Endothelial-to-Mesenchymal Transition in Pulmonary Hypertension

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Abstract

Pulmonary arterial hypertension (PAH) is an incurable disease characterized by an intense thickening of the pulmonary arteries leading to their progressive obliteration. This vascular remodeling is a direct consequence of an aberrant accumulation of α -smooth muscle actin (α -SMA)-positive cells in the intravascular area. This process is multifactorial and complex. Recent studies have demonstrated that part of α -SMA-positive cells from intimal and plexiform lesions have an endothelial origin through a process called endothelial-to-mesenchymal transition (EndoMT). Interestingly most of molecular pathways implicated in PAH are known inducers of EndoMT. This review describes how EndoMT appears to play a crucial role in PAH progression.

Keywords

Pulmonary arterial hypertension · Endothelial-to-mesenchymal transition · Vascular remodeling · Endothelial dysfunction

6.1 Pulmonary Hypertension

Pulmonary arterial hypertension (PAH) is histologically characterized by an aberrant remodeling of the pulmonary arterial bed that includes adventitial, medial and intimal thickening as well as neo-muscularization of pre-capillary arterioles. These

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modifications of the pulmonary vessels increase the vascular resistance and ultimately lead to the progressive occlusion of the lung vasculature. PAH is multifactorial and the underlying mechanisms that lead to the pathological accumulation of α -smooth muscle actin (α -SMA)-positive cells implicated in the remodeling process are yet under investigation. Among the possible explanations, it has been reported that the pathologic α -SMA cells were derived from circulating bone marrow-derived cells, pericytes or mesenchymal stem cells [1]. Growing evidence also suggest that part of α -SMA-positive cells may have an endothelial origin via a trans-differentiation mechanism called endothelial-to-mesenchymal transition (EndoMT).

6.2 Endothelial-to-Mesenchymal Transition

EndoMT is a key process in vascular development and tissue regeneration. In healthy vessels, the endothelial cells (ECs) are bound in a monolayer fashion by tight and adherens junctions that control EC polarity and barrier functions. During the EndoMT process, ECs lose their junctions and polarity to the endothelium and progressively switch from endothelial to mesenchymal markers. This phenotype switch is accompanied with invasive and proliferative properties that allow EndoMT cells to colonize the surrounding environment. The resultant mesenchymal cells become pluripotent and give rise to various lineages. EndoMT is crucial during embryonic development, angiogenesis and tissue regeneration but is also implicated in pathological process like cancer development, atherosclerosis and fibrotic diseases [2].

EndoMT is regulated by key transcription factors Snail/Slug/ZEB/Twist [3]. When expressed, these transcription factors primarily repress endothelial junctions. These junctions are composed of transmembrane proteins (such as VE-cadherin, CD31, Claudins and occludin) that are bound to the cytoskeleton and stabilized by cytoplasmic scaffold proteins (such as p120-catenin, β -catenin or ZO-1) [4]. EndoMT-related transcription factors down-regulate the junction proteins, inducing the dissolution of the cell-cell junctions and the loss of EC polarity in the early stages of transition [3]. The disruption of the junctions also releases free scaffold proteins in the cytoplasm that, if not degraded, trigger secondary pathways promoting ECs proliferation and motility. For example, the inhibition of Claudins and occludin induces the release of ZO-1 from tight junction which promotes cell proliferation via CD1 and PCNA up-regulation [5]. Similarly, the inhibition of VE-cadherin leads to accumulation of free p120-catenin that promotes invasiveness by inhibiting RhoA activity, up-regulating mesenchymal cadherin and activating Rac1/MAPK and Ras/MAPK signaling pathways [6]. Therefore, the chronic activation of EndoMT pathways causes the loss of endothelium integrity (which directly regulates vascular homeostasis, angiogenesis and inflammation) and the invasion of the sub-endothelial area by proliferative mesenchymal cells.

6.3 EndoMT in PAH Pathogenesis

6.3.1 EndoMT in PAH Vascular Remodeling

Aberrant vascular remodeling with occlusive α -SMA-positive cell accumulation forming a neo-intima and endothelial dysfunction are hallmarks of PAH pathogenesis. Both features can be the results of an unresolved EndoMT process as suggested by Arciniegas and colleagues [7]. Our laboratory was the first to confirm the occurrence of EndoMT in situ in idiopathic PAH [8]. Using immunofluorescence, transmission electron microscopy and correlative light and electron microscopy, we provided phenotypic and ultrastructural evidences of ongoing EndoMT in remodeled PAH arteries. We were able to observe ongoing EndoMT in intimal and plexiform lesions where α -SMA-positive cells' accumulation contributed to vessel obliteration. These findings were associated with a loss of endothelial p120-catenin, which is essential for adherens junction and with an overexpression of Twist in PAH lungs. Similar results were observed in two established animal models of pulmonary hypertension (PH) induced by monocrotaline and Sugen/hypoxia. EndoMT was also observed in systemic sclerosis-associated PAH where cells expressing both endothelial and mesenchymal markers were found in 4% of the remodeled vessels [9]. Since then various studies have confirmed the occurrence of an ongoing EndoMT process in PAH with the overexpression of transcription factors Twist/Snail/Slug [10–12].

6.3.2 Molecular Pathways of EndoMT in PAH

The mechanisms that regulate EndoMT are complex and not fully understood but appear to be close of those involved in the epithelial-to-mesenchymal transition (EMT). Interestingly the main characteristics of PAH pathological environment are known inducers of EndoMT and EMT. Indeed, the ECs in PAH are locally exposed to chronic inflammation, hypoxia, mechanical stress and loss of endothelial junction. Inflammation is highly associated with EMT-associated tumor progression and EndoMT. Tumor necrosis factor (TNF)- α , interleukin (IL)-6, transforming growth factor (TGF)- β or IL-8 promote ZEB/Snail/Twist expression via NF κ B and JAK/STAT signaling. Cells undergoing mesenchymal transition also induce local inflammation via increased secretion of cytokines such as IL-6, IL-8 and TNF- α [3, 9, 13]. Hypoxia is also a well-described inducer of EndoMT though hypoxia-inducible factor 2 α (HIF-2 α) activation. Tang et al. demonstrated that PAH ECs display an increased expression of HIF-2 α even under normoxic conditions, and this leads to the up-regulation of Snail/Slug and therefore aberrant EndoMT [12]. Of note, HIF-1 α , that is found up-regulated in PAH smooth muscle cells, induces overexpression of Twist. The increased pressure and shear stress due to disturbed blood flow are known to trigger EndoMT to initiate adaptative muscularization of vessels but are also linked to neointimal hyperplasia in other vasculopathies like

atherosclerosis [14]. Finally, the shear stress resulting from increased vascular resistances further damages the endothelial junctions. As presented above, it triggers EndoMT to overcome the mechanical stress by increasing vascular thickness. Nevertheless, as EndoMT reduces the overall endothelium integrity and increases the vascular remodeling, it may enter in a vicious circle of unresolved EndoMT. The pathological environment of chronic inflammation and increased pressure may then lead to an undesirable feedback loop leading to further endothelial dysfunction, inflammation and shear stress as the vascular remodeling progresses.

The main molecular pathways implicated in PAH development are also related to EndoMT molecular mechanisms. The TGF- β signaling pathways play a crucial role in PAH. TGF- β is found increased in serum and lungs from PAH patients and most of the genetic mutations related to PAH occur in genes encoding receptors of the TGF- β superfamily [1]. Interestingly TGF- β signaling is a key actor of EndoMT either via Smad-dependent or Smad-independent pathways. The activation of TGF- β receptors initiates Smad2/3/4 cascade which up-regulates Snail/Slug/Twist/ZEB. TGF- β also promotes EndoMT through various Smad-independent pathways leading, for example, to the inactivation of GSK-3 β , a repressor of Snail- and β -catenin-induced EndoMT or to the PI3K/AKT-mediated up-regulation of Twist/Snail/Slug/ZEB expression through mTOR and NF- κ B activation. Finally autocrine feedback loops appear in TGF- β -induced EndoMT since Twist overexpression in human pulmonary ECs also up-regulates TGF- β expression [11].

In addition, deficient signaling through bone morphogenetic protein receptor type 2 (BMPR-II), a member of the TGF- β receptor superfamily, plays a critical role in PAH. Indeed, mutations in the *BMPR2* gene are found in 70% of cases of familial PAH and in 10–40% of cases of idiopathic PAH. Moreover, in the absence of a *BMPR2* mutation, reduced expression of pulmonary vascular BMPR-II has also been observed in non-hereditary PAH. We created a rat strain with monoallelic mutations on *BMPR2*, which develops spontaneously PAH. In this animal model, altered BMPR-II signaling was associated with increased pulmonary Twist expression and EndoMT [8]. This link between the main genetic risk factor for PAH and unregulated EndoMT was confirmed by Hopper and colleagues as they found that reduced BMPR-II also promotes Slug overexpression via HMGA1 up-regulation [10].

The TGF- β pathways are not fully characterized, have feedback loops, talk to each other and have interactions with others signaling such as Notch, Wnt and receptor tyrosine kinases (RTKs), all involved in PAH pathogenesis and EndoMT [3, 15–20]. Notch1 was reported to be increased in lung from idiopathic PAH patient and Sugen/hypoxia rats [20], and Notch1/3 were found up-regulated in hypoxia- and MCT-induced PH [21]. Notch signaling also interacts with PAH-associated pathways such as HIF-1 α and IL-6 [3, 15–19]. Notch activation up-regulates Snail/Slug expression [3]. In PAH, both canonical Wnt and non-canonical Wnt were found up-regulated [22, 23]. In the first one, activation of Wnt leads to GSK-3 β inhibition and Snail- and β -catenin-induced EndoMT [3, 15, 16]. In the non-canonical one, Wnt activates GTPases Rac/Rho/cdc42, all involved in migration and

cytoskeleton rearrangement [24]. Several growth factor signaling that act through RTKs are found up-regulated in PAH, including epidermal growth factor, fibroblast growth factor, vascular endothelial growth factor and platelet-derived growth factor [1]. Their roles are incompletely understood due to feedback loop regulations among given growth factor signaling and because of contradictory effects on pulmonary vasculature when used in vivo probably due to their numerous isoforms of receptor and ligand as well as the structural similarities and signaling versatility between different RTKs. Some RTK inhibitors like imatinib or sorafenib have been shown to exert beneficial effects in animal models of PH whereas others like dasatinib or Sugen are known inducers of PAH [25]. Interestingly the activation of most of those RTK signalings initiates the PI3K/AKT/NF- κ B and Ras/MAPK pathways that ultimately up-regulate Twist/Snail/Slug/ZEB. RTKs signaling also cross talks with other EndoMT pathways like TGF- β -, β -catenin or Wnt-induced EndoMT via the common PI3K/AKT-dependant inhibition of GSK-3 β [3]. Nevertheless despite improvements in haemodynamics and exercise capacity, the serious adverse events associated with RKT inhibitors in PAH therapies currently outweigh their beneficial role observed in animal models [1].

Other molecular actors of PAH pathogenesis might also contribute to EndoMT. For example, endothelin-1, which is overexpressed in PAH and contributes to vasoconstriction and vascular remodeling [1], has also been reported to induce EMT. It can trigger transdifferentiation either by activating both ET_A/ET_B receptors and subsequent PI3K/ILK/GSK-3 β and AMPK pathways, or by repressing miR-300, a negative regulator of Twist or by inducing TGF- β overexpression [26–28]. Another hallmark of PAH is an increased level of serotonin in the patients' serum [25]. This neurotransmitter was reported to induce EMT in renal proximal tubular epithelial cells [29] and inhibition of its receptors 5-HT1B/5-HT1D down-regulates ZEB/Snail expression and undergoing EMT in human pancreatic cancer cells [30]. Finally, angiotensin-II which is increased in patients' blood with increased signaling through its type 1 receptor in PAH ECs [31] has also been implicated in EMT and EndoMT in cancer and diabetes-related cardiovascular diseases [32–34].

6.4 Conclusion

PAH has a multifactorial and complex pathobiology. Growing evidences support that an unresolved EndoMT process is actively implicated in the vascular remodeling and endothelial dysfunction involved in the disease progression. EndoMT can be the direct consequence of PAH pathological environment and dysregulated molecular pathways. As presented, EndoMT mechanisms are yet incompletely understood but their cross talk with most of PAH hallmarks leads us to believe that EndoMT might be a crucial actor in PAH progression and a promising therapeutic target.

6.5 Future Direction and Clinical Implications

EndoMT is a crucial actor in embryonic development and tissue regeneration in adult tissues. Numerous studies have demonstrated that the same cellular mechanisms of EndoMT are also implicated in the progression of cancers and cardiovascular diseases. Growing evidences support that EndoMT might play a central role in PAH pathogeny. These findings open exciting opportunities in the study of the initial triggers and the vascular remodeling process of PAH. A better understanding of EndoMT in PAH could lead to the development of novel anti-remodeling strategies in the management of PAH with potentially limited side effects on healthy tissues.

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