Circulating fibrocytes: a potent cell population in antigen-presenting and wound healing

FAN Xia 范霞 and LIANG Hua-ping 梁华平*

【Abstract】Fibrocytes are bone marrow-derived mesenchymal progenitors that co-express hematopoietic cell antigens and markers of monocytic lineage as well as fibroblast products. During wound healing, fibrocytes have been found to possess the ability of antigen-presentation to naive T cells in the inflammatory phase. Moreover, they can promote the endothelial cell proliferation, migration and angiogenesis by secreting several proteins. Fibrocytes can further differentiate into mature mesenchymocyte lineage, such as fibroblasts, myofibroblasts and adipocytes, and they may represent the systemic source of myofibroblasts that exert a contractile force required to close tissue wounds. A deep understanding of the mechanism involved in fibrocyte migration and differentiation may lead to the development of a novel theory of normal physiology and pathology.

Key words: Wound healing; Antigen-presenting cells; Angiogenesis inducing agents

Following trauma and disruption of tissue integrity, several overlapping events will be involved, including inflammation, cell proliferation, migration, angiogenesis and extracellular matrix molecule (ECM) production. In the inflammatory phase, various circulating cells are recruited into the injury sites, such as neutrophils and macrophages. While in the proliferative phase, the migration and proliferation of keratinocytes, fibroblasts and endothelial cells result in epithelialization and tissue granulation. At last, excess collagens in the wound is degraded by proteolytic enzymes, leading to the completion of tissue repair in the remodeling phase. Fibrocytes are a novel cell population that is derived from peripheral blood mononuclear cells and displays fibroblast-like properties. This review focused on the maturation, differentiation, migration of fibrocytes and their roles in antigen-presenting and wound healing.

The discovery, phenotype and origin of fibrocytes

In 1994, Bucala et al investigated the cell population presented in subcutaneous wound chamber consisting of short length of sponge-filled, silastic tubings and found a new population of cell type which showed properties of fibroblasts and expressed instinct leukocyte cell surface markers, CD34 and collagen I. And then they termed the new leukocyte subpopulation as “fibrocytes”. After the discovery of fibrocytes, the phenotypic characteristics of these cells were further evaluated in many studies. The markers of human fibrocytes are shown in Table 1. It was found that fibrocytes co-express hematopoietic stem cell antigens and markers of the monocye lineage, such as CD45, MHCII, CD34, CD13, CD11b, CD32 and CD64. They also express fibroblast products including collagen I and fibronectin. However, the fibrocytes do not express the markers of T cells (CD3, CD4, CD8, CD25 and CD56) or the B cell antigen CD19. Therefore, the expression of CD45 or one of hematopoetic (CD34) or myeloid antigens (CD13, CD11b) is considered as sufficient criteria to distinguish fibrocytes from leukocytes, dendritic cells, endothelial cells and tissue resident fibroblasts in vivo and in vitro. In addition, human fibrocytes also express several CCRs and CXC chemokine receptors, including CCR3, CCR5, CCR7 and CXCR4.

The precise origin of fibrocytes has puzzled many researchers. So far there are two hypothesis. Based on the number of fibrocytes isolated from peripheral...
blood, the first hypothesis states that fibrocytes comprise 0.1%-0.5% of nonerythrocytic cells. But the second hypothesis indicates that human fibrocytes are not present in peripheral blood and originate from a circulating precursor. In other words, there is a mediate stage between monocytes and fibrocytes, termed as fibrocyte precursors. Abe and his colleagues showed that the fibrocytes isolated from blood are differentiated from CD14+CD16 monocytes. However, it should be noted that these monocytes contain a mixture of other precursors which can differentiate into a number of cells different from macrophages and dendritic cells, such as myoblasts, osteoblasts, epithelial cells and so on. Therefore, these fibrocyte precursors may be present in a small portion of monocyte subset, which is an immature group presenting in the circulation and termed as “inflammatory monocytes”. In absence of inflammation, they may serve to replenish the tissue-resident macrophages and dendritic cell populations after an initial differentiation into different subtypes of monocytes before entering the tissue. During the inflammatory process, they are released in large amount from bone marrow into peripheral blood and directly migrate to inflamed sites.

Maturation and differentiation of fibrocytes

The CD14+CD16 monocytes are immature cells which can be differentiated into mature fibrocytes under certain permissive condition. In vitro studies demonstrated that the mature fibrocytes are developed from peripheral blood monocytes after 10-14 days of culture in media containing serum and after 3 days in serum-free media. There are several factors affecting the process of maturation. Direct contact between peripheral blood CD14+ monocytes and T lymphocytes in co-culture and stimulation of peripheral blood CD14+ monocytes with TGF- β increases the production of fibrocytes. Platelet-derived growth factor (PDGF), interleukin (IL) -4 and IL-13 also promote the differentiation of CD14+ monocytes into fibrocytes. However, aggregated IgG or serum amyloid P (SAP) inhibits the development of mature fibrocytes from the CD14+ monocytes. SAP, a member of the pentraxin family of proteins, circulates in blood as stable pentamers. Pro-inflammatory cytokines like interferon- γ (IFN- γ ) and IL-12 also inhibit the differentiation of fibrocytes from CD14+ monocytes. IL-1 β can not affect the process, but it induces the proliferation of mature fibrocytes and reduces the release of collagens from these cells. It is also reported that exogenous administration of leukotriene (LT) D4 induces proliferation of murine and human fibrocytes in a dose-dependent manner.

When fibrocytes have finished their maturation from CD14+ monocytes, they will possess the plasticity to differentiate into fibroblasts and myofibroblasts, which is promoted by transforming growth factor (TGF) β2 or endothelin-1 (ET-1). Hong and his colleagues demonstrated that TGF- β drives fibrocyte-to-myofibroblast differentiation through activating Smad 2/3 and SAPK/JNK MAPK pathway, which in turn stimulates α-smooth muscle actin expression. But in this process of fibrocytes differentiation, the expression of CD34 and CD45 will be down-regulated. Moreover, the profibrotic cytokines, IL-4 and IL-13, promote fibrocyte differentiation to α-SMA positive cells, such as myofibroblasts, from PMBCs without inducing proliferation, whereas the antifibrotic cytokine, IFN- γ , inhibits fibrocyte differentiation. In addition to the formation of fibroblasts and myofibroblasts from fibrocytes, the fibrocyte-to-adipocyte differentiation also occurs and is driven by the peroxisome proliferator-activated receptor (PPAR) γ agonist troglitazone, which is associated with cytoplasmic lipid accumulation and induction of a P2 (this process is inhibited by TGF- β). Thus, it appears that the maturation and differentiation of fibrocytes are influenced by a complex profile of cytokines within the local microenvironment of tissue injury.

Migration of fibrocytes

According to previous reports, fibrocytes are accumulated in subcutaneously implanted wound chambers in mice and in early human cutaneous scar tissues. However, the mechanism by which peripheral blood fibrocytes migrate to specific sites of tissue injury is obscure. As we know, numerous circulating cells, including monocytes, neutrophils and lymphocytes, migrate to tissue location as the result of chemokine-chemokine receptor interactions. So the chemokine receptors expressed on the surface of fibrocytes are studied. It is reported that human fibrocytes express several chemokine receptors, including CCR3, CCR5, CCR7 and CXCR4 (Table 1). Using both in vitro and in vivo fibrocyte chemotaxis techniques, Abe and colleagues found that fibrocytes migrate in response to secondary lymphoid chemokine (SLC), the ligand for CCR7. Interestingly, the expression of SLC at sites of
inflammation has been described. So the CCR7-SLC interaction may play a role in the migration of fibrocytes.

Among these interactions between chemokines and chemokine receptors, the CXCR 4-CXCL12 axis plays an important role in homing of bone marrow-derived progenitor cells.24 In a mouse model of pulmonary fibrosis, fibrocytes are found to express CXCR4 and migrate in response to CXCL12 in vitro and in the setting of bleomycin-induced pulmonary fibrosis in vivo. In the bleomycin model, antibody-mediated neutralization of CXCL12 results in reduced fibrocytes recruitment to lung and decreases collagen deposition.25 The CXCR4-CXCL12 axis is also important in the trafficking of human fibrocytes in the setting of living fibrosis. According to the studies of patients with fibrotic interstitial lung disease, the numbers of CD45, collagen I, CXCR4 positive fibrocytes were higher than those in healthy controls. Moreover, the CXCL12 ligand expression was also markedly elevated in the lung and plasma of patients with lung fibrosis.26 Those findings taken together suggest that fibrocytes express several chemokine receptors and contribute to the migration to wound sites, likely through the CXCR-CXCL12 axis.

### Fibrocytes as potent antigen presenting cells (APCs)

The skin is a vital barrier to infection or tissue invasion and plays a major role in host immunity.27 If the barrier is damaged, pathogenic bacteria can easily invade. At this time, APCs should be recruited to specific injury sites to initiate the antigen-specific responses by a class II major histocompatibility complex (MHC)-dependent pathway. Isolated human fibrocytes express the cell surface molecules required for antigen presentation, including MHC molecules (HLA-DP and HLA-DR), costimulatory molecules (CD80 and CD86) as well as adhesion molecules (CD11a, CD54 and CD58).28 The expression levels of these markers by fibrocytes are similar to those of monocytes. Moreover, human fibrocytes localized to cutaneous scar tissues express high levels of HLA-DR in situ, suggesting that fibrocytes may play an early and critical role in the initiation of antigen-specific immunity and significantly expand the importance of these cells in the host response to tissue injury, because HLA-DR expression is considered as a prerequisite for antigen presentation in vivo.29

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<th>Table 1. The markers of human fibrocytes</th>
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*"-"represents no expression; "+/"-represents being expressed or not; "++" represents the increasing level of expression.
Chesney and colleagues tested the capacity of both human and mouse fibrocytes to present antigens and stimulate antigen-specific T lymphocytes in vitro. Human fibrocytes induced antigen-presenting cell-dependent T cell proliferation when cultured with tetanus toxoid, suggesting that fibrocytes play a role in the initiation of antigen-specific immunity. The antigen-presenting capacity in vitro of fibrocytes was higher than that of monocytes and lower than dendritic cells when assessed by peak antigen-dependent T cell proliferation. In addition, fibrocytes secrete MIP-1α and MIP-1β, potent chemoattractant molecules for CD4+ T cells. These CD4+ T cells are considered essential for the generation of an antigen-specific response in vivo.28 Thus, fibrocytes may contribute to the host defense response during tissue injury by recruiting and activating T lymphocytes to sites of injury.

Fibrocytes can present antigen to CD4+ T lymphocytes, but no information is available about their capacity to stimulate CD8+ cytolytic T lymphocyte (CTL) responses. So Balmelli and colleagues isolated fibrocytes from porcine blood and found that primary fibrocytes endocytose and degrade antigens efficiently. But in absence of exogenous stimuli, endocytosis and MHC II expression are lost. Furthermore, fibrocytes can stimulate CD8+ T lymphocyte proliferation even at low Fb/T lymphocyte ratios, at which dendritic cells were less efficient.30 It is demonstrated from their experiment that fibrocytes can stimulate the proliferation of CD8+ T lymphocytes in addition to being capable of stimulating antigen-specific CD4+ T lymphocytes.

The role of fibrocytes in wound healing

Fibrocytes promote angiogenesis In the normal process of wound healing, angiogenesis is a critical event, by which the cell debris can be easily removed and it can form a wound bed for the development of granulation tissue in vivo.29 And the wound-related angiogenesis appears to be regulated by the interaction of endothelial cells and extracellular matrix within the wound space.30 Although numerous mediators, such as growth factors, cytokines and several enzymes, have been identified to promote angiogenesis, how the microenvironment of cells affect the angiogenesis is still obscure. Afterwards, based on the investigation of fibrocytes, it can be found that fibrocytes secrete VEGF, PDGF, IL-1β, hematopoietic growth factors and bFGF, which can promote the endothelial cell proliferation.32 Furthermore, culture supernatants obtained from fibrocytes promote the migration and differentiation of endothelial cells in vitro.32

The proteolysis of the basement membrane is also involved in the invasion stage of angiogenesis. Previous studies have shown that matrix metaloproteinases (MMPs) mediate the dissolution of basement membrane during early tissue repair and initiate angiogenesis.22 MMP-9 is the main MMP found in wound fluid with peak activity expressed between 2 and 4 days after injury.33 Consistent with these observation, fibrocytes home to cutaneous wound sites in vivo within 1-4 days, and ex vivo cultured fibrocytes constitutively express MMP-9 messenger RNA and secrete high levels of active MMP-9.3,32 Besides both autologous fibrocytes and fibrocyte-conditioned media were found to promote blood vessel formation in vivo. These data taken together suggest that cultured fibrocytes secrete factors that promote an angiogenic phenotype in endothelia cells in vitro and angiogenesis in vivo.

Fibrocytes secret extracellular matrix molecules and are the important source of myofibroblasts in wound During wound healing, it is essential for reparative cells to secret several ECMs in promoting the healing process. In vitro fibrocytes express numerous extracellular matrix molecules, including vimentin, fibronectin and collagen I. But it should be noted that the secreting process of extracellular matrix must be regulated by several mediators. If the process is out of control, the wound healing will be delayed, causing scars and fibrosis diseases, such as nephrogenic systemic fibrosis,34-37 interstitial pulmonary fibrosis,38-40 scleroderma41 and so on.

In addition, it is reported that myofibroblasts transiently found at the sites of tissue injury are believed to play a pivotal role in wound healing, but their origin is poorly understood. Abe and colleagues investigated the ex vivo cultured precursor fibrocytes and found that they have the capacity to differentiate into α-SMA, TGF-β1-responsive fibrocytes that exhibit characteristics similar to those of myofibroblasts, which exert a contractile force for wound closure. Moreover, Luca and his colleagues examined the phenotype of fibrocytes and myofibroblasts present in wound skin of mice and found that 61.4% of fibrocytes became α-SMA-positive between 4 and 7 days after injury, indicating that
the circulating fibrocytes contribute to the myofibroblasts population in the wound skin. Therefore, fibrocytes may be the main source of the myofibroblasts within wound space.

**Conclusion**

A growing body of literature over the last decade has revealed that human fibrocytes may play an important role in wound repair. A complex profile of cytokines within the local microenvironment of tissue injury promote or inhibit the maturation of fibrocytes and their differentiation into mesenchymocyte lineage, such as fibroblasts, myofibroblasts and adipocytes. The fibrocytes reach the injury sites with the interaction between the chemokines and chemokin receptors. They may also promote endothelial cell proliferation, migration and angiogenesis or contribute to wound closure by secreting extracellular matrix component. If tissue infection occurs in wound healing process, fibrocytes can be potent APCs which are able to recruit and activate T cells. However, it should be noted that too many fibrocytes within the damaged tissue may result in fibrosis and scarring. Therefore, additional research is needed to determine the check point between the wound healing and pro-fibrotic abilities of the fibrocytes. Moreover, the mechanism by which fibrocytes migrate to the injured sites is also need to be further investigated, which may contribute to the improvement of the abilities of tissue wound repair in vivo.

**REFERENCES**

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