



The endothelial cell adhesion mediated by integrins, the recruitment and activation of c-Src, EGFR, ErbB2 and involvement of endocan, influence crucial stages of endothelial-mesenchymal transition

La adhesión endotelial mediadas por integrinas, el reclutamiento y la activación de c-Src, EGFR, ErbB2 así como la presencia de endocan, influyen en las etapas cruciales de la transición endotelio-mesénquima

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Abstract

Endothelial mesenchymal transition (EndoMT), a newly recognized type of cellular transdifferentiation, is an essential process that occurs during embryonic development and participates in a number of adult pathologies. Also, the adhesion of cells to the extracellular matrix (ECM) is known as a critical requisite to generate various cellular changes related to EndoMT.

We will review recent findings describing novel signaling mechanisms implicated in the progression of EndoMT that involves changes in distribution and organization of tyrosine kinases receptors (RTKs) that include EGFR and ErbB2/Neu and non-RTKs such as c-Src as well as ECM arterial wall proteoglycans such as endocan, which is associated to soluble growth factors (EGF, TGF- α) or inflammatory cytokines (TNF- α , IL-1 β). The potential role of endocan is also discussed. Exploration of the signaling mechanisms of EndoMT may provide novel therapeutic strategies for treating pathologies.

Key words: endothelial – mesenchymal transition, integrins, c-Src, EGFR, ErbB2, endocan

Resumen

La transición endotelio mesénquima (EndoMT), recientemente reconocida como un tipo de transdiferenciación celular, es un proceso esencial que ocurre durante el desarrollo embrionario y participa en un número importante de patologías del adulto. También, la adhesión de las células a la matrix extracelular (ECM) es conocida como un requisito crítico para generar cambios celulares relacionados a la EndoMT.

En este artículo revisaremos recientes hallazgos describiendo mecanismos de señalización novedosos implicados en la progresión de la EndoMT, los cuales involucran cambios en la distribución y organización de receptores tirosinas quinazas (RTKs) que incluyen a EGFR y ErbB2/Neu y no-RTKs tales como c-Src; así como también proteoglicanos de la pared arterial tales como endocan, este último asociado a factores de crecimiento solubles (EGF, TGF- α) o citoquinas inflamatorias (TNF- α , IL-1 β). La exploración de los mecanismos de señalización en la EndoMT puede proveer nuevas estrategias terapéuticas para el tratamiento de algunas patologías.

Endothelial-mesenchymal transition (EndoMT) in the vascular endothelium appears to be a complex process by which endothelial cells lose their endothelial characteristics and gain a spindle shape mesenchymal-like phenotype^{1,2}. Recently, several studies have shown that EndoMT occurs not only during embryonic development¹ but also in adult life in the angiogenesis³ and a variety of pathologies that include pulmonary hypertension^{2,4,5}, atherosclerosis and restenosis^{6,7}, portal hypertension⁸, intestinal⁹ and kidney fibrosis¹⁰, diabetic nephropathy¹¹, hypertrophic scarring¹², inflammation¹³, and during cancer progression¹⁴. As occur in the epithelial-mesenchymal transition (EMT), the endothelial transition progress through a series of important steps such as cross reactivity of a variety of growth factors and inflammatory cytokines, activation of receptor tyrosine kinases (RTKs) and non-RTKs, loss of endothelial cell-cell contacts and endothelial cell shape and polarity involving reorganization of some cytoskeleton proteins, caused by frictional forces, proteases secretion, extracellular matrix (ECM) remodeling, cell separation, detachment and migration, and differentiation^{1,15}. However, how these factors and signals can cause endothelial transformation is still debated². Interestingly, a functional cooperation between integrins and RTKs is currently regarded as crucial not only during normal vascular development and vascular diseases¹⁶, but also in various pathologies^{1,2,15}.

In this context, *in vitro* studies in epithelial cells have revealed multiple signaling pathways that are involved in directing EMT¹⁷. Also, they have shown that during cell-ECM adhesion, members of the epidermal growth factor (EGF) receptor family such as EGFR/ErbB1 and ErbB2/Neu, can be partially activated by association with some integrins thereby regulating important processes such as adhesion, cell proliferation, differentiation, migration, apoptosis and survival in the absence of their ligands^{18,19}. However, the implications of the interaction between integrins and these RTKs in particular, require a deeper investigation.

In the cell-ECM adhesion some non-tyrosine kinases such as c-Src can act facilitating the expansion, migration, differentiation and transcription^{20,21}. Importantly, in the absence of growth factors, activation of c-Src occurs due to its direct interaction with some integrins and once is activated, it phosphorylates EGFR and Erb2^{19,22-26}. Nevertheless, the mechanism involved in the interaction of c-Src, EGFR and ErbB2, and its implications have not been fully clarified.

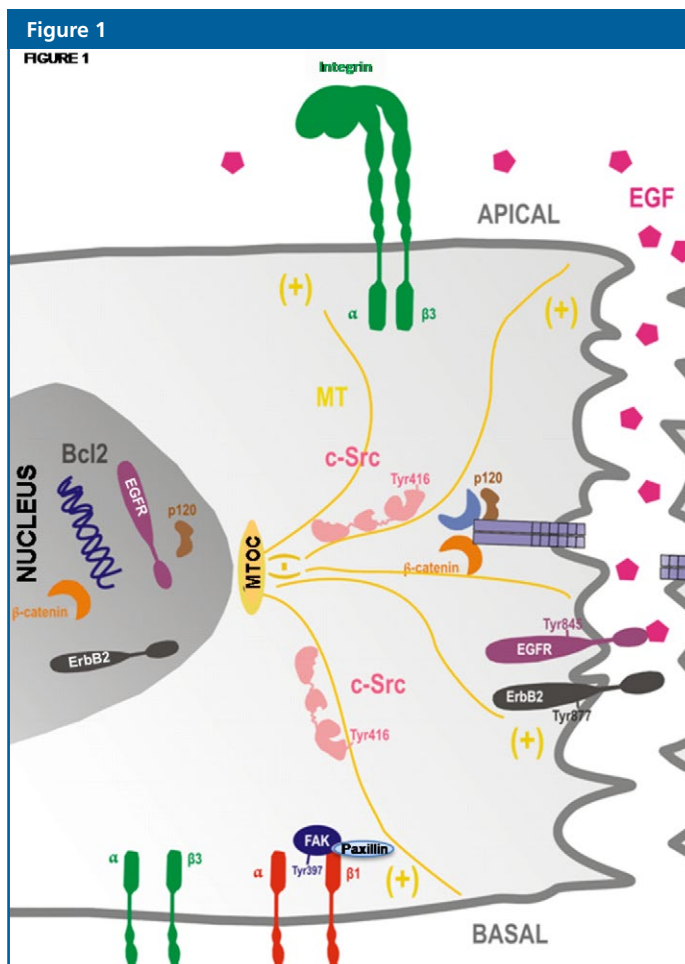
Respect to the growth factors, recent reports indicate that in EMT cellular changes can be facilitated as a result of the

influence of growth factors such as EGF that may affect the function of integrins through alteration of cell-cell interaction and modulation of cell-ECM contacts²⁷. However, molecular and cellular aspects by which EGF and its receptors exert these actions remain to be elucidated.

In the context of endothelial cells, there are reports showing that disruption of endothelial cell-cell contacts or adherens junctions (AJs) and changes in the organization of microtubules (MTs) and actin cytoskeleton may occur in response to mechanical injury, shear stress and/or cross talk of a variety of growth factors including transforming growth factor- β (TGF- β), insulin-like growth factor II (IGF II), and fibroblast growth factor-2 (FGF-2) and inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and IL-1 β , and that such events are necessary in the EndoMT¹. Interestingly, it has been demonstrated that EGF secreted by endothelial cells induces EMT and endow carcinoma cells with enhanced motility and stemness²⁸. However, how these factors and intracellular signals induce the endothelial transformation is still matter of debate^{2,16,29}. It is known that EGF or transforming growth factor- α (TGF- α) acts by binding to EGFR leading to the receptor homo or hetero-dimerization, activation of its RTK cytosolic domain and autophosphorylation on tyrosine residues initiating various important signal transduction pathways that could collaborate in the adhesion, proliferation, migration, differentiation and survival³⁰. Upon binding of EGF, receptors clustering and endocytosis take place followed by recycling back of ligand-receptor complex to the cell surface or degradation. After internalization, nuclear translocation of the receptors may also occur to regulate gene expression^{31,32}. Formation of heterodimers between EGFR and Erb2 in response to ligand growth factors are considered essential for several biological processes³⁰⁻³². Notably the expression of growth factors EGF, TGF- α and heparin-binding EGF (HB-EGF) and their respective receptors EGFR and ErbB2 has been detected in atherosclerotic lesions, whereas a very low or no expression of these molecules is observed in cells of healthy vessels³³. However, there are very few studies related to the specific role of the EGF as well as the possible signaling pathways generated in the process of EndoMT.

In this respect, we have recently suggested that the dimerization, activation and phosphorylation of EGFR and ErbB2, initiated by integrin β_3 , would be favored by the presence of EGF or TGF- α and that the internalization and nuclear translocation of these receptors could be accompanied by microtubules remodeling which induces the activation of c-Src and phosphorylation of β -catenin and p120-catenin to promote loss of endothelial cell polarity and disruption of endothelial cell-cell contacts, facilitating cell spreading, separation, detachment, and migration, cellular events considered as essentials in the progression of EndoMT¹⁶ (Figure 1). Also, we proposed that stimulation with EGF or TGF- α provokes the formation of an activa-

ted FAK-paxillin-c-Src signaling complex which affects not only the assembly and disassembly of focal adhesions, but also promotes cell migration and disruption of AJs^{16,34} (Figure 1). Additionally, we showed that EGF, TGF- α as well as their receptors EGFR and ErbB2 were present in stages of aorta embryonic development where the intimal thickening are clearly evident and EndoMT is an active process. These findings are important considering that in vivo expression of EGF, TGF- α and their receptors have been detected in the intimal thickening and medial smooth muscle cells of atherosclerotic lesions³³, and that expression of activated ErbB2 and ErbB3 has been demonstrated during heart cushion development³⁵, suggesting an important contribution for these receptors in the initiation of atherosclerosis and in the development of cardiac valves, respectively. Based in these findings, we suggested that integrin-mediated endothelial cell adhesion and activation and translocation of c-Src, EGFR and ErbB2 as well as the presence of their ligands are required for EndoMT¹⁶.



Adhesion of endothelial cells to fibronectin (FN) in the presence of EGF produces the dimerization, activation, and phosphorylation of EGFR and ErbB2 in the tyrosine residues Tyr845 and Tyr877, respectively leading to the internalization and nuclear translocation of both receptors which could be accompanied by microtubules (MTs) remodeling inducing the activation of c-Src and phosphorylation of β -catenin and p120 catenin to promote loss of endothelial cell polarity and disruption of adherens junctions (AJs), facilitating cell spreading, separation, detachment, and migration. Also, stimulation with EGF activates FAK-paxillin signaling complex which affect the focal adhesion, promoting cell migration and disruption of AJs.

As mentioned, in addition to permanent interaction with other cells, endothelial cells also need to interact with the ECM. It is well established that most ECM molecules participate in tissue remodeling by promoting cell adhesion provoking cytoskeletal organization, and regulating certain cellular events that include cell proliferation, migration and differentiation in response to mechanical stimuli and / or cross talk of a variety of growth factors and cytokines³⁶. Some of the most studied ECM components in the vessel wall belong to the family of proteoglycans (PGs), which are actually recognized by its role in several cellular processes either via interaction with ECM receptors like integrins or via signaling through RTKs³⁶. Endocan, also called endothelial cell-specific molecule-1, is a small dermatan sulfate PG (DSPG) that is mainly expressed by endothelial cells and regulated in vivo by inflammatory cytokines such as tumor necrosis factor- α , FGF-2, and vascular endothelial growth factor (VEGF)³⁷. Interestingly, overexpression of endocan has been observed in breast, brain, lung, liver, kidney, ovary tumors, suggesting an important role of endocan in angiogenesis and in the development and progression of cancer³⁷. However, the factors regulating the synthesis and secretion as well as their functions, have not yet fully elucidated^{37,38}. Our laboratory has investigated the possible involvement of the endocan in EndoMT process and based on our observations we believe that endocan might be cooperating with integrins to promote the formation of focal adhesions thus influencing the separation of endothelial cells and their migration, seen as crucial steps in the process of EndoMT³⁸.

Conclusions

Given that changes in cell shape, interactions with the ECM, and acquisition of migratory capabilities has been proposed as critical steps during EndoMT process, we suggest that the experimental findings reviewed here represent an important frame of reference for a better understanding of the EndoMT process. Studies are necessary to gain insight into mechanisms that regulate the endothelial cell migration and the transition into mesenchymal cells.

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