

# **Epithelial endothelial transition and endothelial mesenchymal transition**

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**Epithelial-endothelial transition and endothelial--mesenchymal transition****Domenico Ribatti**

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**Abstract.** The movement of continuous sheets of epithelial cells occurs during embryonic development, tissue repair, and cancer. Common to cellular and molecular principles of collective cell migration, invading cancers seem to reactivate embryonic pathways and patterns of cell movement. Epithelial cells possess the capability to become mesenchymal cells in a process called epithelial mesenchymal transition (EMT), which has been extensively studied and described. The aim of this article is to summarize the most recent literature data concerning less known epithelial-endothelial transition and endothelial-mesenchymal transition.

**Keywords.** Epithelial-mesenchymal transition; epithelial-endothelial transition; endothelial-mesenchymal transition; morphogenesis; tumor growth.

## Introduction

The movement of continuous sheets of epithelial cells occurs during embryonic development, tissue repair, and cancer [Friedl and Gilmour, 2009]. The different strategies of epithelial cell migration are likely to be related to physiological differences between cell types and are influenced by the environment through which cells must migrate. Epithelial cells have a clear apico-basal polarity with cell–matrix adhesion at their basal side and cell–cell adhesion at their apical side, and when epithelial cells undergo collective migration, they maintain part of their epithelial characteristics. Collective migration is one of the hallmarks of embryonic morphogenesis, while collective invasion is prevalent in many cancer types. Common to cellular and molecular principles of collective cell migration, invading cancers seem to reactivate embryonic pathways and patterns of cell movement.

Epithelial cells possess the capability to become mesenchymal cells in a process called epithelial mesenchymal transition (EMT) [Ribatti, 2017]. During EMT, stable cell–cell junctions are disassembled, apico-basal polarity is lost, and migratory capabilities are enhanced. EMTs are classified in three types, including type 1, which occurs during embryonic development; type 2, which is associated with adult tissue repair; type 3, which is involved in cancer progression [Kalluri and Weinberg, 2009; Zeisberg and Neilson, 2009]. Cancers exhibit some degree of EMT during their progression, and epithelial tumors are the result of an EMT process, in which tumor cells lose their epithelial features, including cell adhesion and polarity, reorganize their cytoskeleton, and acquire a mesenchymal morphology and the ability to migrate.

Endothelial cell migration is essential to vasculogenesis and angiogenesis, and is directionally regulated by different stimuli, involving degradation of the extracellular matrix to enable progression of the migrating cells. It requires the activation of several signaling pathways that converge on cytoskeletal remodeling, and different regulatory mechanisms and factors control this process, including include gradients of soluble factors, extracellular matrix–cell interaction and cell–

cell interaction. Three types of cells make up the new vasculature: tip cells, which migrate in response to gradients of vascular endothelial growth factor (VEGF), stalk cells, which proliferate and extend the vessels, and phalanx cells, which are quiescent and support the sprout [Ribatti and Crivellato, 2012]. The aim of this article is to summarize the most recent literature data concerning the study of epithelial/endothelial transition and endothelial/mesenchymal transition by means of different *in vitro* and *in vivo* models, including cellular models, such as the use of genetic lineage tracing technology enabling to follow endothelial cell lineage conversion *in vivo*, murine models and patient bioptic specimens' analysis.

### **Epithelial/endothelial transition**

Epithelial endothelial transition (EET), a subtype of EMT, is a process of transformation of tumor epithelial cells in endothelial cells. Tumor cells lose their polarity and tight junctions, involving decreased expression of E-cadherin and occludin, and increased expression of vimentin, VE-cadherin, fibronectin, and vitronectin [Sun et al., 2016]. EET is involved also in kidney allograft fibrosis associated to tubular atrophy [Granata et al., 2020].

EET occurs during vasculogenic mimicry (VM), i.e., cancer cell-derived channels like blood vessels, acting as alternative source of nutrient and oxygen supply and involved in tumor growth and invasion. In 1999, Maniotis and coworkers described for the first time this process in human melanoma, showing trans-differentiation of melanoma cells, which results in the formation of a chimeric vasculature composed of melanoma and endothelial cells [Maniotis et al., 1999]. Since then, VM has been demonstrated in renal cell carcinoma, breast cancer, ovarian cancer, primary gallbladder cancer, esophageal squamous cell carcinoma, mesothelioma, alveolar rhabdomyosarcoma, and hepatocellular carcinoma [Cao and Qian, 2020].

During VM epithelial-derived tumor cells differentiate into cell types expressing some endothelial markers, including VE-cadherin and vimentin. VM formation is promoted by TGF $\beta$  and CXCL12/Stromal cell derived factor-1 (SDF-1) secreted by cancer associated fibroblasts (CAFs), which are involved in EET [Yang et al., 2016].

### **Molecular pathways involved in EET**

Hypoxia induces the EET of cancer stem cells (CSCs) and EET is also associated to the promotion of VM. An EMT transcription factor Twist 1, is involved also for EET [Sun et al., 2011]. Under hypoxia, Twist1 translocate to the nucleus and bind to VE-cadherin promoter to induce EET and VM in hepatocellular carcinoma [Sun et al., 2010]. Similarly, hypoxia-associated Twist1 overexpression upregulates VE-cadherin in MDA-MB-231 triple negative breast cancer (TNBC) cells, and induces these cells to generate CSCs and promote VM in Matrigel [Zhang et al., 2014]. In hepatocellular carcinoma, the Bcl2/Twist1 complex leads to transcriptional activation of different genes that induce EET and VM [Sun et al., 2011]. Moreover, in hepatocellular carcinoma, overexpression of Twist1 under hypoxic conditions increased matrix metalloproteinases- 2 and -9 (MMP-2 and MMP-9) expression and VM [Sun et al., 2011]. Also protease-activated receptor-1 (PAR-1) promotes EET through Twist1 in hepatocellular carcinoma by up-regulating Twist1 both *in vitro* and *in vivo* through thrombin binding [Xiao et al., 2018]. Overall, these data indicate that a significant correlation between the expression level of VM-related proteins, including VEGF receptor-1 and -2 (VEGFR-1 and VEGFR-2), vascular endothelial-cadherin, vimentin, MMP-2 and MMP-9, and protease activated receptor-1 (PAR-1), has been found in hepatocellular carcinoma bioptic specimens [Xiao et al., 2018].

## **Endothelial/mesenchymal transition**

Endothelial cells may de-differentiate into mesenchymal stem-like cells (MSCs) and acquire the characteristics of multipotent cells [Medici and Kalluri, 2014]. This process has been defined as endothelial to mesenchymal transition (EndoMT). During EndoMT, endothelial cells lose endothelial markers and acquire mesenchymal markers (Table 1).

EndoMT takes place during embryogenesis [Timmermann et al., 2003], aorta development, pulmonary artery development, cardiogenesis and vasculogenesis [Arciniegas et al., 1989; 2005].

During cardiogenesis, endothelial cells undergo EndoMT, invade the cardiac jelly, and generate the cardiac cushion (precursors of the semilunar valves) [Kovavic et al., 2012]. During the formation of heart valves, endocardial cells show morphological alterations, including cellular hypertrophy, lateralization of the Golgi apparatus and loss of cell polarity [Markwald and Fizharris, 1975]. During the formation of the endocardial cushion in the chick embryo, cardiac endothelial cells show phenotypic changes correlated with alpha smooth actin expression ( $\alpha$ SMA) [Nakajima et al., 1997]. Epicardial cells also undergo EMT to give rise to smooth muscle cells, interstitial cardiac stromal cells and potentially a sub-population of endothelial cells [Kovavic et al., 2012]. EndoMT contributes to the vascular remodeling and neo-intimal formation that arises following vein graft transplantation into the arterial circulation [Cooley et al., 2014].

EndoMT is involved in different pathological conditions including pulmonary hypertension, transplant arteriopathy, vascular malformations, myocardial infarction, vascular calcification, fibrodysplasia ossificans progressive, cardiac, and renal fibrosis, and atherosclerosis (Table 2). Moreover, EndoMT is involved in different types of cancers (Table 3). Tumor-induced EndoMT is associated with the activation of pro-inflammatory pathways in endothelial cells (Nie et al., 2014). Endothelial cells undergoing tumor induced EndoMT express higher levels of the VEGG gene (Hog

et al., 2018). Moreover, EndoMT contributes to metastatic extravasation and intravasation (Dudley et al., 2012).

### **Molecular pathways involved in EndoMT**

Several signaling pathways are involved in EndoMT, including Notch, transforming growth factor beta (TGF $\beta$ ), WNT, fibroblast growth factor (FGF) and epidermal growth factor (EGF) [Man et al., 2018]. TGF $\beta$  is a potent activator of the EndoMT program in developmental and pathological settings [Arciniegas et al., 1992; Pardoli et al., 2017; Xiao et al., 2015; Mai et al., 2021]. Notch promotes TGF $\beta$ -mediated EndoMT in embryonic heart through the induction of Snail-1 expression and a down-regulation of VE-cadherin expression [Timmerman et al., 2003]. Induction of EndoMT through TGF $\beta$  involves a pathway leading to an increase of Snail-1 through convergence of Smad-dependent and Smad independent signaling [Medici et al., 2011]. TGF- $\beta$ 2 drives EndoMT through a Smad-dependent activation of the myocardin-related transcription factor-A (MRTF-A) (Mihira et al., 2012). Snail up-regulation is delayed following TGF $\beta$  activation of EndoMT in cultured endothelial cells [Sobierajska et al., 2020]. Smad independent pathways include MAPK/ERK/JNK [Medici et al., 2011; Heldin and Moustakas, 2011]. Snail is also required for TGF $\beta$ -induced EndoMT of embryonic stem cell-derived endothelial cells [Kokudo et al., 2008]. Snail acts as a transcription factor of EndoMT induced by Smad-dependent and PI3K/p38-dependent signaling pathways (Medici et al., 211). HGF/cMet signaling prevents TGF $\beta$ -1-induced EndoMT in cardiac fibrosis [Okayama et al., 2012; Wang et al., 2018].

FGF-2 inhibits EndoMT through miRNA-20a-mediated repression of TGF $\beta$  signaling [Correia et al., 2015]. Otherwise, FGF-2 promotes TGF $\beta$ -mediated EndoMT through regulation of let-7 miRNA



expression [Chen et al., 2012]. TNF $\alpha$  enhances TGF $\beta$ -induced EndoMT through TGF $\beta$  signal augmentation [Yoshimatsu et al., 2020].

During sprouting angiogenesis, an EndoMT is activated in endothelial cells to support the acquisition of mesenchymal features. VEGF and TGF $\beta$  may antagonize one another: exogenous VEGF treatment prevents TGF $\beta$ -induced EndoMT during cardiac fibrosis [Illigens et al., 2017]. Otherwise, human pulmonary valve progenitor cells exhibit EndoMT in response to VEGF-A and TGF $\beta$ -2 [Paruchuri et al., 2006]. Both Slug and Snail are involved in sprouting angiogenesis [Welch-Reardon et al., 2015]. Slug is the primary initiator of this process, whereas the induction of Snail occurs later.

Hypoxia is an inducer of EndoMT through the regulation of the expression of TGF $\beta$ -1, -2, and -3 [Caniggia et al., 2000; Hung et al., 2013]. Hypoxia inducible factor 1 alpha (HIF-1 $\alpha$ ) induces EndoMT of human coronary endothelial cells and Snail is a direct target of HIF-1 $\alpha$  [Xu et al., 2015]. Hypoxia induces the expression of Endo-MT-associated transcription factors Snail and Slug [Zhang et al., 2003].

### **Therapeutic approaches**

As CSCs and EET promote VM in malignant tumors, doxycycline as an inhibitor of EMT and VM in hepatocellular carcinoma, prevents also EET through methylation of the E-cadherin gene and down-regulation of vimentin and VE-cadherin [Meng et al., 2014].

A conjugate of Temozolomide and perillyn alcohol inhibits EndoMT and reverts the mesenchymal phenotype of tumor associated brain endothelial cells in glioblastoma [Marin-Ramos et al., 2019]. Resistance to cisplatin and Gefitinib in lung tumor spheroid model is reduced when EndoMT in endothelial cells is reversed, implying EndoMT as a resistance factor [Kim et al., 2019]. Several drugs with anti-EndoMT properties have been approved for treatment of idiopathic pulmonary fibrosis, such as Nintedanib, a tyrosine kinase inhibitor of PDGF, FGF, and VEGF [Tsutsumi et al., 2019] and

diabetic kidney disease, such as Losartan, an inhibitor of TGF $\beta$ -/Smad 2-3 pathway [Yao et al., 2018]. EndoMT in systemic sclerosis induced by endothelin-1 (ET-1) and TGF $\beta$  may be blocked by Macitentan, a dual ET-1 receptor antagonist (Cipriani et al., 2015). Vildagliptin, an anti-diabetic drug, ameliorates pulmonary fibrosis in lipopolysaccharide-induced lung injury by inhibiting EndoMT (Suzuki et al., 2017). Calcitriol, an active form of vitamin D3, reduces TGF $\beta$ -Smad2-mediated EndoMT and fibroblast to myofibroblast transition (Tsai et al., 2019).

### **Concluding remarks**

EET is involved in transformation of tumor epithelial cells in endothelial cells and occurs during VM, i.e., cancer cell-derived channels like blood vessels, acting as alternative source of nutrient and oxygen supply and involved in tumor growth and invasion. Otherwise, EndoMT is involved in embryogenesis, tumor development, and contributes to resistance to cancer treatment. For example, in glioblastoma multiforme, chemoresistance is related to c-met-mediated EndoMT (Huang et al., 2016).

EET and EndoMT are controlled by complex signaling pathways. Different evidence suggests the existence of a complex signaling network involving TGF $\beta$ , Wnt/ $\beta$ -catenin and Notch pathways involved in the control of EndoMT. In this context, modulation of both EET EndoMT may contribute to counteract tumor progression and the molecular regulators of these two processes are potential targets and prognostic indicators. Single cell analysis of tumor cells may allow to analyze the different pathways involved in EET and EndoMT and to discriminate between the molecular alterations underlying tumor progression and the different response to therapeutic agents.

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Table 1.

<b>Loss of endothelial markers and acquisition of mesenchymal markers during EndoMT</b>	
<b>Endothelial markers</b>	CD31
	Platelet endothelial cell adhesion molecule-1 (PECAM-1)
	Tie-2
	Vascular endothelial (VE)-cadherin
<b>Mesenchymal markers</b>	N-cadherin
	Fibroblast specific protein-1 (FSP-1)
	Alpha smooth muscle actin ( $\alpha$ SMA)
	Types I/III collagen

**Table 2.**

Pathological conditions in which EndMT is involved	References
<b>Pulmonary arterial hypertension</b>	Arcinegas et al., 2007; Hopper et al., 2016; Tuder et al., 1994
<b>Atherosclerosis</b>	Souilhol et al., 2018
<b>Cardiac fibrosis</b>	Widyantoro et al., 2010; Zeisberg et al., 2007a
<b>Dermal fibrosis</b>	Manetti et al., 2017
<b>Radiation-induced rectal fibrosis</b>	Mintet et al., 2015
<b>Myocardial infarction</b>	Tombor et al., 2020
<b>Pulmonary fibrosis</b>	Hashimoto et al., 2010
<b>Renal fibrosis</b>	Li et al., 2009; Xavier et al., 2014; Zeisberg et al., 2008
<b>Cancer</b>	Fan et al., 2017; Zeisberg et al., 2007b
<b>Systemic sclerosis-associated interstitial lung disease</b>	Mendoza et al., 2015
<b>Diabetes mellitus</b>	Li et al., 2009; Cao et al., 2014

**Table 3.**

<b>Different types of cancers in which EndoMT is involved</b>	<b>References</b>
<b>Melanoma</b>	Zeisberg et al. 2007 b
<b>Colorectal cancer</b>	Fan et al., 2018; Yamada et al., 2019; Wawro et al., 2018
<b>Pancreatic cancer</b>	Fan et al., 2019
<b>Lung cancer</b>	Choi et al., 2018; Kim et al., 2019
<b>Glioblastoma</b>	Huang et al., 2016, 2020; Liu et al., 2018
<b>Esophageal cancer</b>	Nie et al., 2014