

Review Article

Endothelial mesenchymal transition in cardiac fibrosis

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Abstract: Cardiac fibrosis is the final common pathway of various heart diseases, characterized by excessive buildup of extracellular matrix proteins by fibroblasts in the myocardial interstitial compartments. Although the study of fibroblasts has been carried out for many years, the source of fibroblasts is still unclear. Recent reports suggest that about 1/3 of these interstitial fibroblasts are derived from the endothelial cells. It is a process in which endothelia lose their endothelial features but gain some mesenchymal phenotypes. The phenotypic switching, termed as endothelial mesenchymal transition (EndMT), becomes the focus of the current research in fibrosis diseases. In this review, we summarize the background knowledge of EndMT and then elucidate the evidence and mechanism of EndMT in cardiac fibrosis.

Keywords: Cardiac fibrosis, EMT, EndMT, fibroblast

Introduction

Cardiac fibrosis is the final common pathway of various heart diseases, including hypertensive heart disease, cardiomyopathies, myocarditis, coronary heart disease, etc [1, 2]. From the current view, cardiac fibrosis refers to the remodeling of heart interstitium, essentially involving excessive deposition of extracellular matrix (ECM) which is mainly produced by cardiac fibroblasts (CFs). Cardiac fibrosis is usually divided into two categories: replacement fibrosis and reactive fibrosis. Replacement fibrosis refers to the type of cardiac fibrosis secondary to cardiomyocyte death, such as myocardial infarction. When myocardial infarction happens, the sudden death of a great number of cardiomyocytes will activate an inflammatory response, which replaces the dead cardiomyocytes with collagen scars [3]. But for the type of reactive fibrosis such as hypertensive heart disease, ECM mainly deposits in the perivascular and perimyosium areas without cardiomyocyte death as shown in the pressure overload model [4]. However, both types of cardiac fibrosis co-exist in most cases. For example, in hypertensive heart diseases, reactive fibrosis

is usually initially observed in areas distant to the initial infarction zone, while replacement fibrosis can be discovered at the late stage. Excessive ECM deposition increases cardiac wall stiffness and then causes cardiac diastolic dysfunction. Meanwhile, the increased deposition of ECM can impair the inter-cardiomyocytes electrical and mechanical coupling, and then results in an increased risk of arrhythmia and weakened cardiac systolic function [5, 6]. Ultimately, ECM remodeling will cause cardiomyocyte loss and cardiac failure.

Despite decades of research, the cellular and molecular mechanisms of cardiac fibrosis are still not fully clarified [7, 8]. Recent studies have indicated cardiac fibrosis is associated with the Endothelial Mesenchymal Transition (EndMT), which can be caused by various kinds of injuries and inflammations [9, 10]. During EndMT, endothelial cells lose their own phenotypes and convert into full functional fibroblasts, which produce more ECM but less ECM-degradation enzymes. To date, EndMT has been discovered in many fibrosis diseases and is becoming a research hotspot for scientists [11, 12]. In this review, we summarize the background knowl-

edge of EndMT and then elucidate the evidence and mechanism of EndMT in cardiac fibrosis.

Origin of activated fibroblasts/myofibroblasts in cardiac fibrosis

Cardiac fibroblasts (CFs), which account for two-thirds of total cardiac cells, play a central role in maintaining the homeostasis of ECM [13]. ECM components are mainly synthesized in CFs, meanwhile, CFs also produce proteolytic enzymes to regulate the degradation of ECM. Normally, the turnover rate of ECM is at a low level of about 5% daily. But under some pathological circumstances such as hypertension or myocardial ischemia, increased mechanical overload and hypoxia can activate CFs, and activated CFs (or myofibroblasts) then synthesize more ECM but less degradation enzymes, which leads to an imbalance of the homeostasis of ECM. In addition, CFs can also interact with other cardiac cells, such as infiltrated inflammatory cells, endothelial cells, cardiomyocytes and smooth muscle cells [1], by secreting lots of bioactive proteins, including growth factors, cytokines, proteases, etc. Therefore, CFs play a pivotal role in the pathogenesis of cardiac fibrosis, and have drawn the interest of many researchers to elucidating the bioactivity and molecular mechanisms of myofibroblasts [14].

The origin of cardiac myofibroblasts is not yet clear, due to the fact that there is no specific and sustained molecular marker [15, 16] identified in cardiac myofibroblasts so far. Traditionally, it was believed that myofibroblasts mainly originate from resident fibroblasts under pathological circumstances [17, 18]. But recently researchers have recognized that cardiac myofibroblasts can be derived from three main sources, including proliferation of resident fibroblasts, migration of bone marrow-derived circulating fibrocytes, and EndMT [13-16, 19, 20]. By using the newly developed fate-mapping technology, epithelial cells in epicardium [21, 22] and pericytes of microvessels [23] can now be observed converting into myofibroblasts in cardiac fibrosis as well. Several studies indicate EndMT accounts for one-third of all fibroblasts and is an important source of the myofibroblasts. EndMT presents only in pathological conditions, but not in normal conditions. Researchers are now trying to find a therapy to relieve fibrosis disorders with-

out affecting physiological tissue repairing. As a result, EndMT has attracted more attention to research of cardiac fibrosis and other fibrosis diseases in recent years [24-27].

Endothelial-mesenchymal transition: a recently identified source of activated mesenchymal cells

The basics of EMT

Epithelial-mesenchymal transition (EMT) is a process in which epithelia lose their epithelial features but gain some mesenchymal phenotypes. EMT is initially described in embryogenesis, which involves the formation of the mesoderm, endoderm and neural crest. In embryonic stage, epithelium and mesenchyme are two basic tissue types [28]. Epithelial cells are of apical-basal polarity and connect with each other through tight junction, and mesenchymal cells are spindle-shaped with more mobility and only temporarily connect with surrounding ECM components. The two basic phenotypes can convert into each other through EMT or MET (Mesenchymal-Epithelial Transition), which are normal phenomena universally occurring during embryogenesis and organogenesis.

Early studies of EMT in embryos have provided basic knowledge of this biological process and its molecular regulatory network. Traditionally, researchers have thought that EMT occurs only in the embryo. But in the last decade, plenty of studies have shown that epithelial cells are also capable of transforming into mesenchymal cells in adult and this transformation plays an essential role in fibrosis and tumor metastasis. In fibrosis diseases, epithelial cells transform into fibroblasts and migrate to the pathological sites for tissue repairing. The researches of tumor metastasis have been a major driving force in the insight of EMT at present [29-32]. According to the consensus of The EMT International Association (TEMTIA) meeting in 2008 [32], EMT can be divided into three subtypes: type 1 (EMT in embryogenesis), type 2 (EMT in fibrosis) and type 3 (EMT in tumor metastasis), due to different biological contexts involved.

In current opinion, the three types of EMT share common features in morphological conversion and molecular signaling but differ in some other aspects. Firstly, they occur under different cir-

cumstances. Type 1 EMT is a normal physiological process, but type 2 and type 3 EMT are pathological conditions which involve inflammation. Secondly, the transformed mesenchymal cells in type 1 and type 3 EMT have stem cell properties, but those in type 2 EMT have not. In general, it seems that type 3 EMT recapture EMT process in embryogenesis and type 2 EMT is more like a compensatory mechanism which partially retakes the program of type 1 EMT in nature.

EMT is a complex and orchestrated process. During EMT in fibrosis, the differentiated epithelial cells lose the adherent and tight junctions, but acquire the morphology and function of fibroblasts. The process of EMT is regulated by a combination of myofibroblast, macrophage, leukocyte and endothelial cell itself, which release a plethora of hormones, growth factors, cytokines, matrix metalloproteinases (MMPs) and ECM components. The mesenchymal phenotype changes start by the binding of various bioactive molecules to their receptors and sending signal to nuclei. Generally, there are four key steps in EMT process [32-34]: (1) The decreased expression of epithelial adhesion molecules, including E-cadherin and ZO-1, leads to epithelial cell-cell and cell-basement membrane dissociation, and epithelial cytoskeleton (such as cytokeratin) changes into mesenchymal cytoskeleton, which leads to the apical-basal polarity loss of epithelial cells. (2) Meanwhile, the mesenchymal cytoskeleton molecules are synthesized, such as vimentin, fibroblast-specific protein 1 (FSP1, also known as S100A4), α -smooth muscle actin (α -SMA), etc. The transformed cells become spindle-shaped. (3) Breakdown of basement membrane via MMPs and other ECM-degrading enzymes. (4) Acquisition of invasive and migratory properties, transition to myofibroblasts and moving to the pathological site. It should be noted that these 4 steps do not necessarily occur in sequence and they overlap with each other.

EndMT: similar to EMT

In the last decade, tremendous progress has been made in providing evidence of EMT almost in every fibrosis disease in organs including kidney [35], liver [36, 37], lung [38, 39], skin, etc, and has provided novel mechanism and therapy for fibrosis diseases. Endothelial cells, spe-

cial kind of epithelial cells, line the interior surface of blood vessels. They undergo mesenchymal transition (EndMT) too. There are many similarities between EMT and EndMT on both cellular and molecular levels [40-42]. As expected, studies have revealed that EndMT plays a significant role in kidney [11, 27], heart, lung [26, 43], cornea [44] and intestine fibrosis [45].

Recent experiments in mice have indicated that microvasculature endothelial cells can also have a process similar to EMT during fibrosis, known as EndMT. In fibrosis, endothelial cells recapture the process which normally occurs during the formation of the endocardial cushion in embryogenesis [46, 47]. Unlike EMT, EndMT not only causes the conversion of endothelial cells to fibroblasts, but also leads to microvessel rarefaction, which results in inadequate perfusion of tissue. In addition, basement membrane that breaks down during EndMT facilitates the infiltration of inflammatory cells and activates thrombosis system. Though there are plenty of researches concerning EMT, a number of studies about EndMT are quite limited. The following sections will focus on the evidence and mechanism of EndMT in cardiac fibrosis.

Evidence and mechanism of EndMT in cardiac fibrosis

In vitro evidence: EndMT in cells

Numerous studies on cultured cells have illustrated that endothelial cells can undergo mesenchymal phenotype conversion [48-51]. In 1992, Arciniegas described using TGF- β 1 to induce bovine aortic endothelial cells to differentiate into SMA positive phenotype [48]. But his result had long been questioned, with the suspicion that the cultured endothelial cells might be polluted by other cells. In 2002, by using of Fluorescence-Activated Cell Sorting (FACS) and Immunomagnetic Purification Techniques, Maria G. Frid purified endothelial cell cultures to be free of contamination, and then confirmed that endothelial cells were able to transform into SMA positive cells [50]. This phenomenon has also been observed in pulmonary arteriolar endothelial cells [52]. In 2008, Krenning reported that human umbilical cord endothelial cells (HUVEC) can convert into smooth muscle-like cells through the EndMT

Common pathological process

Table 1. EndMT in different cardiac fibrosis models

Diseases	Animal	Models	Percentage	Inhibitors	References
Hypertensive heart disease	Mouse	Pressure overload	27-35%	BMP7	Ref. 24
AMI	Mouse	Ligation of LAD	25%		Ref. 55
Diabetic cardiomyopathy	Mouse, rat	Streptozotocin induced diabetes	15% to 20%		Ref. 25 Ref. 58
Graft cardiac fibrosis	Mouse	Heterotopic heart transplantation		BMP7	Ref. 24
Myocarditis	Mouse	Coxsackie virus B3 induced myocarditis		BMP7	Ref. 61

induced by TGF- β [53]. These studies on cultured cells lay the cornerstones for researching the role of EndMT in vivo.

In vivo evidence: EndMT in different cardiac fibrosis models

Although EndMT is well described in cultured cells and in cardiac embryogenesis, it remains to be illuminated whether such events can occur in adult cardiac fibrosis. As described below, cardiac injuries from almost any cause can lead to the activation of cardiac repairing and fibrosis (**Table 1**). EndMT seems to be a common process in cardiac fibrosis, though it needs to be confirmed with further studies in different models as well as human bodies.

Hypertensive heart disease

Hypertensive heart disease (HHD) is the most important organ damage in hypertension diseases, including primary and secondary hypertension. Cardiac interstitial fibrosis and cardiomyocyte hypertrophy is the two main features of HHD. During hypertension, overload pressure directly increases the collagen synthesis and proliferation of fibroblasts through mechano-sensory mechanisms, the ways of mechanical stimulus signaling in cells. Simultaneously, mechanical load induces the expression of profibrotic factors, including TGF and Ang II [54]. Then, cardiac fibrosis is mediated by the combined effect of mechanical tension and the bioactivity factors. In recent years, researches have indicated that, the profibrotic factors could transform the endothelial cells into fibroblasts and this is the essential origin of fibroblasts in pressure overload model. In 2007, Zeisberg and colleagues published a milestone research of EndMT in cardiac fibrosis [24]. They confirmed the EndMT during pressure overload-induced cardiac fibrosis in two genetic models. One is the *Tie1Cre; R26RstoplacZ* double-transgenic mice, in which endothelial cells are

permanently tagged with lac Z. Compared with the markers used before, such as VE-cadherin, CD31, etc, the expression of lac Z is constant in endothelium-originated cells despite the phenotype transition. The other is FSP1-GFP transgenic mice, in which green fluorescent protein (GFP) shares the promoter of fibroblast-specific protein 1 (FSP1) and provides a more specific fibroblast marker. With the ingenuity method, the authors revealed that 27-35% of all fibroblasts originated from endothelial cells through EndMT. Analogous to EMT, EndMT is mediated by TGF- β 1/smad pathway in cardiac fibrosis. This is the first research convincingly proving EndMT in cardiac fibrosis of pressure overload model. But there is no evidence about EndMT in other hypertensive model and patients with hypertension.

AMI

Acute myocardial infarction (AMI) is one of the leading causes of death in the world. Occlusion of coronary artery triggers a series of events that involve cardiomyocyte necrosis and myocardial repair. The death of cardiomyocytes initiates an inflammatory cascade to clear the dead cells and matrix debris. Then, profibrotic factors activate cardiac cells, which deposit more collagen to fill the void left by dead cardiomyocytes, and simultaneously growth factors mediate the angiogenesis in infarct area. The two processes interact with each other to maintain the integrity of cardiac structure and function. It should be noted that post-AMI cardiac fibrosis includes not only replacement fibrosis in infarcted area but also general active fibrosis in non-infarcted area. As a result, the entire cardiac chambers are involved, which eventually leads to cardiac dysfunction. One group recently reported that EndMT may be a key player for neovascularization and myofibroblast recruitment after acute myocardial infarction [55]. They indicated that the percentage of double-positive cells, CD31⁺/SMA⁺, is 25% of the total

cells in 7 days post-MI heart, but only less than 1% in normal heart. Furthermore, they confirmed EndMT after AMI is mediated by canonical Wnt signaling, which is an important pathway in angiogenesis as well.

Diabetic cardiomyopathy

The prevalence of diabetes mellitus (DM) is rapidly increasing in both developing and developed countries. The number of patients with DM will be 300 million by 2025 as a major health burden in the world [56]. Traditionally, it is believed that DM affects cardiac function through accelerated atherosclerosis and hypertension. Increasing evidence of epidemiology suggests that the increased risk of heart failure is involved in DM patients after considering other cardiovascular risk factors and significant correlation between DM and diastolic dysfunction. The results above indicate that DM directly stimulates the change of cardiac cells, independently of hypertension and atherosclerosis. Further studies have revealed that the three pathological conditions, hyperlipidemia, hyperinsulinemia and hyperglycemia, play a crucial role in the phenotype alternation in diabetes [57]. These conditions cause cardiomyocytes apoptosis and fibroblast activation through complex effectors and signal pathways [31]. Using double immunofluorescence staining, Widyantoro's group provided evidence of EndMT in diabetic cardiomyopathy model, and suggested that approximately 15-20% of fibroblasts are from endothelial cells [25]. Furthermore, they demonstrated that ET1-induced EndMT is mediated by activation of TGF- β signaling in ET-1 knockout model. In addition, Ri-Ning Tang showed that angiotensin receptor blocker (ARB) could reduce cardiac fibrosis through preventing EndMT in diabetic cardiomyopathy [58].

Graft cardiac fibrosis

Cardiac transplantation is the only established surgical therapy to patients with end-stage heart failure, but it has limited efficacy due to graft rejection. Chronic heart rejection refers to the progressive dysfunction of transplanted heart occurring months or years after transplantation. It is associated with progressive heart fibrosis which is caused by immune injury between host and graft. In a mouse model of class II major histocompatibility difference and

chronic heart rejection, EndMT is also observed in transplant hearts. Using the EndMT inhibitor BMP-7 leads to 50% reduction of cardiac fibrosis, and BMP-7 antagonists abolish the anti-fibrosis effect [24].

Myocarditis

There are few researches on cardiac fibrosis model with myocarditis. Myocarditis refers to the pathological immune processes in the heart with many causes, such as virus, radiation, chemicals, etc [59, 60]. The immune reaction in the heart causes inflammatory cell infiltration, which in turn leads to cardiac fibrosis. A most recent study illustrated that EndMT participated in the process of cardiac fibrosis in acute viral myocarditis [61] and this was blocked by BMP7.

EndMT as a therapeutic target in cardiac fibrosis

Cardiac fibrosis involves many cells and cytokines, which form a very complex regulatory network. There are many studies on antifibrotic drugs, but few have clinical approval. The real excitement for EndMT is the possibility of discovering previously unrecognized mechanisms that may lead to novel therapeutic strategies for cardiac fibrosis. EndMT only occurs in the pathological condition in organ fibrosis. In the past decade, understanding of signaling pathways in EndMT reveals novel insights into the pathogenesis of cardiac fibrosis, which would translate to applications in clinical medicine. Moreover, many traditional anti-fibrosis drugs have been proven to inhibit EMT/EndMT, such as ARB [58], statins [62], peroxisome proliferator-activated receptor agonists (PPAR)- γ [63], etc.

New drugs directly targeting EMT/EndMT have been revealed in many studies. BMP7 and HGF (hepatocyte growth factor) have been shown to be endogenous inhibitors that effectively block EMT both in vitro and in vivo. BMP7, a new drug directly aiming at EMT/EndMT, is a member of TGF- β superfamily and regulates a multitude of cellular functions such as differentiation, apoptosis, proliferation and migration. Recombinant human BMP7 (rhBMP7) can block or even reverse TGF- β 1-induced EMT in kidney fibrosis. Studies have shown that rhBMP7 increases the E-cadherin expression possibly through tran-

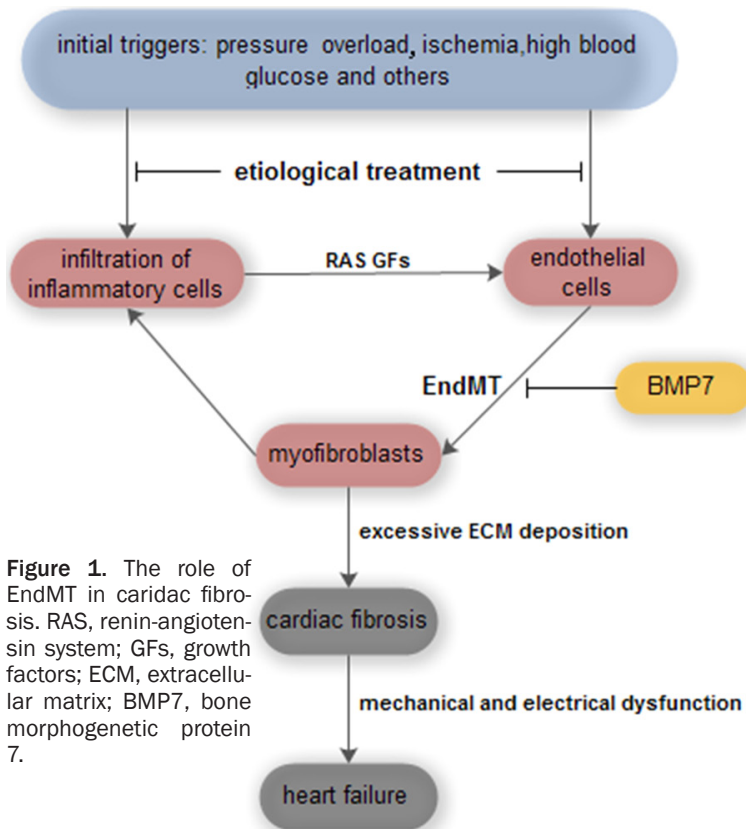


Figure 1. The role of EndMT in cardiac fibrosis. RAS, renin-angiotensin system; GFs, growth factors; ECM, extracellular matrix; BMP7, bone morphogenetic protein 7.

scription factors SIP1 and Snail [64]. In vivo and in vitro studies have also demonstrated that rhBMP7 can block EndMT in cardiac fibrosis. In human coronary endothelial cells, rhBMP7 prominently inhibits TGF- β 1-induced EndMT. In vivo, rhBMP7 reduced the cardiac fibrosis in chronic rejection and pressure overloaded mice. BMP7 also inhibit EMT in kidney fibrosis [35]. Of note, there are no clinical studies of BMP7 in fibrosis disease. HGF is an important protective factor in fibrosis disease. In vitro studies showed that HGF reversed all phenotypic conversion of EMT triggered by TGF- β 1 [65]. Consistently, in vivo studies provided the same result that HGF attenuated renal interstitial fibrosis via blocking of EMT in obstructive nephropathy [66]. But there are no studies of HGF in cardiac fibrosis. Attempts to use general inhibitors of TGF- β have encountered some obstacles, because TGF- β is involved in many critical physiological conditions [67]. In summary, the underlying signaling pathways of EndMT could be extremely complex, with innumerable cross-talks and feedbacks. It is important to identify “entral switch” factors in the complicated processes of EndMT in the future.

Conclusion and perspective

After more than 30 years of hard work, we have known much about EndMT. We now know that EndMT plays a crucial role in the origin of fibroblasts and during the whole course of cardiac fibrosis (Figure 1). The regulatory signal transduction from extracellular factors to post-translation is a multi-level and highly collaborative network. Although a great number of studies concerning EMT/EndMT have already been done, it still has a long way to go in terms of fully clarifying the molecular mechanism of EMT/EndMT and truly applying it to clinical practice. There are several aspects yet needing to be further explored. First is the need for new cell fate-mapping technologies. The ideal approach to confirm EMT/EndMT would be a real-time observation of epithelial cells transiting

into fibroblasts and migrating to interstitial tissues. Due to technical limitations, we currently could only capture some snapshots with immunofluorescence methods, which led to a heated debate in 2010 [68-70]. The second question is what is the difference in biological and molecular mechanisms between different origins of fibroblasts. Insights into the distinct contribution of each fibroblast subgroup to cardiac fibrosis may provide novel therapeutic targets in the future. Third, it is difficult to biopsy the heart of a cardiac disease patient, as is widely being done for kidney disease. Several non-invasive imaging techniques, such as Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET) and PET Magnetic Resonance Imaging (PET-MRI), ignite the possibility of tracking EMT in human body [10]. The last question is the timing of intervention in EndMT process. EMT is a rapid and effective way to recruit fibroblasts in physiological tissue repairing and a compensatory mechanism in pathological conditions. Early intervention of EMT may lead to undesirable consequences. As a result, the timing of intervention is needed to be confirmed by further studies. Furthermore, the role of miRNAs in cardiac fibrosis may be a field to be concerned.

Disclosure of conflict of interest

None.

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References

- [1] Nicoletti A, Michel JB. Cardiac fibrosis and inflammation interaction with hemodynamic and humoral factors. *Cardiovasc Res* 1999; 41: 532-543.
- [2] Berk BC, Fujiwara K and Lehoux S. ECM remodeling in hypertensive heart disease. *J Clin Invest* 2007; 117: 568-575.
- [3] Daskalopoulos EP, Janssen BJ and Blankesteijn WM. Myofibroblasts in the infarct area: concepts and challenges. *Microsc Microanal* 2012; 18: 35-49.
- [4] Javier Díez M. Mechanisms of cardiac fibrosis in hypertension.pdf. *J Clin Hypertens* 2007; 9: 547-550.
- [5] Kong P, Christia P and Frangogiannis NG. The pathogenesis of cardiac fibrosis. *Cell Mol Life Sci* 2014; 71: 549-74.
- [6] Tian Y and Morrissey EE. Importance of myocyte-nonmyocyte interactions in cardiac development and disease. *Circ Res* 2012; 110: 1023-1034.
- [7] Wynn TA and Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med* 2012; 18: 1028-1040.
- [8] Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; 214: 199-210.
- [9] von Gise A and Pu WT. Endocardial and epicardial epithelial to mesenchymal transitions in heart development and disease. *Circ Res* 2012; 110: 1628-1645.
- [10] Kovacic JC, Mercader N, Torres M, Boehm M and Fuster V. Epithelial-to-mesenchymal and endothelial-to-mesenchymal transition: from cardiovascular development to disease. *Circulation* 2012; 125: 1795-1808.
- [11] Zeisberg EM, Potenta SE, Sugimoto H, Zeisberg M and Kalluri R. Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J Am Soc Nephrol* 2008; 19: 2282-2287.
- [12] Liu Y. New insights into epithelial-mesenchymal transition in kidney fibrosis. *J Am Soc Nephrol* 2010; 21: 212-222.
- [13] Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmoulière A, Varga J, De Wever O, Mareel M, Gabbiani G. Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. *Am J Pathol* 2012; 180: 1340-1355.
- [14] Souders CA, Bowers SL and Baudino TA. Cardiac fibroblast: the renaissance cell. *Circ Res* 2009; 105: 1164-1176.
- [15] Fan D, Takawale A, Lee J, Kassiri Z. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. *Fibrogenesis Tissue Repair* 2012; 5: 15.
- [16] Zeisberg EM and Kalluri R. Origins of cardiac fibroblasts. *Circulat Res* 2010; 107: 1304-1312.
- [17] Yano T, Miura T, Ikeda Y, Matsuda E, Saito K, Miki T, Kobayashi H, Nishino Y, Ohtani S, Shimamoto K. Intracardiac fibroblasts, but not bone marrow derived cells, are the origin of myofibroblasts in myocardial infarct repair. *Cardiovasc Pathol* 2005; 14: 241-246.
- [18] Cucoranu I, Clempus R, Dikalova A, Phelan PJ, Ariyan S, Dikalov S, Sorescu D. NAD(P)H oxidase 4 mediates transforming growth factor-beta1-induced differentiation of cardiac fibroblasts into myofibroblasts. *Circulation Res* 2005; 97: 900-907.
- [19] Mital S. Biomarkers of cardiac fibrosis: New insights. *Prog Pediatr Cardiol* 2011; 32: 35-36.
- [20] Krenning G, Zeisberg EM and Kalluri R. The origin of fibroblasts and mechanism of cardiac fibrosis. *J Cell Physiol* 2010; 225: 631-637.
- [21] Limana F, Bertolami C, Mangoni A, Di Carlo A, Avitabile D, Mocini D, Iannelli P, De Mori R, Marchetti C, Pozzoli O, Gentili C, Zacheo A, Germani A, Capogrossi MC. Myocardial infarction induces embryonic reprogramming of epicardial c-kit(+) cells: role of the pericardial fluid. *J Mol Cell Cardiol* 2010; 48: 609-618.
- [22] Duan J, Gherghe C, Liu D, Hamlett E, Srikantha L, Rodgers L, Regan JN, Rojas M, Willis M, Leask A, Majesky M, Deb A. Wnt1/betacatenin injury response activates the epicardium and cardiac fibroblasts to promote cardiac repair. *EMBO J* 2012; 31: 429-442.
- [23] Diaz-Flores L, Gutiérrez R, Madrid JF, Varela H, Valladares F, Acosta E, Martín-Vasallo P, Díaz-Flores L Jr. Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. *Histol Histopathol* 2009; 24: 909-969.
- [24] Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, Chandraker A, Yuan X, Pu WT, Roberts AB, Neilson EG, Sayegh MH, Izumo S, Kalluri R. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* 2007; 13: 952-961.
- [25] Widyantoro B, Emoto N, Nakayama K, Anggrahini DW, Adiarto S, Iwasa N, Yagi K, Miyagawa K, Rikitake Y, Suzuki T, Kisanuki YY,

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- Yanagisawa M, Hirata K. Endothelial cell-derived endothelin-1 promotes cardiac fibrosis in diabetic hearts through stimulation of endothelial-to-mesenchymal transition. *Circulation* 2010; 121: 2407-2418.
- [26] Arciniegas E, Frid MG, Douglas IS, Stenmark KR. Stenmark Perspectives on endothelial-to-mesenchymal transition: potential contribution to vascular remodeling in chronic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2007; 293: L1-L8.
- [27] Li J, Bertram JF. Review: Endothelial-myofibroblast transition, a new player in diabetic renal fibrosis. *Nephrology (Carlton)* 2010; 15: 507-512.
- [28] Hay ED. The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. *Dev Dynam* 2005; 233: 706-720.
- [29] Zheng H, Kang Y. Multilayer control of the EMT master regulators. *Oncogene* 2014; 33: 1755-63.
- [30] De Craene B, Bercx G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer* 2013; 13: 97-110.
- [31] Potter JD. Morphogens, morphostats, microarchitecture and malignancy. *Nat Rev Cancer* 2007; 7: 464-474.
- [32] Kalluri R and Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009; 119: 1420-1428.
- [33] Guarino M, Tosoni A and Nebuloni M. Direct contribution of epithelium to organ fibrosis: epithelial-mesenchymal transition. *Hum Pathol* 2009; 40: 1365-1376.
- [34] Kalluri R and Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* 2003; 112: 1776-1784.
- [35] Carew RM, Wang B and Kantharidis P. The role of EMT in renal fibrosis. *Cell Tissue Res* 2012; 347: 103-116.
- [36] Zeisberg M, Yang C, Martino M, Duncan MB, Rieder F, Tanjore H, Kalluri R. Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition. *J Biol Chem* 2007; 282: 23337-23347.
- [37] Gressner OA, Weiskirchen R and Gressner AM. Evolving concepts of liver fibrogenesis provide new diagnostic and therapeutic options. *Comparat Hepatol* 2007; 6: 7.
- [38] Milara J, Peiro T, Serrano A and Cortijo J. Epithelial to mesenchymal transition is increased in patients with COPD and induced by cigarette smoke. *Thorax* 2013; 68: 410-20.
- [39] Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, Chapman HA. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci U S A* 2006; 103: 13180-13185.
- [40] Piera-Velazquez S, Jimenez SA. Molecular mechanisms of endothelial to mesenchymal cell transition (EndoMT) in experimentally induced fibrotic diseases. *Fibrogenesis Tissue Repair* 2012; 5 Suppl 1: S7.
- [41] Lin F, Wang N and Zhang TC. The role of endothelial-mesenchymal transition in development and pathological process. *IUBMB Life* 2012; 64: 717-723.
- [42] Piera-Velazquez S, Li Z, Jimenez SA. Role of endothelial-mesenchymal transition (EndoMT) in the pathogenesis of fibrotic disorders. *Am J Pathol* 2011; 179: 1074-1080.
- [43] Hashimoto N, Phan SH, Imaizumi K, Matsuo M, Nakashima H, Kawabe T, Shimokata K, Hasegawa Y. Endothelial-mesenchymal transition in bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2010; 43: 161-172.
- [44] Lee JG and Kay EP. FGF-2-mediated signal transduction during endothelial mesenchymal transformation in corneal endothelial cells. *Exp Eye Res* 2006; 83: 1309-1316.
- [45] Rieder F, Kessler SP, West GA, Bhilocha S, de la Motte C, Sadler TM, Gopalan B, Stylianou E, Fiocchi C. Inflammation-induced endothelial-to-mesenchymal transition: a novel mechanism of intestinal fibrosis. *Am J Pathol* 2011; 179: 2660-2673.
- [46] Eisenberg LM and Markwald RR. Molecular regulation of atrioventricular valvuloseptal morphogenesis. *Circ Res* 1995; 77: 1-6.
- [47] Kinsella MG and Fitzharris TP. Origin of cushion tissue in the developing chick heart: cinematographic recordings of in situ formation. *Science* 1980; 207: 1359-1360.
- [48] Arciniegas E, Sutton AB, Allen TD, Schor AM. SCHOR Transforming growth factor1 promotes the differentiation of endothelial cells into smooth muscle-like cells in vitro. *J Cell Sci* 1992; 103: 521-529.
- [49] Ishisaki A, Hayashi H, Li AJ and Imamura T. Human umbilical vein endothelium-derived cells retain potential to differentiate into smooth muscle-like cells. *J Biol Chem* 2003; 278: 1303-1309.
- [50] Frid MG, Kale VA and Stenmark KR. Mature vascular endothelium can give rise to smooth muscle cells via endothelial-mesenchymal transdifferentiation: in vitro analysis. *Circ Res* 2002; 90: 1189-1196.
- [51] Beranek JT. Vascular endothelium-derived cells containing smooth muscle actin are present in restenosis. *Lab Invest* 1995; 72: 771.
- [52] Zhu P, Huang L, Ge X, Yan F, Wu R, Ao Q. Transdifferentiation of pulmonary arteriolar endothelial cells into smooth muscle-like cells

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- regulated by myocardin involved in hypoxia-induced pulmonary vascular remodelling. *Int J Exp Pathol* 2006; 87: 463-474.
- [53] Krenning G, Moonen JR, van Luyn MJ and Harmsen MC. Vascular smooth muscle cells for use in vascular tissue engineering obtained by endothelial-to-mesenchymal transdifferentiation (EnMT) on collagen matrices. *Biomaterials* 2008; 29: 3703-3711.
- [54] Jill E, Bishop GL. Regulation of cardiovascular collagen synthesis by mechanical load. *Cardiovasc Res* 1999; 42: 27-44.
- [55] Aisagbonhi O, Rai M, Ryzhov S, Atria N, Feoktistov I, Hatzopoulos AK. Experimental myocardial infarction triggers canonical Wnt signaling and endothelial-to-mesenchymal transition. *Dis Models Mechan* 2011; 4: 469-483.
- [56] Perez-Pomares JM and Munoz-Chapuli R. Epithelial-mesenchymal transitions: a mesodermal cell strategy for evolutive innovation in Metazoans. *Anatom Record* 2002; 268: 343-351.
- [57] Lobo MV, Santander RG, Cuadrado GM, Alonso FJ. Cytochemical localization of calcium in perfusion myoblasts from the chick embryo myotome. *Histochem J* 1999; 31: 347-355.
- [58] Tang RN, Lv LL, Zhang JD, Dai HY, Li Q, Zheng M, Ni J, Ma KL, Liu BC. Effects of angiotensin II receptor blocker on myocardial endothelial-to-mesenchymal transition in diabetic rats. *Int J Cardiol* 2011; 162: 92-99.
- [59] Sagar S, Liu PP, Cooper LT Jr. Myocarditis. *Lancet* 2012; 379: 738-747.
- [60] Leuschner F, Katus HA and Kaya Z. Autoimmune myocarditis: past, present and future. *J Autoimmun* 2009; 33: 282-289.
- [61] Hua JY, Zhang ZC, Jiang XH, He YZ and Chen P. [Relationship between endothelial-to-mesenchymal transition and cardiac fibrosis in acute viral myocarditis]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2012; 41: 298-304.
- [62] Patel S, Mason RM, Suzuki J, Imaizumi A, Kamimura T, Zhang Z. Inhibitory effect of statins on renal epithelial-to-mesenchymal transition. *Am J Nephrol* 2006; 26: 381-387.
- [63] Chen Y, Luo Q, Xiong Z, Liang W and Chen L. Telmisartan counteracts TGF-beta1 induced epithelial-to-mesenchymal transition via PPAR-gamma in human proximal tubule epithelial cells. *Int J Clin Exp Pathol* 2012; 5: 522-529.
- [64] Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, Strutz F, Kalluri R. BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med* 2003; 9: 964-968.
- [65] Yang J and Liu Y. Blockage of tubular epithelial to myofibroblast transition by hepatocyte growth factor prevents renal interstitial fibrosis. *J Am Soc Nephrol* 2002; 13: 96-107.
- [66] Yang J, Dai C and Liu Y. Systemic administration of naked plasmid encoding hepatocyte growth factor ameliorates chronic renal fibrosis in mice. *Gene Ther* 2001; 8: 1470-1479.
- [67] Hinz B and Gabbiani G. Fibrosis: recent advances in myofibroblast biology and new therapeutic perspectives. *F1000 Biol Rep* 2010; 2: 78.
- [68] Kriz W, Kaissling B and Le Hir M. Epithelial-mesenchymal transition (EMT) in kidney fibrosis: fact or fantasy? *J Clin Invest* 2011; 121: 468-474.
- [69] Zeisberg M and Duffield JS. Resolved: EMT produces fibroblasts in the kidney. *J Am Soc Nephrol* 2010; 21: 1247-1253.
- [70] Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, Duffield JS. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol* 2010; 176: 85-97.