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When the endothelium scores an own goal: endothelial cells actively augment metastatic extravasation through endothelial-mesenchymal transition

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Endothelial-mesenchymal transition (EndMT) is an important mechanism during organ development and in certain pathological conditions. For example, EndMT contributes to myofibroblast formation during organ fibrosis, and it has been identified as an important source of cancer-associated fibroblasts, facilitating tumor progression. Recently, EndMT was proposed to modulate endothelial function during intravasation and extravasation of metastatic tumor cells. Evidence suggests that endothelial cells are not passive actors during transendothelial migration (TEM) of cancer cells, as there are profound changes in endothelial junctional protein expression, signaling, permeability, and contractility. This review describes these alterations in endothelial characteristics during TEM of metastatic tumor cells and discusses them in the context of EndMT. EndMT could play an important role during metastatic intravasation and extravasation, a novel hypothesis that may lead to new therapeutic approaches to tackle metastatic disease.

endothelial-mesenchymal transition; transendothelial migration; metastatic extravasation

Endothelial-Mesenchymal Transition: Role During Development and Disease

The inner lining of blood and lymphatic vessels is composed of a single compact layer of endothelial cells. Just like an epithelium, an organized endothelial layer is formed by endothelial cells showing apicobasal polarity, bound to each other by strong intercellular junction complexes. In a similar process to epithelial-mesenchymal transition (EMT) endothelial cells can undergo changes toward acquiring a mesenchymal, myofibroblast-like phenotype. This form of endothelial cell plasticity is termed endothelial-mesenchymal transition (EndMT). During EndMT, endothelial cells lose their specific markers and intercellular contact proteins (e.g., VE-cadherin), start expressing fibroblast-specific and mesenchymal proteins [e.g., fibroblast-specific protein-1 (FSP1), plasminogen activator inhibitor-1, vimentin] and to synthesize extracellular matrix proteins (e.g., fibronectin), ultimately differentiating into α-smooth muscle actin (SMA)-positive myofibroblasts. EndMT follows a well-defined and sequentially orchestrated chronology: downregulation of the endothelial program and activation of the fibrogenic-mesenchymal and myogenic program (62). The resulting cells acquire a fibrogenic phenotype, become motile, and are capable of migrating into surrounding tissues.

EndMT is an embryonic program switched off in adult organisms, yet under pathological conditions, it can be reactivated.

EndMT was first described in an embryonic environment. For example during heart development, EndMT leads to the development of mesenchymal cells that are required for the formation of endocardial cushion tissue during early embryonic chick heart development (18). Endothelial cells surrounding the atrioventricular canal and the outflow tract transform into myofibroblasts capable of invading surrounding tissues while forming the heart valves and septa in a transforming growth factor-β (TGF-β)-dependent manner (64). Further, in vivo observations demonstrated that embryonic pulmonary endothelial cells undergoing an EndMT participate in intimal thickening formation and pulmonary vascular remodeling (3).
Recently, EndMT was shown to contribute to the formation of cerebral cavernous malformations due to loss-of-function mutations of the CCM1 gene. In vivo experiments revealed that, mechanistically, EndMT in endothelial cells of CCM1-deficient mice is induced by the upregulation of bone morphogenetic protein 6 (BMP6), which, in turn, activates the BMP and TGF-β signaling pathways. The correct communication of the endothelium with astrocytes is recovered by inhibiting BMP and TGF-β signaling (60).

EndMT gained fresh spotlight in recent years when landmark studies demonstrated the implication of EndMT during pathological processes, such as fibrosis or cancer. EndMT was shown to have a major role during kidney fibrosis. Activated fibroblasts and myofibroblasts accumulate in fibrotic kidney through the contribution of EndMT, as evidenced in mouse models of streptozotocin-induced diabetic nephropathy (STZ-DN), unilateral ureteral obstructive nephropathy, and a model of Alport syndrome (104). Up to 50% of fibroblasts in the fibrotic kidneys were found to originate from endothelial cells. In later work by the same group, the contribution of EndMT to kidney fibrosis was estimated to be lower (10%) (50); nevertheless, the concept of EndMT involvement during renal fibrosis was documented by other studies as well (55). Moreover, inhibition of EndMT reduced the early development of STZ-DN (54). Cardiac fibrosis is also a scene for EndMT-derived fibroblasts. Elegant in vivo studies demonstrated that TGF-β1-driven EndMT of endothelial cells is a major contributor to cardiac fibrosis. Moreover, BMP-7 significantly inhibited EndMT and the progression of cardiac fibrosis in mouse models of chronic allograft rejection and pressure overload (105). Inhibition of EndMT through TGF-β blockade suppressed fibrogenic reaction in corneal endothelium in vivo (88). Lung endothelial cells can represent a significant source of fibroblasts through EndMT, as found in a bleomycin-induced lung fibrosis model (25). EndMT may contribute to abnormal vascular remodeling and fibrogenesis in systemic sclerosis (11), whereas the EndMT of human intestinal microvascular endothelial cells contribute to fibrosis in inflammatory bowel disease, as evidenced by in vivo findings in human microvessels of inflammatory bowel disease mucosa and in a mouse model of experimental colonic fibrosis (80).

During cancer, EndMT contributes to the formation of cancer-associated fibroblasts (CAFs). CAFs are known to facilitate tumor progression and are key components of tumor stroma. In mouse models of cancers, up to 40% of CAFs originated through EndMT (103). An EndMT may also explain the heterogeneity of cell types within Kaposi sarcoma lesions as a consequence of Kaposi sarcoma herpesvirus infection (10).

EndMT is similar to EMT and is a phenomenon characterized by analogous steps and signaling mechanisms. The EndMT and EMT programs can be regulated by several factors, yet the most important of these is the TGF-β family of growth factors (96). TGF-β induces EndMT and EMT through Smad, ERK, and p38 MAPK signaling pathways (63, 82). RhoA and Rac1 small GTPases (66, 84) contribute to the acquisition of the myofibroblast phenotype along the concerted function of several common transcription factors, like TWIST, ZEB-1, Snail, or LEF-1 (62). Rho/ROCK-dependent pathways are well known to regulate expression of the myofibroblast marker protein SMA during EMT and EndMT (66). Similarly to EMT (83), MRTF-A drives EndMT and SMA expression in MS-1 mouse pancreatic microvascular endothelial cells (66). Pathways involving Rac1, PAK, and p38 regulate contact-dependent induction of EMT (83). Focal adhesion kinase (FAK) activation is required for both EMT and EndMT (84, 90), FAK mediating TGF-β1-induced EMT (16). JAK/STAT signaling is also involved in regulating EMT (12), as activation of JAK2/STAT3 results in TWIST expression (41). Finally, matrix metalloproteases (such as MMP-2) are required to degrade the basal lamina (33).

Are Endothelial Cells Actively Augmenting Transendothelial Migration of Cancer Cells by Undergoing an EndMT?

Cancer-related mortality is overwhelmingly a consequence of metastatic events (85). The metastatic cascade describes the defined sequence of events occurring during cancer progression and metastasis. In the first step, primary tumor cells need to invade the local extracellular matrix, then these dislocated cells intravasate into the lumina of blood vessels. These circulating tumor cells (CTCs) can then extravasate from the vasculature into the surrounding tissue. Surviving extravasated cells can form micrometastasis and, later, can generate macroscopic metastases in the invaded tissues (21, 95). The survival of metastatic cells is limited by the damage induced by hemodynamic shear stress and the eradication by the innate immune system. The metastatic cascade is a highly inefficient process. Despite this fact, rather large numbers of CTCs can undergo extravasation (59).

Cancer cell extravasation typically occurs in the small capillaries of the microvasculature. CTCs have diameters larger than small capillaries; therefore, they will be trapped in the first capillary bed encountered (8). At this stage, two scenarios are possible for metastasis. These trapped cells could start growing in the microvascular lumina and form a colony, which later breaks the walls of the vessels and, then, metastatic cells can invade the tissue parenchyma (1). The other more likely scenario is that carcinoma cells engage in extravasation by penetrating the endothelial layer and vessel lumina to reach the surrounding tissues (39, 86). Most tumor cells use the paracellular route of transendothelial migration (TEM), when cancer cells squeeze between endothelial cells by disrupting the junctions between neighboring endothelial cells (79) and disrupt the basal membrane. Still, the endothelial cells are not passively enduring the intruders; they interact with the tumor cells.

During the extravasation step, tumor cells engage first with the cells in the endothelial layer of the vessels; however, the exact mechanisms of this interplay are less known. Under physiological conditions, the vessels are very stable and compact. During cancer metastasis, the endothelial cells at the site of extravasation are activated, as cancer cells secrete certain growth factors and cytokines to permeabilize the vasculature, and the compact endothelial layer undergoes several changes during metastatic extravasation (cytoskeletal remodeling, enhanced contractility, cell contact disruption, and decreased stiffness), which allow and enhance extravasation of metastatic tumor cells (98). Similar observations in experiments in which endothelial cells were cocultured with cancer cells with high metastatic potential have been reported (65).

In many aspects, tumor cell TEM is similar to leukocyte extravasation (67). Metastatic cells attach to endothelial cells,
which is the first step of the extravasation. For this, the expression of certain endothelial cell contact proteins needs to be remodeled to ensure the presence of cognate ligands on the surface of endothelial cells that would bind to surface proteins expressed by the tumor cells. Rho-(71) and FAK- (28) dependent expression of E-selectin is required for an initial weak adhesion between tumor and endothelial cells. N-cadherin expression is characteristic of neural and mesenchymal cells; however, N-cadherin is expressed by metastatic cells as well. The presence of N-cadherin ensures the attachment of tumor cells to the endothelium, and it is involved in melanoma cell adhesion between tumor and endothelial cells. N-cadherin expression is required for tumor cell adhesion. The interaction and attachment of endothelial and metastatic cells require the expression of endothelial β1-integrin and fibronectin, since β1-integrin expressed by cancer cells is important for extravasation. Endothelial β1-integrin expression in human umbilical vein endothelial cells (HUVECs) was shown to be required for metastatic cell adhesion (77). Metastatic tumor cells express integrins. By binding to its ligand fibronectin, β1-integrin contributes to the adhesion between endothelial cells and prostate cancer cells (5).

The integrity of the endothelium is ensured by the compact organization of interendothelial tight and adherens junctions. Tight junctions (such as claudins, occludin, zonula occludens proteins ZO-1 and ZO-2, and junctional adhesion molecules) are transmembrane proteins that render strong binding between endothelial cells and development and maintenance of tight junctions being supported by adherens junctions. Two adjacent endothelial cells are bound by the extracellular domains of the adherens junction protein, VE-cadherin. The intracellular part of VE-cadherins is complexed to a network of catenins and other proteins linking VE-cadherin to the actin cytoskeleton. During metastasis, CTCs must disrupt these intercellular junctions to extravasate. Tight junction proteins are degraded by tumor cell-derived proteolytic enzymes. VE-cadherin complexes are altered by tyrosine phosphorylation and subsequently internalized and cleaved. This causes interendothelial junctional complex disintegration, leads to reduced endothelial barrier function, and increased vascular permeability, modulating metastatic cell migration through the openings at the endothelial cell junctions during extravasation (15, 20, 23, 49). Phosphorylation of VE-cadherin leads to Rac1 activation and localization of activated Rac1 at the leading edge of migrating cells; an intact barrier function is rendered by functional VE-cadherin suppressing Rac1 activation (26).

Cytoskeletal reorganization during TEM is accompanied by increased actomyosin contractility and subsequent signaling events, which are regulated by small GTPases. Lung cancer cell attachment to brain endothelial cells and their subsequent TEM activate endothelial RhoA. Rho/ROCK participate in the TEM of lung cancer cells through regulating actin cytoskeleton reorganization, and inhibition of ROCK prevents cancer cell TEM (53). Moreover, inhibition of the Rho/ROCK pathway rescues tight junction integrity in brain endothelial cells (22). Activation of Rac1 also regulates endothelial barrier function during TEM by stimulating stress fiber-mediated tension on adherens junctions (7).

FAK is activated by both growth factors and integrins in the control of vascular permeability, and activated FAK contributes to VE-cadherin-β-catenin dissociation and to the subsequent breakdown of interendothelial junctions (9). Small-molecule inhibition of endothelial FAK has been shown to prevent tumor progression in mice (81), and inhibition of FAK activity has been shown to prevent tumor metastasis by enhancing endothelial barrier function (32).

Adhesion of colon cancer cells to endothelial cells results in an increase in the activity of ERK and p38 mitogen-activated protein kinases. Transendothelial permeability and migration of HT-29 cells are enhanced by the activation of endothelial ERK and p38. The mechanisms by which these two MAPK contribute to TEM are different. ERK-dependent signaling events lead to the opening of interendothelial spaces by the dissociation of the VE-cadherin/β-catenin complex. The p38-dependent effects result in formation of stress fibers, an effect mediated through phosphorylation of its downstream effector, myosin light chain (MLC) (93). MLC-kinase and downstream myosin II are activated by MDA-MB231 breast cancer cells invasion of calf pulmonary artery endothelial cells, leading to an increase in endothelial contractility (38). In HUVECs, p38 mediated melanoma-induced VE-cadherin junction disassembly, while activation of p38 increased VE-cadherin-mediated gap formation, facilitating melanoma TEM (37).

In vivo experiments also led to the conclusion that vascular permeability and metastasis are dependent on p38 and the JAK2-STAT5 pathway, which play a pivotal role in extravasation of colon carcinoma cells to mouse lungs (101). p38 activation is regulated by Rac1-dependent signaling via PI3K. Inhibition of PI3K prevented lateral junctional separation and the activation of another downstream effector of PI3K, JNK, which, in turn, regulated VE-cadherin phosphorylation and junctional separation (72).

Local tissue remodeling is essential to enable tumor cell invasion and metastasis. The expression of matrix metalloproteases is upregulated at the premetastatic niche. MMP9 expression is specifically increased in endothelial cells in the premetastatic lung (29). Besides the necessity of intracellular junction openings, tumor cells need to pass through the basal membrane, as well to successfully extravasate. Basal membrane can be degraded by MMPs. As such, MMP-2 that is expressed by endothelial cells augment the TEM of breast cancer cells through the microvascular barrier. Kargozaran et al. (36) observed that inhibition of MMP-2 expression or activity in endothelial cells significantly reduced MDA-MB-231 cell TEM across an endothelial monolayer barrier grown on a reconstituted basement membrane.

Blood-brain barrier (BBB) represents a highly impermeable obstacle; nevertheless, the endothelial overexpression of Snail1 alone lead to junction disruption and BBB permeability. Snail1 expression in endothelial cells was dependent on the ERK signaling cascade. Interestingly, it was not tumor cell extravasation that led to these observations: Group B Streptococcus infection induced Snail1 expression in murine and zebrafish models and required Snail1 expression to promote BBB permeability to allow bacterial passage (40).

Also noteworthy is the fact that endothelial expression of FSP-1, a well-known marker of EMT and EndMT, impairs endothelial function by increased permeability, increased expression of adhesion molecules, and decreased expression of junction molecules, whereas in vivo, ROCK1 activation and FSP-1 expression were detected in diabetic mice (58) (Fig. 1).
These findings indicate that during metastatic TEM and increased microvascular/endothelial permeability, endothelial cells suffer a series of changes in their signaling mechanisms and expressional profile; cells undergo cytoskeletal remodeling, become contractile, and lose stiffness. Disruption of intercellular junctions and basal membrane allows endothelial permeability and augments tumor cell metastasis (Fig. 2). Yet all of these changes in morphology, intercellular junctions, signaling, permeability, and contractility lead to an increased plasticity and endothelial remodeling, which are very similar to a transition toward a mesenchymal, myofibroblast-like phenotype. We hypothesized that these changes induced by TEM could be a result of an endothelial-mesenchymal transition. These mechanisms suggest that cancer cell TEM requires the active role of endothelial cells, and the endothelial cells participate in the metastatic process by allowing and enhancing TEM by undergoing a partial or complete EndMT. As such, EndMT seems to be a necessary contributor to successful metastatic TEM under certain conditions.

We demonstrated earlier that primary brain endothelial cells undergo EndMT upon TGF-β1 treatment in vitro by characterizing EndMT using specific markers. Endothelial cells responded to TGF-β1 stimulation by downregulation of occludin, claudin-5, VE-cadherin, and by expressing β1-integrin, N-cadherin, and fibronectin: endothelial cells lost their junctional proteins, rendering intercellular contacts and started expressing proteins, which would ensure adhesion of cancer cells. Further, endothelial cells started expressing calponin and SMA as a response to this stimulus, proteins involved in enhanced contractility. Importantly, activated cancer cell line-conditioned medium (ACM) was sufficient to induce EndMT of brain endothelial cells, and this effect occurred in a TGF-β-dependent manner. Moreover, stimulation of endothelial cells with ACM resulted in TGF-β-dependent decrease of transendothelial electrical resistance (TEER), increase in adhesion between metastatic and endothelial cells. As a logical consequence of a decrease in TEER and an increase in the number of adhering melanoma cells, preconditioning of the endothelial layer with ACM caused a significant increase in the number of transmigrating melanoma cells. When stimulating with ACM, the observed effects were specific to TGF-β, since the presence of a specific chemical inhibitor mitigated the effects of ACM. Moreover, stimulating endothelial cells with conditioned medium alone did not induce EndMT. EndMT was observed only following stimulation with heat-activated conditioned medium, a step necessary to activate TGF-β. Further, ACM stimulation induced Smad2 and Smad3 phosphorylation, a key step during TGF-β-dependent signaling. Finally, coculture with melanoma cells induced the expression of specific EndMT markers in HUVECs (46).

Importantly, in vivo studies also revealed that tumor-conditioned media enhance metastatic potential. Pretreatment of mice with tumor-conditioned medium accelerated spontaneous metastasis in breast cancer (51). This effect was mostly detectable in lymph nodes and, more intriguingly, in lungs. In this model, a potential explanation was the ability of tumor-conditioned medium to induce lymphangiogenesis, yet this particular mechanism is less plausible in the case of the lungs: lungs...
are metastasized via blood vessels (27). More likely tumor-conditioned medium affected endothelial integrity and resulted in endothelial plasticity corresponding to an endothelial-mesenchymal transition that may have facilitated extravasation and may have led to the observed increase in metastatic load into the lungs, but this aspect was not explored in this work.

EndMT might play a more relevant role during tumor cell extravasation in organs with a more compact organization of the endothelial cells. The sinusoid capillaries in the bone marrow (43) or the capillaries of the liver (48) are lined with fenestrated endothelia. Such fenestrated endothelial layers could represent weaker, and, as such, more permissive obstacles for the metastatic cells. Nevertheless, liver metastasis occurs with the active contribution of endothelial cells: the luminal surface develops cytoplasmic projections that attach to and later cover the tumor cell surface, and only after this interaction do tumor cells reach the extr sinusoidal space (75). On the other hand, capillaries of lungs and the endothelial cells of the BBB are tightly compact and, together with the surrounding basement membrane, present a more restrictive environment for metastasis (69). To infiltrate into such tissues, metastatic cells may need specialized, additional functions. In brain or lungs, one such potential function could be the induction of an EndMT. This could be a prerequisite step for tumor cell extravasation and TEM. Along EndMT, other mechanisms could also contribute to extravasation. For example, tumor cells are known to release proteolytic enzymes, such as seprase (20). Importantly, TGF-β controls seprase expression (94).

Studies in mouse models revealed that EndMT is required for tumor cell intravasation as well, as a consequence of endoglin deficiency in the tumor vasculature (2). An EndMT-like process was described in lymph vascular cells during the intravasation of breast carcinoma cells. This process required the function of ZEB1, a well-known inducer of EMT (97). The extent and role of EndMT during intravasation could be less dramatic than in the context of extravasation. Generally, the primary tumor vasculature consists of leaky newly formed blood vessels with weaker endothelial cell-cell junctions (17) through which metastatic cells can easily enter the circulation.

Regulation of EndMT During Metastatic TEM

TGF-β is recognized as the classical inducer of EMT and EndMT. TGF-β could have a dual role during metastatic progression; tumor cells acquire an invasive phenotype as a result of TGF-β-driven EMT, and, in parallel, TGF-β “preconditions” certain endothelial foci via EndMT to enhance the extravasation of metastatic cells. In the context of TEM and extravasation of metastatic cells, the role of TGF-β is inevitable. Cancer cells express TGF-β1 (31, 74). Patients with malignant melanoma have elevated serum TGF-β1 and TGF-β2 levels (44). Breast cancer cell lines are also known to express different TGF-β isoforms (34). Moreover, sudden elevations of serum TGF-β1 levels were observed at the time point of metastasis initiation (13). The adhesion between melanoma and endothelial cells was found to be influenced by TGF-β1 treatments and activated TGF-β in cancer cell-conditioned medium in vitro, and endothelial morphology was changed when challenged by TGF-β1 stimulation (92).
Metastatic cells could induce EndMT during their extravasation by three potential scenarios, but probably a combination of these would orchestrate an efficient metastatic TEM (Fig. 2). First, metastatic cells, by expressing TGF-β, could exert such an effect locally following an initial contact and a potential weak adhesion step, or during CTC arrest in the microvasculature (4). Notably, in the case of brain metastasis, the duration of TEM by cancer cells in vitro is significantly shorter (6 to 18 h) than that required for extravasation in vivo (3 to 5 days) (57), a time window that would allow the development of local effects. A second potential mechanism that could play an important role in EndMT induction is also local; during the initial arrest of cancer cells in the vasculature, platelet-tumor cell microthrombi may form and platelets may release significant amounts of TGF-β at the site of extravasation (47). Platelets are a major source of TGF-β in the circulation, and platelet-released TGF-β could have a dual effect to promote metastasis: first by the induction of an EMT in the tumor cell itself (47) but also by stimulating local endothelial cells to undergo EndMT. The third potential, cumulative mechanism could be the result of the systemic release of TGF-β originating from the primary tumors. By secreting a plethora of factors, primary tumors can modulate distant microenvironments of the premetastatic niche and the endothelium before the homing of metastatic cells. To find the least resistant areas of the endothelial layer, tumor cells need to screen the endothelial surface of the vessels to find the optimal sites for extravasation (86). Metastatic cells can then localize to such regions of vascular hyperpermeability (28). EndMT may be the pivotal mechanism leading to these areas of decreased resistance suitable for vessel penetration (Fig. 3).

EndMT could be one of the mechanisms playing a role in the formation of the premetastatic niche. The premetastatic niche is formed in the parenchyma of the target organ by several cytokines and growth factors, as well as by different cell types. For example, bone marrow-derived cells migrate to and cluster into the parenchyma at common sites of metastasis before tumor cells extravasate. These cells preferentially localize to sites containing increased levels of fibronectin. Excessive levels of fibronectin can be newly synthesized by resident fibroblasts and fibroblast-like cells. The vascular endothelial growth factor receptor 1-positive (VEGFR1+) bone marrow-derived hematopoietic progenitor cells, along with fibronectin and associated stromal cells, alter the local microenvironment. As a consequence, integrins and chemokines are activated. These factors enhance the attachment, survival, and growth of extravasated metastatic cells. It has also been observed that tumor cell-conditioned media induced fibronectin expression and bone marrow-derived cell cluster formation at the sites of the premetastatic niche (35).

The modulation of the premetastatic niche is pivotal for early metastatic colonization, and EndMT may play a further leading role in this preconditioning of the niche: the expression of extracellular matrix proteins periostrin and tenascin C is also required for successful metastasis (61, 73). Importantly, perioserin expression was detected following developmental EndMT in vivo (68, 70). Myofibroblasts express tenasin C in a TGF-β-dependent manner (14), and tenasin C is also expressed during EndMT in the embryonic mouse heart (106).

Several factors produced by the primary tumor can orchestrate the formation of the premetastatic niche. For example, VEGF promotes newly synthesized fibronectin in the lung. VEGF is also known to alter endothelial and vascular permeability by inducing dissociation of VE-cadherin-dependent endothelial junctions (15). Importantly, VEGF induced vascular permeability increase is dependent on p38 activity (6). Moreover, VEGFR1 activation stimulates the activity of Snail, TWIST, and Slug in the primary tumors (102). These transcription factors are key regulators of EMT, and it is plausible that it could have similar effects on endothelial cells by contributing to EndMT. From the signaling perspective, the involvement of VEGF is putting EndMT at the crossroads between angiogenesis and endothelial permeability during metastatic TEM. Partial EndMT could be an important mechanism during angiogenesis as well (99, 100). A central regulation of endothelial permeability via EndMT could be rendered the TGF-β-VEGF axis: TGF-β induces VEGF expression (45), and these two stimuli could together modulate vascular permeability by inducing an EndMT. Tumor cells, upon adhesion to endothelium, initiate 12(S)-HETE biosynthesis (30), and 12(S)-HETE induced EndMT of lymphendothelial cells during intravasation (97). In microvascular endothelial cells 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] altered cell junctions and induced cytoskeletal remodeling in a reversible manner (91). Nevertheless, 12(S)-HETE-induced EndMT is not independent of TGF-β, since these pathways cross-talk and activate each other; in mesangial cells, TGF-β significantly increased the levels of 12(S)-HETE, and 12(S)-HETE also induced TGF-β expression (42). Interestingly, the endothelial chemokine signaling-dependent extravasation (101) could also be a result of

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Fig. 3. EndMT is required for metastatic transendothelial migration (TEM). Areas of endothelial remodeling correspond to the areas of hyperpermeability, which could be used by metastatic tumor cells as gates of exit during extravasation and TEM. Similar mechanism could be involved in metastatic intravasation as well, where similar areas could be used by metastatic cells as gates of entry.
a CCR2-TGF-β signaling cross-talk; in breast cancer cell lines, CCL2 enhanced cell migration and survival by phosphorylation of Smad3 and ERK proteins independently of TGF-β (19). Nevertheless, under certain circumstances, CCL2 may enhance TGF-β expression (24), and even TGF-β may modulate cellular migration via CCR2 (52).

In conclusion, herewith we propose EndMT as one of the mechanisms playing a leading role in cancer cell metastasis. Extravasation and, to some extent, intravasation occurs with the active involvement of endothelial cells, which respond via EndMT-like features to several signals that are known to regulate the premetastatic niche and TEM of metastatic tumor cells. Currently, the majority of efforts toward developing therapeutic options against the metastatic disease target properties of the tumor cells. Because of their active role during intravasation and extravasation, endothelial cells represent potential novel targets of antimetastatic therapeutics. For example, interfering with TGF-β signaling had beneficial effects on TEM of cancer cells: fenofibrate, known to suppress TGF-β1/Smad3 signaling pathways during tubulointerstitial fibrosis in diabetic nephropathy in vivo (56), enhances barrier function of endothelium in the metastatic niche (76). Moreover, inhibition of ROCK attenuates microvascular endothelial hyperpermeability induced by the Rho-ROCK-MLC-actin stress fiber formation pathway (89). Considering these aspects, the addition and combination of drugs that prevent or inhibit EndMT could efficiently delay the progress of the metastatic disease.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

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