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The Role of Endothelial-to-Mesenchymal Transition (EndoMT) in Organ Fibrosis

Qing-Si Wen[1#] Qing Guan[1#] Yue Zhou[2] Yan Shi[1] Yu Sun[1] Yu Xue[1] Jing-Chun Pan[1] Qing-Nan Zhu[1] Yu-Ye Li[1] Ze-Wen Yan[1] Jian-Guo Xie[1,2*] Da-Peng Wang [1,2*]

1. College of Integrated Chinese and Western Medicine, Dalian Medical University, Dalian, China
2. The First Affiliated Hospital, Dalian Medical University, Dalian, China

*Correspondence: Jian-Guo Xie, Da-Peng Wang.
1. Hospital of Dalian Medical University
2. Zhongshan Road, Xigang District, Dalian City, Liaoning Province, China

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ABSTRACT

Endothelial cells and mesenchymal cells are two distinct cell types that have different forms and functions. A growing number of evidence that endothelial cells could differentiate into myofibroblasts (EndoMT) during the development of organ fibrosis. In this review, we discuss the role of EndoMT in renal fibrosis, cardiac fibrosis and pulmonary fibrosis and summarize representative signaling pathways involved in the process of EndoMT. Understanding the role and mechanisms of EndoMT in organ fibrosis will clear the therapeutic potential of targeting this process.

Keywords: EndoMT, fibrosis, TGF-β, Notch, Wnt/β-Catenin

Introduction

Endothelial cells (ECs) and mesenchymal cells are two distinct cell lineages and derived from the mesoderm. Endothelial cells are a heterogeneous cell population in different tissues [1,2]. Mesenchymal cells (e.g., myofibroblasts and smooth muscle cells) play an important role in organ function [3,4] .They lack attachments and tight junctions and have a spindle or stellate shape that allows the cells to move freely in the extracellular matrix . EndoMT is the process of endothelial to mesenchymal cell differentiation [5], which is an important pathological process that causes chronic inflammation and fibrosis in blood vessels. During this process, endothelial cells gradually lose endothelial-specific markers and acquire a mesenchymal phenotype [6]. The expression of mesenchymal-specific factors are increased [7].

Under inflammation-related pathological conditions, Endothelial cells have a higher incidence of EndoMT, such as myocardial infarction, portal hypertension, and pulmonary hypertension [8].EndoMTs also promote the development of ath-

erosclerotic lesions [9]. The mechanism of EndoMT is complex because multiple transcription factors and signaling pathways are involved [10], and many signaling pathways also cross-influence each other. We highlight some of the typical organ fibrosis and pathways that are being targeted as potential therapies for a variety of human diseases.

EndoMT and renal fibrosis

Renal fibrosis is characterized by myofibroblast aggregation, excessive deposition of extracellular matrix (ECM) [11], which is closely associated with tubular and interstitial capillary loss [12]. Therefore, myofibroblasts are considered to be important players in the process of renal fibrosis.LeBleu VS et al. used multiple genetically engineered mice to track, fate map and ablate cells to determine the source and function of myofibroblasts in kidney fibrosis, experimentally showed that 10% of myofibroblasts in fibrotic kidneys were derived from EndoMT [13].

In 2008, Zeisberg et al [14] demonstrated that EndoMT is an important mechanism leading to the aggregation of activated fibroblasts and myofibroblasts in renal fibrosis. And they identified the key role of EndoMT in renal fibrosis through three mouse models: (1) Unilateral ureteral obstructive nephropathy, (2) streptozotocin-induced diabetic nephropathy, and (3) a model of Alport renal disease.In their study, they demonstrated that in all three models, a variety of myofibroblasts can co-express the endothelial marker CD31, myofibroblast marker α-smooth muscle actin (α-SMA), and fibroblast-specific protein-1 (FSP-1). They claimed that 40-50% of activated fibroblasts are derived from EC. The data confirmed that EndoMT promotes the proliferation of myofibroblasts in the early stage of renal fibrosis and a considerable number of myofibroblasts of endothelial origin in fibrotic kidneysusing diabetic mouse model.

EndoMT and cardiac fibrosis

Zeisberg et al. showed that during cardiac fibrosis, endothelial cells join the total group of cardiac fibroblasts via EndoMT, which make up 27-35% of cardiac fibroblasts[15]. During cardiac development, endocardial cardiomyocytes are the main source of coronary vascular cardiomyocytes, and these cardiomyocytes via EndoMT produce mesenchymal cells with plasticity and migratory properties [16]. Under normal physiological conditions, this cell fate conversion is required for the normal formation of cardiac valves in the developing heart [17] . However, EndoMT is also involved in the development of several cardiovascular diseases (CVDs) such as atherosclerosis, adult valvular disease, myocardial fibrosis, and pulmonary arterial hypertension (PAH)[18] . Endothelial cells acquire a fibroblast phenotype and then migrate to the myocardial layer to produce collagen type I (collagen I) thus leading to myocardial fibrosis. Numerous studies have shown that inhibiting of EndoMT can slow the progression of these cardiovascular diseases.

EndoMT and Pulmonary Fibrosis

Viruses, bacteria, drugs, dust, etc. can cause pulmonary fibrosis, which often has an insidious onset, progressive exacerbation, and respiratory failure in the late stage.EndoMT is associated with various environmental or signaling responses involved in pulmonary arterial hypertension PAH [19], and plays an important role in pulmonary vascular remodeling of PAH.

Hashimoto et al [20] are the first to find that En-

doMT is involved in bleomycin-induced pulmonary fibrosis and associated with rat sarcoma virus oncogene homolog (RAS) and transforming growth factor-beta (TGF-beta) activation.EndoMT is also involved in pulmonary fibrosis induced by mechanical ventilation, which induces NLRP3 activation in lung tissue. Direct mechanical stretching of primary mouse lung vascular endothelial cells in vitro leads to similar NLRP3 activation and formation of EndoMT, which can be prevented by knockdown of the NLRP3 gene [21].

Mechanisms

In recent years, it has been a popular trend to explore the regulatory mechanisms and cytokine changes of EndoMT. Many EndoMT-related signaling pathways have been identified, including the TGF-β signaling pathway, the Notch signaling pathway, the Wnt signaling pathway, and the Hedgehog (Hh) signaling pathway.

TGF-β (Transforming Growth Factor-β)

TGF-β-mediated signaling pathways, especially the TGF-β/Smads signaling pathway, is the most important and classical signaling pathway for EndoMT in physiological and pathological conditions [22].There are three isoforms of TGF-β ligands (TGF-β1, 2, and 3). TGF-β receptors are classified into type I and type II, and TGF-β ligands bind to type II receptors to form a complex, which then activates TGF-β type I receptors and turns on downstream signaling [23].TGF-β1 has been the most studied in pathological EndoMT, while TGF-β2 is more involved in developmental EndoMT [24]. Both in vitro and in vivo studies have confirmed TGFβ signaling-induced EndoMT [25, 26]

In vivo, TGF-β is secreted as inactive precursor proteins. Its constituents, LAP and LTBP are activated by cleavage by fibrinolytic enzymes, integrin β6 matrix metalloenzymes, etc. TGF-β regulates endothelial mesenchymal transdifferentiation through Smad-dependent and Smad-independent pathways, respectively [27,28].

Additionally, in the process of EndoMT TGF-β signaling pathway also interacts with other signaling pathways, such as Wnt, MAPK and Notch, which is extremely complex.Deficiency of negative regulators of the TGF-β1 pathway promotes renal fibrosis, and up-regulation of the expression of these negative regulators can be in the improvement of renal fibrosis [29].

Notch

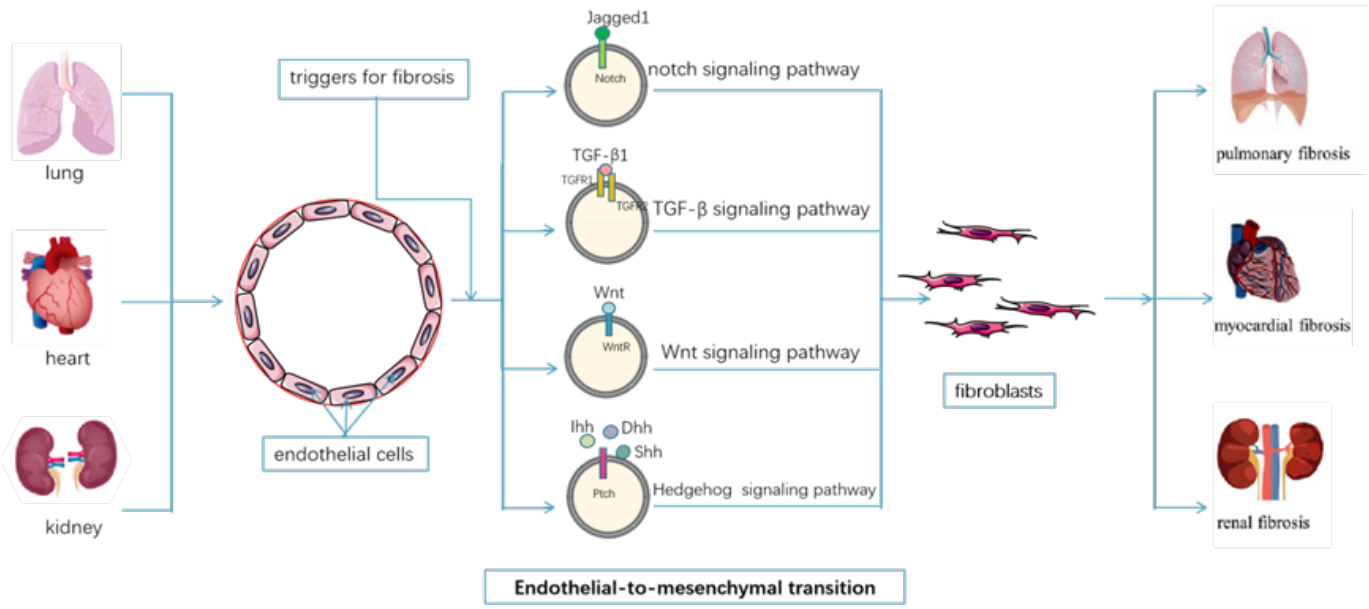
The Notch signaling pathway is critical for the development and homeostasis of several organs(e.g. kidney [30]). Therefore its abnormalities can lead to a variety of pathological changes, such as abnormal organ development or fibrotic diseases.The Notch signaling pathway consists of Notch receptors, Notch ligands, intracellular effector molecule-binding proteins, and target genes.This signaling pathway can regulate the processes of cell proliferation, cell differentiation, and apoptosis. EndoMT occurs in human umbilical vein endothelial cells and microvascular endothelial cells in a high Na+ environment and is regulated by Notch signaling [31].

Wnt/β-Catenin

Wnt ligands are a family of secreted glycoproteins with highly complex receptor signaling pathways, which present during embryonic development and in cardiovascular, inflammatory, autoimmune, and fibrotic diseases.The Wnt signaling pathway is a process that activates relevant target genes via intranuclear β-catenin (β-catenin). In a mouse model of myocardial infarction, classical Wnt signaling

pathway is activated, which then induces EndoMT in subendocardial endothelial cells. And this conclusion is consistent with other findings of decreased expression of VE-cadherin and increased expression of fibronectin caused by β-catenin accumulation in the nucleus [32].

Until now, researchers have identified 19 different Wnt ligands in mammals. Among them, the most common receptors for Wnt ligands includes the ten Frizzleds (FZD(1-10)), the co-receptor lipoprotein-related receptors 5 and 6 (LRP5/6), the RYK family, and tyrosine kinase receptors. Under normal physiological and pathological conditions, the combined action of Wnt ligands and their specific receptors activates various intracellular pathways. In a typical Wnt signaling pathway, binding of the Wnt ligand Wnt-3a to LRP5/6 and FZD1-10 triggers an intracellular signaling cascade that leads to the inactivation of GSK-3β, which is a multiprotein complex that phosphorylates and ubiquitinates proteases to degrade β-catenin. If GSK-3β is inactivated, β-catenin could not be degraded and would accumulate and translocate to the nucleus. In the nucleus, it acts on the expression of TCF/LEF family transcription factors to regulate downstream signaling.Researchers observed expression of the Wnt/β-catenin pathway in fibroproliferative disorders of renal and hepatic tissues, confirming that the Wnt signaling pathway promotes renal fibrosis through epithelial-mesenchymal transition in chronic kidney disease [33].



Hh (Hedgehog)

The Hh signaling pathway also plays a key role in embryonic development, tissue and organ physiology. Activated Hh signaling pathway is present in fibrotic diseases of liver, lung and kidney. In mammals, there are three Hh ligands (Dhh, Ihh, and Shh), and Hh signaling involves two transmembrane proteins, Patched (Ptch) and Smoothened (Smo). In the absence of Hh ligand, Ptch protein inhibits Smo activity. If Hh ligand binds to Ptch protein, Smo activates the transcription factor Gli, which translocates to the nucleus, regulates the expression of fibrogenic genes(e.g. Snail) and induces epithelial/endothelial mesenchymalization and production of extracellular matrix (ECM). In a mouse model of renal fibrosis, Li L et al. Found that the expression of Ptch1 and Gli1 was upregulated in the epithelium of renal cortex and medullary tubules. On the other hand, using Smo antagonist IPI-926 (saridegib) inhibited Gli expression

and delayed renal fibrosis, which suggests the Hh signaling pathway has a role in the course of chronic kidney disease [34].

Hypoxia

Oxygen plays an important role in physiological functions because it participates in cofactor/substrate for many enzymes. Hypoxia-inducible factor (HIF), a central regulator of oxygen detection at the cellular level, is a heterodimeric transcription factor composed of HIF-1α or HIF-2α and HIF-1β/ARNT subunits.Under normoxic conditions, prolyl hydroxylases, such as PHD2 and PHD3 can degrade HIF-1α, and the hypoxic environment inhibits prolyl hydroxylase activation [35]. As a transcription factor, HIF-1α binds to the hypoxia-responsive element of the Twist1 promoter and regulates the expression of EndoMT-associated genes such as TGF-β and Twist [36,37].

Endothelial cells are one of the main targets influenced by hypoxia, which activates the receptor for advanced glycation end products (RAGE) and stimulates p38 mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) signalling to accelerate renal disease[38]. In this process, endothelial cells differentiate into myofibroblasts, which then increase the production of ECM and lead to severely hypoxia in the kidney[39].

MicroRNAs

MicroRNAs (miRNAs) are short (20-23 nucleotides) and conserved non-coding RNAs, which regulate the expression of protein-coding genes by base-pairing with the complementary sequences of the target mRNAs [40]. MiRNAs are involved in the processes of cell proliferation, cell differentiation and cell death .

In recent years, miRNAs have been found to be able to regulate EndoMT[41].Moskalik A et al. suggest that the supernatant from miR-31-5p-modified RAW 264.7 could downregulate the mRNA expression for genes regulating endothelial-to-mesenchymal transition (EndoMT) and fibrosis in LECs[42]. MiR-200c-3p has the ability to promote EndoMT, while using miR-200c-3p inhibition could reduce EndoMT[43].

Conclusions

The occurrence of EndoMT in vascular endothelial cells is closely related to renal fibrosis, cardiac fibrosis and pulmonary fibrosis. And this process is associated with various of signaling pathways, including TGF-β signaling pathway, Notch signaling pathway, Wnt signaling pathway, and Hedgehog (Hh) signaling pathway, etc. The intersection of the above signaling pathways forms a complex signaling network. This review summarizes the recent research findings, and aims to provide a basis for future intervention targets.

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