Endothelial to mesenchymal transition

Lung diseases, such as pulmonary hypertension and pulmonary fibrosis, are life-threatening diseases and have common features of vascular remodeling. During progression, extracellular matrix protein deposition and dysregulation of proteolytic enzymes occurs, which results in vascular stiffness and dysfunction. Although vasodilators or anti-fibrotic therapy have been mainly used as therapy owing to these characteristics, their effectiveness does not meet expectations. Therefore, a better understanding of the etiology and new therapeutic approaches are needed. Endothelial cells (ECs) line the inner walls of blood vessels and maintain vascular homeostasis by protecting vascular cells from pathological stimuli. Chronic stimulation of ECs by various factors, including pro-inflammatory cytokines and hypoxia, leads to ECs undergoing an imbalance of endothelial homeostasis, which results in endothelial dysfunction and is closely associated with vascular diseases.

1. Introduction

Endothelial cells (ECs), a monolayer composed of the inner cellular lining of the vascular lumen, play an important role in various physiological processes to maintain vascular homeostasis [1][2][3]. These cells are involved in the regulation of vascular tone, permeability, and inflammatory responses [4]. However, endothelial injury by stimuli, such as hypoxia, pro-inflammatory cytokines and abnormal mechanical forces, can induce endothelial-to-mesenchymal transition (EndMT), resulting in endothelial dysfunction and destruction of homeostasis [2][5]. EndMT is the process by which ECs lose their cellular features and acquire mesenchymal characteristics [6]. EndMT-derived cells gain migration potential by losing endothelial markers, such as cluster of differentiation 31 (CD31) and vascular endothelial cadherin (VE-cadherin), which are involved in cell-to-cell contact [7][8]. Concomitantly, the expressions of mesenchymal markers, such as fibronectin, alpha-smooth muscle actin (SMAα), smooth muscle protein 22 alpha, vimentin, and neural cadherin (N-cadherin), are upregulated [7][8]. The morphology of ECs undergoing EndMT changes from a cobblestone monolayer to an elongated phenotype [9]. This phenomenon mainly occurs during embryonic cardiac development, but is also involved in various lung diseases, such as pulmonary arterial hypertension (PAH) and pulmonary fibrosis (PF) (Figure 1) [7][10][11][12][13].

Figure 1. A schematic representation of endothelial-to-mesenchymal transition (EndMT) involved in lung diseases. Endothelial cells stimulated by transforming growth factor-β (TGFβ), interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNFα), and hypoxia undergo EndMT. EndMT is characterized by phenotypic change from a cobblestone into an elongated shape, loss of endothelial markers, and the acquisition of mesenchymal markers. EndMT contributes to the pathogenesis of lung diseases, including pulmonary arterial hypertension (PAH), radiation-induced pulmonary fibrosis (RIPF), and idiopathic pulmonary fibrosis (IPF). Various mediators and transcription factors are identified in this process.

Pulmonary hypertension (PH) is categorized into five groups: PAH, PH due to left heart disease, PH due to lung diseases and/or hypoxia, PH due to pulmonary arterial obstructions, and PH with unclear and/or multifactorial mechanisms [14][15][16]. PAH has been defined as pulmonary artery pressure (PAP) ≥25 mmHg at rest and occurs as a result of multiple causes, such as heritable factors (mainly bone morphogenic protein receptor-2 (BMPR2) mutations), drugs and toxins, as well as association with other diseases; however, PAH without known causes is known as idiopathic PAH (IPAH) [14][17]. Vascular remodeling in PAH is characterized by the aberrant proliferation of pulmonary arterial ECs (PAECs) and smooth muscle cells (SMCs), which form occlusive neointima and vascular structural changes [18][19][20]. These progressive changes cause excess vasoconstriction and right ventricle hypertrophy and, ultimately, death [18][19][20]. Endothelial dysfunction is a key player in the pathogenesis of PAH [21]. Growing evidence suggests that EndMT potentially contributes to endothelial dysfunction and the vascular remodeling of PAH [7][11][22][23]. Indeed, many studies have demonstrated that various signaling pathways and mediators, including transforming growth factor beta (TGFβ), nuclear factor kappa B (NF-κB), Notch, and microRNA, are involved in the EndMT of PAH [24][25]. It has been reported that the endothelial-specific loss of BMPR2, known as the principal mutation factor of heritable PAH, induces EndMT in vitro and in vivo [7][11][23]. In

addition, exposure to hypoxia or chronic stimulation with proinflammatory cytokines or TGFβ also induce EndMT in vitro and in vivo [26][27][28][29][30]. However, the contribution of EndMT to disease progression is not fully understood [2]. Current therapies for PAH, such as phosphodiesterase-5 inhibitors, prostacyclin analogues, and endothelin receptor antagonists, can help relieve symptoms and slow progression, but there is no effective treatment [21][31]. Thus, targeting EndMT is emerging as a novel therapeutic approach by alleviating vascular remodeling and the PAH phenotype in vitro and in vivo [7][29][32][33][34][35][36].

Idiopathic PF (IPF) is chronic, progressive, and the most common interstitial lung disease without a definite etiology [37][38]. Various cell types, such as epithelial cells, pneumocytes, ECs, pericytes, fibrocytes, resident fibroblasts, and mesenchymal cells, are associated with the pathogenesis of IPF [25]. The injured epithelial cells, through aging, genetic susceptibility and repetitive microinjury, release fibrogenic factors and cytokines, resulting in the recruitment of contractile myofibroblasts, which are key cellular mediators of fibrosis [38]. Recruited myofibroblasts undergoing activation and proliferation induce extracellular matrix expansion, which consequently results in aberrant vascular remodeling in the lung [38]. The myofibroblasts are derived not only from the proliferation of resident mesenchymal cells, circulating fibrocytes, lung interstitium pericytes, epithelial–mesenchymal transition, but also EndMT. [38][39][40]. Many studies have demonstrated that EndMT occurs in the lung tissue of IPF patients and animal models, suggesting EndMT may play an important role in pathological processes in PF [25][41][42]. In addition, emerging evidence indicates that inhibiting EndMT can also be a therapeutic strategy in PF in vivo [41][43][44][45].

2. EndMT in Pulmonary Hypertension

PH is characterized by the muscularization of arterioles, medial thickening, plexiform region formation, intimal fibrosis, and the hyperproliferation of ECs and SMCs [15][16][46][47]. Most studies have identified EndMT by analyzing the co-expression of endothelial markers and mesenchymal markers in the lung tissue of patients and experimental PH animal models. EndMT has been observed in pathological lesions in the lungs of PH patients [7][30][32][48][49]. Endothelial (CD31, CD34, and VE-cadherin) and mesenchymal marker (SMAα) double-positive cells were observed in intimal and plexiform lesions in the lung tissue of PAH patients [7]. Another group also demonstrated that neointimal and plexiform lesions in the lung tissue of human PAH patients contain endothelial markers, CD31 or von Willebrand factor (vWF), and SMAα co-expressing cells [48]. Isobe et al. reported that the CD44 spliced variant form (CD44v) results from EndMT, and its positive cells also expressed vWF and SMAα in neointimal lesions of IPAH patients [32]. The 4 ± 1% of pulmonary arterioles in systemic sclerosis (SSc)-PAH patients showed vWF/SMAα co-localization [30]. CD31 and SMAα co-expressing cells were detected in endarterectomized tissues from patients with chronic thromboembolic pulmonary hypertension (CTEPH) [49].

In addition to performing the double staining of endothelial and mesenchymal markers, ECs isolated from the lung have also been used for studying EndMT [49][50]. Endothelial-like cells isolated from the vascular tissue of patients with CTEPH underwent disruption of the endothelial monolayer and abnormal growth even after sorting with CD31 [49]. In addition, conditioned media from myofibroblast-like cells isolated from CTEPH patients induced phenotypic changes and mesenchymal marker expression in pulmonary microvascular ECs (PMVECs) [49]. Pulmonary vascular ECs (PVECs) isolated from patients with IPAH exhibited molecular characteristics of EndMT and a spindle-shaped morphology, which was similar to that of normal PVECs treated with TGFβ1, a well-known factor of EndMT [50]. Pulmonary arteries isolated from PAH patients showed increased mRNA levels of mesenchymal markers and EndMT-related factors, which also supports EndMT [7].

Animal models have also been used to demonstrate EndMT. Monocrotaline (MCT) injection causes endothelial injury and pulmonary vascular remodeling, and is commonly used to induce severe PH [50][51]. Several groups observed the reduction of endothelial markers and the induction of mesenchymal markers, as well as the co-staining of SMAα and endothelial marker (CD31 or CD34), in the lung tissue of MCT-induced PH rats [7][28][29][50][52]. Zhang et al. found that changes in endothelial and mesenchymal cell marker expressions occurred in a time-dependent manner during MCT-induced PAH development [51]. Chronic hypoxia also contributes to the vascular remodeling of small pulmonary arteries [27][53]. With this, it has been demonstrated that three weeks of hypoxia induces EndMT in the pulmonary arteries of rats and mice [26][53]. EndMT was further identified within the intimal layer of small pulmonary arteries, but not in large arteries, in chronic hypoxia-induced PH rats [27]. The combination of SU5416, a vascular endothelial growth factor receptor antagonist, and a chronic hypoxia model (SuHx) has been used for severe PH owing to the similarity of pathological lesions to plexiform lesions of human PAH [53]. In the lung of the SuHx model that had over 80 mmHg of right ventricular systolic pressure (RVSP), transitions of vWF+ vimentin− ECs to vWF− vimentin high mesenchymal-like cells were observed in pulmonary vascular lesions [7]. Tie2+ vimentin+ and Tie2+ SMAα+ cells were also found in occlusive lesions [7]. In addition, 6 ± 1% of pulmonary vessels had vWF/SMAα double-positive ECs, which contrasts with normal tissues having only 1% transitional EndMT cells in SuHx mice [30].

In general, endothelial and mesenchymal marker double-positive cells are considered EndMT-induced cells. However, this approach has the limitation of not being able to distinguish complete EndMT (cEndMT), where there are lost endothelial markers, and partial EndMT (pEndMT) cells. To overcome this problem, several studies have used endothelial-specific fluorescence transgenic animals [48][54]. Qiao et al. established VE-cadherin Cre or Tie2 Cre-mTomato/mGFP lineage-tracing mice [48]. Histological analysis identified SMAα-expressing neointima in an experimental PH animal model derived from the endothelium in VE-cadherin Cre or Tie2 Cre-mTomato/mGFP lineage-tracing mice [48]. Furthermore, cEndMT cells isolated from SuHx-induced Cdh5-Cre/CAG-GFP double-transgenic mice showed a spindle-like morphology and were characterized by mesenchymal-like functions, such as high proliferation and migration ability [54]. Additionally, conditioned media from cEndMT had a paracrine effect on the proliferation and migration of non-endothelial mesenchymal cells, suggesting that EndMT contributes directly and indirectly to the vascular remodeling of PAH [54].

3. EndMT in Pulmonary Fibrosis

IPF characterizes matrix deposition and fibrotic tissue remodeling, and it has been demonstrated that fibroblasts are involved in pathogenesis; thus, efforts to identify the origin of fibroblasts have been made [42][55]. In the lung tissue of radiation-induced pulmonary fibrosis (RIPF) patients and radiation-exposed mouse models, the co-localization of CD31 and SMAα was significantly elevated compared to that of the control group, indicating EndMT [41]. The same group also reported endothelial heat shock protein beta 1 (HSPB1)-dependent EndMT in the PF of lung cancer [45]. The bleomycin-induced PF in animal models is the most commonly used model to study human IPF by causing damage to epithelial cells and alveolar inflammation [56][57]. Another group reported significant alterations of EC markers in the lungs of bleomycin-treated endothelial-specific autophagy-related 7 (ATG7) knockout mice compared to bleomycin-treated WT mice [58]. Hashimoto et al. established a Tie2-Cre/CAG-CAT-LacZ double transgenic mice model to track endothelial-derived fibroblasts in bleomycin-induced PF [42]. The 16.2% of lung fibroblasts isolated from bleomycin-treated mice were X-gal-staining-positive and 14.8% of X-gal-positive cells were SMAα- and Collagen I-double positive (myofiboblast), while the other 85.2% were SMAα-negative and Collagen I-positive, suggesting that a significant number of fibroblasts are EC-derived [42]. Suzuki et al. demonstrated that PVECs isolated from lipopolysaccharide (LPS)-induced mouse lungs undergo EndMT using the double staining of CD31 and SMAα or S100A4 [59]. Flow cytometry analysis showed that the number of SMAα + PVECs and S100A4 + PVECs increased, while the total number of PVECs decreased [59].

Taken together, EndMT may play a key role in the pathogenesis of lung diseases. Many studies describe EndMT based on the evidence of co-expression of EC markers and mesenchymal markers in the lung tissue of animal disease models or human patients, which has a primary limitation because EndMT is a switching process; thus, the underlying molecular mechanisms are not yet fully understood. The methods to clarify partial and complete EndMT processes have been improved using endothelial-specific fluorescence transgenic mice; however, further investigation with human samples is needed. Thus, the clinical relevance of EndMT should be thoroughly assessed.

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