansion of the fibroblast population in multiple organs and tissues, including skin, stomach and esophagus using mouse transplantation models, and in human liver fibrosis (Direkze et al 2003, 2004 and Forbes et al 2004). Regarding the lung, a pioneer work published in 2004 described that the collagen-producing fibroblasts in experimental pulmonary fibrosis are derived from BM progenitor cells (Hashimoto et al., 2004). While these studies documented the BM origin of at least part of the tissue fibroblasts during injury, they did not resolve whether these BM derived fibroblasts were from hematopoietic stem cells (HSCs) or mesenchymal stem cells. Later, through a model of transplantation of clones of cells derived from a single HSC from transgenic enhanced green fluorescent protein (EGFP) mice, it was clearly demonstrated that fibrocytes are derived from HSCs (Ebihara et al., 2006).

The circulating fibrocyte was first described in 1994 by Bucala, in a model of wound healing response, with the surgical implantation of wound chambers into the subcutaneous tissues of mice. The implantation resulted in a rapid influx of peripheral blood cells such as neutrophils, monocytes, and lymphocyte subpopulations within 24 hr. They noticed that 10% of the cells present in the wound chamber, were spindle shaped cells and expressed collagen I, and CD34, (Bucala et al., 1994). The idea that these cells were of circulating origin arose from the observation that their arrival in to the wound chamber was much faster than would be expected by entry of fibroblasts from the surrounding tissue, since the fibroblasts would have to migrate across the permeable plastic layer, enter the wound chamber, and begin matrix deposition, (Bucala, 2008). Hence, the entrance of large numbers of fibroblast-like cells simultaneously with circulating inflammatory cells suggested that this cell population was from peripheral blood origin and not exclusively by slow migration from adjacent connective tissue (Bucala et al., 1994). This new leukocyte sub-population was termed “fibrocytes”, which combines the greek “kytos” referring to cell, and “fibro”, which is from the latin denoting fiber. This nomenclature may lead to some overlap as the term “fibrocyte” is also used in histopathologic literature as a synonym for “mature” fibroblasts, and to name a cell constituent of the inner ear spiral ligament, (Quan et al., 2004).

Now it is known that fibrocytes are a hematopoietic stem cell source of fibroblasts/myofibroblasts that participate in the mechanisms of wound healing and fibrosis in many organs (Schmidt et al., 2003; Mori et al., 2005; Ebihara et al., 2006; Andersson-Sjöland et al., 2008; El-Asrar et al., 2008; Strieter et al., 2009).

3. Purification and culture

The current methods and techniques employed for the isolation and characterization of peripheral blood fibrocytes are based mainly in the derivation of these cells from the buffy coat of peripheral blood obtained from human or animal sources. Circulating fibrocytes comprise the ~0.1-0.5% of the non-erythrocytic cells in the peripheral blood and they can be quantified and analyzed by flow cytometry (Bucala et al., 1994, Moeller et al., 2009).

Fibrocytes can be obtained and/or differentiated in vitro from the complete peripheral blood mononuclear cell (PBMC) population as well as from an enriched CD14+ population (Abe et al., 2001, Pilling et al., 2009, García-de-Alba et al., 2010). Accordingly, fibrocytes represent one of the variety of cell types that can differentiate from monocytes, including macrophages, osteoclasts and dendritic cells (Wu & Madri 2010; Seta et al., 2010; Castiello et al., 2011).

The fibrocytes obtained from human or mouse blood, either from PBMCs or CD14+ enriched cells, are grown commonly in Dubelcco´s Modified Eagle Medium (DMEM) supplemented with 20% human AB serum (HAB) or fetal calf or bovine serum without the addition of any other growth factors. Some authors have reported the use of RPMI instead of DMEM with good results (Curnow et al., 2010). The resulting fibrocyte population (≥95% pure) is then characterized based on the combined expression of extracellular surface markers including cluster of differentiation (CD) antigens, major histocompatibility complex (MHC)-like molecules, extracellular matrix protein (ECM) markers, and chemokines receptors expression patterns (Metz, 2003) (table 1).

Marker type	Function
Extracellular matrix proteins	
Collagen I and III	Extracellular matrix
Type I pro-collagen	Collagen I precursor
Prolyl-4-hydroxilase	Collagen hydroxiproline
α-smooth muscle actin	Contractile element
Vimentin	Intermediate Filament
CD markers	
CD11a (LFA-1)	L subunit of integrin LFA-1, adhesion molecule
CD11b (Mac 1)	M subunit of integrin CR3, adhesion molecule
CD13	Pan-myeloyd antigen
CD34	Hematopoietic stem cell antigen, endothelial cell
CD45	Leukocyte common antigen
CD54 (ICAM)	Intracellular adhesion molecule binds LFA-1 and Mac-1
CD58 (LFA-3)	Adhesion molecule, binds CD2
CD80 (B7-1)	Co-stimulatory molecule binds CD28
CD86 (B7-2)	Co-stimulatory molecule binds CD28 and CTLA-4
MHC-related markers	
MHC class II	
HLA-DP	Major histocompatability molecule for antigen
HLA-DQ	presentation
HLA-DR	
Chemokine receptors	
CCR3	Receptor for secondary lymphoid chemokine (SLC)
CCR5 (CD195)	Receptor for RANTES (CCL5), MIP-1α and MIP-1β
CCR7 (CD197)	Receptor for CCL19 and CCL21
CXCR4	Receptor for CXCL12 (SDF-1α)

Table 1. Human fibrocytes surface and intracellular phenotype. Reviewed in Bucala et al., 1994, Chesney et al., 1998, Abe et al., 2001, Hartlapp et al., 2001

It was previously reported that the differentiation of fibrocytes is inhibited by serum amyloid P (SAP), a major constituent of serum, (Pilling et al., 2003) and more recently it was described that in the absence of serum the process of differentiation of fibrocytes can be accelerated with cells with the spindle-shaped morphology appearing in culture after only ~2-5 days, compared to ~8-14 days when fibrocytes are cultured with serum supplemented medium (Curnow et al., 2010). They also reported a difference in the ability of serum free and serum complemented fibrocytes to differentiate from PBMC and CD14+ peripheral blood cells, with more efficient generation of fibrocytes from PBMC cultured without serum, and from CD14+ cells when these were cultured in the presence of serum complemented medium (Curnow et al., 2010).

Cell population obtained regardless of the initial method for enrichment (PBMC or CD14+ enriched cell culture) are cells expressing a combination of CD45 or other haematopoietic markers (CD34, CD11b), as well as collagen I and III, with an elongated spindle-shaped morphology, making clear that the cells differentiated under both conditions can be classified as fibrocytes, based on the current definition: spindle-shaped cell that expresses both haematopoietic and mesenchymal cell markers, (Bucalla et al., 1994, C. Metz 2003),

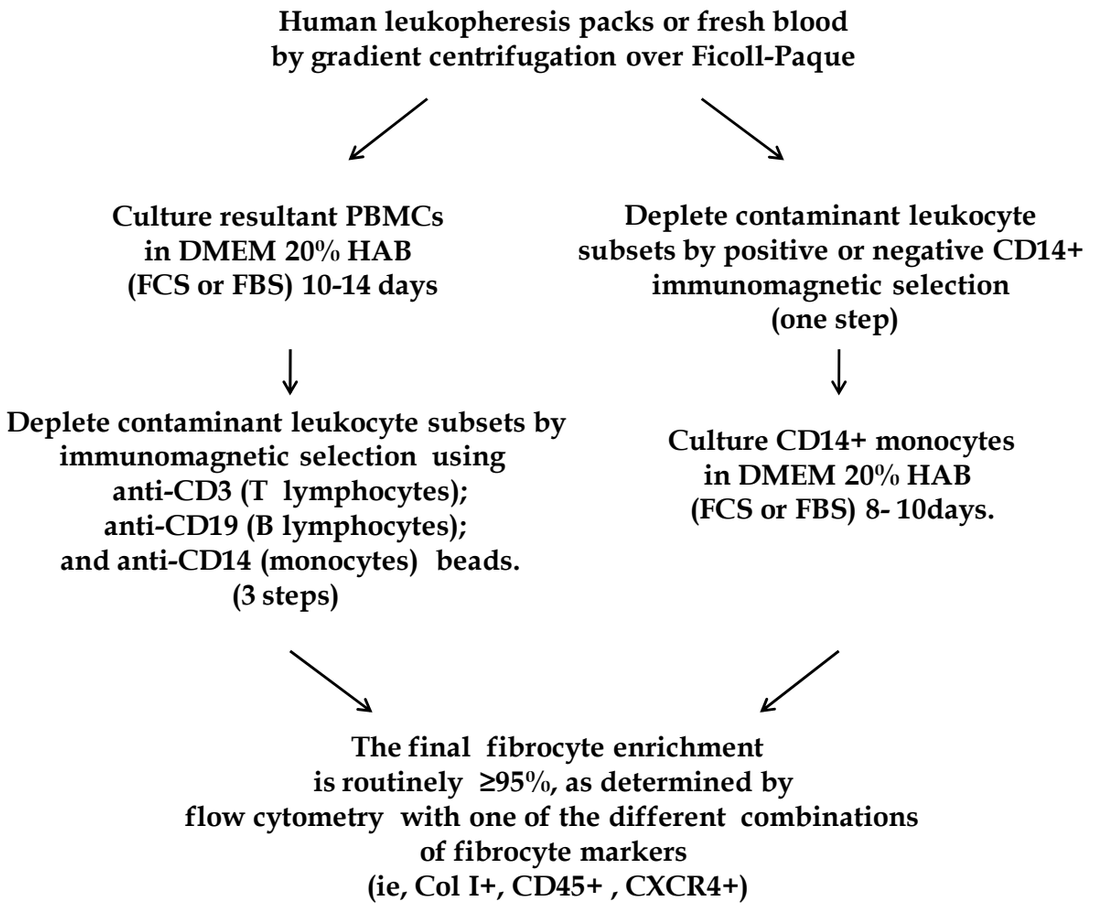

Fig. 1. Schematic description of the two most common methods for fibrocytes culture and enrichment.

3.1 Flow cytometry analysis

Flow cytometry is a critical technique for the characterization and quantification of circulating fibrocytes after their enrichment and in vitro differentiation as well as for fibrocytes obtained directly from fresh blood samples.

Cell preparation. Flow analysis requires a single cells suspension. Ice cold 0.05% EDTA in PBS or trypsin-EDTA 0.05% are recommended to detach the cells from the plastic surface, just covering it for 1-2 min at 37° C. Since trypsin is toxic for the cells, they must be observed closely to adjust and change the timing of the trypsin digestion. Immediately, media complemented with 10% serum is added to neutralize the enzymatic activity of the trypsin present in the buffer (normal human AB serum, FCS or FBS). Horizontal shear force can be applied, or cells can be gently scraped if needed for harvesting and they are immediately washed in cold PBS. The number of dead cells should be estimated by trypan blue exclusion.

Number of cells required for staining. Approximately 2.5-5 x 10^5 cells with a minimum volume of 300ul of staining buffer (1% BSA-PBS) in polystrene tubes 12X75 are needed for the analysis of the in vitro cultured fibrocytes; fewer cells mean longer collection time and potentially more background noise. For the analysis of circulating fibrocytes from fresh blood samples, ~ 0.5- 1 × 10^6 cells in 300ul of staining buffer (1% BSA-PBS) in polystrene tubes 12X75 are needed, since the normal percentage of this cells in the circulation is 0.1-0.5% of the total leukocytes it is better to analyze at least 50,000 events, the use of high performance flow cytometers is recommended.

Protocol for staining cell surface and intracellular antigens for fibrocytes analysis. The following steps are the same for both cell types (fresh PBMC´s or cultured cells).

Cells are centrifuged and resuspended in staining buffer (1% BSA-PBS). The optimum amount of buffer to incubate depends on the protocol suggested by the antibody´s manufacturer technical data sheet, commonly 100µl is an adequate volume. Cells are incubated with the corresponding fluorochrome-labeled antibodies for surface markers (i. e., CD45, CD34, CD11b, and CXCR4) and then fixed and permeabilized with a commercial kit recommended for this purpose (i.e., BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit, BD Biosciences) prior to staining with the anti-collagen antibody or its corresponding isotype control. It is important to consider that isotype controls are critical in the analysis of these cells, since they have to be permeabilized and fixed and a high percentage of nonspecific binding can occur. Also non-stained cells treated with the same process are required as control to discriminate collagen fibers autofluorescence. The number of markers that can be analyzed depend on the capacity (i.e., lasers, filters) of the cytometer to be used, at least 2- 3 fibrocyte markers (i.e., CD45+/Collagen I or CD45+/CXCR4+/Collagen I) are needed to meet the minimum criteria of the fibrocytes definition.

3.2 Fibrocyte to myofibroblast differentiation

Fibrocytes increase the expression of α-smooth muscle actin (α-SMA) spontaneously in culture, and gradually loose the expression of CD34 and CD45 over time, which likely reflects terminal differentiation or other phenomena related specifically to a particular tissue microenviroment (Schmidt et al., 2003, Mori et al., 2005, Bucala, 2008). The differentiation of

fibrocytes into myofibroblasts can be enhanced by transforming growth factor (TGF)-β or endothelin-1, which results in an increment in the synthesis of collagen and the myofibroblast marker α–SMA (Schmidt et al., 2003; Bucala, 2008).

For myofibroblast differentiation as described in the literature (Hong et al., 2007): a population of enriched fibrocytes has to be previously obtained with one of the techniques described above. The percentage of enrichment needs to be verified by flow cytometry in each culture to ensure reproducibility of the results.

Fibrocytes are treated with serum-free DMEM with 10 ng/ml TGF-β1 for 3 weeks, refreshing TGF-β1 supplemented medium every 48-72hs. If the objective is to analyze changes in the pattern of gene and protein expression, time curves should be performed previously since these effects might be different depending on the gene or protein of interest.

The signaling pathways that are activated by TGF-β1 to induce α-SMA transcription and thus fibrocyte differentiation to myofibroblast-like cells include Smad2/3 and stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK) - mitogen-activated protein kinase (MAPK). Interestingly, it was reported that treatment with troglitazone (TGZ, a synthetic agonist of peroxisome proliferator-activated receptor gamma: PPARγ), inhibits TGF-β1 induced α-SMA expression and this effect is modulated through attenuation of the SAPK/JNK activity leading to decreased Smad2/3 levels and transactivation activity, (Hong et al., 2007).

3.3 Adipocyte differentiation

Hong et al., demonstrated in a model of differentiation of human circulating adipogenic progenitors to adipocytes in SCID mice, that fibrocytes, in the presence of specific environmental characteristics can give rise to adipocytes. By gene microarray analysis they found a significant up-regulation of specific mature adipocyte genes and proteins after fibrocyte differentiation to adipocyte, including fatty acid binding protein 4 (FABP4), leptin, and PPARγ; remarkably certain genes, such as those involved in cell motility, chemotaxis, or metalloproteinase activity where also upregulated in the process of differentiation to adipocytes. These findings indicate that fibrocytes may retain unique functions for motility and chemoattractive activity that might allow them to participate in migration and trafficking despite their differentiation into adipocytes (Hong et al., 2005). Differentiation of fibrocytes into adipocytes appears to be mediated by PPARγ that leads to lipid accumulation and induction of aP2 gene expression (Rival et al., 2004). By contrast, this process is inhibited by TGF-β through SAPK/JNK pathway activation (Hong et al., 2007).

For adipocyte differentiation: fibrocytes are treated with PBM culture media (Cambrex Bio Science) supplemented with 10 M troglitazone. Culture media has to be changed every 48 h for 21 days. Following 21 days in culture, the cells accumulate lipids in intracellular vacuoles. Oil Red O Staining can be used to confirm fibrocytes differentiation to adipocytes (Hong et al., 2007).

3.4 Osteoblast and chondrocyte differentiation

Osteoblasts and chondrocytes, which are derived from a common mesenchymal precursor cell, are critical in bone and cartilage formation respectively (Knothe et al., 2010). It has

recently been reported that fibrocytes possess the ability to differentiate into chondrocytes and osteoblasts in vitro when the appropriate combination of cytokines and growth factors are used (Choi et al., 2010). These findings, taken together with their capacity to differentiate into myofibroblasts and adipocytes, indicate that fibrocyte may differentiate toward several types of mesenchymal cell types and that this process is influenced by a complex profile of cytokines within the local microenvironment of the host tissue or tissue injury.

Induction of the differentiation of fibrocytes to osteoblasts: Purified fibrocytes are seeded at a concentration of 1×10^5 cells/well in a fibronectin-coated 12 well plate, they are treated with osteogenic basal media (this media is commercially available) supplemented with dexamethasone, ascorbate, mesenchymal cell growth supplement (MCGS), l-glutamine, 1× Penicillin/Streptomycin, and β-glycerophosphate. β-glycerophosphate is critical to stimulate calcified matrix formation in combination with the effects of dexamethasone and ascorbate. Cells are cultured during 21 days with media replacement every 3 days (Choi et al., 2010).

Induction of the differentiation of fibrocytes to chondrocytes: Purified fibrocytes are seeded at the concentration of 5×10^4 cells/tube in 15ml sterile polypropylene tubes, followed by centrifugation at ~300×g for 10min to form pellets. Supernatant has to be carefully removed in order not to disrupt the fibrocyte micromass pellet. Fibrocytes are additioned with chondrogenic differentiation cocktail: basal chondrogenic media (also commercially available) supplemented with 1 x 10^{-7}M dexamethasone, 0.1 M ascorbate, l-glutamine, Penicillin/Streptomycin, 1 M sodium pyruvate, proline and 10 ng/ml of TGF-β3. Cells are cultured for 21 days with media replacement every 2 - 3 days (Barry et al., 2001; Choi et al., 2010). Fig. 2.

4. Fibrocytes participation in repair processes

Wound repair is a complex process that results from the coordinated release of cytokines, chemokines, and growth factors, leading successively to the recruitment and activation of different cells into the injured site from the very initial phases of repair (Gurtner GC et al., 2008). Fibrocytes have been postulated as important players of the tissue repair process since they have the ability to rapidly home to sites of tissue together with the infiltrating inflammatory cells that act to prevent infection and degrade damaged connective tissue components (Bucala et al., 1994).

Fibrocytes secrete proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin (IL)-6, IL-8, IL-10, macrophage inflammatory protein-1α/β (MIP-1α/β) CC-chemokine ligands (CCL) -3 and -4 in response to IL-1β which is an important mediator of wound healing response (Chesney et al., 1998). The fibrocyte products MIP-1α , MIP-1β, and monocyte chemotactic protein-1 (MCP-1) are potent T cell chemoattractants and may act to specifically recruit CD4+ T cells into the tissue repair microenvironment; moreover, the fibrocytes increase the cell surface expression of leukocyte adhesion molecules, such as intercellular adhesion molecule 1 (ICAM1), which would enhance leukocyte trafficking (Chesney et al., 1998). Interestingly, in addition to these functions, fibrocytes may play an early and important role in the initiation of antigen-specific immunity. Thus, it has been demonstrated that peripheral blood fibrocytes: express the surface proteins required for antigen presentation, including class II major histocompatability complex molecules: HLA-

DP, -DQ, and –DR; the costimulatory molecules CD80 and CD86, and the adhesion molecules CD11a, CD54, and CD58. Fibrocytes are potent stimulators of antigen-specific T cells in vitro, and migrate to lymph nodes and sensitize naïve T cells in situ (Chesney et al., 1997). Likewise, fibrocytes may also participate in the development of the innate immune response; in porcine models, specific in vitro stimulation of fibrocytes for TLR 2, 4, 7 or TLR3 leads rapidly to the translocation of the NF-kB transcription factor and the production of high levels of IL-6 (Balmelli et al., 2007); on the other hand, exposure to innate immune stimulation in the form of TLR agonists induces an increased expression of MHC class I and II molecules and of the co-stimulatory proteins CD80 and CD86 on fibrocytes, which enables these cells to function as antigen-presenting cells for the activation of cytotoxic CD8+ T cells. All these findings indicate that fibrocytes may recognize a large variety of pathogens such as viruses or bacteria and could be part of the initiation of innate immune responses (Balmelli et al., 2005 and 2007).

Blood vessel formation during normal physiological processes, such as wound healing, is highly regulated by a delicate balance between pro- and antiangiogenic factors. As mentioned, circulating fibrocytes have been shown to migrate to early wound sites where angiogenesis occurs, fibrocytes produce and secrete active matrix metalloproteinase 9 and 2 (MMP-9: gelatinase B; MMP-2: gelatinase A) (Hartlapp I et al., 2001, García-de-Alba et al., 2010), which are implicated in the proteolysis of the basement membrane early during the invasion stage of angiogenesis. In addition, cultured fibrocytes constitutively secrete vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), insulin growth factor (IGF-I) and hematopoietic factors as granulocyte monocyte-colony stimulating factor (GM-CSF) that induce endothelial cell migration, proliferation, and alignment of endothelial cells into tubular-like structures in vitro. In like manner cultured fibrocytes (and fibrocyte-conditioned media) showed the ability to promote angiogenesis in vivo using a Matrigel implant model, (Hartlapp I et al., 2001).

Interestingly, it has been reported that Th2 cytokines (IL-4 and IL-13) induce, whereas Th1 cytokines (IFN-γ and IL-12) inhibit the CD14+ monocyte to fibrocyte differentiation. When added together the profibrocyte activities of IL-4 and IL-13 and the fibrocyte-inhibitory activities of IFN-γ and IL-12 counteract each other in a concentration-dependent form. By contrast, the fibrocyte-inhibitory activity of the plasma protein serum amyloid P (SAP) dominates over the profibrocyte activities of IL-4 and IL-13. These results might indicate that the complex mix of cytokines and plasma proteins present in inflammatory lesions, wounds, and fibrosis will influence fibrocyte differentiation (Shao et al., 2008). Consistent with this data, it was recently reported that CD14+ monocytes can differentiate in vitro into two different subtypes of fibrocytes depending on the presence or absence of serum in the culture media, which could resemble the changes in serum protein concentrations that occur during tissue repair, inflammation and its resolution (Curnow et al., 2010).

Fibrocytes also contribute to normal wound healing by serving as the contractile force of wound closure via α-smooth muscle actin expression (Abe et al., 2001; Metz, 2003), and secreting components of the extracellular matrix (collagen I, collagen III, fibronectin) (Abe et al., 2001; Bucala et al., 1994). Interestingly, it has been reported that the capacity to produce collagen of fibrocytes from normal subjects or from burn patients is less than that of fibroblasts (dermal and lung fibroblasts) (Wang et al., 2007; García-de-Alba et al., 2010),

which raises the question if fibrocytes main contribution to the process of tissue repair is only a direct participation in the production of ECM components. In this context, it is important to emphasize that fibrocytes secrete paracrine growth factors such as connective tissue growth factor (CTGF), PDGF, FGF and TGF-β1 that induce proliferation, migration and differentiation of fibroblasts to myofibroblasts in culture (Chesney et al., 1998, Wang et al., 2007). These findings suggest that the predominant role of fibrocytes in scarring could be the regulation of the functions of local fibroblasts.

Proteins secreted by fibrocytes	**Pattern of Expression**
Growth factors	
Platelet-Derived Growth Factor A (PDGF-α)	Constitutive
Fibroblast Growth Factor basic (bFGF)	
Granulocyte-Monocyte Colony Stimulating Factor (GM-CSF)	
Insulin Growth Factor 1 (IGF1)	
Vascular Endothelial Cell Growth Factor (VEGF)	
Transforming Growth Factor-beta1 (TGF-β1)	
Connective Tissue Growth Factor (CTGF)	
Hepatocyte Growth Factor (HGF)	
Cytokines	
Tumoral Necrosis Factor-alpha (TNF-α)	Induced by IL-1β stimulation
IL-6	Induced by IL-1β or TNF-α stimulation
IL-10	Induced by IL-1β or TNF-α stimulation
IL-1α	Constitutive
Chemokines	
IL-8	Constitutive; ↑ with TGF-β1 or IL-1β
GROα	
MIP-1α	
MIP-1β	
MCP-1	
MMPs	
MMP-2	Constitutive; ↑ with TGF-β1
MMP-9	
MMP-7	Constitutive; ↓ with TGF-β1
MMP-8	

Table 2. Fibrocytes pattern of expression for diverse proteins.

4.1 Migration and homing

Fibrocytes trafficking from the bone marrow and circulation to the organs or site of lesion is given through several chemokines. Human fibrocytes express diverse chemokine receptors, including CCR3, CCR5, CCR7, and CXCR4; whereas mouse fibrocytes express CXCR4, CCR7, and CCR2 (Abe et al., 2001; Phillips et al., 2004; Moore et al., 2005; Mehrad et al., 2009). Secondary lymphoid tissue chemokine (SLC/CCL21) and its receptor CCR7 was the first chemokine-chemokine receptor system described to induce the recruitment of fibrocytes as a mechanism of migration to wound sites (Abe et al., 2001). In humans as in mice CCL21 is constitutively abundant in lymphoid tissues, particularly in the lymph nodes and spleen but it is also expressed at lower levels in some non-lymphoid tissues, including the kidneys and lungs (Gunn et al., 1998; Abe et al., 2001; Sakai N et al., 2006).

CXCR4 is an important chemokine receptor for stem and immune cell migration, high levels of CXCL12, which is the only known ligand for CXCR4, were found in the lungs and plasma of patients with IPF and these levels correlated with circulating fibrocyte concentrations (Mehrad B et al., 2007; Andersson-Sjöland A et al., 2008).

Recently (Mehrad et al., 2009) reported that most (but not all) freshly isolated human fibrocytes expressed CXCR4, whereas 46% expressed CCR2 and 9% expressed CCR7. Approximately 30% were CCR2/CXCR4+ and most CCR7+ cells also expressed CCR2, but there was no overlap between CXCR4+ and CCR7+ receptors.

It has been reported an association between serum concentration of MCP-1 and high levels of CD45/pro-Col-I+ fibrocytes in the circulation of scleroderma patients with interstitial lung disease (ILD) or in healthy aging subjects, suggesting that MCP-1 may be also involved in mobilization of fibrocytes into the peripheral blood. (S. Mathai et al., as cited in Herzog & Bucala , 2010),

Thus, fibrocytes can use different chemokine–chemokine receptor axis for tissue homing and this might be related to the type of process (acute or chronic) or to the organs involved; however, the mechanisms implicated in the migration through basement membranes and extracellular matrix and subsequent tissue homing remain unclear. In this context, it was recently reported that fibrocytes express several MMP's (MMP- 2, 7, 8 and 9) (Fig 3) that may facilitate the process of migration of fibrocytes from the circulation to the tissues in response to chemokine gradients (Garcia-de-Alba et al., 2010). In this work it was showed that fibrocytes transmigration towards CXCL12 or PDGF through collagen I coated migration chambers, was highly associated with the collagenase MMP8, while migration through a combination of proteins of basal membrane was facilitated by gelatinases MMP2 and MMP9. Thus, these MMPs may ease cell migration by breaking down matrix barriers or affecting the state of cell-matrix interactions and also may play an important role in the remodelling of ECM. Interestingly, PDGF showed to be a more potent chemotactic agent when migration was given through collagen I coated chambers, possibly indicating that when fibrocytes have arrived to lung interstitium, PDGF plays an important role as a chemoattractant through lung parenchyma.

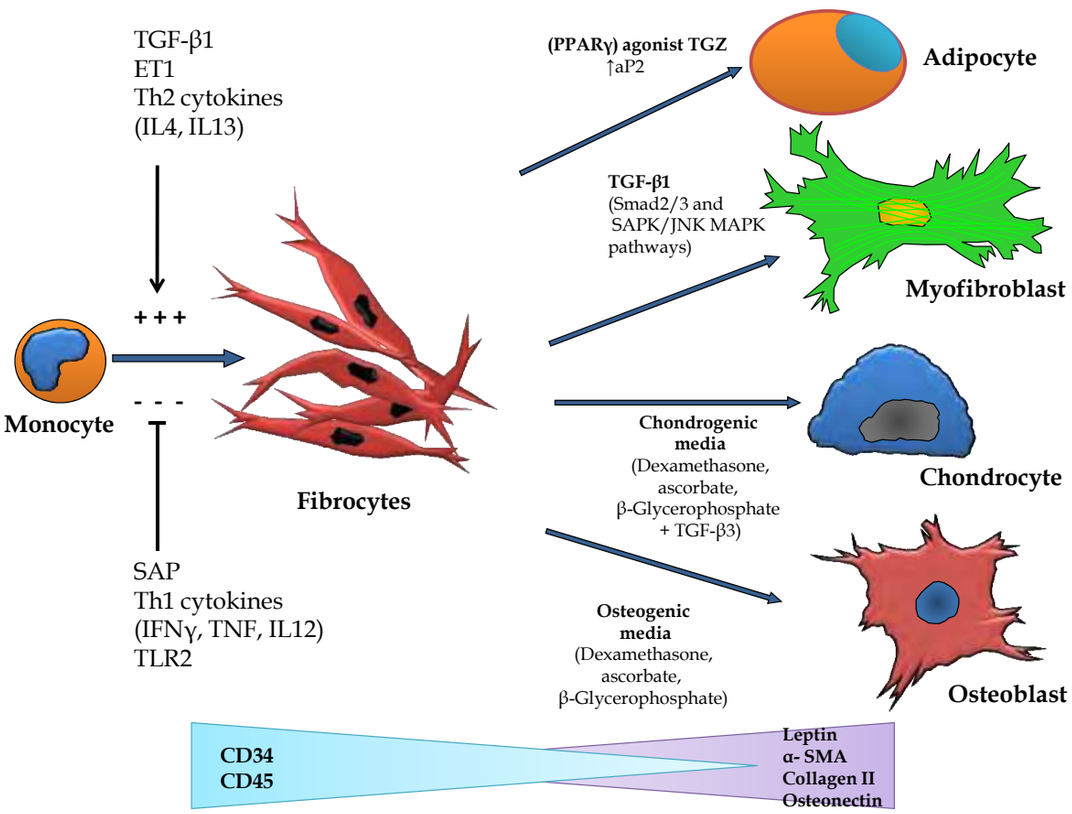

Fig. 2. Schematic summary of the mediators and inhibitors of CD14+monocyte to fibrocyte differentiation, and fibrocytes differentiation to other mesenchymal cells. TGZ: trogliotazone. A crosstalk between PPARγ and TGF-β1 exists, where they can strongly inhibit each other signaling, making clear that a complex and critical balance exists between both of them. It is noteworthy that the expression of hematopoietic markers decrease as fibrocytes differentiate into other mesenchymal cells, while specific markers for that given cell increase their expression during differentiation.

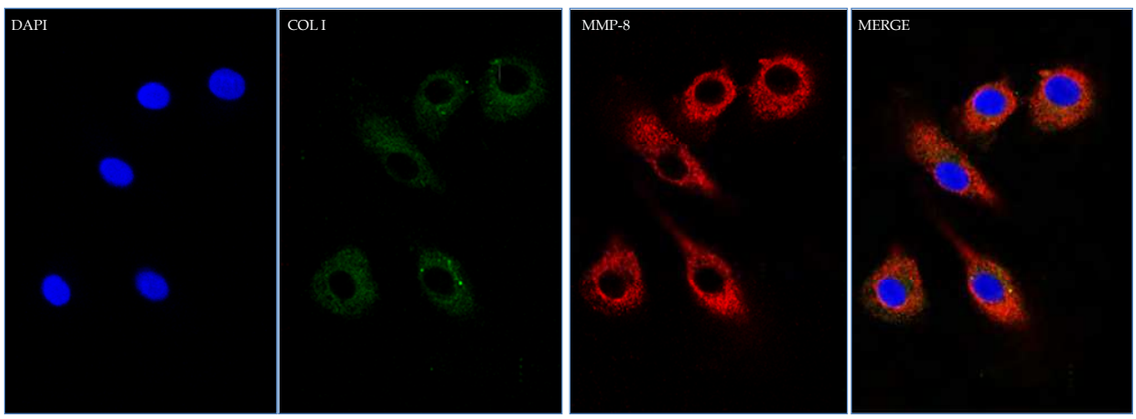

Fig. 3. Fluorescent immunocytochemistry showing a group of fibrocytes positive for collagen I and MMP-8 staining.

5. Role of fibrocytes in the pathogenesis of fibrotic disorders

In contrast to acute inflammatory reactions, which are characterized by rapidly resolving events; fibrosis typically results from chronic unsolved inflammation or aberrant epithelial activation (King, Pardo & Selman, 2011). Despite having distinct etiological and clinical manifestations, fibrotic remodelling is characterized by fibroblast/myofibroblast activation, and excessive extracellular matrix accumulation leading to scarring formation and progressive dysfunction of a given organ.

Fibrocytes have become the focus of research of a wide variety of focal and diffuse fibrosing disorders in diverse organs including lung, heart, liver, and kidney (Barth et al., 2005; Sakai et al., 2006, 2008, 2010; Andersson-Sjöland et al., 2008; Scholten et al., 2011); primarily because of their ability to home into tissues and secret extracellular matrix components. More recently however, a large and varied amount of new knowledge about fibrocytes biology has emerged, rising new hypothesis that have enriched the understanding of these cells and their participation in fibrotic diseases.

5.1 Pulmonary fibrosis

Pulmonary fibrosis is the final result of a numerous and heterogeneous group of disorders known as interstitial lung diseases (ILD). Lung fibrotic remodeling is characterized by fibroblast/myofibroblast activation, and excessive extracellular matrix accumulation leading to progressive destruction of the lung architecture and usually terminal outcome (Pardo & Selman, 2002). Idiopathic pulmonary fibrosis (IPF), the most common form of the idiopathic interstitial pneumonias, is a chronic, progressive, irreversible, and usually lethal lung disease of unknown cause (King, Pardo & Selman 2011). IPF is characterized by the presence of clusters of fibroblasts and myofibroblasts circumscribed from surrounding cells (fibroblastic foci), which represent sites of active fibrogenesis (Selman, King & Pardo, 2001).

During a long time, proliferation of local (resident) fibroblasts and differentiation to myofibroblasts were considered the main source of extracellular matrix deposition in pulmonary fibrosis. The first report of the possible participation of mesenchymal stem cells in the pathogenesis of pulmonary fibrosis, described that collagen-producing cells with fibroblast characteristics were derived from BM progenitor cells, in a model of bleomycin induced pulmonary fibrosis (Hashimoto et al., 2004). The mice in this model were engrafted with BM from GFP transgenic mice that allow to easily follow the fate of these BM-derived cells. Though this group did not prove that these cells were actually fibrocytes, they recognize the possibility of this premise. Not much later, a work that showed that human CD45+Col I+CXCR4+ circulating fibrocytes were able to migrate to the lung of mice treated with bleomycin was published (Phillips et al., 2004). These authors also described that maximal intrapulmonary recruitment of CD45+Col I+CXCR4+ fibrocytes directly correlated with increased collagen deposition in the lungs. Likewise, they identified a second fibrocyte population that is CD45+Col I+CCR7+ and also traffics to the lungs of bleomycin-treated mice; interestingly the absolute number of CCR7+ fibrocytes found in the fibrotic lung was two to three fold lower than the number of CXCR4+ fibrocytes present under similar conditions, indicating that CXCR4 predominates for the recruitment of fibrocytes to injured lungs (Phillips et al., 2004).

Fibrocyte recruitment to damaged lungs has been proved to be mediated by several chemokine/chemokine receptor interactions. Thus, in a model of fluorescein isothiocyanate (FITC)-induced lung fibrosis, it was demonstrated that significantly higher numbers of fibrocytes are present in the airspaces of fluorescein isothiocyanate-injured $CCR2^{+/+}$ mice compared to $CCR2^{-/-}$ mice (Moore et al., 2005; 2006). Fibrocytes isolated from the lung expressed CCR2 and migrated toward CCL2 and CCL12 ligands. Interestingly, CCL2 stimulated collagen secretion by lung fibrocytes, which differentiated towards a myofibroblast phenotype, transition that was associated with loss of CCR2 expression (Moore et al 2005).

Importantly, interruption of the chemokine axis attenuated both fibrocyte accumulation and pulmonary fibrosis (Phillips et al., 2004; Moore et al., 2006), strengthening the notion that these chemokine/chemokine receptor axis are the main responsible of fibrocytes trafficking to the lungs; however, under which biological/pathological conditions one or other chemokine/chemokine receptor system is activated, or if they represent redundant mechanisms, yet remains to be elucidated.

Recently several independent research groups have identified fibrocytes in different forms of fibrotic human lung disease. In an initial study, it was reported that circulating fibrocytes expressing CXCR4 and both lung and plasma levels of CXCL12 were elevated in IPF patients (Mehrad 2007). CXCL12 levels showed a positive correlation with higher number of circulating fibrocytes in the peripheral blood of these patients. Later, Andersson-Sjöland et al., evaluated the presence of fibrocytes in the lung of patients with idiopathic pulmonary fibrosis by immunofluorescence and confocal microscopy. Fibrocytes were identified with different combinations of markers in most fibrotic lungs; interestingly, no fibrocytes were identified in normal lungs. They also found a positive correlation between the abundance of fibroblastic foci and the amount of lung fibrocytes and a negative correlation between plasma levels of CXCL12 with lung function tests (lung diffusing capacity for carbon monoxide and oxygen saturation on exercise) (Andersson-Sjöland et al., 2008). These findings indicate that circulating fibrocytes may contribute to the expansion of the fibroblast/myofibroblast population in idiopathic pulmonary fibrosis.

On the other hand, as mentioned earlier in this chapter, fibrocytes constitutively synthesize and release to the medium important amounts of MMP-2, MMP-7, MMP-8, and MMP-9 (García-de-Alba et al., 2010).

MMPs consist of a large family of zinc endoproteases, collectively capable of degrading all ECM components (Pardo et al., 2006). However, ECM represents only a fraction of their proteolytic targets, and moreover, a given MMP can act on various proteins and, in turn, affect a variety of processes. Gelatinases (MMP-2 and MMP-9) have been found up-regulated in human pulmonary fibrosis and animal models of lung fibrosis (Swiderski et al., 1998; Selman et al., 2000; Oikonomidi et al., 2009). The overexpression of MMP-2 and MMP-9 has been mainly associated with their capacity to provoke disruption of alveolar epithelial basement membrane and enhanced fibroblast invasion into the alveolar spaces (Ruiz et al., 2003; Pardo et al., 2006). In the case of fibrocytes, these MMPs may facilitate the process of migration from the circulation to the interstitial and alveolar spaces in response to SDF-1/CXCL12 synthesized by alveolar epithelial cells (Andersson-Sjöland et al., 2008; García-de-Alba et al., 2010). TGF-β1–stimulated fibrocytes significantly increase gene and protein

expression of both MMP-2 and MMP-9 in vitro. Another putative pathogenic role of these two enzymes is that cell surface localized MMP-2 and MMP-9 can activate latent TGF-β, and this constitutes a mechanism that may operate in normal tissue remodeling as well as in fibrosis, tumor growth, and invasion (Yu et al., 2000). Fibrocytes also synthesize MMP-7 and MMP-8; the presence of MMP-7 is interesting because this metalloproteinase has been associated with pulmonary fibrosis since is one of the most up-regulated genes in IPF and display several profibrotic activities (Zuo et al., 2002, Pardo et al., 2005). Moreover, MMP-7 and MMP-1 have been related to alveolar and bronchiolar cell migration over different matrices during IPF lung remodeling (Oikonomidi, 2009). In addition, MMP-7 cleave E-cadherin, which may influence several aspects of cell behavior, such as epithelial-to-mesenchymal transition, which is a well-recognized event that recently has gained importance as a mechanism in the pathogenesis of fibrosis (Lochter et al.,1997; Noe et al., 2001; Hinz et al., 2007). MMP-8 or collagenase-2 specifically degrades fibrillar collagen types I, II and III, and is known to play an important regulatory role in both acute and chronic inflammation (Prikk et al., 2001). In the context of fibrocytes it seems to have an important role in the transmigration of these cells through collagen I (García-de-Alba et al., 2010).

Recently it has been suggested that circulating fibrocytes could have a role as biomarkers for disease severity in IPF; Moeller and coworkers quantified circulating fibrocytes from patients with IPF and found that high percentages of these cells in blood were predictive of poor clinical outcomes; they compared fibrocyte levels in peripheral blood from patients with idiopathic pulmonary fibrosis (stable and during an exacerbation), patients with acute respiratory distress syndrome, and normal controls. Fibrocytes were significantly elevated in patients with stable idiopathic pulmonary fibrosis compared with normal controls, but showed a prominent increase during acute exacerbations of the disease. The number of fibrocytes in patients with acute respiratory distress syndrome was not significantly different from patients with stable idiopathic pulmonary fibrosis or normal controls (Moeller et al., 2009). These data suggest that serial measurements of fibrocyte percentages may predict acute exacerbations (Moore, 2009). This work was the first to bring up the notion that fibrocytes measurements may be a useful biomarker in this disease but larger studies are needed to confirm this hypothesis.

Finally, a recently published work exploring senescence-accelerated prone mice found increased levels of CXCR4 expressing fibrocytes in the blood of these mice when compared to wild type controls. The senescence-prone mice also displayed increased lung fibrosis when exposed to intratracheal bleomycin, suggesting the possibility that the increased number of fibrocytes contributed to disease. This is an interesting observation since IPF is considered an age related disease (Selman M et al., 2010). Actually, unpublished data from Mathai et al (Mathai et al., as cited in Herzog & Bucala 2010) indicates that the blood of healthy aged individuals contain increased concentrations of CD45+/Col-1+ fibrocytes and high circulating levels of MCP-1 and IL-13, suggesting that fibrocytes may be associated with certain aging processes.

5.2 Asthma

Asthma is an inflammatory disorder of the conducting airways which undergo distinct structural and functional changes, leading to non-specific bronchial hyper-responsiveness

(BHR) and airflow obstruction. It is among the commonest chronic conditions in Western countries affecting 1 from 7 children and 1 from 12 adults (Holgate et al., 2009).

It has long been known that architectural and structural remodeling occur in the airways of asthmatic patients. These changes include increased collagen (type III and IV) and fibronectin deposition, increased thickness of subepithelial basement membrane, angiogenesis, and fibrosis. All these processes collectively contribute to severe alterations of the normal bronchial architecture in response to the inflammatory tissue injury, leading to progressive airway obstruction and a permanent impairment in respiratory function (Holgate et al., 2009, Hamid & Tulic 2009). Pathologic examination of these tissues demonstrates subepithelial fibrosis and myofibroblast accumulation. Fibrocytes have been identified in the airways of patients with asthma, and it has been reported that allergen exposure induced an increment of fibrocyte-like cells in the bronchial mucosa of patients with allergic asthma (Shmidt et al., 2003). In a mouse model of allergic asthma, fibrocytes were recruited into the bronchial tissue following allergen exposure and differentiated into myofibroblasts providing evidence for the first time that these cells might be a source of myofibroblasts in allergic asthma (Shmidt et al., 2003). Nihlberg and his group showed that fibrocytes in patients with mild asthma were primarily localized, either individually or in clusters, close to the epithelium and to blood vessels. Fibrocyte numbers correlated to the thickness of the basement membrane, supporting that these cells may participate in airway wall remodeling. The increase number of fibrocytes expressing α-SMA seen in patients with increment in the basement membrane thickness may indicate a more differentiated phenotype (Nihilberg et al., 2006). More recently, in two different works, fibrocytes percentages in peripheral blood were shown to be increased in patients with asthma with chronic airway obstruction and severe refractory asthma (Saunders et al., 2008; Chun-Hua et al., 2009). Additionally, a yearly decline in lung function has been significantly associated with the percentage of circulating fibrocytes in patients with chronic obstructive asthma (Saunders et al., 2008).

5.3 Renal fibrosis

Renal tubulo-interstitial fibrosis is a non-specific process, representing the common end-stage for kidney diseases, regardless of their etiology. The histological characteristics include the presence of tubular atrophy and dilation, interstitial leukocyte infiltration, accumulation of fibroblasts, and increased interstitial matrix deposition (Strutz et al., 2006). Fibrocytes have also been implicated in the pathogenesis of renal fibrosis in diverse models. For example, in an experimental model of unilateral ureteral obstruction, fibrocytes appeared in injured parenchyma in a time dependent fashion. Thus, a remarkable number of fibrocytes dual-positive for CD45 or CD34 and type I collagen infiltrated the interstitium, reaching a peak at day 7. Morphological interstitial fibrosis and collagen content were reduced by almost 50% in mice treated with anti-CCL21 antibodies 7 days after ureteral ligation. A similar reduction was observed in CCR7-null mice (Sakai et al., 2006). Interestingly, most fibrocytes were positive for CCR7 and CCL21, and the blockade of CCR7 reduced the number of infiltrating fibrocytes indicating that for this organ, CCR7/CCL21 might be the main recruitment axis. The same investigators showed later that fibrocytes might contribute to fibrosis by an angiotensin II dependent pathway (Sakai et al., 2008). Using two models of renal fibrosis (unilateral ureteral obstruction and chronic angiotensin II infusion), angiotensin II type 2 receptor (AT2R)-deficient mice developed increased renal fibrosis and fibrocyte infiltration and a concomitant

upregulation of procollagen type I compared with wild-type mice. Pharmacologic inhibition of angiotensin II type 1 receptor (AT1R) with valsartan reduced the degree of renal fibrosis and the number of fibrocytes in both the kidney and the bone marrow. In isolated human fibrocytes, inhibition of AT2R signalling increased the angiotensin II-stimulated expression of type I collagen, whereas inhibition of AT1R decreased collagen synthesis. These results suggest that AT1R/AT2R signalling may contribute to the pathogenesis of renal fibrosis by at least two fibrocytes-related mechanisms: by regulating the number of fibrocytes in the bone marrow, and by activation of these cells in the tissues (Sakai et al. 2008).

More recently, the presence of fibrocytes was investigated by immunohistochemistry in kidney biopsy specimens from 100 patients with chronic disease; in addition 6 patients with thin basement membrane disease were studied as a disease control. In patients with chronic kidney disease, the infiltration of fibrocytes was observed mainly in the interstitium and their numbers were higher than that in patients with thin basement membrane disease. Moreover, there was an inverse correlation between the number of interstitial fibrocytes and kidney function at the time of biopsy (Sakai et al., 2010). These results suggest that fibrocytes may be involved in the pathogenesis of human chronic kidney disease though the mechanisms involved in their participation are yet to be studied.

CD34+spindle-shaped cells have also been detected in tubulointerstitial lesions in patients with renal interstitial fibrosis. Although in this work the complete phenotype corresponding to fibrocytes was not documented, it is possible that the described CD34+ cells were actually fibrocytes (Okona et al., 2003).

5.4 Liver fibrosis

Hepatic fibrogenesis represents a wound-healing response of liver to a variety of insults. The net accumulation of extracellular matrix (ECM) in liver injury arises from increased synthesis by activated hepatic stellate cells and other hepatic fibrogenic cell types, as well as from bone marrow and circulating fibrocytes (Guo & Friedman, 2007).

Fibrocytes participation in liver fibrosis is a growing field of research and has been assessed in different models. In a murine model of bile duct ligation-induced liver fibrosis, investigators found bone marrow derived collagen-expressing GFP+ cells in the liver of chimeric mice (Kisseleva et al., 2006). The majority of these bone marrow derived cells co-expressed collagen-GFP+ and CD45+, suggesting that collagen-producing fibrocytes were recruited from the bone marrow to the damaged liver (Kisseleva et al., 2006). Later, fibrocyte migration in response to liver injury was investigated using bone marrow (BM) from chimeric mice expressing luciferase (Col-Luc-wt) or green fluorescent protein (Col-GFP-wt) under control of the α1(I) collagen promoter and enhancer, respectively. Migration of CD45+Col I+ fibrocytes was regulated by chemokine receptors CCR2 and CCR1. In addition to CCR2 and CCR1, egress of BM CD45+Col I+ cells was regulated by TGF-β and liposaccharide in vitro and in vivo. Interestingly, development of liver fibrosis was also increased in aged mice and correlated with high numbers of liver fibrocytes (Kisseleva et al., 2011). However, it is unknown what proportion of tissue myofibroblasts/fibrocytes are derived from bone marrow or circulating fibrocytes, whether myofibroblasts of these origins transition through a stellate cell phenotype, and what happens to activated myofibroblasts from various sources when liver injury resolves (Guo & Friedman, 2007).

5.5 Cardiovascular disease

Deposition and remodeling of connective tissue in the heart plays a critical role in cardiac repair and response to injury. Fibrosis also occurs on a reactive basis around coronary vessels (perivascular fibrosis) and in the interstitial space (Haudek et al., 2006). It is generally considered that both reactive and reparative fibrosis may contribute to adverse remodeling. A number of studies have supported the contribution of bone marrow progenitor cells or fibrocytes to remodeling in diverse areas of the cardiovascular system where fibrotic response seems to be a common feature.

In a mouse model of fibrotic ischemia/reperfusion cardiomyopathy (I/RC) it was observed a prolonged elevation of MCP-1, and concomitantly a population of small spindle-shaped fibroblasts with a distinct phenotype appeared in the sites of lesion. These cells were highly proliferative and expressed collagen I and α-smooth muscle actin as well as CD34, and CD45; these cells represented 3% of all non myocyte live cells. Haudek and coworkers confirmed the bone marrow origin of these cells creating a chimeric mice expressing lacZ; I/RC injury resulted in a large population of spindle-shaped fibroblasts containing lacZ. Interestingly, the administration of SAP in vivo markedly reduced the number of proliferative spindle-shaped fibroblasts and completely prevented I/RC-induced fibrosis and global ventricular dysfunction (Haudek et al., 2006). Similar results were reported later, in a model induced by Ang-II. Ang-II infusion resulted in the appearance of bone marrow-derived CD34+/CD45+ fibroblasts that expressed collagen type I and the cardiac fibroblast marker DDR2 while local fibroblasts were CD34−/CD45−. Genetic deletion of MCP-1 (MCP-1-deficient mice) prevented the Ang-II-induced cardiac fibrosis and the appearance of CD34+/CD45+ fibroblasts. Interestingly, Ang-II-treated hearts showed induction of types I and III collagens, TGF-β1, and TNF mRNA expression. Apparently the differentiation of a CD34+/CD45+ fibroblast precursor population in the heart is induced by Ang-II and mediated by MCP-1 (Haudek et al., 2010).

Neointimal hyperplasia is a common feature of various cardiovascular diseases such as atherosclerosis, postangioplasty restenosis and transplant arteriopathy. Neointima usually consists of smooth muscle cells and deposited extracellular matrix. In an in vivo ovine model of carotid artery synthetic patch graft, circulating leukocytes were shown to express collagen and α-SMA. Importantly, these cells also expressed markers unique to fibrocytes (CD34, CD45, vimentin; Varcoe et al., 2006), suggesting an association between intimal hyperplasia and fibrocyte migration. In other work performed in a rat model of transplant vasculopathy, accelerated transplant vasculopathy was associated with increased levels of host-endothelial chimerism and increased neointimal smooth muscle cell proliferation; moreover, accelerated transplant vasculopathy was associated with increased frequency of circulating CD45+vimentin+ fibrocytes (Onauta et al., 2009).

CD34+ fibrocyte-like cells are detectable in normal mitral valves. In cases of myxomatous degeneration CD34+ fibrocytes make up the majority of mitral valve stromal cells (Barth et al., 2005). Since major factors in the development of myxomatous valve degeneration are the MMP-9 and collagen I and III, which are secreted by CD34+ fibrocytes, they propose that these cells might be involved in the pathogenesis of myxomatous mitral valve (Barth et al., 2005).

5.6 Skin disease

Fibrocytes are thought to play a role in skin repair by several mechanisms such as the secretion of ECM, antigen presentation, cytokine production, angiogenesis, and wound closure (Metz, 2003). After the original work by Bucala, several groups examined the participation of fibrocytes in the wound healing process. Mori and coworkers examined the phenotype of fibrocytes and myofibroblasts present in the wounded skin of BALB/c mice and observed that during wound healing, between 4 and 7 days post-wounding, more than 50% of the cells present at the site of injury were CD13+/collagen I+ fibrocytes that could be isolated at an early stage of the healing process from digested fragments of wounded tissue by fluorescence-activated cell sorting (Mori et al., 2005). Fibrocytes have been identified in postburn hypertrophic scar tissue but were absent from normal skin, moreover, the number of fibrocytes was higher in hypertrophic than in mature scar tissue (Yang et al., 2005). It is noteworthy that over time the expression of CD34 on fibrocytes present in these wounds decreases, whereas the expression of proline-4-hydroxylase (an enzyme involved in collagen synthesis) increases in both hypertrophic or keloid scars (Aiba and Tagami, 1997). This finding has been corroborated by other authors (Abe et al., 2001; Phillips et al., 2004) and it's an important feature to be considered for the analysis of these cells in organ fibrosis. In other words, it seems that fibrocytes, once in the tissues, progressively lose their typical markers and can be difficult to identify.

Also, the participation of fibrocytes in wound healing of human skin has been postulated as a useful marker for wound age determination in the legal pathology area. In an interesting study (Ishida et al., 2009) a double-color immunofluorescence analysis was carried out using anti-CD45 and anti-collagen type I antibodies to examine the time-dependent appearance of fibrocytes in 53 human skin wounds with different wound ages. Fibrocytes were initially observed in wounds aged 4 days, and their number increased in lesions proportionally with advances in wound age. These findings imply that human skin wounds containing fibrocytes are at least 4 days old. Moreover, a fibrocyte number of over 10 indicates a wound age between 9 and 14 days. Fibrocytes numbers, evaluated with these markers (CD45+/Col I+) showed a marked decrease from day 17 to 21 which was the longest time of evaluation, exposing the need to use other parameters to confirm the wound ages since fibrocytes numbers in day 4 were similar to numbers in day 17-21.

Yang and his group reported high percentages of fibrocytes present in the cultures of peripheral blood mononuclear cells obtained from burn patients compared with controls (89.7 +/- 7.9% versus 69.9 +/- 14.7%, $p < 0.001$) and this percentages were consistently higher in patients with more than 30% extent of burn; moreover, they found a positive correlation between the levels of serum TGF-β1 and the percentage of fibrocytes developed in the cultures of PBMC derived from these patients (Yang et al., 2002). Interestingly, it has been postulated that the principal role of fibrocytes in burn injury as well as in hypertrophic scars is the regulation of the function of local fibroblasts. Thus, dermal fibroblasts treated with conditioned medium obtained from burn patient fibrocytes, but not by those derived from normal subjects, showed an increase in cell proliferation and migration (Wang et al., 2007). Furthemore, it has been suggested that fibrocytes can be reprogrammed by changes in the culture media, and that this reprogrammed fibrocytes have the ability to increase cell proliferation and MMP-1 expression in dermal fibroblasts (Medina, A & Ghahar, A. 2010). These findings have opened a new research line worthy of follow up.

5.7 Nephrogenic systemic fibrosis

Fibrocytes have been also identified in the skin of patients with cutaneous fibrosing diseases, such as nephrogenic systemic fibrosis. Nephrogenic systemic fibrosis (NSF) is a recently described cutaneous fibrosing disorder that exhibits pathologic similarities with scleroderma but occurs exclusively in patients with renal insufficiency who have received gadolinium containing magnetic resonance contrast agents. The onset of the disease varies from days to several months following exposure to gadolinium-based contrast. It is a debilitating disease characterized by the development of discolored plaques on the skin of the extremities and trunk. Over time, contractures develop and complete loss of range of motion can occur (Cowper & Bucala, 2003; Cowper et al., 2008). Skin biopsies from patients with this disease have revealed an important accumulation of CD34, pro-Col-I+ fibrocytes in the dermis with abundant connective tissue matrix production; it is noteworthy that in vitro studies revealed that gadolinium may decrease the ability of endogenous mediators, such as SAP and IL-12, to inhibit fibrocyte outgrowth (Vakil et al., 2009). The reason for why fibrocytes are present in high numbers and are such a prominent feature of the dermatopathology of NSF remains unclear, but may be due to the acute and abrupt development of skin fibrosis (Bucala, 2008).

6. Opportunities for research and therapeutic targets

The study of fibrocytes and their participation in the pathogenesis of chronic inflammation and fibroproliferative diseases presents both important challenges and opportunities for researchers. To advance this field, detailed molecular characterization of these cells and establishment of defined experimental strategies in animals and humans will be necessary to catalyze progress in this area of investigation. Recent studies and emerging concepts have significantly improved our understanding of the participation of fibrocytes in health and disease and so have opened the door to new hypotheses and approaches aimed at therapeutic targets and strategies.

One of the main therapeutic targets, suggested since the initial works on fibrocyte biology research, was the serum amyloid P (SAP), a member of the pentraxin family of proteins. In this context, it was first demonstrated that SAP could inhibit the differentiation of monocytes into fibrocytes (Pilling et al., 2003). SAP binds to Fcγ receptors through which apparently mediates its anti-fibrotic activities affecting peripheral blood monocyte differentiation and activation states (Lu et al., 2008). In a rat model of bleomycin-induced lung injury it was shown that purified rat SAP could suppress development of lung fibrosis which correlated with reduced fibrocyte numbers within the lung tissue (Pilling et al., 2007). More recently, SAP ability to reduce fibrosis was tested in models of renal and lung fibrosis where this therapeutic potential was confirmed. In both models, the mechanisms through which SAP exerts its antifibrotic effect seemed to be independent of monocyte to fibrocyte differentiation (Casraño et al., 2009; Murray 2010). Further analysis of this molecule and its potential as antifibrogenic therapy is needed to identify all the mechanisms involved in its effect as well as the feasibility of its use in human disease.

Several chemokines are abundantly expressed in experimental models of fibrosis and in the human disease (Agostini & Gurrieri 2006). Regarding fibrocytes, several studies have focused on the role of chemokines in recruiting these cells to the injured lung. In human IPF, the

CXCL12/CXCR4 axis may be of particular significance (Andersson-Sjoland et al., 2008). As mentioned human circulating fibrocytes express CXCR4 and α-SMA, and can traffic toward the unique CXCR4 ligand, CXCL12 (Mehrad et al., 2007; Andersson-Sjoland et al., 2008). Supporting a major role of this axis in the lung disease, it was demonstrated that the administration of neutralizing anti-CXCL12 antibodies to bleomycin-treated mice resulted in a significant reduction of fibrocyte lung homing and collagen deposition, but interestingly without affecting the numbers of other leukocyte populations in the lungs (Phillips et al., 2004). These data suggest that blocking or interfering with chemokine/chemokine receptor networks may help to diminish or stop fibrocyte recruitment in fibrotic lung disorders. Recently, two groups have explored this hypothesis. Xu et al., 2007 used an antagonist of the receptor CXCR4 (TN14003) in a model of bleomycin-induced pulmonary fibrosis. Intraperitoneal treatment of mice with TN14003 attenuated the development of lung fibrosis and blocked in vitro migration of bone marrow derived stem cells towards CXCL12 or lung homogenates of bleomycin treated mice. Likewise, Song and coworkers showed that intraperitoneal treatment of mice with AMD3100 (Plerixafor, which is a small synthetic specific inhibitor of CXCR4), resulted in decreased levels of CXCL12 in the bronchoalveolar fluid and decreased numbers of fibrocytes in the lungs of mice treated with bleomycin (Song et al 2010). Collagen deposition and pulmonary fibrosis were also attenuated by treatment with AMD3100 (Song et al., 2010). Though the initial results seem to be optimistic, this is still an area of active research, and further studies are needed to elucidate whether pharmacologic inhibition of the CXCR4/CXCL12 axis could modify the lung fibrotic process in human disease.

The potential use of circulating fibrocytes as biomarkers in fibrosing diseases is a window of opportunity that has to be explored; diverse groups have reported differences in the percentages of circulating fibrocytes between healthy controls and patients (Mehard et al., 2007; Moeller et al., 2008; Chun-Hua et al., 2008). An increase in the percentages of circulating fibrocytes was demonstrated in a cohort of 51 patients with stable IPF, compared to healthy controls, but more important, a huge increase was observed during an acute exacerbation, a highly lethal process in IPF. Moreover, the number of fibrocytes returned to the values of stable IPF in the few patients that recovered. In general, fibrocyte numbers were an independent predictor of early mortality (Moeller et al., 2008).

However, higher number of patients should be evaluated and larger longitudinal studies should be done in order to establish if differences in percentages of circulating fibrocytes as well as changes in the percentages of circulating fibrocytes in a given patient with a given disease may predict outcome. The possibility of using differences in the percentages of circulating fibrocytes as biomarkers for disease diagnostic, outcome, or therapeutic response is an important biomedical area of research that needs attention.

Fibrocytes are progenitor cells capable to differentiate not only into myofibroblasts but also in other mesenchymal cells (Hong et al., 2005 and 2007; Choi et al., 2010). The ability of fibrocytes to undergo differentiation to osteoblasts and chondrocytes like cells when treated with specific cytokines and defined media raises the opportunity of their use for regenerative therapy related to bone or articular cartilage repair. Hypothetically, circulating fibrocytes could be isolated from the patient's own blood, processed for differentiation into osteoblasts or chondrocytes, followed by transplantation into the damaged tissue. Tissue engineering is a growing field in the biomedical sciences, and the role of fibrocytes in regenerative therapy has to be assessed with future studies in the area.

7. References

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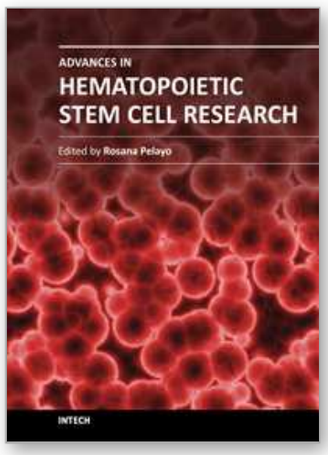

Advances in Hematopoietic Stem Cell Research
Edited by Dr. Rosana Pelayo

ISBN 978-953-307-930-1
Hard cover, 464 pages
Publisher InTech
Published online 27, January, 2012
Published in print edition January, 2012

This book provides a comprehensive overview in our understanding of the biology and therapeutic potential of hematopoietic stem cells, and is aimed at those engaged in stem cell research: undergraduate and postgraduate science students, investigators and clinicians. Starting from fundamental principles in hematopoiesis, Advances in Hematopoietic Stem Cell Research assemble a wealth of information relevant to central mechanisms that may regulate differentiation, and expansion of hematopoietic stem cells in normal conditions and during disease.

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