Experimental Cell Research 353 (2017) 1–5

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Epithelial-mesenchymal transition in morphogenesis, cancer progression and angiogenesis

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ARTICLE INFO

Keywords:
Angiogenesis
Epithelium
Organogenesis
Mesenchyme
Metastasis
Tumor growth

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].

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http://dx.doi.org/10.1016/j.yexcr.2017.02.041
Received 22 January 2017; Received in revised form 22 February 2017; Accepted 27 February 2017
Available online 28 February 2017

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

administration of amino bisphosphonates could revert EMT [52].

IL-27 promotes the expression of epithelial markers, inhibits cell migration and the production of angiogenic factors in human NSCLC through a STAT1 dominant pathway [53]. IL-27-treated lung cancer cells show increased expression of epithelial marker (E-cadherin and γ-catenin), decreased expression of Snail (transcriptional repressor of E-cadherin), and decreased expression of mesenchymal marker (N-cadherin and vimentin). In addition, IL-27 treatment suppressed in vitro cell migration [53].

1.6. EMT and angiogenesis

The same factors that drive epithelial cells toward a mesenchymal phenotype may also drive endothelial cells toward a pro-angiogenic phenotype. Angiogenesis is defined as a new blood vessel sprouting from pre-existing vessels. This can be accomplished through endothelial sprouting or non-sprouting (intussusceptive) microvascular growth (IMG) [54]. The first description of sprouting angiogenesis in tumor growth was reported by Ausprunk and Folkman in 1977 [55], which indicated the following stages: i) The basement membrane is locally degraded on the side of the dilated peritumoral postcapillary venule situated closed to the angiogenic stimulus; ii) Interendothelial contacts are weakened and endothelial cells migrate into the connective tissue; iii) A solid cord of endothelial cells form; iv) Lumen formation occurs proximal to the migrating front, contiguous tubular sprouts anastomose to form functionally capillary loops, parallel with the synthesis of the new basement membrane and the recruitment of pericytes. Initiation of sprouting requires the specification of endothelial cells into tip and stalk cells bearing different morphologies and functional properties [56]. Endothelial tip cells primarily migrate but proliferate only minimally, in contrast to endothelial stalk cells, which do proliferate. Tip cell is migratory and polarized, while stalk cell proliferates during sprout extension and forms the nascent vascular lumen cell. During angiogenesis, the tip cells lack apical-basal polarity, degrade basement membrane and extracellular matrix, and become migratory. The phenotypic specialization of endothelial cells as tip or stalk cells is very transient and reversible, depending on the balance between pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and Jagged-1 (JAG-1), and suppressors of endothelial cell proliferation, such as delta –like ligand 4 (Dll4)-Notch activity [57–60]. Tip cells express high levels of Dll-4, platelet derived growth factor-b (PDGF-b), unc-5 homolog b (UNC5b), VEGF receptor-2 (VEGFR-2), and VEGFR-3/Flt-4, and have low levels of Notch signaling activity [61–65].

The necessity of Dll-4/Notch-1 signaling in the endothelium has been well established as loss of a single copy of Dll-4 or deletion of Notch-1 causes vascular defects and embryonic lethality [66,67]. Notch-1 and Notch-4 and three Notch ligands, JAG-1, Dll-1, and Dll-4 are expressed in endothelial cells for the selection of endothelial tip and stalk cell during sprouting angiogenesis [68]. A loss of Notch signaling induces while its activation reduces sprouting. Cells dynamically compete for the tip position utilizing differential VEGFR levels, as cells with higher VEGFR signaling produces more Dll4 and therefore inhibit their neighboring cells. In this context, a fine tuned feed-back loop between VEGF and Notch/Dll4 signaling pathways is established and a cross-talk between these pathways is essential for proper patterning of the vasculature [69]. Blockade of Notch leads to widespread Flt4 expression, increases filopodia and sprouting, and promotes tip cell activity [64,70]. Endothelial cells with higher levels of VEGF increase Dll4 expression which further increases the cell sensitivity to VEGF, and this cell becomes the tip cell selected for outward migration for the parent vessel [62,64,71–73]. Stalk cells have high levels of Notch signaling activity and elevated expression of JAG-1 [74]. Stalk cell JAG-1 antagonizes Dll4 activity, reducing the induction of Notch signaling in the adjacent tip cell, which therefore maintains its responsiveness to VEGF stimulation and migrates outward to establish a new branch [74].

During the final steps of capillary development, endothelial cell migration comes to a halt and endothelial cells form a lumen and re-establish functional adherens junctions [75]. Adherens junction formation is associated with the inhibition of endothelial cell migration in monolayers. This process was shown to be mediated by vascular endothelial cell cadherin (VE-cadherin) [75,76], exclusively expressed in endothelial cells and facilitating their homotypic interaction [77,78]. VE-cadherin is strictly required for the polarization of endothelial cells in vitro and in vivo, and VE-cadherin-based junctions are subjected to continuous reorganization, which renders them highly dynamic and sensitive to extracellular stimuli [79,80].

Transcription factors Snail and Slug are expressed and regulated by endothelial cells during in vitro angiogenesis [81]. Moreover, inhibition of Snail or Slug expression resulted in a reduced capacity of endothelial cells to migrate through extracellular matrix [81]. During endothelial mesenchymal transition upregulation of endothelial cell Slug by TGFβ results in increased migration and invasion in extracellular matrix [81]. Notch can suppress (or activate) gene expression directly or through upergulation of Snail and Slug in both epithelial or endothelial cells [82,83]. In sprouting angiogenesis, slug is the primary initiator of this process, whereas the induction of Snail occurs at much later time [81].

EMT and angiogenesis have emerged as integral processes in promoting carcinogenesis [84]. Rojas-Puentes et al. [85] demonstrated that VEGF and EGFR expression correlated with hangs in TWIST2 levels and loss of E-cadherin expression. They concluded that the presence of EMT markers was associated with proliferative and pro-angiogenic protein expression and influenced the prognosis of cervical cancer. The cross-talk between Notch and VEGF pathways in the context of hypoxic tumors promotes endothelial mesenchymal transition in angiogenic tumor endothelial cells [69]. Studies on xenografts in pre-invasive cells demonstrated that the addition of VEGF induces the appearance of EMT markers [86,87].

2. Concluding remarks

The importance of epithelial-mesenchyme interactions in embryogenesis and tissue formation was suspected even before its significance in malignancy can be appreciated. During normal development, transition of epithelial to mesenchyme occurs in gastrulation, and mesenchyme can differentiate into new epithelia in kidney development. In the meantime, the loss of epithelial character in malignant carcinomas, resulting in the appearance of invasive and motile cells, is fundamental in tumor progression. Angiogenesis involves adhesion, degradation of extracellular matrix, migration, proliferation, and differentiation of vascular endothelial cells, a process similar to cancer metastasis. In this context, angiogenesis plays also an integral role in the stability of the epithelium and the interstitial matrix.

Intriguing parallels between the process of normal development, malignant growth and angiogenesis have been established. Some of the same cytokines, growth factors, proteolytic enzymes, cell adhesion molecules, and components of the extracellular matrix, as it has been shown in this article, have been implicated in these processes.

EMT creates a pro-angiogenic context, and although the cellular pathways leading to EMT and their contribution to enhanced invasive properties of tumor cells have been extensively studied, the impact of EMT programs on the crosstalk between tumor cells and the tumor microenvironment are still not completely clarified and should be further investigated.

Therefore, inhibition of EMT might be a rational strategy to prevent metastasis. For example, whilst originally identified and optimized for their anti-proliferative effects, evidence suggests that some of the targeted small molecule inhibitors may also inhibit EMT initiation or sustenance, since the EMT program is modulated by similar signaling pathways for which these molecules have been generated [88]. More recently, it has been established that extracellular vesicles, including

exosomes, are involved in EMT and metastasic process [89–91]. In this context, exosomes may be also utilized as system for drug delivery, such as doxorubicin to tumor tissue leading to inhibition of growth without overt toxicity [92].

References

[1] P. Friedl, D. Gilmour, Collective cell migration in morphogenesis, regeneration and cancer, Nat. Rev. Mol. Cell. Biol. 10 (2009) 445–457.
[2] R. Kalluri, R.A. Weinberg, The basics of epithelial-mesenchymal transition, J. Clin. Invest. 119 (2009) 1420–1428.
[3] M. Zeisberg, E.G. Neilson, Biomarkers for epithelial-mesenchymal transitions, J. Clin. Invest. 119 (2009) 1429–1437.
[4] H. Haegel, L. Larue, M. Ohsugi, L. Fedorov, K. Herrenknecht, R. Kemler, Lack of beta-catenin affects mouse development at gastrulation, Development 121 (1995) 3529–3537.
[5] P.H. Strobl-Mazzulla, M.E. Bronner, Epithelial to mesenchymal transition: new and old insights from the classical neural crest model, Semin. Cancer Biol. 22 (2012) 411–416.
[6] J.P. Thiery, H. Acloque, R.Y.J. Huang, M.A. Nieto, Epithelial-mesenchymal transitions in development and disease, Cell 139 (2009) 871–890.
[7] J. Xu, S. Lamouille, R. Derynck, TGF-β-induced epithelial to mesenchymal transition, Cell Res. 19 (2009) 156–172.
[8] K.K. Kim, M.C. Kugler, P.J. Wolters, L. Robillard, M.G. Galvez, A.N. Brumwell, D. Sheppard, H.A. Chapman, Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix, Proc. Natl. Acad. Sci. USA 103 (2006) 13180–13185.
[9] M.M. Giacomini, M.A. Travis, M. Kudo, D. Sheppard, Epithelial cells utilize cortical actin/myosin to activate latent TGF-β through integrin αvβ6-dependent physical force, Exp. Cell. Res. 318 (2012) 716–722.
[10] S. Grünert, M. Jechlinger, H. Beug, Opinion: diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis, Nat. Rev. Mol. Cell. Biol. 4 (2003) 657–665.
[11] E.D. Hay, An overview of epithelio-mesenchymal transformation, Cells Tissues Organs 154 (1995) 8–20.
[12] M.A. Huber, N. Kraut, H. Beug, Molecular requirements for epithelial–mesenchymal transition during tumor progression, Curr. Opin. Cell. Biol. 17 (2005) 548–558.
[13] H.W. Lo, S.C. Hsu, W. Xia, X. Cao, J.Y. Shih, Y. Wei, J.L. Abbruzzese, G.N. Hortobagyi, M.C. Hung, Epidermal growth factor receptor cooperates with signal transducer and activator of transcription 3 to induce epithelial-mesenchymal transition in cancer cells via up-regulation of TWIST gene expression, Cancer Res. 67 (2007) 9066–9076.
[14] D. Warburton, M. Schwarz, D. Tefft, G. Flores-Delgado, K.D. Anderson, W.V. Cardoso, The molecular basis of lung morphogenesis, Mech. Dev. 92 (2000) 55–81.
[15] K. Lin, S. Baritaki, L. Militello, G. Malaponte, Y. Bevelacqua, B. Bonavida, The role of B-RAF mutations in melanoma and the induction of EMT via dysregulation of the NF- B/snail/RKIP/PTEN circuit, Genes Cancer 1 (2010) 409–420.
[16] M. Nakamura, Y. Tokura, Epithelial–mesenchymal transition in the skin, J. Dermatol. Sci. 61 (2011) 7–13.
[17] E. Batlle, E. Sancho, C. Francí, D. Domínguez, M. Monfar, J. Baulida, A. García de Herreros, The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells, Nat. Cell. Biol. 2 (2000) 84–89.
[18] M. Iwatsuki, K. Mimori, T. Yokobori, H. Ishi, T. Beppu, S. Nakamori, H. Baba, M. Mori, Epithelial-mesenchymal transition in cancer development and its clinical significance, Cancer Sci. 101 (2010) 293–299.
[19] E.A. Carver, R. Jiang, Y. Lan, K.F. Oram, T. Gridley, The mouse snail gene encodes a key regulator of the epithelial-mesenchymal transition, Mol. Cell. Biol. 21 (2001) 8184–8188.
[20] V.C. Garside, A.C. Chang, A. Karsan, P.A. Hoodless, Co-ordinating notch, BMP, and TGF-β signaling during heart valve development, Cell. Mol. Life Sci. 70 (2012) 2899–2917.
[21] D.S. Micalizzi, S.M. Farabaugh, H.L. Ford, Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression, J. Mammary Gland Biol. Neoplasia 15 (2010) 117–134.
[22] A. Wells, C. Yates, C.R. Shepard, E-cadherin as an indicator of mesenchymal to epithelial reverting transitions during the metastatic seeding of disseminated carcinomas, Clin. Exp. Metastasis 25 (2008) 621–628.
[23] E. Scarpa, A. Szabó, A. Bibonne, E. Theveneau, M. Parsons, R. Mayor, Cadherin switch during EMT in neural crest cells leads to contact inhibition of locomotion via repolarization of forces, *Dev Cell* 34 (2015) 421–434.
[24] M.S. Samuel, J.I. Lopez, E.J. McGhee, D.R. Croft, D. Strachan, P. Timpson, J. Munro, E. Schroder, J. Zhou, V.G. Brunton, N. Barker, H. Clevers, O.J. Sansom, K.I. Anderson, V.M. Weaver, M.F. Olson, Actomyosin-mediated cellular tension drives increased tissue stiffness and beta-catenin activation to induce epidermal hyperplasia and tumor growth, Cancer Cell 19 (2011) 776–791.
[25] M. Affolter, S. Bellusci, N. Itoh, B. Shilo, J.P. Thiery, Z. Werb, Tube or not tube: remodeling epithelial tissues by branching morphogenesis, Dev. Cell 4 (2003) 11–18.
[26] B. Lubarsky, M.A. Krasnow, Tube morphogenesis: making and shaping biological tubes, Cell 112 (2003) 19–28.
[27] B.L. Hogan, P.A. Kolodziej, Organogenesis: molecular mechanisms of tubulogenesis, nature reviews, Genetics 3 (2002) 513–523.
[28] D. Ribatti, M. Santoiemma, Epithelial-mesenchymal interactions: a fundamental developmental biology mechanism, Int. J. Dev. Biol. 58 (2014) 303–306.
[29] R. Montesano, G. Schaller, L. Orci, Induction of epithelial tubular morphogenesis in vitro by fibroblast-derived soluble factors, Cell 66 (1991) 697–711.
[30] R. Montesano, K. Matsumoto, T. Nakamura, L. Orci, Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor, Cell 67 (1991) 901–908.
[31] V. Brinkmann, H. Foroutan, M. Sachs, K.M. Weidner, W. Birchmeier, Hepatocyte growth factor/scatter factor induces a variety of tissue-specific morphogenic programs in epithelial cells, J. Cell. Biol. 131 (1995) 1573–1586.
[32] K.M. Weidner, G. Hartmann, L. Naldini, P.M. Comoglio, M. Sachs, C. Fonatsch, H. Rieder, W. Birchmeier, Molecular characteristics of HGF-SF and its role in cell motility and invasion, EXS 65 (1993) 311–328.
[33] F. Carrozzino, Inducible expression of snail selectively increases paracellular ion permeability and differentially modulates tight junction proteins, AJP: Cell Phys. 289 (2005) C1002–C1014.
[34] M.S. Pepper, K. Matsumoto, T. Nakamura, L. Orci, R. Montesano, Hepatocyte growth factor increases urokinase-type plasminogen activator (u-PA) and u-PA receptor expression in Madin-Darby canine kidney epithelial cells, J. Bio. Chem. 267 (1992) 20493–20496.
[35] J.V. Soriano, M.S. Pepper, T. Nakamura, L. Orci, R. Montesano, Hepatocyte growth factor stimulates extensive development of branching duct-like structures by cloned mammary gland epithelial cells, J. Cell. Sci. 108 (Pt 2) (1995) 413–430.
[36] M.S. Pepper, J.V. Soriano, P.A. Menoud, A.P. Sappino, L. Orci, R. Montesano, Modulation of hepatocyte growth factor and c-met in the rat mammary gland during pregnancy, lactation, and involution, Exp. Cell. Res. 219 (1995) 204–210.
[37] J.V. Soriano, L. Orci, R. Montesano, TGF-β1Induces morphogenesis of branching cords by cloned mammary epithelial cells at subpicomolar concentrations, Biochem. Biophys. Res. Commun. 220 (1996) 879–885.
[38] R. Montesano, Bone morphogenetic protein-4 abrogates lumen formation by mammary epithelial cells and promotes invasive growth, Biochem. Biophys. Res. Commun. 353 (2007) 817–822.
[39] R. Montesano, Retinoids induce lumen morphogenesis in mammary epithelial cells, J. Cell Sci. 115 (2002) 4419–4431.
[40] R. Montesano, Tumour necrosis factor confers an invasive, transformed phenotype on mammary epithelial cells, J. Cell Sci. 118 (2005) 3487–3500.
[41] H. Hugo, M.L. Ackland, T. Blick, M.G. Lawrence, J.A. Clements, E.D. Williams, E.W. Thompson, Epithelial–mesenchymal and mesenchymal–epithelial transitions in carcinoma progression, J. Cell Physiol. 213 (2007) 374–383.
[42] T.K. Lee, R.T.P. Poon, A.P. Yuen, M.T. Ling, W.K. Kwok, X.H. Wang, Y.C. Wong, X.Y. Guan, K. Man, K.L. Chau, S.T. Fan, Twist overexpression correlates with hepatocellular carcinoma metastasis through induction of epithelial-mesenchymal transition, Clin. Cancer Res. 12 (2006) 5369–5376.
[43] X. Ye, R.A. Weinberg, Epithelial–mesenchymal plasticity: a central regulator of cancer progression, Trends Cell. Biol. 25 (2015) 675–686.
[44] S. Kase, K. Sugio, K. Yamazaki, T. Okamoto, T. Koga, K. Ondo, M. Yamaguchi, F. Shoji, K. Sugimachi, Expression of E-cadherin and beta-catenin in human non-small cell lung cancer: clinical significance and prognosis, Lung Cancer 29 (2000) 196.
[45] R.T. Pirinen, Reduced expression of alpha-catenin, beta-catenin, and gamma-catenin is associated with high cell proliferative activity and poor differentiation in non-small cell lung cancer, J. Clin. Pathol. 54 (2001) 391–395.
[46] M. Sato, D.S. Shames, Y. Hasegawa, Emerging evidence of epithelial-to-mesenchymal transition in lung carcinogenesis, Respirology 17 (2012) 1048–1059.
[47] H. Peinado, D. Olmeda, A. Cano, Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype?, Nat. Rev. Cancer 7 (2007) 415–428.
[48] A. Satelli, S. Li, Vimentin in cancer and its potential as a molecular target for cancer therapy, Cell. Mol. Life Sci. 68 (2011) 3033–3046.
[49] Z. Wang, Y. Li, D. Kong, S. Banerjee, A. Ahmad, A.S. Azmi, S. Ali, J.L. Abbruzzese, G.E. Gallick, F.H. Sarkar, Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway, Cancer Res. 69 (2009) 2400–2407.
[50] Y.-G. Jiang, Y. Luo, D.-l. He, X. Li, L.-l. Zhang, T. Peng, M.-C. Li, Y.-H. Lin, Role of Wnt/β-catenin signaling pathway in epithelial-mesenchymal transition of human prostate cancer induced by hypoxia-inducible factor-1α, Int. J. Urol. 14 (2007) 1034–1039.
[51] Q. Zhang, X. Bai, W. Chen, T. Ma, Q. Hu, C. Liang, S. Xie, C. Chen, L. Hu, S. Xu, T. Liang, Wnt/-catenin signaling enhances hypoxia-induced epithelial-mesenchymal transition in hepatocellular carcinoma via crosstalk with hif-1 signaling, Carcinogenesis 34 (2013) 962–973.
[52] S. Sangaletti, C. Tripodo, A. Santangelo, N. Castioni, P. Portararo, A. Gulino, L. Botti, M. Parenza, B. Cappetti, R. Orlandi, E. Tagliabue, C. Chiodoni, Mario P. Colombo, Mesenchymal transition of high-grade breast carcinomas depends on extracellular matrix control of myeloid suppressor cell activity, Cell Rep. 17 (2016) 233–248.
[53] P. Kachroo, M.-H. Lee, L. Zhang, F. Baratelli, G. Lee, M.K. Srivastava, G. Wang, T.C. Walser, K. Krysan, S. Sharma, S.M. Dubinett, J.M. Lee, IL-27 inhibits epithelial-mesenchymal transition and angiogenic factor production in a STAT1-dominant pathway in human non-small cell lung cancer, J. Exp. Clin. Cancer Res. 32 (2013) 97.
[54] D. Ribatti, Genetic and epigenetic mechanisms in the early development of the vascular system, J. Anat. 208 (2006) 139–152.
[55] D.H. Ausprunk, J. Folkman, Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis, Microvasc. Res. 14 (1977) 53–65.
[56] D. Ribatti, E. Crivellato, “Sprouting angiogenesis”, a reappraisal, Dev. Biol. 372

(2012) 157–165.
[57] H.M. Eilken, R.H. Adams, Dynamics of endothelial cell behavior in sprouting angiogenesis, Curr. Opin. Cell. Biol. 22 (2010) 617–625.
[58] I. Geudens, H. Gerhardt, Coordinating cell behaviour during blood vessel formation, Development 138 (2011) 4569–4583.
[59] J.J. Tung, I.W. Tattersall, J. Kitajewski, Tips, stalks, tubes: notch-mediated cell fate determination and mechanisms of tubulogenesis during angiogenesis, Cold Spring Harb. Perspect. Med. 2 (2011) (a006601-a006601).
[60] A. Wacker, H. Gerhardt, Endothelial development taking shape, Curr. Opin. Cell. Biol. (2011).
[61] S. Claxton, M. Fruttiger, Periodic delta-like 4 expression in developing retinal arteries, Gene Expr. Patterns 5 (2004) 123–127.
[62] M. Hellström, L.-K. Phng, J.J. Hofmann, E. Wallgard, L. Coultas, P. Lindblom, J. Alva, A.-K. Nilsson, L. Karlsson, N. Gaiano, K. Yoon, J. Rossant, M.L. Iruela-Arispe, M. Kalén, H. Gerhardt, C. Betsholtz, Dll4 signalling through notch1 regulates formation of tip cells during angiogenesis, Nature 445 (2007) 776–780.
[63] X. Lu, F. le Noble, L. Yuan, Q. Jiang, B. de Lafarge, D. Sugiyama, C. Bréant, F. Claes, F. De Smet, J.-L. Thomas, M. Autiero, P. Carmeliet, M. Tessier-Lavigne, A. Eichmann, The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system, Nature 432 (2004) 179–186.
[64] A.F. Siekmann, N.D. Lawson, Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries, Nature 445 (2007) 781–784.
[65] S. Suchting, C. Freitas, F. le Noble, R. Benedito, C. Breant, A. Duarte, A. Eichmann, The notch ligand delta-like 4 negatively regulates endothelial tip cell formation and vessel branching, Proc. Natl. Acad. Sci. USA 104 (2007) 3225–3230.
[66] N.W. Gale, M.G. Dominguez, I. Noguera, L. Pan, V. Hughes, D.M. Valenzuela, A.J. Murphy, N.C. Adams, H.C. Lin, J. Holash, G. Thurston, G.D. Yancopoulos, Haploinsufficiency of delta-like 4 ligand results in embryonic lethality due to major defects in arterial and vascular development, Proc. Natl. Acad. Sci. USA 101 (2004) 15949–15954.
[67] F.P. Limbourg, Essential role of endothelial notch1 in angiogenesis, Circulation 111 (2005) 1826–1832.
[68] T. Kume, Novel insights into the differential functions of Notch ligands in vascular formation, J. Angiogenes. Res. 1 (2009) 8.
[69] M.T. Holderfield, C.C.W. Hughes, Crosstalk between vascular endothelial growth factor, notch, and transforming growth factor- in vascular morphogenesis, Circ. Res. 102 (2008) 637–652.
[70] G. Thurston, I. Noguera-Troise, G.D. Yancopoulos, The delta paradox: DLL4 blockade leads to more tumour vessels but less tumour growth, Nat. Rev. Cancer 7 (2007) 327–331.
[71] I.B. Lobov, R.A. Renard, N. Papadopoulos, N.W. Gale, G. Thurston, G.D. Yancopoulos, S.J. Wiegand, Delta-like ligand 4 (Dll4) is induced by VEGF as a negative regulator of angiogenic sprouting, Proc. Natl. Acad. Sci. USA 104 (2007) 3219–3224.
[72] R.C.A. Sainson, Cell-autonomous notch signaling regulates endothelial cell branching and proliferation during vascular tubulogenesis, FASEB J. (2005).
[73] T. Tammela, G. Zarkada, E. Wallgard, A. Murtomäki, S. Suchting, M. Wirzenius, M. Waltari, M. Hellström, T. Schomber, R. Peltonen, C. Freitas, A. Duarte, H. Isoniemi, P. Laakkonen, G. Christofori, S. Ylä-Herttuala, M. Shibuya, B. Pytowski, A. Eichmann, C. Betsholtz, K. Alitalo, Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation, Nature 454 (2008) 656–660.
[74] R. Benedito, C. Roca, I. Sörensen, S. Adams, A. Gossler, M. Fruttiger, R.H. Adams, The Notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis, Cell 137 (2009) 1124–1135.
[75] E. Dejana, Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis, J. Clin. Investig. 98 (1996) 1949–1953.
[76] M.G. Lampugnani, The molecular organization of endothelial cell to cell junctions: differential association of plakoglobin, beta-catenin, and alpha- catenin with vascular endothelial cadherin (VE-cadherin), J. Cell. Biol. 129 (1995) 203–217.
[77] U. Cavallaro, S. Liebner, E. Dejana, Endothelial cadherins and tumor angiogenesis, Exp. Cell. Res. 312 (2006) 659–667.
[78] M. Labelle, H.J. Schnittler, D.E. Aust, K. Friedrich, G. Baretton, D. Vestweber, G. Breier, Vascular endothelial cadherin promotes breast cancer progression via transforming growth factor signaling, Cancer Res. 68 (2008) 1388–1397.
[79] M.G. Lampugnani, F. Orsenigo, N. Rudini, L. Maddaluno, G. Boulday, F. Chapon, E. Dejana, CCM1 regulates vascular-lumen organization by inducing endothelial polarity, J. Cell Sci. 123 (2010) 1073–1080.
[80] B. Strilić, T. Kučera, J. Eglinger, M.R. Hughes, K.M. McNagny, S. Tsukita, E. Dejana, N. Ferrara, E. Lammert, The molecular basis of vascular lumen formation in the developing mouse aorta, Dev. Cell 17 (2009) 505–515.
[81] K.M. Welch-Reardon, S.M. Ehsan, K. Wang, N. Wu, A.C. Newman, M. Romero-Lopez, A.H. Fong, S.C. George, R.A. Edwards, C.C.W. Hughes, Angiogenic sprouting is regulated by endothelial cell expression of slug, J. Cell Sci. 127 (2014) 2017–2028.
[82] K. Niessen, Y. Fu, L. Chang, P.A. Hoodless, D. McFadden, A. Karsan, Slug is a direct Notch target required for initiation of cardiac cushion cellularization, J. Cell Biol. 182 (2008) 315–325.
[83] C. Sahlgren, M.V. Gustafsson, S. Jin, L. Poellinger, U. Lendahl, Notch signaling mediates hypoxia-induced tumor cell migration and invasion, Proc. Natl. Acad. Sci. USA 105 (2008) 6392–6397.
[84] I. Sánchez-García, The crossroads of oncogenesis and metastasis, N. Engl. J. Med. 360 (2009) 297–299.
[85] L. Rojas-Puentes, A.F. Cardona, H. Carranza, C. Vargas, L.F. Jaramillo, D. Zea, L. Cetina, B. Wills, E. Ruiz-Garcia, O. Arrieta, Epithelial-mesenchymal transition, proliferation, and angiogenesis in locally advanced cervical cancer treated with chemoradiotherapy, Cancer Med. 5 (2016) 1989–1999.
[86] S. Desai, S. Laskar, B.N. Pandey, Autocrine IL-8 and VEGF mediate epithelial–mesenchymal transition and invasiveness via p38/JNK-ATF-2 signalling in A549 lung cancer cells, Cell Signal. 25 (2013) 1780–1791.
[87] O. Gonzalez-Moreno, J. Lecanda, J.E. Green, V. Segura, R. Catena, D. Serrano, A. Calvo, VEGF elicits epithelial-mesenchymal transition (EMT) in prostate intraepithelial neoplasia (PIN)-like cells via an autocrine loop, Exp. Cell. Res. 316 (2010) 554–567.
[88] J.P. Thiery, J.P. Sleeman, Complex networks orchestrate epithelial–mesenchymal transitions, Nat. Rev. Mol. Cell. Biol. 7 (2006) 131–142.
[89] J. Kim, T.Y. Kim, M.S. Lee, J.Y. Mun, C. Ihm, S.A. Kim, Exosome cargo reflects TGF-β1-mediated epithelial-to-mesenchymal transition (EMT) status in A549 human lung adenocarcinoma cells, Biochem. Biophys. Res. Commun. 478 (2016) 643–648.
[90] Z. Wang, A. von Au, M. Schnolzer, T. Hackert, M. Zöller, CD44v6-competent tumor exosomes promote motility, invasion and cancer-initiating cell marker expression in pancreatic and colorectal cancer cells, Oncotarget 7 (2016) 55409–55436.
[91] B. Dos Anjos Pultz, F. Andrés Cordero da Luz, S. Socorro Faria, L. Peixoto Ferreira de Souza, P. Cristina Brígido Tavares, V. Alonso Goulart, W. Fontes, L. Ricardo Goulart, M. José Barbosa Silva, The multifaceted role of extracellular vesicles in metastasis: priming the soli for seeding, Int J. Cancer (2017). http://dx.doi.org/10.1002/ijc.30595 (Epub ahead of print).
[92] Y. Tian, S. Li, J. Song, T. Ji, M. Zhu, G.J. Anderson, J. Wei, G. Nie, A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy, Biomaterials 35 (2014) 2383–2390.