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Epithelial-mesenchymal transition in morphogenesis, cancer progression and angiogenesis

Domenico Ribatti[a,b,*]

[a] Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari Medical School, Bari, Italy
[b] National Cancer Institute "Giovanni Paolo II", Bari, Italy

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ABSTRACT

All organs consist of an epithelium and an associated mesenchyme, so these epithelial-mesenchymal intercations are among the most important phenomena in nature. The aim of this article is the summarize the common mechanisms involved in the establishment of epithelial mesenchymal transition in three biological processes, namely organogenesis, tumor progression and metastasis, and angiogenesis, apparently independent each from other. A common feature of these processes is the fact that specialized epithelial cells lose their features, including cell adhesion and polarity, reorganize their cytoskeleton, and acquire a mesenchymal morphology and the ability to migrate.

1. Introduction

Epithelial tissues can acquire mesenchymal features during development, tissue repair, wound healing, and cancer invasion [1]. Epithelial-mesenchymal transitions (EMTs) are classified in three types [2,3]: type 1, which occurs during embryonic development; type 2, which is associated with adult tissue repair; type 3, which is involved in cancer progression. The first developmental EMT occurs at gastrulation [4] and a central component of the neural crest migration is programmed EMT [5,6].

Approximately, 90% of cancers exhibit some degree of EMT during their progression, and epithelial tumors are the result of an EMT process [6]. After activation of EMT, tumor cells lose their epithelial features, including cell adhesion and polarity, reorganize their cytoskeleton, and acquire a mesenchymal morphology and the ability to migrate [6]. Transforming growth factor beta (TGF-β) is the best known inducer of EMT and acts through Smads to induce EMT-related transcription factors [7]. Extracellular matrix composition has profound effect on the regulation of EMT via TGF-β availability. $\alpha_v\beta_6$ integrin engages fibronectin and activates latent TGF-β to induce EMT [8], while $\alpha_v\beta_6$-mediated TGF-β activation can be blocked by inhibitors of Rhokinase (ROCK) [9].

Moreover, EMT may be induced by hepatocyte growth factor (HGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF) overexpression [10–12]. EGF receptor (EGFR) can be involved in EMT via up-regulation of TWIST gene expression [13]. In the developing lung, FGF acts as a chemoattractant for epithelial cells which express FGF receptors and elongate towards spatially sources of FGF [14].

EMT is coordinated by a group of transcription factors, including Snai1/Snail, Snai2/Slug, Twist, and ZEB1, and is characterized by increased expression of the mesenchymal markers vimentin and N-cadherin and downregulation of the E-cadherin gene, an epithelial marker and potent suppressor of tumor cell invasion and metastasis [15,16]. Transcriptional repression of E-cadherin by Snail is closely correlated with EMT and the loss of E-cadherin expression is a hallmark of EMT [17,18], and Snail-mutant mice do not survive beyond gastrulation because E-cadherin is unexpressed and cells cannot undergo EMT [19]. Snail is sufficient to induce EMT in tissue culture, and transfection of Snail into epithelial cell lines results in their mesenchymalization associated with a downregualtion of E-cadherin expression [17]. Notch is involved in the EMT associated with embryonic heart development [20].

EMT is characterized by the breakdown of adherens junctions and loss of epithelial markers, including cytokeratins and E-cadherin, and by the overexpression of mesenchymal markers, including fibronectin, N-cadherin, and vimentin, as well as the acquisition of an invasive fibroblastoid phenotype [20–22]. During the EMT of neural crest formation, a switch from E-cadherin to N-cadherin promotes adherens junction disassembly [23], and adherens junctions destabilization allows for the release of associated proteins, including β-catenin, which can upregulate EMT genes [24].

* Correspondence address: Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari Medical School, Policlinico - Piazza G. Cesare, 11, 70124 Bari, Italy.
E-mail address: domenico.ribatti@uniba.it.

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1.1. Mechanisms underlying tubulogenesis

The formation of branched tubes from initially unbranched epithelial buds is a fundamental morphogenetic process in the development of different organs, including the pancreas, liver, mammary gland, lung and kidney [25,26]. Tubules can arise through the invagination of cells from an epithelial sheet, as occurs in the formation of the neural tube or through the organization of initially unpolarized cells into cord-like structures that invade the surrounding mesenchyme, forming branched, hollow tubules lined by polarized cells [27]. It is important underline that embryonic epithelia fail to undergo branching morphogenesis if separated from the adjacent mesenchyme, and that morphogenesis resumes when the components are recombined in vitro [28].

1.2. Fibroblast-derived soluble factors induce morphogenesis of branching tubules by kidney epithelial cells and HGF is a paracrine mediator of morphogenetic epithelial-mesenchymal interactions

The morphogenetic properties of the Madin-Darby canine kidney (MDCK) cells may be influenced by diffusible factors released by neighboring mesenchymal or stromal cells. MDCK cells suspended within a collagen gel contiguous to a fibroblast-populated gel layer form branching tubules instead of the spherical cysts that develop in the absence of fibroblasts; MDCK cells grown as a monolayer on a cell-free collagen gel cast layer invade the underlying collagen matrix, within which they form a network of branching tubules; fibroblast-conditioned medium mimics the effect of co-culture by eliciting tubule formation by MDCK cells [29].

MDCK cells grown in collagen gels in the presence of HGF formed linear or branching tubular structures, while MDCK cells grown in the presence of fibroblast-conditioned medium that had been pre-incubated with specific anti-HGF antibodies exclusively formed spherical cysts similar to those observed in the absence of conditioned medium; anti-HGF antibodies suppressed tubulogenesis in co-cultures of MDCK cells and fibroblasts [30]. Overall, these data demonstrated that the fibroblast-derived factor that induces tubule formation by MDCK cells is HGF. HGF was identified as the fibroblast growth factor that stimulates epithelial cells derived from a variety of different organs to form tubule-like extensions when seeded in three-dimensional matrices [31,32].

In a further study, the role of the transcription factor Snail was investigated on epithelial properties of MDCK cells [33]. The inducible expression of Snail does not result in overt EMT, but selectively reduces the expression of claudin-3, -4 and –7 and increases paracellular ionic conductance without affecting tight junction permeability [33].

Moreover, epithelial tubulogenesis is dependent on extracellular plasmin-dependent tubulogenesis. When MDCK cells were grown in fibrin gels, HGF-induced tubule formation was prevented by the addition of serine proteinase inhibitors [29]. Conditioned medium from fibroblasts increased urokinase plasminogen activator (uPA) activity and mRNA by about 5-fold and this effect was completely inhibited by preincubation of conditioned medium with anti-HGF antibodies; exogenously added recombinant HGF induced a comparable increase in uPA activity and mRNA in MDCK cells; both fibroblast-conditioned medium and HGF induced a more than 30-fold increase in uPAR mRNA in MDCK cells [34].

1.3. Paracrine epithelial-mesenchymal interactions, HGF and TGF-β1 play a role in mammary gland morphogenesis in vivo

Diffusible factors released by fibroblasts could promote the formation of duct-like structures by mammary gland epithelial cells embedded in collagen gels [35]. Moreover, the effect of fibroblast-conditioned medium was completely abrogated by antibodies to HGF, whereas the addition of exogenous HGF to the cultures mimicked the tubulogenic activity of conditioned medium. The levels of both HGF and its receptor c-met mRNA progressively reduced during pregnancy, were undetectable during lactation, but increased during the involution phase up to pre-pregnancy levels [36]. Moreover, after 3 days of lactation both HGF and c-met transcripts were once again reduced to undetectable levels in the mothers, and prolactin significantly reduced the levels of c-met mRNA in mammary cells, thus providing a possible mechanism for c-met down-regulation in the rat mammary gland during lactation [36].

Low concentrations of TGF-β1 promote the elongation and branching of mammary cells, whereas high levels have inhibitory effects [37]. Mammary epithelial cells grown in collagen gels in chemically defined medium form spherical cysts, while the addition of acidified fetal calf serum (FCS) to the defined medium induced the formation of branching tubes [37,38]. Moreover, the effect of acidified FCS was replicated by the addition of exogenous TGF-β1, suggesting that, at low concentrations, TGF-β1 can activate a morphogenetic program resulting in the formation of epithelial tubes. Tube formation was suppressed by a recombinant tissue inhibitor of matrix metalloproteinase-2 (MMP-2) and by a selective inhibitor of MMP-9, indicating that this morphogenetic process requires the activity of MMP-9.

1.4. Retinoids induce lumen formation, whereas tumor necrosis factor alpha (TNF-α) and bone morphogenetic protein-4 (BMP-4) confer an invasive and transformed phenotype to cultured mammary epithelial cells

Retinoic acid induces the formation of cysts in cultured mammary epithelial cells and lumen formation was abrogated by the addition of the synthetic MMP inhibitor BB94 [39]. TNF-α causes multicellular colonies of mammary epithelial cells to disaggregate and induces cells grown on top of a collagen gel to invade the underlying matrix [40]. Moreover, TNF-α confers to mammary epithelial cells several additional properties characteristics of malignantly transformed cells, including proliferation in the absence of exogenously added growth factors, anchorage-independent growth and the loss of contact-mediated inhibition of proliferation [40]. Finally, BMP-4 disrupts cyst organization in a concentration-dependent manner, causing lumen obliteration, the extension of invading cell cords, and three-dimensional cell scattering [38].

1.5. EMT in cancer

The importance of EMT in driving carcinogenesis has been shown in lung, breast, prostate, pancreatic, and liver cancers [41,42] and activation of the EMT programs serves as a major mechanism for generating cancer stem cells (CSCs) [43].

Factors such as E-cadherin, catenins, vimentin, and Snail have all been correlated with clinical and pathological features in non-small-cell lung cancer (NSCLC), [44–46], where the expression of E-cadherin and catenins is reduced [44,45]. In human carcinomas, Snail plays a major role in inducing EMT, whereas Zeb ½ and twist are mainly involved in maintain the invasive mesenchymal phenotype [47]. In addition, vimentin is over-expressed in many epithelial cancers, including lung cancer, and its overexpression correlates with tumor growth, invasion, and poor prognosis [48]. Notch is implicated in the acquisition of EMT and cancer stem-like phenotypes in pancreatic cancer cells [49]. In human prostate cancer, the expression and nuclear activity of β-catenin correlates with the level of hypoxia inducible factor 1 alpha (HIF-1α)-induced EMT [50]. The degree of hypoxia-induced EMT can also be enhanced by Wnt3a-induced activation of β-catenin in hepatic carcinoma [51].

In high-grade breast cancer, high SPARC expression identifies tumors with increased EMT, reduced treatment response, and poor prognosis. The ability of SPARC to induce EMT dependon the localization and suppressive function of myeloid cells and inhibition of the suppressive function of myeloid derived stem cells (MDSCs) by