The endothelial-to-mesenchymal transition process

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Abstract

Endothelial-to-mesenchymal transition is a process through which certain endothelial cells lose endothelial characteristics and transform into mesenchymal cells or smooth muscle-like cells. Rapidly emerging data suggest that this process may have crucial roles during vascular development, cardiac fibrosis and intimal thickening formation observed in atherosclerosis and restenosis as well as in complex lesions associated with pulmonary hypertension, and during cancer progression. EndoMT entails growth factors and cytokines synthesis and secretion, activation of receptors, cytoskeleton reorganization, disassembly of the adherence junctions or loss of endothelial cell-cell contacts, change in cell shape and polarity, proteases secretion, extracellular matrix remodeling, cell detachment and cell migration and differentiation.

Here, we briefly review EndoMT keeping in mind that several questions still remain without answer.

Key words: endothelial-mesenchymal transition, vascular remodeling, intimal thickening, growth factors, β-catenin

Introduction

Blood vessels are not static structures. The vasculature undergoes continuous remodeling as part of its normal ageing and physiological function. Vascular remodeling, more precisely wall remodeling, is a dynamic process of structural and architectural alterations that occurs during the embryonic and the vasculature development as well as in the progression of arterial disease. This process involves cell growth, cell migration, apoptosis and degradation and reorganization in the extracellular matrix (ECM) composition and is regulated by forces that are associated with blood flow (hemodynamic forces), as well as by environmental and genetic factors[1,2]. Pulmonary vascular remodeling is a process generally associated with pulmonary arterial hypertension that is characterized by perturbations of hemodynamic forces, including shear stress to which the endothelium is exposed. This process includes intimal thickening or intimal hyperplasia, medial thickness, and plexiform lesions, this last being the most complicated form of intimal thickening[3,4]. In general, intimal thickening is defined as an increase in cell number residents within the innermost region of the vessel wall (intima) which occurs concomitantly with the degradation and reorganization of ECM components. It may also involve microenvironmental factors (e.g. cytokine and growth factors), cell migration and proliferation, and gene expression modulation[5,6]. Currently, there is controversy regarding not only the nature and origin or the source of vascular smooth muscle cells (SMCs) involved in development[7] and the cells that conform the intimal thickening[8], but also regarding the mechanisms contributing to their formation. In this respect, recent studies have proposed different sources for intimal cells that include circulating hematopoietic cells or local vascular SMCs[9]. Other sources could be the endothelium by an endothelial-mesenchymal transition (EndoMT) process[10] or epicardial cells[7]. Therefore, it is our intention to briefly review EndoMT, keeping in mind that several problems still remain without answer.

EndoMT process:

EndoMT is a process through which certain endothelial cells lose endothelial characteristics and transform into mesenchymal cells or SM-like cells (Fig. 1). EndoMT is now recognized as a phenomenon that occurs during cardiac fibrosis[11] and neointimal thickening formation observed in atherosclerosis and restenosis[10], and that also occurs in complex lesions associated with pulmonary hypertension[3,4] and with idiopathic portal hypertension[12]. In addition to this role, recent evidence has also demonstrated that EndoMT is critical in heart and vascular development[10,13], hyperthrophic scarring[14], kidney fibrosis[15], and during cancer progression[16]. As in the transition of epithelial cells to an mesenchymal phenotype (EMT), EndoMT seems to progress through a series of important steps whose interdependence and order are not clear as yet[10,17]. This complex process entails growth factors and cytokines synthesis and secretion, activation of receptors, cytoskeleton reorganization, disassembly of the adherence junctions or loss of endothelial cell-cell contacts, change in cell shape and polarity, proteases secretion, ECM remodeling, cell detachment and cell migration and differentiation[10]. Outstandingly, excessive signaling of growth factors or aberrant activation of tyrosine kinase as well as loss of epithelial cell-to-cell contacts have been consistently reported at EMT sites during development and disease[17]. Regarding the vascular endothelium, endothelial cell-to-cell contacts are maintained by adherens junctions mainly composed of vascular endothelial cadherin (VE-cadherin)[18,19]. This transmembrane adhesive protein is linked to the actin cytoskeleton via cytoplasmic proteins called catenins, in particular β-catenin and p120-catenin[18,19]. These molecules are generally related to the maintenance of cell-cell contacts and regulation of the intracellular signaling pathways that are implicated in cell growth, apoptosis, matrix and cytoskeletal remodeling[18,19]. The architecture of this complex may be disrupted by tyrosine phosphorylation of both VE-cadherin and β-catenin involving specific tyrosine kinases[18-20]. In this respect, there are reports showing that loss of endothelial cell-cell contacts and cell shape and polarity may occur in response to mechanical injury, shear stress, and / or cross talk of a variety of growth factors including fibroblast-growth factor-2 (FGF-2), transforming growth factor-β (TGFβ), and insulin-like growth factor II (IGFII)[21-23] and that such events are necessary in the progression of EndoMT (Fig. 2). Nevertheless, how these factors and signals generated may cause the endothelial transformation is still matter of debate[24]. As mentioned, in the EMT and EndoMT processes some soluble growth factors participate actively. For instance, IGFII which, apart from being considered important in wound healing, cancer progression, pulmonary fibrosis, angiogenesis, and atherosclerosis lesions[25-28] is also considered to participate during embryonic development[29]. This growth factor binds the insulin-like growth factor receptor I (IGFIR) initiating several signaling cascades that would lead to the dissociation of β-catenin from VE-cadherin allowing its relocation to the cytoplasm and the nucleus where it modulates gene transcription[23,24]. The interpretation of these results is complex, but we can speculate that IGFIR activation might be involved in the regulation of endothelial cell-cell contacts and cell polarity and the eventual activation of some transcription factors contributing to the generation of signaling cascades that govern the EndoMT process[24]. Notably, the activation of nuclear factor kappa light chain gene enhancer in B cells (NFκB), a transcriptional factor that regulates the expression of hundred genes and whose activation proceeds rapidly and depends on the type and intensity of the stimulus has been correlated with the loss of VE-cadherin and redistribution of β-catenin and subsequent expression of α-smooth muscle actin (α-SM actin) during the embryonic aortic wall remodeling and the EndoMT[30]. A link between such activation and the expression of some metalloproteinases (MMPs) during the EndoMT was also proposed[30]. This assumption was based on studies related to hemodynamic alterations which had demonstrated that an increased expression of MMP2 and MMP9 occurred during intimal thickening formation, some of them indicating that the translocation of NFκB correlates with the expression and activation of certain MMPs in endothelial cells[31]. However, little work has been done examinating specifically the role of these molecules in the EndoMT process.

Another important growth factor is epidermal growth factor (EGF) which acts by binding to specific receptor tyrosine kinases, the EGF receptor family (EGFR), provoking its homo and/or heterodimerization and tyrosine phosphorylation and initiating various important signal transduction pathways[32]. Upon binding of EGF, receptor clustering and endocytosis take place followed by recycling back of the ligand-receptor complex to the cell surface or degradation. After internalization, nuclear translocation of the receptors may also occur to regulate gene expression[34]. EGF ligands include: EGF-like growth factor,transforming growth factor-α (TGF-α), heparin binding EG-like growth factor (HBEGF), amphiregulin (AREG), betacellulin (BTC), epiregulin (EREG), epigen (EPGN), and four neuregulins (NREGS)[32,33]. As mentioned, these ligands are recognized by four members of the EGFR family: EGFR (HER1), Neu (ErbB2, HER2), ErbB3 (HER3) and ErbB4 (HER4)[32,33]. These receptors regulate important cellular processes, including cell proliferation, apoptosis, cell polarity, differentiation, migration and invasion[35]. Importantly, regulation of cell-cell contacts by EGF/ErbB1 via E-cadherin and cytoskeleton has been proposed to correlate with tumor progression and metastasis and EMT[36,37]. Overexpression of EGFR has also been suggested to contribute to the development of arterial hypertension[38]. Also, overexpression of EGF and activation of NFκB has been documented[39]. Moreover, expression of EGF and HB-EGF as well as of their respective ligands, ErbB1 and ErbB2, has been detected in the intimal thickening and medial SMCs of atherosclerotic lesions, whereas little or not presence of these molecules is observed in the cells of healthy vessels[40,41]. Of note, a study in transformation of endocardial endothelial cells into mesenchymal cells demonstrated the presence of an ErbB2-ErbB3 heterodimer suggesting that this complex is required for the normal endocardial cushion tissue development[42]. Nevertheless, the cellular and molecular mechanisms by which EGF / EGFR exert these actions remain to be elucidated.