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Review Article

Transdifferentiation of endothelial cells to smooth muscle cells play an important role in vascular remodelling

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Abstract: Pulmonary artery remodelling it is a major feature of pulmonary hypertension (PH). It is characterised by cellular and structural changes of the pulmonary arteries causing higher pulmonar vascular resistance and right ventricular failure. Abnormal deposition of smooth muscle-like (SM-like) cells in normally non-muscular, small diameter vessels and a deregulated control of endothelial cells are considered pathological features of PH. The origin of the SM-like cells and the mechanisms underlying the development and progression of this remodelling process are not understood. Endothelial cells within the intima may migrate from their organised layer of cells and transition to mesenchymal or SM-like phenotype in a process called endothelial-mesenchymal transition (EnMT). Traditionally, Waddington's epigenetic landscape illustrates that fates of somatic cells are progressively determined to compulsorily follow a downhill differentiation pathway. EnMT induces the transformation of cells with stem cell traits, therefore contrasting Waddington's theory and confirming that cell fate seems to be far more flexible than previously thought. The prospect of therapeutic inhibition of EnMT to delay or prevent PH may represent a promising new treatment modality.

Keywords: Endothelial to mesenchymal transition, remodelling, endothelial cells, pulmonary hypertension, cellular reprogramming

Pulmonary hypertension

Pulmonary hypertension (PH) is a complex and progressive disease characterised by increased blood pressure in pulmonary arteries. Hemodynamically, it is defined by a *mean* pulmonary artery pressure at rest exceeding 25 mmHg [1]. Pulmonary vessel remodelling, which consists of intimal, medial and adventitial hypertrophy, leads to a reduction of the vascular lumen [2]. This causes an increase in pulmonary resistance, severe PH, right ventricular failure, and early death [3]. Currently, there is no cure for PH, but available treatments can lessen symptoms and improve quality of life. PH can occur due to genetic or sporadic causes, but the exact basis of PH remain unknown [4, 5].

Pulmonary arterial remodelling

Pulmonary artery remodelling it is a major feature of PH. It is characterised by cellular and structural changes affecting all three layers of the vessel wall of the pulmonary arteries [6, 7]. Common pulmonary vascular remodelling changes in PH include increased intimal and/or medial stiffening and thickening, elevated expression of smooth muscle α-actin, collagen synthesis/deposition, and inflammation [8]. Abnormal deposition of smooth muscle-like (SM-like) cells in normally non-muscular, small diameter vessels and a deregulated control of endothelial cells are considered important pathological features of PH [9]. Also, increased production of extracellular matrix proteins, with deposition of collagen and elastin contribute to lumen narrowing and PH [10]. It is this remodel-

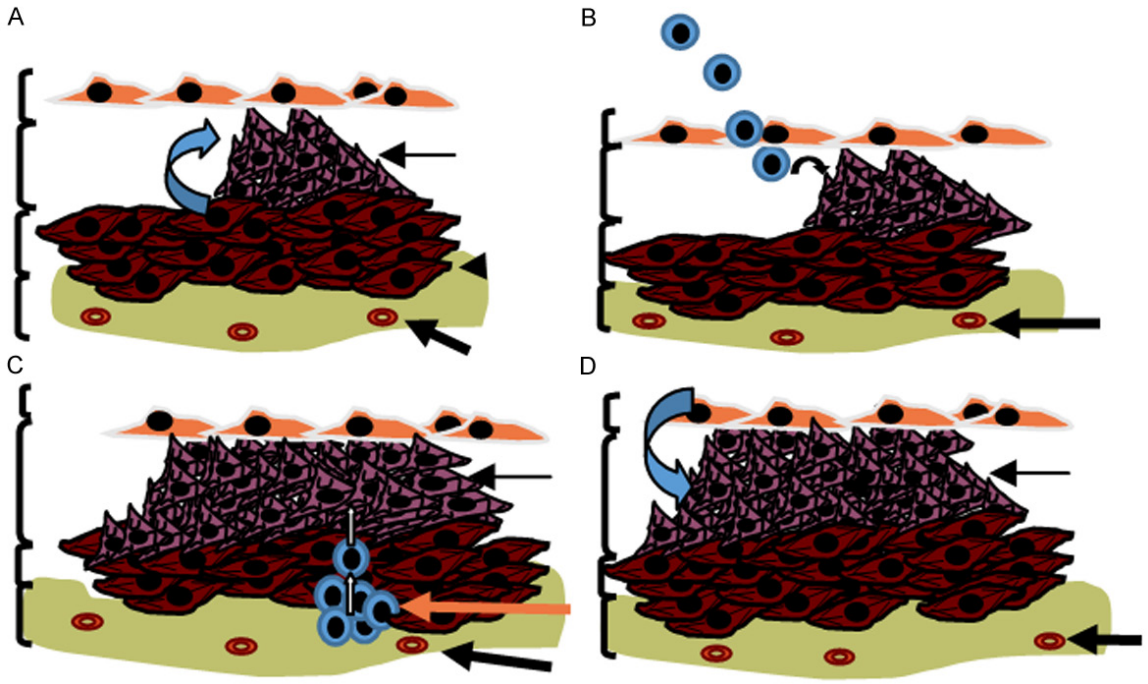

Figure 1. The origin of the SM-like cells in pulmonary arterial remodelling. Resident vascular smooth muscle cells (SMCs) from the medial layer retain high cell plasticity and, under specific circumstances, undergo phenotypic switch towards a synthetic or "de-differentiated" state (A). Differentiated SMCs become highly proliferative and migratory. Circulating progenitor cells could be recruited to sites of vascular injury and assume a SM-like phenotype (B). Resident progenitor cells in the adventitia may also serve as a source of SM-like cells and contribute to the pathophysiological changes in vascular structure (C). Additionally, Endothelial cells within the intima may migrate from their organised layer of cells and transition to mesenchymal or SM-like phenotype in a process called endothelial-mesenchymal transition (EnMT) (D).

ling process inside the pulmonary vessels that is responsible for elevation of pulmonary vascular resistance, progressive PH, right ventricular failure and finally death [11].

The origin of the SM-like cells and the mechanisms underlying the development and progression of this remodelling process are not completely understood. It is been thought that muscularisation of the intimal layer of the vessel wall is caused by proliferation of resident vascular smooth muscle cells of the medial layer which migrate to the intima [12, 13] (**Figure 1A**). Smooth muscle cells of the medial layer retain high cell plasticity and, under specific circumstances, can undergo phenotypic switch towards a synthetic or "de-differentiated" state. De-differentiated SMCs express high levels of extracellular matrix components and reduced expression of SMC contractile proteins. In contrast to differentiated SMCs, de-differentiated SMCs become highly proliferative and migratory [14, 15]. Recently, other possible sources of SM-like cells in the intimal layer of pulmonary vessels have been postulated. Circulating progenitor cells have been shown to be recruited to sites of vascular injury and assume a SM-like phenotype [16-19] (**Figure 1B**). Resident progenitor cells present in the adventitia have also been postulated to be involved in vascular remodelling [20] (**Figure 1C**). Additionally, resident endothelial cells within the intima may delaminate from their organised layer of cells in the vessel lining, transition to mesenchymal or SM-like phenotype in a process called endothelial-mesenchymal transition (EnMT) and migrate to their underlying tissue [13, 21] (**Figure 1D**). *In vitro*, these altered cells have an indistinguishable morphology from de-differentiated SMCs and express abundant extracellular matrix proteins. In this review we will go over the evidences for EnMT of vascular endothelial cells and its potential implications in PH.

Endothelial-mesenchymal transition (EnMT)

Endothelial cells may contribute to vascular remodelling through EnMT. This is a process of endothelial cell "*transformation*" into mesen-

chymal cells, by which endothelial cells lose their endothelial characteristics and gain a spindle shaped mesenchymal-like phenotype [22, 23]. EnMT is a central process during embryonic development [24-26]. In heart development, endocardial cells with a clearly endothelial phenotype are able to give rise to mesenchymal heart cushion cells through a process of EnMT [27-29]. Arciniegas *et al,* have also shown that EnMT is an important event in aortic and pulmonary artery development [30] and in the development of the normal arterial intima [31]. Moreover, morphological studies in human embryos suggest that EnMT also occurs during the maturation of both arteries and veins [32].

Recently, some studies have shown that EnMT could also happen in adult life in a variety of pathologic settings, including PH [33, 34], atherosclerosis [35] and wound healing [36]. In 1997, Romero *et al* showed that capillary endothelial cells could undergo mesenchymal transition in response to chronic inflammatory stimuli [37]. Additionally, a number of *in vitro* studies have demonstrated that endothelial cells from a variety of vascular beds retain the ability to transition into mesenchymal or SM-like cells under specific culture conditions [38, 42].

A loss of cell-to-cell contact seems to be a triggering step in the development of EnMT [43]. Cell-cell adhesion glycoprotein, VE-cadherin, plays an important role in vascular biology by controlling the cohesion and organization of cell-cell junctions. The loss of expression of VE-cadherin, consistently preceded endothelial phenotype downregulation and SM-like transformation [44].

Signalling during EnMT

There are several key signalling pathways contributing to the remodelling process and to date, a number of studies have demonstrated the induction of EnMT *in vitro*. It has been suggested that the TGF-β1 signalling pathway is involved in EnMT [45]. Both mouse and human endothelial cells cultured in the presence of TGF-β1 have shown SM-like cell morphology and an up-regulation of α-SM actin [46-48]. Recently, studies have shown that both TGF-β1 and Wnt-signalling pathways could synergize in the EnMT process [49, 50]. Moreover, in addition to TGF-β1 and Wnt-signalling pathways, the Notch pathway has also been shown to be involved in both vascular development and intimal lesion formation processes [51-53]. Although it is known that all these signalling pathways contribute to EnMT, it is not clear whether Notch, Wnt, TGF-β1, or the combination of all pathways, provide the initiation signal for EnMT *in vivo* [54]. Furthermore, it is likely that other signalling pathways such as VEGF, NFAT and BMP, which interact with TGF-β1 and Notch signalling, also mediate EnMT [55].

Despite these signalling studies, the transcriptional networks which mediate EnMT remain largely unidentified. Recently, the role of the Snail family of transcriptional repressors has been highlighted in control of different transcriptional programs of mesenchymal stem cell differentiation [56]. Snail proteins including Slug are involved in a broad spectrum of biological functions including epithelial to mesenchymal transition (EMT), cell differentiation, cell motility, cell-cycle regulation and apoptosis. In the context of EnMT, Snail and Slug play a critical role in disrupting cell-cell junctions and down-regulating VE-cadherin gene expression [57-59].

EMT/EnMT

EnMT is related to the more generally recognized mechanism of somatic cell plasticity; epithelial-to-mesenchymal transition (EMT) [60]. EMT involves a phenotypic cellular switch, in this case, from an epithelial to mesenchymal phenotype [61]. EnMT, EMT and its reverse pathway, mesenchymal to epithelial transition (MET) are key biological processes that occur naturally during embryonic life [62, 63]. Cellular switching from an epithelial to mesenchymal phenotype (EMT), and conversely from a mesenchymal to epithelial phenotype (MET) are important biologic programs fundamental to the generation of several complex body patterns throughout evolution [64, 65]. EMT plays a central role in germ layer specification (endoderm, ectoderm and mesoderm) and a range of different recurrent EMT/MET cycles occur before final organ formation [66]. In lungs for example, EMT is a natural process that exists to allow airway branching during fetal development. During cardiac development, EMT/MET is crucial in valve formation and heart septation [67]. In other organs, such as the kidney, suc-

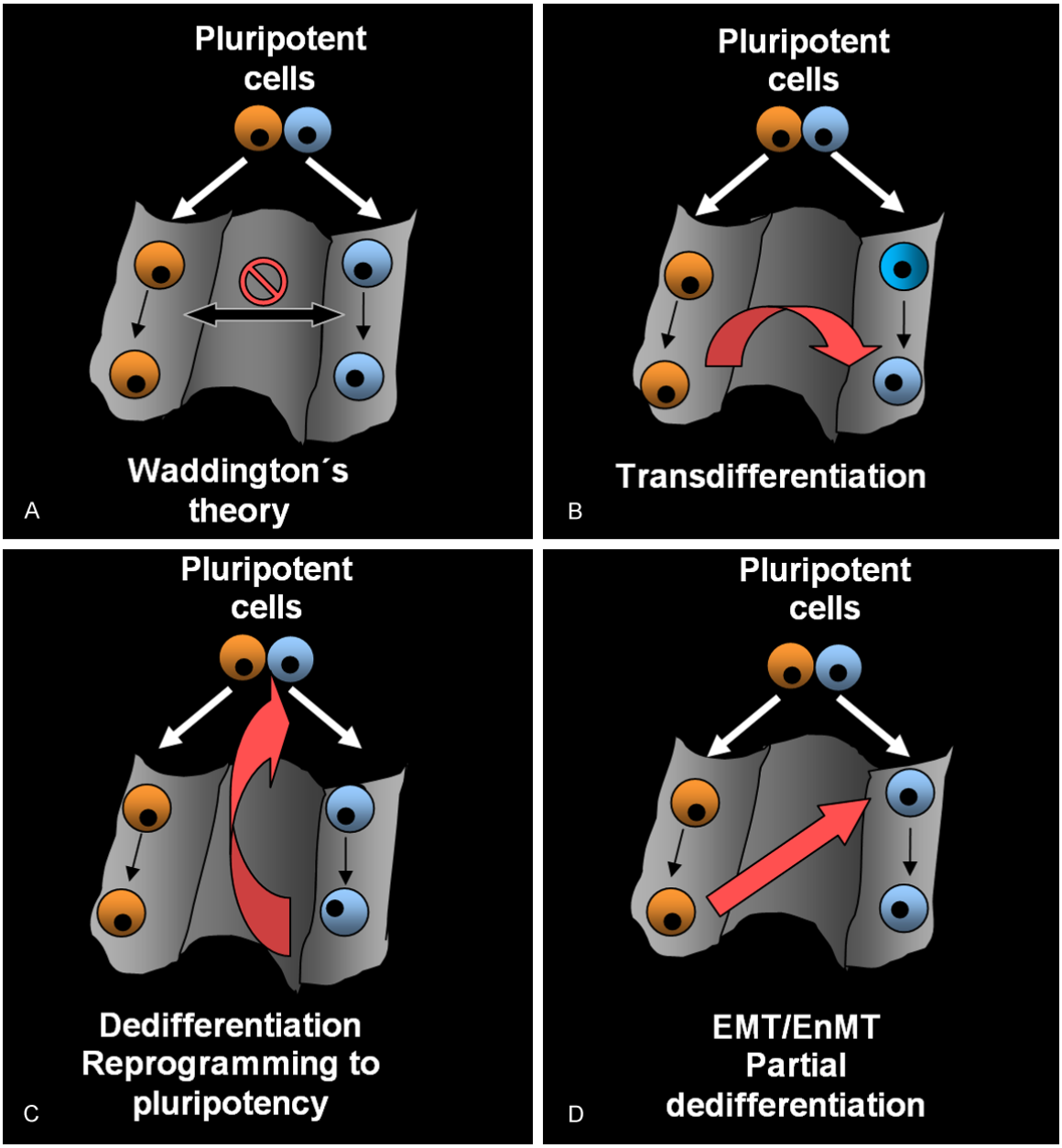

Figure 2. Cellular differentiation and reprogramming patterns. Waddington's epigenetic landscape. Somatic cells take on a specific fate by compulsorily progressing from the pluripotent state to a terminal differentiated state (A). Transdifferentiation. A mature cell switches its phenotype and function to that of another mature differentiated cell type without undergoing an intermediate pluripotent state or becoming a progenitor cell (B). Dedifferentiation of reprogramming to pluripotency. Differentiated cells return to an immature state and regain pluripotency (C). EMT/EnMT; partial dedifferentiation. Cells change their specific fate, dedifferentiate and acquire a more immature, proliferative phenotype from another somatic lineage without converting to a pluripotent cell (D).

cessive phases of EMT/MET are necessary to ultimately give rise to nephrons or nephric ducts [68]. Moreover in the adult, EMT contributes to pathology of tissue fibrosis, tumour progression, and tumour metastasis, which results when cells delaminate from the primary tumour, allows them to migrate [69, 70].

Cellular reprogramming

Traditionally, Waddington's epigenetic landscape illustrates that fates of somatic cells are progressively determined to compulsorily follow a downhill differentiation pathway [71] (**Figure 2A**). As such any reversal of cell differentiation

status would require external intervention in nuclear function such as nuclear transfer [72, 73] or the introduction of several transcription factors [74]. The plasticity of somatic cell fate is a complex concept that has evolved through decades of research. It is currently known that some somatic cells seem to possess a greater plasticity when exposed to certain stimuli. Smith *et al,* showed in 1998 that Oct4 transcription factor is required for maintaining mouse embryonic stem cell pluripotency [75], and in 2006 Takahashi and Yamanaka *et al*, were able to reprogram mouse fibroblasts to induced pluripotent stem cells (iPS) using 4 transcription factors (Sox2, Klf4, Oct4 and c-Myc) [76]. EMT and EnMT are examples of remarkable somatic cell plasticity occurring naturally though organogenesis. There is current evidence that cells undergoing EMT/EnMT acquire stem cell properties [77, 78] and it has recently been postulated that MET is a key cellular mechanism required for transforming somatic cells toward the generation of induced pluripotent stem cells [79]. Li *et al,* showed that while Sox2/Oct4 upregulation suppresses the EMT mediator Snail, c-Myc downregulates TGF-β1 and TGF-β receptor 2, whilst, Klf4 induces the expression of epithelial genes including E-cadherin [80]. These recent findings are important and raise many questions. They oppose Waddington's epigenetic view in which a fully differentiated cell had completed a downhill journey into a valley from which it could not then escape. Cell fate seems now to be far more flexible than previously thought [81].

Transdifferentiation refers to a process where one mature cell switches its phenotype and function to that of another mature differentiated cell type without undergoing an intermediate pluripotent state or becoming a progenitor cell [82] (**Figure 2B**). Davis *et al,* showed in 1987, that mouse embryonic fibroblasts could transdifferentiate directly into mature myoblasts [83]. EMT/EnMT induces the generation of cells with stem cell traits, thus it does not fulfil Takahashi's definition of transdifferentiation. Dedifferentiation is a process which induces cell rejuvenation. It refers to a process where cells travel back up their differentiation path, to become more immature and finally convert into a pluripotent cell [84] (**Figure 2C**). EMT/EnMT could be considered an initial stage of cellular dedifferentiation or reprogramming processes where cells dedifferentiate and acquire a more immature, proliferative phenotype but do not convert to a pluripotent cell. Cellular reprogramming and change of cell fate decisions through EMT/EnMT provides new ways to traverse across Waddington's epigenetic landscape (**Figure 2D**). As EMT/EnMT are essential and occurs frequently during embryonic development one could postulate that cellular reprogramming occurs spontaneously in the embryo and in certain adult pathological conditions.

Reversibility of EMT/EnMT. Future perspectives

During embryogenesis, EMT and its reverse process, MET, occur spontaneously during organ morphogenesis [85]. However, less is known about the reversibility of EnMT [86]. It has been suggested that endothelial cells transformed to a more SM-like cell phenotype could be restored by administration of FGF [87]. Recently our group have shown that *in vitro*, following withdrawal of TNF-α, SM-like cells acquired a normal phenotype (manuscript submitted). However, more studies are necessary to determine the reversibility of this EnMT process *in vitro* and *in vivo*.

It is accepted that EMT/EnMT occurs not only during embryonic development but also in the pathogenesis of various cardiovascular diseases such as heart failure and PH. The prospect of therapeutic manipulation of EnMT/EMT in the treatment of these conditions is particularly attractive [88, 89]. In particular, therapies directed at inhibiting EnMT to delay or prevent PH, may represent a promising new treatment modality. Nevertheless, additional studies are needed to identify the precise molecular mechanism of EnMT in disease, to provide novel insights into the mechanisms of such diseases and to determine which signalling components might be viable therapeutic targets.

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