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Epithelial-endothelial transition and endothelial-mesenchymal transition

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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 on their basal side and cell-cell adhesion on 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 into three types: type 1, which occurs during embryonic development; type 2, which is associated with adult tissue repair; and 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 gradients of soluble factors, extracellular matrix-cell inter-

Abbreviations used in this paper: αSMA, alpha smooth actin; CAFs, cancer associated fibroblasts ; CSCs, cancer stem cells; EET, epithelial endothelial transition; EGF, epidermal growth factor; EMT, epithelial mesenchymal transition; EndoMT, endothelial mesenchymal transition; ET-1, endothelin-1; FGF, fibroblast growth factor; FSP-1, fibroblast specific protein-1; HGF, hepatocyte growth factor; HIF-1α, hypoxia inducible factor 1 alpha; MRTF-A, myocardin-related transcription factor-A; MMP, matrix metalloproteinase ; MSCs, mesenchymal stem cells; PAR, protease activated receptor; PDGF, platelet derived growth factor; PECAM-1, platelet endothelial cell adhesion molecule-1; SCDF, stromal cell derived factor; TGFβ, transforming growth factor beta ; TNFα tumor necrosis factor alpha; TNBC, triple negative breast cancer; vascular endothelial cadherin, VE cadherin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor; VM, vasculogenic mimicry.

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action and cell- cell interaction. Three types of cells constitute 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 making it possible to follow endothelial cell lineage conversion *in vivo*, murine models, and patient bioptic specimen 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, vascular endothelial (VE)-cadherin, fibronectin, and vitronectin (Sun *et al.*, 2016). EET is also involved in kidney allograft fibrosis associated with 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 co-workers described this process in human melanoma for the first time, 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 transforming growth factor beta (TGFβ), 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

An EMT transcription factor, Twist1, is also involved in EET (Sun *et al.*, 2011). Under hypoxia, Twist1 translocates to the nucleus and binds 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 cancer stem cells (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 MMP9) expression and VM (Sun *et al.*, 2011). In addition, 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). Finally, 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), VE-cadherin, vimentin, MMP-2 and MMP-9, and PAR-1, has been found in hepatocellular carcinoma bioptic specimens (Xiao *et al.*, 2018).

TABLE 1

LOSS OF ENDOTHELIAL MARKERS AND ACQUISITION OF MESENCHYMAL MARKERS DURING ENDOMT

Endothelial markers	CD31
	Platelet endothelial cell adhesion molecule-1 (PECAM-1)
	Tie-2
	Vascular endothelial (VE)-cadherin
Mesenchymal markers	N-cadherin
	Fibroblast specific protein-1 (FSP-1)
	Alpha smooth muscle actin (αSMA)
	Types I/III collagen

Endothelial-mesenchymal transition

Endothelial cells may de-differentiate into mesenchymal stem-like cells (MSCs) and acquire the characteristics of multipotent cells (Medici and Kalluri, 2012). This process has been defined as endothelial to mesenchymal transition (EndoMT). During EndoMT, endothelial cells loss endothelial markers and acquire mesenchymal markers (Table 1).

EndoMT takes place during embryogenesis (Timmerman *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) (Kovacic *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 (αSMA) (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

TABLE 2

PATHOLOGICAL CONDITIONS IN WHICH ENDMT IS INVOLVED

Pathological conditions	References
Pulmonary arterial hypertension	Arcinegas *et al.*, 2007; Hopper *et al.*, 2016; Tuder *et al.*, 1994
Atherosclerosis	Souilhol *et al.*, 2018
Cardiac fibrosis	Widyantoro *et al.*, 2010; Zeisberg *et al.*, 2007a
Dermal fibrosis	Manetti *et al.*, 2017
Radiation-induced rectal fibrosis	Mintet *et al.*, 2015
Myocardial infarction	Tombor *et al.*, 2021
Pulmonary fibrosis	Hashimoto *et al.*, 2010
Renal fibrosis	Li *et al.*, 2009; Xavier *et al.*, 2014; Zeisberg *et al.*, 2008
Cancer	Fan *et al.*, 2017; Zeisberg *et al.*, 2007b
Systemic sclerosis-associated interstitial lung disease	Mendoza *et al.*, 2015
Diabetes mellitus	Li *et al.*, 2009; Cao *et al.*, 2014

TABLE 3

DIFFERENT TYPES OF CANCERS IN WHICH ENDOMT IS INVOLVED

Types of cancers	References
Melanoma	Zeisberg *et al.* 2007b
Colorectal cancer	Fan *et al.*, 2018; Yamada *et al.*, 2019;
Pancreatic cancer	Fan *et al.*, 2019
Lung cancer	Choi *et al.*, 2018; Kim *et al.*, 2019
Glioblastoma	Huang *et al.*, 2016, 2020; Liu *et al.*, 2018
Esophageal cancer	Nie *et al.*, 2014

of endothelial cells (Kovacic *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 (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 VEGF 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, TGFβ, WNT, fibroblast growth factor (FGF) and epidermal growth factor (EGF) (Man *et al.*, 2018). TGFβ is a potent activator of the EndoMT program in developmental and pathological settings (Arciniegas *et al.*, 1992; Pardali *et al*., 2017; Xiao *et al.*, 2015; Ma *et al.*, 2021). Notch promotes TGFβ-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 TGFβ involves a pathway leading to an increase of Snail-1 through convergence of Smad-dependent and Smad-independent signaling (Medici *et al.*, 2011). TGF-β2 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 TGFβ 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 TGFβ-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.*, 2011). Hepatocyte growth factor (HGF)/cMet signaling prevents TGFβ-1-induced EndoMT in cardiac fibrosis (Okayama *et al.*, 2012; Wang *et al.*, 2018).

FGF-2 inhibits EndoMT through miRNA-20a-mediated repression of TGFβ signaling (Correia *et al.*, 2015). Otherwise, FGF-2 promotes TGFβ-mediated EndoMT through regulation of let-7 miRNA expression (Chen *et al.*, 2012). Tumor necrosis factor alpha (TNFα) enhances TGFβ-induced EndoMT through TGFβ 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 TGFβ may antagonize one another: exogenous VEGF treatment prevents TGFβ-induced EndoMT during cardiac fibrosis (Illigens *et al.*, 2017). Otherwise, human pulmonary valve progenitor cells exhibit EndoMT in response to VEGF-A and TGFβ-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 TGFβ-1, -2, and -3 (Caniggia *et al.*, 2000; Hung *et al.*, 2013). Hypoxia inducible factor 1 alpha (HIF-1α) induces EndoMT of human coronary endothelial cells and Snail is a direct target of HIF-1α (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 also prevents EET through methylation of the E-cadherin gene and downregulation 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 Getifinib 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 platelet derived growth factor (PDGF), FGF, and VEGF (Tsutsumi *et al.*, 2019) and diabetic kidney disease, or Losartan, an inhibitor of TGFβ-/Smad 2-3 pathway (Yao *et al.*, 2018).

EndoMT in systemic sclerosis induced by endothelin-1 (ET-1) and TGFβ 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 TGFβ-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 TGFβ, Wnt/β-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 permit analysis of the different pathways involved in EET and EndoMT and differentiation between the molecular alterations underlying tumor progression and the different response to therapeutic agents.

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