Review Article

Role of Circulating Fibrocytes in Cardiac Fibrosis

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Abstract

Objective: It is revealed that circulating fibrocytes are elevated in patients/animals with cardiac fibrosis, and this review aims to provide an introduction to circulating fibrocytes and their role in cardiac fibrosis.
Data Sources: This review is based on the data from 1994 to present obtained from PubMed. The search terms were "circulating fibrocytes" and "cardiac fibrosis".
Study Selection: Articles and critical reviews, which are related to circulating fibrocytes and cardiac fibrosis, were selected.
Results: Circulating fibrocytes, which are derived from hematopoietic stem cells, represent a subset of peripheral blood mononuclear cells exhibiting mixed morphological and molecular characteristics of hematopoietic and mesenchymal cells ($CD34^+/CD45^+$/collagen I^+). They can produce extracellular matrix and many cytokines. It is shown that circulating fibrocytes participate in many fibrotic diseases, including cardiac fibrosis. Evidence accumulated in recent years shows that aging individuals and patients with hypertension, heart failure, coronary heart disease, and atrial fibrillation have more circulating fibrocytes in peripheral blood and/or heart tissue, and this elevation of circulating fibrocytes is correlated with the degree of fibrosis in the hearts.
Conclusions: Circulating fibrocytes are effector cells in cardiac fibrosis.

Key words: Aging; Atrial Fibrillation; Circulating Fibrocytes; Coronary Heart Disease; Fibrosis; Heart Failure; Hypertension

Introduction

Cardiac fibrosis is present in many pathological conditions, including hypertension, coronary heart disease (CHD), and heart failure.[1] Cardiac fibrosis has adverse effects on cardiac function. It culminates in increased stiffness of the heart, impairing diastolic function of the heart. It is also a mechanism involved in cardiac arrhythmia: (a) fibrous tissue can cause conduction slowing, leading to increased conduction heterogeneity, conduction block, or reentry,[2] (b) inappropriate cardiomyocyte-fibroblast couplings may form in fibrous hearts, predisposing individuals to cardiac arrhythmia.[3] Cardiac fibrosis is characterized by excessive deposition of extracellular matrix (ECM) and is now considered a result of exaggerated activity of fibroblasts, therefore researchers have done substantial work to inhibit the proliferation, differentiation, and oxidative stress of fibroblasts.[4,5] Previously, this deposition is thought to be produced by resident fibroblasts. New evidence suggests that other cells are also involved in this process. These cells include endothelial cells (undergoing endothelial-to-mesenchymal transition),[6] mesenchymal stem cells (differentiating into fibroblasts),[7] and circulating fibrocytes. "Circulating fibrocytes" were first described in 1994 as a subpopulation of leukocytes. They are derived from hematopoietic cells and can further differentiate into cells such as fibroblasts and adipocytes.[8,9] They are spindle-shaped cells co-expressing hematopoietic and mesenchymal markers, such as CD34, CD45, α-smooth muscle actin (α-SMA), and collagen I.[10,11] Circulating fibrocytes, producers of ECM, have been extensively reported to contribute to the development of pulmonary fibrosis and renal fibrosis.[12,13] Evidence accumulated in recent years shows that circulating fibrocytes also play important roles in cardiac fibrosis. They are significantly increased in the hearts when the hosts are subject to cardiac ischemia,[14] hypertension,[15] heart failure,[16]

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and atrial fibrillation (AF) [Table 1].[17] Similar results were obtained when compared the number of circulating fibrocytes in aging heart to that of younger individuals [Table 1].[23] This novel insight into the pathogenesis of cardiac fibrosis suggests that circulating fibrocyte may be a potential target for treatment of cardiac fibrosis. The remainder of this article describes the features of circulating fibrocytes and their contribution to cardiac fibrosis.

Features of Circulating Fibrocytes

Circulating fibrocytes are of hematopoietic origin

It was reported more than 150 years ago that there were a population of fibroblast-like cells in peripheral blood. However, it was not until 1994 when the term fibrocyte was used for the first time to define these blood-borne fibroblast-like cells.[10] Nevertheless, this term is not specifically used to describe these cells, and it also refers to quiescent fibroblast and a cell in inner ear. Therefore, circulating fibrocyte or CD34+ fibrocyte is preferred. In the initial report, using wound-healing models, it was found that an unexpectedly large number of fibroblast-like cells were present in wound chambers.[10] Further examinations of these cells revealed that they co-expressed CD34, CD45, vimentin, and collagen I, which were reminiscent of leukocytes and fibroblasts.[10] In addition, cells expressing the same markers were found in peripheral blood.[10] Combining these findings together, researchers defined these blood-borne fibroblast-like cells as circulating fibrocytes and supposed that these cells circulated in the blood stream, mobilized to wound sites, and contributed to wound healing.[10] It was initially thought that circulating fibrocytes were derived from hematopoietic stem cells (HSCs). However, chimera studies conducted by the same group failed to prove that circulating fibrocytes were of hematopoietic origin, which may be associated with the inability of irradiation with 800 rads (1 rad = 0.01 Gy) to kill all the HSCs.[10] Later, *in vivo* and *in vitro* experiments with different methods to trace circulating fibrocytes confirmed that these cells originate from HSCs.[37-39]

In vitro experiments suggested that fibrocytes differentiated from monocytes.[40] In this monocyte to fibrocyte differentiation, monocytes first differentiate into M1 macrophages, the latter become M2 macrophages, and finally M2 macrophages give rise to fibrocytes. This transition can be modulated by many factors. T-cells are indispensable in monocyte to fibrocyte differentiation, and deficiency in T-cells impairs this process as can be seen in a study that nude rats undergoing myocardial infarction (MI) showed few circulating fibrocytes within the myocardium.[40,41] Many cytokines are involved in this differentiation. For example, Th1 cytokines (interferon-γ and interleukin-12 [IL-12]) suppress this process while Th2 cytokines (IL-4 and IL-13) promote this differentiation.[42] In addition, epigenic modulation can also be a way to regulate this differentiation.

Table 1: Results of previous studies showing that circulating fibrocytes are elevated in cardiac fibrosis

Species	Disease/animal model	Sample	Cell markers	Authors	Year
Mouse	I/RC	Heart tissue	CD45 and collagen I	Haudek *et al.*[18]	2006
eGFP chimeric mouse	MI	Heart tissue	GFP and vimentin	Möllmann *et al.*[14]	2006
eGFP chimeric mouse	MI	Heart tissue	GFP and vimentin/GFP and α-SMA	Fujita *et al.*[69]	2007
hPLAP chimeric rat	MI	Heart tissue	hPLAP and vimentin/hPLAP and α-SMA	Odörfer *et al.*[20]	2008
eGFP chimeric mouse	MI	Heart tissue	GFP and α-SMA	van Amerongen *et al.*[21]	2008
eGFP chimeric mouse	MI	Heart tissue	GFP and vimentin/GFP and α-SMA	Chu *et al.*[16]	2010
Mouse	Ang II-induced cardiac fibrosis	Heart tissue	CD45 and collagen I	Haudek *et al.*[22]	2010
Mouse	Aging	Heart tissue	CD45 and collagen I	Cieslik *et al.*[23]	2011
Mouse	Ang II-induced cardiac fibrosis	Heart tissue	CD45 and α-SMA	Qi *et al.*[24]	2011
Mosue	Ang II-induced cardiac fibrosis	Heart tissue	CD45 and collagen I	Xu *et al.*[25]	2011
Human	Hypertensive heart disease	Peripheral blood	CD45 and collagen I	Keeley *et al.*[26]	2012
Mouse	Ang II-induced cardiac fibrosis	Heart tissue	ED, α-SMA, and CD133	Sopel *et al.*[27]	2012
eGFP chimeric mouse	Aging	Heart tissue	GFP and SMemb	Szardien *et al.*[28]	2012
Mouse	Ang II-induced cardiac fibrosis	Heart tissue	CD34, CD45 and collagen I	Duerrschmid *et al.*[29]	2013
Mouse	Ang II-induced cardiac fibrosis	Heart tissue	CD45 and collgen I	Falkenham *et al.*[30]	2013
Human	Hypertrophic cardiomyopathy	Peripheral blood	CD34, CD45, and collagen I	Fang *et al.*[15]	2013
eGFP chimeric mouse	TAC	Heart tissue	CXCR4 and fibronectin	Kazakov *et al.*[31]	2013
Pig	MI	Heart tissue	CD34 and α-SMA	Lei *et al.*[32]	2013
eGFP chimeric mouse	Ang II-induced cardiac fibrosis	Heart tissue	GFP and collagen I	Rosin *et al.*[33]	2013
eGFP chimeric mouse	Ang II-induced cardiac fibrosis	Heart tissue	GFP and FSP1	Markó *et al.*[34]	2014
Mouse	Ang II-induced cardiac fibrosis	Left ventricle and peripheral blood	CD34, CD45, and collagen I/CD34, CD45, and α-SMA	Williams *et al.*[35]	2014
Human	AF	Left atrial tissue and peripheral blood	CD45 and pro-collagen I	Xie *et al.*[17]	2014
Mouse	Ang II-induced cardiac fibrosis	Heart tissue	CD34, CD45, and collagen I	Duerrschmid *et al.*[36]	2015

I/RC: Ischemia/reperfusion cardiomyopathy; eGFP: Enhanced green fluorescent protein; MI: Myocardial infarction; α-SMA: Alpha-smooth muscle actin; FSP: Fibroblast specific protein; hPLAP: Human placental alkaline phosphatase; Ang II: Angiotensin II; TAC: Transverse aortic constriction; AF: Atrial fibrillation.

Inhibition of Class I histone deacetylases was shown to suppress differentiation of monocytes to circulating fibrocytes.[35]

Of note, circulating fibrocytes represent a part of stromal cells in embryos,[43] therefore although circulating fibrocytes are recruited to certain sites in disease state, their presence in normal tissues was also reported.[44,45]

Identification of circulating fibrocytes

Morphologically, circulating fibrocytes are 50–200 μm long spindle-shaped cells with ellipsoid nuclei, and there are prominent projections on the surface of them.[10] They express markers of both hematopoietic cells (CD34, CD45, and leukocyte-specific protein 1) and mesenchymal cells (collagen I, procollagen-I).[40,46] Therefore, a combination of hematopoietic and mesenchymal markers is widely used in identifying circulating fibrocytes [Table 1]. To confirm the hematopoietic origin of circulating fibrocytes, green fluorescent protein (GFP)-transgenic mice are frequently used in the tracing of circulating fibrocytes [Table 1]. Bone marrow cells of these GFP-transgenic mice are injected into experimental mice. Then, double staining of mesenchymal markers with GFP indicates the existence of circulating fibrocytes.

Functions of circulating fibrocytes

In general, circulating fibrocytes are polyfunctional. They can produce ECM[33] and a variety of cytokines, such as tumor necrosis factor-α, platelet-derived growth factor-A, transforming growth factor-β1 (TGF-β1), macrophage colony-stimulating factor, and matrix metalloproteinases.[45,47] Circulating fibrocytes are regarded as precursors of fibroblasts/myofibroblasts. Once differentiated into fibroblasts/myofibroblasts, the production of ECM can even be enhanced.[48] During this process, circulating fibrocytes will lose their expression of CD34 and CD45, and express α-SMA, a marker of myofibroblasts.[49,50] This differentiation can be spontaneous when isolated fibrocytes are cultured *in vitro*[51] and will be enhanced when cytokines such as endothelin-1, TGF-β1, and connective tissue growth factor are added to the culture medium.[33,48,52] In addition to tissue remodeling, circulating fibrocytes also participate in a variety of processes, including antigen presentation and angiogenesis,[53,54] which will not be extensively discussed here.

Recruitment of circulating fibrocytes

It is estimated that circulating fibrocytes comprise 0.1–1% of nonerythrocytes in peripheral blood,[55] and this proportion is relatively stable unless injuries such as inflammation and hypoxia occur. For example, in patients with interstitial lung disease (ILD), the proportion of circulating fibrocytes goes up to 6–10%,[56] and during exacerbation of ILD, up to 15% of nonerythrocytes in the blood are circulating fibrocytes.[57] The alteration of the amount of circulating fibrocytes is probably mediated by chemokine ligand/chemokine receptor axis. Chemokines are chemotactic cytokines that control the migratory patterns and positioning of cells expressing chemokine receptors, which are G protein-coupled receptors and are expressed on the surfaces of circulating fibrocytes.[46] The binding of chemokines to chemokine receptors directs the mobilization of circulating fibrocytes according to the gradient of chemokines.[58] In response to injuries such as inflammation and hypoxia, the concentrations of chemokines in both peripheral blood and organs are elevated, and this elevation leads to accumulation of circulating fibrocytes in blood stream and injured organs.[59,60] This has been shown in many studies. For example, CXC-chemokine ligand 16 (CXCL16) (a transmembrane CXC chemokine) knockout mice administrated with angiotensin II (Ang II) were found to have significantly less circulating fibrocytes in injured kidney compared with these in wild-type mice,[61] indicating CXCL16 is one of the mediators involved in circulating fibrocyte recruitment.

Circulating Fibrocytes in Cardiac Fibrosis

Accumulating evidence has indicated that circulating fibrocytes are involved cardiac fibrosis, when hosts are subject to hypertension, aging, cardiac ischemia, AF, and heart failure.

Circulating fibrocytes in hypertensive heart disease

Hypertension, defined as a usual blood pressure of 140/90 mmHg (1 mmHg = 0.133 kPa), is now a leading cause of death and is recognized as a risking factor for MI, aortic dissection, and cardiac fibrosis. In hypertension-induced cardiac fibrosis, circulating fibrocytes may play a role. It has been shown that patients with hypertensive heart disease had more circulating fibrocytes in peripheral blood, which is also correlated with left ventricular mass.[26] In experimental models, it was found that Ang II infusion or transverse aortic constriction which resulted in a significant increase of blood pressure, causes a significant increase of fibrocytes in the hearts, resulting in enhanced deposition of collagen in the affected hearts.[24,25,31,62] Interestingly, it was shown in a study that NaCl, an excessive intake of which will lead to hypertension, can potentiate monocyte to fibrocyte differentiation, enhancing ECM production.[63]

Circulating fibrocytes in heart failure

Heart failure is one of the most common and costly disabling diseases worldwide. It is a heterogeneous syndrome caused by the inability of the heart to pump sufficient blood to meet the requirement of metabolizing tissues. Cardiac fibrosis is commonly seen in patients with heart failure,[64] and circulating fibrocytes may be involved in this process. Using Mst1 mice, which are animal models of chronic heart failure without acute ischemia (a common cause of fibrocyte recruitment), it was found that cardiomyocytes in chronic heart failure can secrete chemotactic factor, stromal-derived factor-1, recruiting circulating fibrocytes to the myocardium, and contributing to the fibrosis of affected hearts.[16]

Circulating fibrocytes in coronary heart disease

CHD, causing 50% of death in developed countries, appears to rank number one in prevalence at present.[65] Typically,

CHD is characterized by atherosclerotic plaques presented in coronary arterial walls. These plaques narrow the lumen of coronary arteries, limiting blood supply to the hearts. Partial or complete coronary artery narrowing leads to acute myocardial infarction (AMI), which is characterized by sudden loss of cardiomyocytes and overwhelming inflammatory response. Thereafter, the infarcted areas are replaced by collagen-rich scars because of the limited regeneration ability of cardiomyocytes.[66] The scar was thought to be the production of resident myofibroblasts.[67] This paradigm is now challenged by many reports. It was shown in a study that a significant circulating fibrocytes were present within the myocardium on day 7 post-MI. Examination of the heart tissue revealed that 24% of myofibroblasts were derived from bone marrow, indicating that circulating fibrocytes contributed to reparative process of the hearts after MI.[21] Similar results were obtained from other studies.[14,20,32,68,69] In addition to acute cardiomyocyte loss, daily, brief coronary occlusion, which does not cause death of cardiomyocytes, leads to increase of circulating fibrocytes in myocardium. It was shown that ischemia/reperfusion cardiomyopathy cause induction of monocyte chemotactic protein-1 (MCP-1, also known as chemokine [C-C motif] ligand-2) in cardiomyocytes, thus contributing to the recruitment of monocytes and circulating fibrocytes and cardiac fibrosis.[18,70] Although animal models of cardiac ischemia have shown that coronary occlusion leads to infiltration of circulating fibrocytes into myocardium, it is reported that patients with AMI had less circulating fibrocytes in peripheral blood.[71] As it was shown that circulating fibrocytes were present in fibrous caps,[72] the decrease of circulating fibrocytes may contribute to instability of atherosclerotic plaques. This result indicated that elevated circulating fibrocytes may not always be unfavorable.

Circulating fibrocytes in atrial fibrillation

AF is the most common sustained cardiac arrhythmia, and its morbidity and mortality are now increasing.[73] Atrial fibrosis is an important contributor to the pathogenesis of AF.[74] It was reported that the number of circulating fibrocytes in peripheral blood was also increased in patients with AF compared to patients with sinus rhythm.[17] Examination of left atrial tissue from the two groups also revealed that cardiac fibrosis was enhanced in tissue from patients with AF, coinciding with 3-fold more fibrocytes presenting in the atrial tissue.[17] In addition, it was shown in the same study that circulating fibrocytes from patients with AF had enhanced ability to produce collagen when cultured in complete medium,[17] further unveiling important role of circulating fibrocytes in the development of atrial fibrosis.

Circulating fibrocytes in aging hearts

Age is a risk factor for many cardiovascular diseases, including heart failure, hypertension, and cardiac arrhythmia. Aging hearts are characterized by loss of cardiomyocytes and progressive fibrosis. Many mechanisms are involved in age-related cardiac fibrosis, including TGF-β unresponsiveness and fibrocyte infiltration.[75] It was found that compared with younger individuals, aging mice had more circulating fibrocytes in the hearts.[23,28] This increase of fibrocytes is correlated with up-regulation of MCP-1, Th2 cytokines (IL-4, IL-13), thereby enhancing infiltration of monocytes and monocyte to fibrocyte differentiation.

Conclusions

Circulating fibrocytes, a unique cell population, are of hematopoietic origin and circulate in peripheral blood. They are reminiscent of leukocytes and fibroblasts because of their co-expression of hematopoietic and mesenchymal markers. Accumulating evidence suggests that circulating fibrocytes are involved in the process of cardiac fibrosis, and it is shown that there are many ways to regulate biological behavior of circulating fibrocytes, such as modulating the recruitment and differentiation of circulating fibrocytes. Findings in the present study indicate that circulating fibrocytes can serve as a potential target for diminishing adverse effect of cardiac fibrosis, thereby improving prognosis of these patients. However, many aspects of circulating fibrocytes remain elusive, for instance, signaling pathway of cytokines in circulating fibrocytes is still unknown. Therefore, more studies are still needed to expand our understanding of circulating fibrocytes.

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Conflicts of interest

There are no conflicts of interest.

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