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Review

Mesenchymal Stem Cell-Like Properties in Fibroblasts

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Key Words
Fibroblasts • Mesenchymal stem cells • Directed differentiation • Epithelial-mesenchymal transition • Mesenchymal to epithelial transition

Abstract
Fibroblasts are biologically dynamic and morphologically heterogeneous and are the most abundant connective tissue cells, with diverse structures depending on their location and activity. The main function of fibroblasts is to maintain the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix. Recent advances in our knowledge on pathophysiologic features of fibroblasts revealed that in some situations epithelial cells can give rise to fibroblasts by epithelial-mesenchymal transition (EMT) and conversely, in some other situations, fibroblasts may give rise to epithelia by undergoing a mesenchymal to epithelial transition (MET). Given an opportunity to differentiate to other cells, fibroblasts may foster a novel clue for *in situ* tissue repair and contribute to cellular mechanisms of mesenchymal stem cell-like features under normal or pathological conditions. They have also been shown to suppress immune responses *in vitro*. Because of these properties, fibroblasts have recently received a very high profile in the literature. This review summarizes our understanding of the origins, mesenchymal stem cell-like characteristics and potency of directed differentiation of fibroblasts. In addition, we also present the evidence that mesenchymal stem cells and fibroblasts share much more in common than previously recognized.

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Introduction

Fibroblasts are the most common type of cells found in connective tissue. Fibroblasts secrete collagens that are used to maintain a structural framework for many tissues. They also play an important role in healing wounds. During the process of the chronic diseases or during normal aging, all kinds of tissues and organs undergo fibrosis gradually, such as fibrosis of skin, lung, liver, kidney and heart etc., which can severely impair our health. Fibrosis is also closely related to proliferation of fibroblasts. Fibroblasts secrete excessive extracellular matrix and collagens during proliferation and thereby replace normal organ structure, leading to functional impairment and scar formation, which may further trigger persistent fibrosis [1, 2].

Fibroblasts have classically been viewed as a uniform cell type with equivalent functions regardless of the origins of tissue [3, 4]. This view has been challenged by data illustrating extensive phenotypic heterogeneity among fibroblasts from different tissues and from a given tissue under different physiologic conditions [5]. Indeed, lung fibroblasts have been shown to be heterogeneous in cell surface marker expression, as well as in their levels of collagen production [6]. Moreover, periodontal fibroblasts also show heterogeneity based on morphology, glycogen pools, and collagen production [7]. Additionally, fibroblasts from different anatomic sites have distinct transcriptional patterns [8]. Under appropriate stimulation, relatively quiescent fibroblasts can acquire an active synthetic, contractile phenotype and express several smooth muscle cell markers, which are not exclusive for fibroblasts [9].

Human hypertrophic scar-derived fibroblasts show the biologic characteristics of mesenchymal stem cells [10]. Multidirectional differentiation induction indicated that the fibroblasts could differentiate into adipogenic, osteogenic and chondrogenic lineages [11-13]. Scientists pay close attention to their function in repair of pathological injury. Despite the dramatic benefits reported in early phase clinical trials, their functions remain poorly understood. Particularly, several questions remain unanswered concerning the origin of mesenchymal stem cells and their relationship to other stromal cells such as fibroblasts. In the following sections, we summarize current insights into fibroblasts origin and their distribution, molecular markers, as well as characteristics of mesenchymal stem cells and potency of directed differentiation, and highlight emerging hypotheses and targets for further research.

Origin and distribution

Originally, fibroblasts were described in late 19th century based solely on their location and morphological criteria [14, 15]. Fibroblasts are typically identified by their spindle-shaped flattened morphology, ability to adhere to culture plates, and in general they lack a basement membrane and tend to have multiple processes or sheet-like extensions. They contain an oval nucleus (with 1 or 2 prominent nucleoli), extensive rough endoplasmic reticulum, a prominent Golgi apparatus, and abundant cytoplasmic granular material. In all tissues, fibroblasts are usually adherent to the fibers which they themselves lay down and thus can form a three-dimensional (3-D) network and become embedded within the fibrillar extracellular matrix (ECM) [14, 16]. Fibroblasts synthesize most of the ECM of connective tissues, including interstitial collagens, proteoglycans, glycoproteins, cytokines, growth factors, and proteases [17]. These components form an elaborate 3-D network that is intimately associated with structure and function [18].

Fibroblasts serve diverse vital distribution during different development states, various organs and different locations. For example, cardiac fibroblasts (CFs) are the predominant cell type in number, but the cardiac myocytes actually occupy the greatest volume [19, 20]. CFs are found throughout the heart in a 3-D network surrounding myocytes and bridging the gaps between myocardial tissues [16, 21]. Myocytes are arranged in laminae bounded

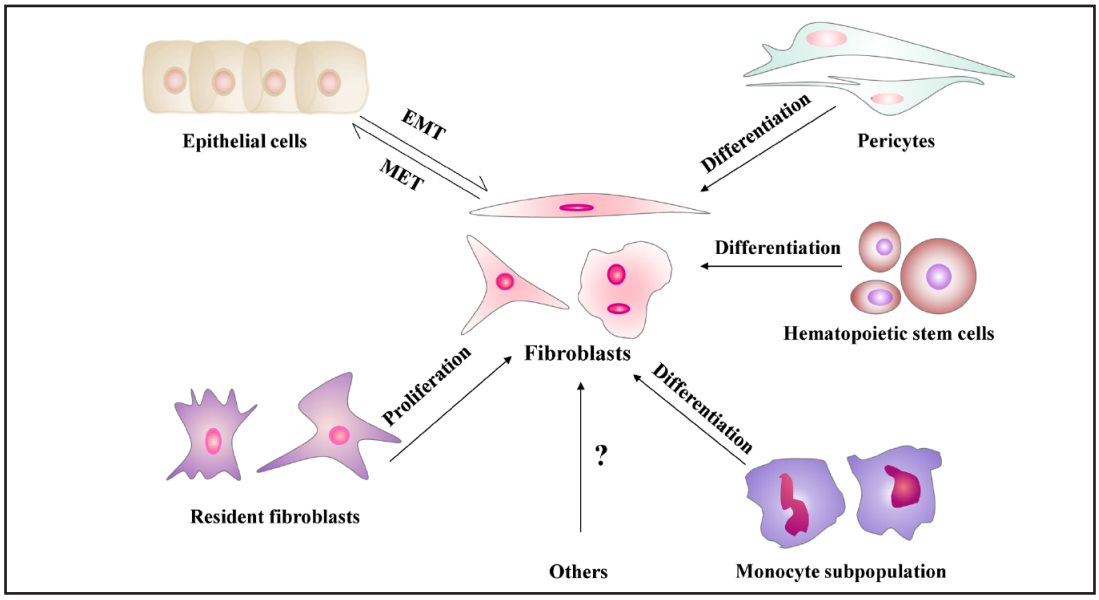

Fig. 1. The possible origin of the fibroblasts. The sources of fibroblast include (1) resident fibroblasts division; (2) epithelial cells through EMT pathway, fibroblasts in some situations may give rise to epithelia by undergoing MET pathway; (3) hematopoietic stem cell origin; (4) pericytes can contribute to the adult interstitial fibroblast population; (5) monocyte subpopulation can migrate into the damaged parts and differentiate into fibroblasts; (6) others have not been recognized.

by endomysial collagen, and CFs lie within this endomysial network [22]. CFs appear coincidentally with ventricular compaction around embryonic of day 12.5 and increase in number steadily through postnatal day one in mouse [23]. CFs have been recently shown to play an important role in proliferation during development of heart. The mammalian heart undergoes a major change in physiological pressures to transition from a fetal to neonatal circulation. Cessation of flow through the ductus arteriosus and increased pulmonary return cause an elevation of ventricular pressure. A robust CF response to the increased neonatal circulatory demands is seen during the first two neonatal weeks when the CF population increases from 10% -20% to70% within a relatively short time [24]. Most studies agree that the first 2 weeks of murine cardiac growth result in at least a doubling of the CF population. However, there is a paucity of reports regarding the relative CF makeup of the adult heart. Early studies on adult rat left ventricle estimated that 65%~70% of the cells were non-cardiomyocytes [25, 26], whereas recent studies analyzing the total mouse heart by FACS and confocal microscopy estimate a much higher number of cardiomyocytes at ~56% and ~44% nonmyocyte content, with only 27% of them are fibroblasts. The densest population of CFs in healthy adult hearts is found around the sinoatrial node [27, 28], thus providing complete electrical insulation. It remains unclear whether CFs are evenly distributed throughout the developing heart and whether they emerge via a uniform or clustered spatiotemporal manner.

Despite the identification of fibroblasts in the late 19th century and the advent of elegant lineage mapping tools such as DiI labeling, zebrafish photoactivatable (caged) fluorescein marking, and murine *loxP/Cre* recombinase-mediated genetic cell-marking techniques, relatively little is known about the origin and development of the fibroblasts. Combining the existing domestic and overseas research results, fibroblasts have different origins at different developmental stages. Fibroblasts are traditionally defined as cells of mesenchymal origin (Fig. 1). The mesenchymal cells that form the fibroblast population are believed to be derived from the EMT. Epithelia cells lose their special molecular markers, such as E-cadherin or zonaoccludens-1, and express the proteins of fibroblasts (fibroblast

Table 1. molecular markers for fibroblasts or myofibroblasts

Molecular markers	Cell types expressed
vimentin	fibroblasts ; telocytes; pancreatic precursor cells; endothelial cells; neurones; sertoli cells; macrophages; neutrophils; leukocytes; mesangial cells; various epithelial cancer cells
DDR2	fibroblasts; osteocytes; leukocytes; tumor cells
CD90/Thy1	fibroblasts; neuronal cells; bone marrow stem cells; thymocytes; glomerular mesangial cells; T-lymphocytes
α8β1 integrin	myofibroblasts; mesangial cells; vascular smooth muscle cells (VSMC); pericytes; visceral smooth muscle cells; kidney mesangial cells; liver stellate cells; lung interstitial cells
FSP1	fibroblasts; hematopoietic cells; endothelial cells; vascular smooth muscle cells; leukocytes; a multitude of cancer cells
smooth muscle α-actin	myofibroblasts; myoepithelial cell; myoid cells
fibroblast-activation protein	myofibroblasts

specific proteins, FSPs) during the process of EMT [29]. Then fibroblasts start to proliferate rapidly for the first several days of the postnatal life. Several studies have illustrated that increased fibroblasts are derived from resident fibroblast division or through EMT pathway. But some researches insist on their hematopoietic stem cell origin, and these multipotent progenitors have the ability to differentiate into a variety of vascular and mesodermal tissues [30]. A variety of studies in the adult suggests that fibroblasts increase slowly, derived from resident fibroblast division or through EMT pathway. Some research teams persist in the belief that progenitor cells, such as pericytes, adventitial cells associated with the vasculature, and mesenchymal stem cells of the bone marrow, can contribute to the adult interstitial fibroblast population [31, 32]. It is conceivable that the proliferating, perivascular fibroblast population in the heart stems from bone marrow-derived circulating progenitors and the pro-epicardial organ [18, 33, 34]. So fibroblasts are thought to arise from various sources at different stages of development.

Fibroblasts start to proliferate rapidly during development and disease, such as cardiac hypertrophy, myocardial infarction (MI), idiopathic pulmonary fibrosis (IPF), kidney fibrosis, hypertrophic scar in skin, and so on, which eventually leads to organ fibrosis. Studies show that fibroblasts in pathological conditions have a wide variety of origins. The fibrosis process is similar in all organs to some degree. In addition to endogenous fibroblast proliferation, circulating monocyte subpopulations can migrate into the damaged parts and differentiate into fibroblasts. But some scientists presume that bone marrow-derived fibroblasts enter in the injured sites. So far, more and more studies afford evidence that EMT pathway is associated with the fibrosis process. Fibroblasts in some situations may give rise to epithelia by undergoing a mesenchymal to epithelial transition (MET). This process is seen in many developmental situations, as well as in wound healing and tumorigenesis. (http://en.wikipedia.org/wiki/Fibroblast)

Taken together, these studies suggest that fibroblasts of various organs are likely to derive from hematopoietic stem cells, pericytes, mesenchymal cells through EMT pathway. But how fibroblasts are generated, which cytokines prompt their migration, transformation, and how cells move to reside in their final position, also remains to be elucidated more comprehensively.

Molecular markers

The lack of a reliable and specific fibroblast marker is a major limiting factor in the study of fibroblasts *in vivo* and is assuredly why they remain so poorly understood in

both molecular and cellular terms [14, 15]. A useful label for fibroblasts is anti-vimentin antibodies that react with the abundant intermediate filaments of fibroblasts [27]. But vimentin is also expressed in endothelial cells of capillaries that often locate very close to fibroblasts. This marker also labels neurons, which contain intermediate filaments (Table 1). Given the characteristic cyto-morphological differences between these cell types, however, anti-vimentin has been a suitable tool for reliable identification of fibroblasts.

More recently, a more specific marker for cardiac fibroblasts is the collagen receptor Discoidin Domain Receptor 2 (DDR2 [19]). DDR represents a relatively novel family of collagen specific receptor tyrosine kinases [35, 36]. Receptor tyrosine kinases are a family of proteins involved in the conversion of extracellular stimuli into cellular responses [37]. These receptors mediate a variety of cell functions, including growth, migration, morphology and differentiation. The tissue distribution of DDR1 and DDR2 varies (and can be mutually exclusive), and DDR2 expression has been detected in both rat and mouse heart, as well as the isolated cardiac fibroblasts [38, 39]. Originally defined as a collagen receptor on mesenchymal cells, DDR2 has also been found on leukocytes, as well as in tumors, but not on cardiomyocytes or cardiac endothelial and smooth muscle cells [19]. Interestingly, DDR2 gives a labeling pattern that is very similar to that obtained using vimentin antibodies [16].

Thy-1 (or CD90) is a small glycoprotein (25–30 kDa) anchored through a glycosylphosphatidylinositol (GPI) tail [40]. Thy-1 is expressed on several cell types: thymocytes, T-lymphocytes, bone marrow stem cells, neuronal cells, glomerular mesangial cells as well as fibroblasts of different origins (Table 1). Thy-1 expression on fibroblasts appears to be variable and seems to reflect the heterogeneity of this cell population [6]. Indeed, several groups have been able to isolate Thy-1^+ and Thy-1^- fibroblasts from mouse and rat lungs or from human orbit and reproductive tract [41-44]. At best, scientists detected less than 10% of Thy-1^- cells in primary culture of cardiac fibroblasts. This may represent contaminating cells. In addition, this level fell below 2% in subsequent passages [45]. Unlike murine or rat lung fibroblasts, rat cardiac fibroblasts appear to form a homogeneous population with only Thy-1^+ cells. Flow cytofluorometry revealed that the cells, at every passage, were positive for DDR2 or α8 integrin and Thy-1. Interestingly, the majority of cardiac fibroblast cells expressed high levels of all three antigens. Interestingly, Thy-1 heterogeneity seems to differently affect their proliferative and synthetic characteristics [46]. Thy-1^+ lung fibroblast phenotype exhibits profibrogenic properties with enhanced collagen deposition [47], whereas the Thy-1^- cell shows more apparent myofibroblastic features, particularly when the cells were stimulated with TGF-β or connective tissue growth factor (CTGF) [48].

Another marker that has been proposed to be a fibroblast-specific marker is fibroblast-specific protein-1 (FSP1) [29, 49], however, other studies in the literature have shown that FSP1 is also expressed in a variety of other cell types, including leukocytes and a multitude of cancer cells [50]. It has also been shown that fibroblast-activation protein, a serine protease, is highly expressed on activated fibroblasts [15, 51].

α8β1 integrin, a cell adhesion receptor that links actin myofilaments to ECM proteins, is also detected on fibroblasts. It can not only mediate mechanical and chemical signals from the matrix, but also regulate cell differentiation, immunization and adhesion through bidirectional membrane transduction. However, it has been recently shown that α8β1 integrin is present not only on fibroblasts but also on vascular smooth muscle cells (VSMC) [52]. An interesting feature of cardiac fibroblasts is their ability to transform into myofibroblasts. The appearance of smooth muscle (SM) α-actin-positive cytoplasmic myofilaments is often used to detect them, and as such SM α-actin is now recognized as a reliable marker of the myofibroblast phenotype. Myofibroblasts also express a larger quantity of α8β1 integrin [52, 53].

The relatively specific molecular markers have been established gradually, which promotes further research on fibroblasts and improves our understanding of their function in normal and patho-physiological states.

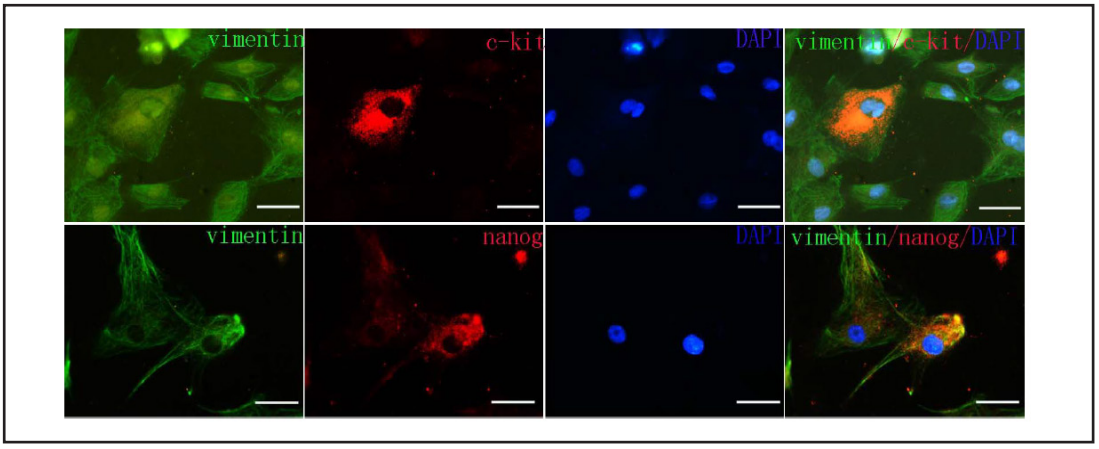

Fig. 2. Immunofluorescence staining of vimentin and stem cell markers (c-kit or nanog) in the cultured neonatal rat cardiac fibroblasts. A and E: general cell appearance revealed by vimentin immunostaining (FITC immunolabeling); B and F: c-kit or nanog expression in some fibroblasts (Cy3 immunolabeling); C and G: cell nuclei immunolabeled with DAPI (blue, florescent DNA dye); D: merge of A, B and C; H: merge of E, F and H. Scale bars: 50 μm.

Characters of mesenchymal stem cell

Mesenchymal stem cells (MSCs)are currently defined as plastic adherent, multipotential fibroblast-like cells expressing CD73, CD105 and negative for the hematopoietic markers CD14, CD34 and CD45 [54, 55], but these properties and markers are also shared by fibroblasts.

Saeed et al. revealed that mouse embryonic fibroblasts(MEF) exhibited surface markers characteristic of the BMSC: Sca-1$^+$, CD73$^+$, CD105$^+$, CD29$^+$, CD44$^+$, CD106$^+$, CD11b$^-$, and CD45$^-$ [56]. Interestingly, compared to BMSC, MEF exhibited a more enhanced differentiation into adipocyte and chondrocyte lineages. Cultured fibroblasts can express the pluripotency marker genes Oct-4 [57, 58], which is a homeodomain transcription factor of the POU family. This protein is critically involved in the self-renewal of undifferentiated embryonic stem cells. As such, it is frequently used as a marker for undifferentiated cells. Researchers have found that adult epidermal cells possess multipotent differentiation capacities of epidermal stem cells under injury stimulation and differentiate into epidermal cells, participating in wound healing.

Fibroblasts can express CD90, detected in many tissues, such as human myometrium, orbital cavity and lung. CD90$^+$ fibroblasts in adult show stem cell characters and high immature potential. These cells are isolated and cultured in the early generations and have the potential to differentiate into other cell types. Our previous results have established that cardiac fibroblasts express nanog, which is also expressed in embryonic stem cells (ESCs) and is thought to be a key factor in maintaining pluripotency. In other words, these cells have the ability to become virtually any cell of any of the three germ layers (endoderm, ectoderm, mesoderm). Fibroblasts in the infarction border in 1 W myocardial infarction rats also express c-kit, one of the important members of tyrosine kinase receptor protein. c-kit plays an important role in cell differentiation and proliferation. We have the similar results in the cultured neonatal rat cardiac fibroblasts (Fig. 2).

In conclusion, fibroblasts in mouse embryonic, epidermis and heart have stem cell characteristics. It remains unknown whether fibroblasts in other organs can express stem cell markers.

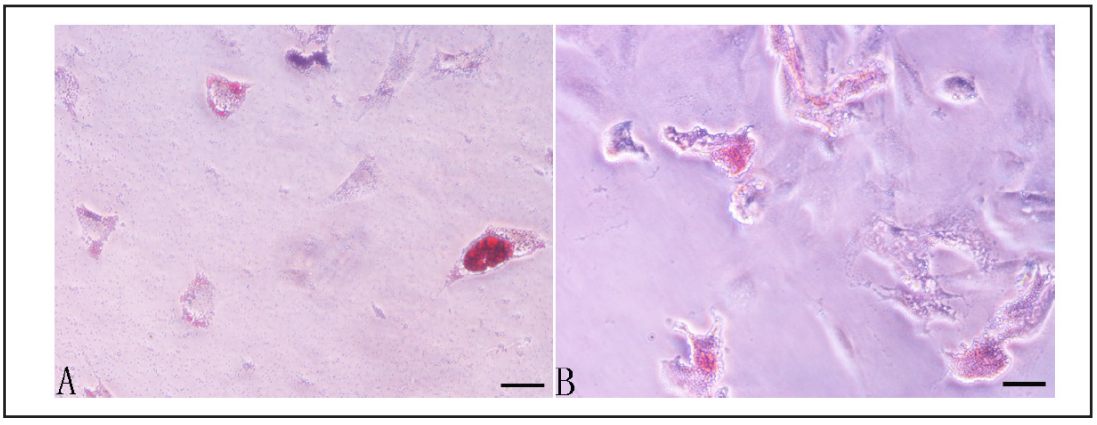

Fig. 3. Adipogenic and osteogenic differentiation of cultured neonatal rat cardiac fibroblasts. A: adipogenic differentiation on the 14th day in culture with adipogenic induction medium, as detected by Oil red O staining; B: osteogenic differentiation on the 21th day in culture with osteogenic induction medium, as detected by Alizarin red staining. Scale bars: 25 μm.

Multipotential characteristics

Takahashi's team demonstrated induced pluripotent stem cells (iPSCs) from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions [59]. It shows that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors. Moreover, iPSCs present several safety concerns such as the genetic and epigenetic aberrations they are carrying, as well as the potential risk for tumor formation [60]. Recently, advances in direct cell lineage conversion have suggested a potential solution to these issues. Moreover, as direct lineage conversion bypasses the pluripotent state, it could theoretically reduce the risk of tumorigenicity after transplantation [61].

Lineage reprogramming describes the conversion of differentiated cells directly into other somatic cell types. This general concept was first clearly established by the conversion of fibroblasts to skeletal myoblasts by the forced expression of MyoD in 1987, and was followed by more examples of lineage reprogramming, including conversion of B lymphocytes to macrophages, inner ear support cells to hair cells, exocrine pancreatic cells to endocrine β-cells, and fibroblasts to neurons [62]. A lineage reprogramming strategy for conversion of fibroblasts to cardiomyocytes was first reported by Ieda et al. in 2010 [63]. Qian et al. [64] described that a combination of three developmental transcription factors (i.e., Gata4, Mef2c and Tbx5) rapidly and efficiently reprogrammed post-natal cardiac or dermal fibroblasts directly into differentiated cardiomyocyte-like cells. Induced cardiomyocytes became binucleate, assembled sarcomeres and had cardiomyocyte-like gene expression. Song et al. [65] reported that addition of a fourth factor, Hand2, could increase the efficiency of the conversion. Some reports prove that expression of Ascl1, Brn2, and Myt1l efficiently converted mouse embryonic fibroblasts (MEFs) and postnatal fibroblasts into functional neurons (induced neurons, or iN cells) [66]. In this regard, the same strategy can be applied to human embryonic and postnatal fibroblasts. By overexpression of the transcription factors Ascl1, Brn2, and Myt1l, human fibroblasts were efficiently converted to functional neurons [67]. Zhang et al. [68] have reported that the conversion of human fibroblasts into retinal pigment epithelium lineage using defined sets of transcription factors.

Osteoblastic, chondrogenic, adipogenic differentiation from fibroblasts has also been described [69]. More recently, hepatocyte differentiation potential of adult human dermal fibroblasts was demonstrated in an *in vivo* model of liver injured immunodeficient mice [70]. Sandrine et al. [71] has published that human dermal fibroblasts (from adult skin or infant foreskin) and bone marrow mesenchymal stem cells (BM-MSCs) share *in vitro* morphology

and many biological properties, but fibroblasts do not have the same capacity as BM-MSCs to differentiate into adipocytes and osteoblasts.

More recently, several reports have demonstrated that dermal fibroblasts are multipotent cells. Dermal skin-derived fibroblasts from rodent and human have been found to exhibit mesenchymal surface antigen immunophenotype and differentiation potential along the three main mesenchymal-derived tissues: bone, cartilage and fat [72, 73]. Rodent dermal fibroblast cell lines were examined for their mesenchymal potential [74-76]. Toma et al. [74] isolated skin-derived precursors (SKPs) from mouse dermis and characterized them as nestin and fibronectin positive, but not as vimentin or cytokeratin expressing cells. These cells could differentiate into cells of both neural and mesodermal lineages, like neurons, glia, smooth muscle cells and adipocytes. Crigler et al. [75] found a murine dermal skin-derived subpopulation that had the capacity to differentiate into osteogenic, adipogenic, chondrogenic and myogenic cell lineages, and even into epidermal cell types.

Recently dermal fibroblasts have been proven to express the markers of mesenchymal stem cells and differentiate into osseous tissue, fat, cartilage, tendon, nerve, pancreatic islet and so on under certain induced medium. Our previous research shows that cardiac fibroblasts in neonate rats have similar capacity to differentiate into osteogenic and adipogenic cell lineages (Fig. 3).

These results give us a clue whether fibroblasts derived from all organs have multipotential characteristics and the ability to differentiate into other cell type.

Conclusions

Data illustrate extensive phenotypic heterogeneity among fibroblasts from different tissues and from a given tissue under different physiological conditions. In fact, cardiac fibroblasts comprise over 50% of all the cells in the heart [8, 15]. Cardiac fibroblasts are fully differentiated somatic cells that provide support structure, secrete signals and contribute to scar formation upon cardiac damage. SAN pacemaker tissue has higher relative fibroblast content than ventricle, occupying some 45% to 75% of SAN volume in man [77, 78].

Although fibroblasts are the key component in the connective tissue, its function in the organism has been neglected. However, fibroblasts have the capacity to synthesize and remodel the extracellular matrix. In addition to their presumed role as scaffolding support, fibroblasts have been directly proven to play roles in regulating self-tolerance, organ development, wound healing, inflammation and fibrosis [6, 79-81]. Under normal physiological conditions, equilibrium between synthesis and degradation is maintained, and under pathological conditions, accumulated ECM results in tissue fibrosis. Inappropriate tissue repair and continued insult can result in chronic inflammation and eventually lead to fibrosis. At the cellular level, accumulation and persistence of myofibroblasts during tissue repair and healing has been proposed as a leading cause of fibrosis [82, 83]. This process is associated with the transformation of granulation tissue into a hypertrophic scar with excessive production of ECM and ratification of the microvasculature.

More recent studies revealed that fibroblasts have much broader functions in any organism. These studies have suggested a “sentinel” role for fibroblasts that is intimately associated with the global response to mechanical, electrical, and chemical signals. Fibroblasts form a complex 3-D network within the connective tissue matrix that they occupy and play important roles in modulating the whole function of the organisms.

Fibroblasts show different functional phenotypes: migratory phenotype, proliferative phenotype, synthetic phenotype and contractile phenotype due to a variety of cytokines involved and in different conditions. Identification of specific molecular markers of fibroblasts should assist in continued efforts to understand this dynamic cell. Their stem cell-like characters and multipotential differentiation could provide new targets for in situ tissue repair and more abundant seed cells for cell therapy in various incurable pathological conditions.

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