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Endothelial-Mural Cell Signaling in Vascular Development and Angiogenesis

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Abstract—Mural cells are essential components of blood vessels and are necessary for normal development, homeostasis, and organ function. Alterations in mural cell density or the stable attachment of mural cells to the endothelium is associated with several human diseases such as diabetic retinopathy, venous malformation, and hereditary stroke. In addition mural cells are implicated in regulating tumor growth and have thus been suggested as potential antiangiogenic targets in tumor therapy. In recent years our knowledge of mural cell function and endothelial-mural cell signaling has increased dramatically, and we now begin to understand the mechanistic basis of the key signaling pathways involved. This is mainly thanks to sophisticated in vivo experiments using a broad repertoire of genetic technologies. In this review, we summarize the five currently best understood signaling pathways implicated in mural cell biology. We discuss PDGFB/PDGFRβ- dependent pericyte recruitment, as well as the role of angiopoietins and Tie receptors in vascular maturation. In addition, we highlight the effects of sphingosine-1-phosphate signaling on adherens junction assembly and vascular stability, as well as the role of TGF-β-signaling in mural cell differentiation. We further reflect recent data suggesting an important function for Notch3 signaling in mural cell maturation. **(*Arterioscler Thromb Vasc Biol*. 2009;29:630-638.)**

Key Words: pericyte ■ PDGF ■ angiopoietin ■ sphingosine-1-phosphate ■ TGF-β

All blood vessels are composed of two distinct cell types: endothelial cells and mural cells. Whereas endothelial cells form the inner vessel wall, the mural cells associate with and coat the endothelial cell tube. Depending on their density, morphology, location, and expression of specific markers mural cell are commonly subdivided in vascular smooth muscle cells and pericytes. Vascular smooth muscle cells are associated with arteries and veins around which they form multiple concentric layers. Pericytes are associated with the smallest diameter blood vessels (arterioles, capillaries, and venules) and share their basal membrane with the endothelium. Pericytes are either solitary associated with the endothelial cell tube or form a single, often discontinuous, cell layer around it. Pericyte morphology and the degree by which they cover the endothelium vary substantially between different tissues. In certain tissues pericytes have acquired specialized functions and names, eg, mesangial cells in kidney glomeruli, and perisinusoidal fat storing cells (Ito-cells) in the liver. In the current literature a number of signaling pathways are implicated in mural cell specification, differentiation, recruitment, or their attachment to endothelial cells.[1,2] The level of analysis varies substantially between the implicated signaling molecules or their genes. Here, we review those signaling pathways for which a detailed in vivo analysis of mouse knockout mutants has been published and a mechanistic understanding of their role in endothelial-pericyte signaling has emerged. Because pericytes and endothelial-pericyte signaling mechanisms have recently come into focus as putative targets in tumor therapy, we also briefly review current ideas and new data concerning the function of tumor pericytes.

Pericyte Recruitment

PDGFB/PDGFRβ Signaling

The ligand/receptor pair platelet-derived growth factor (PDGF)B/PDGF receptor-beta (PDGFRβ) are factors with relatively clearly defined roles during pericyte recruitment (reviewed in[3]). PDGFB is secreted as a homodimer from the endothelium of angiogenic sprouts where it serves as an attractant for comigrating pericytes, which in turn express PDGFRβ[4] (See Figure). In addition, PDGFB stimulates proliferation of vascular smooth muscle cells and induces mural cell fate in undifferentiated mesenchymal cells.[5–8] Ablation of PDGFB or PDGFRβ in mice causes mural cell deficiency leading to widespread vascular leakage and perinatal lethality. Also, kidney glomerular tufts do not form in these mice because of an absence of mesangial cells.[9]

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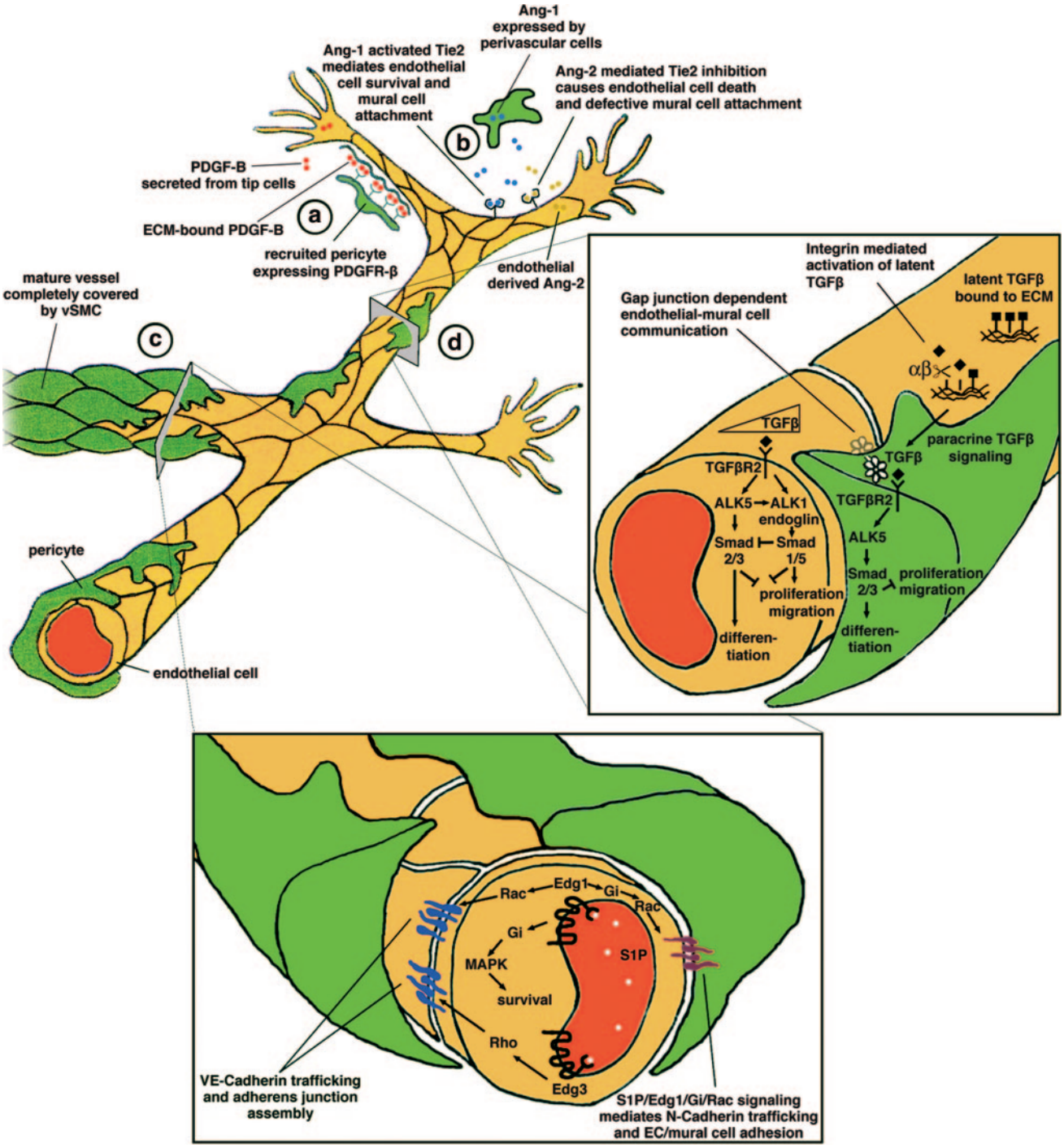

Figure. Signaling pathways involved in vascular smooth muscle cell/pericyte differentiation, recruitment, and in vascular maturation. a, PDGFB is secreted from tip cells and retained in close proximity to the growing endothelial vessel by heparan sulfate proteoglycans where it serves as an attractant for comigrating pericytes expressing PDGFRβ. b, Ang-1, expressed by perivascular cells binds to and activates the Tie2 receptor, thereby stimulating endothelial cell survival, angiogenesis, and subsequent mural cell attachment. Endothelial-derived Ang-2 is inhibitory to Tie2 signaling. c, S1P released from platelets or hematopoietic cells binds to and activates S1P/Edg receptors on endothelial cells. $S1P_1$/Edg1 signals via a G_i/MAPK cascade to stimulate cell survival and via G_i/Rac to mediate N-Cadherin trafficking into adherens junctions, thereby stabilizing endothelial/mural cell adhesion. In addition, S1Preceptors signal G_i independent via Rac ($S1P_1$/Edg1) and Rho ($S1P_3$/Edg3) to stimulate VE-Cadherin–based interendothelial cell adhesion. d, TGF-β produced in endothelial cells induces SMC differentiation in adjacent perivascular cells via Alk5/Smad2/3. In endothelial cells TGF-β can signal via Alk5/Smad2/3 to promote differentiation and via Alk1/endoglin/Smad1/5 to promote proliferation and migration. Which of these pathways prevail depends on the duration of TGF-β stimulation as well as on TGF-β levels. Alk1 signaling is inhibitory to Alk5 signaling, whereas Alk5 signaling is required for full Alk1 activity. Integrin-mediated latent TGF-β activation as well as gap junction communication between endothelial and mural cells is critical for TGF-β paracrine signaling. ECM indicates extracellular matrix; vSMC, vascular smooth muscle cell; EC, endothelial cell.

Once secreted, PDGFB is bound to heparan sulfate proteoglycans on the cell surface or in the extracellular matrix through its C-terminal "retention" motif.[10] Extracellular retention limits the range of action of PDGFB,[11] which is necessary for tight adhesion of pericytes to the vessel wall.[6,12] Knockout of the PDGFB retention motif in mice results in defective investment of pericytes in the microvessel wall and delayed formation of the glomerular mesangium. Although mutants live into adulthood, they develop glomerulosclerosis, proteinuria, and severe retinopathy.[12] Reduced N-sulfation of heparan sulfate proteoglycans caused by knockout of the N-deacetylase/N-sulfotransferase-1 gene (*ndst-1*) attenuates signaling by several heparan sulfate-binding growth factors including PDGFB and causes pericyte detachment and delayed pericyte migration similar to the PDGF retention motif knockout.[13] Interestingly, C-5-epimerase knockouts in which heparan sulfate proteoglycans are extensively N- and 6-O-sulfated but lacks 2-O-sulfated L-iduronic acid residues show only minor temporal pericyte recruitment defects. Thus, PDGFB retention and pericyte recruitment require sufficiently extended and correctly spaced N-sulfated heparan sulfate domains, whereas the detailed sequence of monosaccharide and sulfate residues does not appear to be critical for this process.[13]

Blood Vessel Maturation

Angiopoietins and Tie Receptors

Whereas the PDGFB/PDGFRβ axis has been defined as a paracrine endothelium-to-mural cells signaling loop, the angiopoietin-Tie receptor axis represents mainly a signaling loop of the opposite orientation—from mural cells to the endothelium. The angiopoietin (Ang) family of secreted growth factors consists of Ang-1, Ang-2, and the orthologes Ang-3 (in mouse) and Ang-4 (in human). All Angs are ligands for the Tie2 receptor (also known as TEK). For long, the related Tie1 receptor remained orphan, but recent evidence suggests that both Ang-1 and 4 can activate Tie1, and that its signal is amplified through heterodimeric interaction with Tie2.[14] In vivo, Ang-1 is considered the main agonistic ligand for Tie2,[15] whereas Ang-2 is antagonistic for Tie2 in most contexts.[16] Ang-3 and -4 are less well understood but have been reported to have antagonistic (Ang-3) and agonistic (Ang-4) effects.[17]

Tie2 is expressed throughout the developing embryonic endothelium and in the quiescent vasculature of the adult.[18,19] In addition, Tie2 is expressed in bone marrow–derived monocytes/macrophages, in certain mesenchymal progenitors,[20] and in cultured retinal pericytes.[21] Tie1 gets expressed slightly later in the embryonic endothelium and its expression persists during adulthood.[22,23]

Ang-1 is predominantly expressed by perivascular and mural cells[19,24,25] suggesting a paracrine mode of action. The major expression site for Ang-2 is the endothelium itself, suggesting an autocrine function,[26] although expression in perivascular cells including vascular smooth muscle cell and pericytes has been reported as well.[16,19] In adult tissues Ang-1 is widely expressed, whereas Ang-2 expression is associated mainly with sites of vascular remodeling.[16]

Tie1, Tie2, and Ang-1 are indispensable for normal vascular development, whereas Ang-2 null mice may survive into adulthood in a permissive genetic background. Mice lacking Tie2 develop cardiac defects, edema, and hemorrhage,[27] are growth retarded and die in utero between E9.5-E10.5.[27,28] They lack the normal hierarchical organization of large and small vessels suggesting impaired angiogenic sprouting or remodeling of the primary vascular plexuses.[28,29] Ang-1 null mice have a similar but slightly weaker phenotype.[15]

Ultrastructural analysis suggests that Tie2-knockout blood vessels lack mural cells.[29] Similarly, a poor association between endothelial cells and surrounding mesenchymal cells and matrix was seen in Ang-1 knockouts, suggesting that Ang-1 mediates adhesion between these components.[15] These in vivo observations are corroborated by recent in vitro analyses showing that matrix-bound Ang-1 induces cell adhesion and specific Tie2 signals at sites of cell–matrix contact.[30]

Human venous malformation, a condition characterized by uneven coverage or complete lack of vascular smooth muscle cell around certain veins, is caused by mutations in the *TIE2* gene.[31] In two independent families the responsible mutation was found to activate the Tie2 kinase. Thus, both inactivation (mouse knockout) and constitutive activation of Tie2 (human venous malformation) result in mural cell loss, which is intriguing but remains to be explained.

Recent reports have challenged the idea that Tie2 plays a direct role in pericyte recruitment (Figure, part b) and have instead suggested that the observed loss of periendothelial cells in mutants is secondary to endocardial and hematopoietic defects or to endothelial cell death.[23,32] A key observation was that initial pericyte coverage is normal in chimeras containing a large proportion of Tie2-deficient cells, suggesting that pericytes get normally recruited to Tie2-deficient endothelial cells in the context of a functional circulation.[23] In such chimeras, Tie2-deficient endothelial cells are rapidly eliminated from E 15.5 onwards.[23] Tie2 loss at later embryonic stages induces endothelial cell apoptosis, demonstrating that after a critical period in embryonic development Tie2 is dispensable for normal heart development but essential for endothelial cell survival.[32] Thus, the pericyte defects observed in Tie2 and Ang-1 knockouts may be secondary to endothelial cell apoptosis.

Tie1 knockouts have a generally milder phenotype than Tie2 knockouts and die between E13.5 and birth. Tie1 has thus far not been implicated in pericyte recruitment or differentiation. Instead available data suggest that Tie1, similar to Tie2, is cell autonomously required for endothelial cells survival.[23,28,29,33,34]

Several recent papers investigate the role of the Ang/Tie2 system in age-dependent vascular degeneration and in diabetic retinopathy. Endogenous Ang-2 is strongly upregulated in the retinas of diabetic rats and injection of recombinant Ang-2 into rat retinas or transgenic overexpression of Ang-2 in the mouse retina leads to pericyte loss.[35,36] This suggests that Ang-2 antagonizing effect on Tie2 may play a role in diabetes-associated pericyte loss and capillary degeneration in the retina.[36] In line with these observations, Ang-2 het-

erozygous mice are somewhat protected from age-dependent retinopathy. These effects were accompanied by an increase in VEGF expression and stabilization of Ang-1 expression, suggesting that the levels and possibly ratio of VEGF and Ang-1 are important for capillary cell survival.[37]

Sphingosine-1-Phosphate Signaling

The bioactive lipid sphingosine-1-phosphate (S1P) is indispensable for vascular maturation.[38] Notably, while many sphingolipid metabolites are known to function intracellularly, extracellular S1P binds to and activates a family of G protein–coupled receptors ($S1P_{1\ to\ 5}$) formerly know as *e*ndothelial *d*ifferentiation *g*ene (Edg)-receptors.[39] Sphingosine, which is produced intracellularly through degradation of ceramide by the enzyme ceramidase,[40] is phosphorylated in mammals by two sphingosine kinases, SphK1 and SphK2,[41] and can be recycled by a S1P specific sphingosine phosphatases.[42] In addition, S1P can be degraded by an S1P specific sphingosine lyase.[43,44] Platelets are considered to be the major source of S1P. They lack the S1P lyase and thus the ability to degrade S1P, and once activated release high amounts of S1P.[45] In addition, S1P is also secreted from hematopoietic cells[46] leading to high S1P concentrations in plasma and serum.[45,47] S1P is therefore not likely a paracrine signal between endothelial and mural cells, but its signaling in endothelial cells has secondary consequences for endothelial-mural interactions, as discussed below.

Complete depletion of S1P through genetic ablation of both SphKs in mice results in severe neurological and vascular defects.[48] SphK1/2 double knockouts appear normal at E9.5 but subsequently develop hemorrhages throughout the body and die between E11.5-E13.5. A more detailed analysis of the vascular defects in these mice revealed a dilated and abnormally developed vascular network at E10.5 and an incomplete coverage of the dorsal aorta by mural cells at E11.5, suggesting that recruitment or stabilization of mural cells is regulated through S1P signaling.[48] Of the five S1P receptors, $S1P_{1-3}$ (Edg1, -5 and -3, respectively) are widely expressed,[49] whereas $S1P_4$ (Edg6) expression is restricted to lymphoid tissue and the lung and $S1P_5$ (Edg8) to the central nervous system.[50] Single, double, or triple knockout combinations of these receptors in mice have revealed that $S1P_1$ and $S1P_3$ function redundantly during vascular development, but that $S1P_1$ is the most significant of these receptors during vessel maturation.[49] $S1P_1$ shows a complex and developmentally highly regulated expression pattern. At midgestation, $S1P_1$ is expressed in arterial and capillary but not in venous endothelium. It is also weakly expressed in vascular smooth muscle cell surrounding the dorsal aorta.[51] $S1P_1$ functions mainly cell-autonomously within endothelial cells to control vascular maturation, as the endothelium-specific knockout of $S1P_1$ phenocopies the vascular defects of the full knockout.[52]

Vasculogenesis and early angiogenesis are largely unaffected in $S1P_1$ mutants, as these appear grossly normal until E11.5. However, between E12.5-E13.5 they develop severe edema and hemorrhages with no embryos surviving beyond E14.5. In wild-type mice, smooth muscle cells usually cover the aorta by E11.5. In contrast, in $S1P_1$ mutants, smooth muscle cells are only present on the ventral side and fail to completely encircle the aorta even by E12.5. In addition, head and limb vessels show a poor association with mural cells. It was concluded that $S1P_1$ directs mural cell recruitment and association to vessels[51,52] and suggested that the lack of supporting mural cells causes the severe vascular leakage in $S1P_1$ mutants. In addition, in the limbs, which are hemorrhagic, edematous, and severely underdeveloped, both endothelial-specific and full $S1P_1$ deletion has been found to noncell autonomously induce expression of hypoxia induced factor (HIF)-1α and VEGF resulting in limb hypervascularization.[53] Conversely, VEGF has been reported to induce $S1P_1$ expression in cultured bovine aortic endothelial cells and markedly enhance subsequent $S1P_1$-dependent activation of nitric oxide synthase (eNOS)—an enzyme in endothelial cells implicated in angiogenic responses.[54] Some insights have been made into the downstream signaling events triggered by S1P. In human umbilical vein endothelial cells S1P activates $S1P_1$ and $S1P_3$ and induces a $S1P_1/G_i$/mitogen activated protein kinase/cell survival pathway, as well as VE-cadherin assembly into adherens junctions via the non–G_i-dependent activation of the small GTPases Rac (through $S1P_1$) and Rho (through $S1P_3$) (See Figure: 3). Both pathways were found important for endothelial cell morphogenesis into capillary-like structures in vitro.[55]

N-Cadherin, a cell adhesion molecule connecting endothelial cells and pericytes, has been shown to be activated and subsequently relocated to polarized plasma membrane domains via $S1P/S1P_1$/Gi/Rac signaling. It was concluded that S1P signaling in endothelial cells is critical for proper trafficking of N-Cadherin to cell adhesions between endothelial and mural cells[56] (Figure, part c). Interestingly, the same $S1P/S1P_1$/Gi/Rac signaling cascade has recently also been shown to modulate vascular barrier function and to reduce vascular permeability via changes in cortical actin and adherens junction stabilization.[57]

Pericyte Differentiation

TGF-β Signaling

Induction of the mural cell phenotype is still incompletely understood, but several studies implicate transforming growth factor-β (TGF-β) signaling in this process. In vitro experiments have demonstrated that TGF-β signals via two distinct TGF-β type I receptors in vascular cells (*A*ctivin receptor-*l*ike *ki*nase [Alk]-1 and Alk-5) triggering discrete intracellular signaling pathways with opposing effects on proliferation, migration, and tube formation.[58] Activation of Alk5 leads to phosphorylation of the receptor-regulated Smads 2/3, which translocate to the nucleus after association with Smad4 and regulates the transcription of specific target genes (eg, SM22α, fibronectin, connexin 37, and plasminogen activator inhibitor-1) that mediate inhibition of cell migration, reduced proliferation, increased vessel maturation, and smooth muscle differentiation. Activation of Alk1 instead leads to phosphorylation of Smad1/5 and induction of distinct target genes (such as inhibitor of differentiation or DNA binding [Id-]1 and Id-2, c-myc, endoglin, Smad6 and 7), which trigger cell migration, proliferation, and inhibit vessel maturation and smooth muscle differentiation.[58–60] Because Alk1 and Alk5

activation have opposing effects, the question remains what determines the cellular response. At least in part it seems that different levels of TGF-β may activate Alk1 and Alk5 differentially.[58] Also, the relative cellular levels of Alk1 to Alk5 expression might determine which pathway gets predominantly activated. Furthermore, a balancing role has been proposed, in which Alk1 signaling via Smad1/5 inhibits Alk5 signaling via Smad2/3,[61,62] whereas Alk5 on the other hand is indispensable for Alk1 signaling.[62] A consensus model (Figure, part d) suggests that both Alk1 and Alk5 get activated upon moderate TGF-β activation. Alk1 subsequently turns on promigratory and proproliferative target genes and inhibit the Alk5 pathway in endothelial cells, which leads to increased proliferation, migration, and tube formation. After strong and prolonged TGF-β stimulation Alk1 signaling decreases and the Alk5 pathway prevails, causing matrix production, growth arrest, and differentiation of mural cells. This model is primarily based on in vitro experiments but lends support also from studies of mouse knockouts of TGF-β pathway components, as further discussed below.

Mouse knockouts of TGF-β1[63] Alk5,[64] and Smad5[65,66] show similar phenotypes and die in utero between E9.5 and E11.5. Knockouts of TGF-β1 or TGF-βRII[67] die because of variable defects in yolk sac hematopoiesis and vasculogenesis. Alk5 mutant mice also display defects in yolk sac vascular development; initial vessel-like structures form but appear enlarged, fragile, and in lack of supporting mural cells. They also show placental defects, with embryonic vessels being unable to sprout into the labyrinthine layer. Interestingly, however, hematopoietic potential appears normal in the absence of Alk5.[64] Genetic inactivation of Alk1 also perturbs yolk sac vascular development, but vasculogenesis appears normal, and instead Alk1 seems to regulate the subsequent vessel remodeling. At E10.5 yolk sacs of Alk1 mutants have become anemic indicating defective hematopoiesis. In contrast to TGF-β1, TGF-βRII, or Alk5, which are widely expressed, Alk1 is specifically expressed in endothelial cells[68] and displays a variety of phenotypes not observed in TGF-β1, TGF-βRII, or Alk5 mutants. Embryos lacking Alk1 form large connections between arteries and veins and downregulate EphB2 (an arterial marker), whereas VEGFR2 and Ang-2 become upregulated. These mice show excessive fusion of capillaries into cavernous structures and larger vessels are often hyperdilated. Also, mural cell development is impaired as judged by the absence or inappropriate association of cells expressing the SM22αlacZ reporter.[61,69]

Endoglin, a Type III TGF-β coreceptor specifically expressed in endothelial cells, modulates Alk1 and Alk5 signaling.[70,71] Endoglin knockouts display a similar but slightly less severe phenotype than Alk1 knockouts. They fail to establish distinct arterio-venous boundaries, but in contrast to Alk mutants they do not show vessel dilation or downregulation of EphB2.[72] In humans, mutations in endoglin or Alk1 cause hereditary hemorrhagic telangiectasia type I and type II, respectively, an autosomal dominant disease characterized by inappropriate fusion of arterioles with venules.[73,74]

Knockout of the endothelial-specific receptors Alk1 and endoglin clearly demonstrates the critical role of TGF-β signaling in endothelial cells for vascular development. It has been more difficult to demonstrate whether the mural cells defects, which are invariably observed in TGF-β pathway mutants, reflect primary effects of TGF-β signaling in mural cells, or occur secondary to the impairment of endothelial functions. By knocking the LacZ reporter into the Alk5 gene, Seki et al[75] demonstrated that Alk5 expression preferentially occurs in vascular smooth muscle cells in vivo, and they also argued, based on reanalysis of the Alk5 knockout phenotype, that the primary defect of these mice is likely to take place in mural cells. A different scenario was suggested by Carvalho et al who showed that endothelial-specific knockout of Alk5 leads to yolk sac vascular defects and embryonic lethality at E10.5 similar to the complete Alk5 knockout.[76] The same authors showed that Alk5 signaling is important also in vascular smooth muscle cells since knockout of the TGF-β type II receptor (TGF-βRII, which is upstream of Alk5) specifically in these cells leads to vascular defects and embryonic lethality at slightly later age (E12.5).[76] Taken together, these studies demonstrate that TGF-β signaling has critical primary roles in both endothelial and mural cells during vascular development. Interestingly, these signals appear to be connected. Endothelial-specific ablation of the Alk5 or TGF-βRII genes, as well as the ablation of endoglin, has been shown to impair TGF-β/Alk5 signaling in the adjacent yolk sac mural (mesothelial) cells by reducing, through an as yet unknown mechanism, the availability or activity of TGF-β1 protein produced and secreted from endothelial cells.[77] These observations lend support to earlier in vitro work that demonstrated that cocultures of endothelial cells and smooth muscle cells promote proteolytic activation of latent TGF-β secreted by either cell type.[78]

It has become apparent that a number of genes/proteins whose knockout lead to vascular smooth muscle cell/pericyte deficiencies influence TGF-β signaling. Examples include connexin43 (Cx43)[79] and connexin45 (Cx45).[80] Connexins are components of gap junctions. During vascular development endothelial cells express Cx43 and mural cells Cx43 and Cx45.[80,81] Genetic ablation of Cx45 causes defects reminiscent of Alk5 and other TGF-β pathway mutants. Experiments on Cx43-deficient mesenchymal cells show that these cells fail to undergo mural cell differentiation when cocultured with endothelial cells and are unable to produce activated TGF-β. However, in response to exogenous TGF-β1 the Cx43-deficient cells were able to differentiate into mural cells and regained their ability to themselves produce TGF-β, indicating that intercellular communications from endothelia cells to adjacent mesenchymal cells via gap junctions is critical for TGF-β activation and subsequent mural cell differentiation[79] (depicted in Figure: 4). Another example is integrin $\alpha_V\beta_8$ knock out mice,[82,83] which display vascular phenotypes that are attributed to an insufficient TGF-β1 activation.[84] Currently, two models are proposed for how integrins contribute to activation of latent TGF-β1. A first model suggests a protease-dependent mechanism in which $\alpha_V\beta_8$ and $\alpha_V\beta_3$ integrins simultaneously bind MMPs and latent TGF-β1, facilitating enzymatic cleavage of the latter and release of active TGF-β1. A second model implies that integrins $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_V\beta_6$, and $\alpha_V\beta_8$ change the conformation of latent TGF-β1 complex by transmitting cell

traction forces, and that this results in liberation of active TGF-β1. This mechanism requires a mechanically rigid ECM[85] (depicted in Figure: 1 to 4).

Notch

Mutations in human Notch3 cause CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) a late-onset disorder causing stroke and dementia, which arises from slowly developing systemic vascular lesions ultimately resulting in the degeneration of vascular smooth muscle cell.[86,87] Mice lacking Notch3 function undergo normal embryonic development, however a closer analysis of adult mice revealed that Notch3 is required for proper maturation of arterial vascular smooth muscle cell.[88] Whereas earlier experiments in zebrafish suggested that Notch signaling is required for arterial-venous differentiation by repressing venous fate in arterial endothelial cells,[89] no such defects are displayed in Notch3 mutant mice. In contrast, maturation but not initial differentiation of arterial vascular smooth muscle cell is defective. Notch3-negative arterial vascular smooth muscle cell are altered in size and shape and fail to properly associate with endothelial cells, whereas proliferation and survival are unaffected. Phenotypic appearance and a reduced expression of arterial vascular smooth muscle cell markers suggest that Notch3-negative arterial vascular smooth muscle cell follow a venous pattern of maturation.[88] Recent work by Jin and colleagues strongly suggests that Notch signaling regulates PDGFRβ expression in vascular smooth muscle cells.[90] In primary vascular smooth muscle cell PDGFRβ was found to be a direct Notch target gene that could be activated by Notch ligands or activated forms of Notch. Newborn Notch3-deficient mice showed reduced PDGFRβ expression in vascular smooth muscle cell. Importantly, vascular smooth muscle cell from a CASADIL patient with a Notch3 missense mutation as well as Notch3 mutant embryonic stem cells failed to upregulate PDGFRβ expression when stimulated with a Notch ligand. This provides a possible mechanistic explanation for the vascular smooth muscle cell defects observed in CASADIL and Notch3 mutant mice suggesting that Notch affects their maturation indirectly via its effects on PDGF signaling in certain vessels.[90]

Pericytes in Tumor Angiogenesis

Could the signaling pathways involved in endothelial-mural signaling crosstalk provide new drug targets? Tumor pericytes have recently attracted attention as potential antiangiogenic targets in tumor therapy. Endothelial cells devoid of pericytes appear more dependent on vascular endothelial growth factor (VEGF-A) signaling for survival,[91] and inhibition of VEGF-A signaling results in tumor vasculature with increased pericyte coverage.[92] This suggests that pericytes may protect endothelial cells from VEGF withdrawal. As in developmental angiogenesis, pericytes are recruited to tumor vessels by PDGFB/PDGFRβ signaling,[6] arguing that tumor pericytes might be targeted by PDGF signaling pathway inhibitory agents. This has provided a rationale for testing the effects of combined inhibition of VEGF signaling in the endothelium and PDGFRβ signaling in mural cells using different kinase inhibitors. Indeed, such combined therapy increased the antitumor effects as compared to VEGF inhibition alone in a variety of experimental models.[93,94] Because kinase inhibitors are not mono-specific, the increased antitumor efficacies are somewhat difficult to interpret, as they might reflect broader actions than combined endothelial-mural cell targeting, such as targeting of the tumor cells themselves or of other types of stromal cell (ie, fibroblasts and inflammatory cells).

Combined anti-VEGF and anti-PDGF pathway inhibition has also been tested clinically. In a trial against human clear cell renal carcinoma, combined VEGF and PDGF pathway inhibition did not improve the therapeutic effect of inhibiting the VEGF pathway alone, and moreover displayed toxicity.[95] These negative results should be considered together with recent reports correlating poor pericyte coverage of the tumor vasculature with increased metastatic events in human colorectal carcinoma.[96] Also in an experimental tumor model of mouse pancreatic cancer, depletion of tumor pericytes elicited metastatic dissemination of tumor cells both to local lymph nodes and distant organs.[97] Further studies in experimental models and human patients are therefore needed to clarify potential benefits and risks associated with pericyte targeting in tumor therapy.

Novel data add additional complexity to the potential role of endothelial/mural crosstalk in tumors. RGS5 was recently proposed as a marker for tumor pericytes.[98,99] Physiological angiogenesis is normal in RGS5-deficient mice,[99] but pancreatic RIP1-tag5 tumors generated on RGS5-deficient background showed increased pericyte coverage.[100] After adoptive T-cell transfer, the RGS5-deficient tumors were massively infiltrated by $CD4^+$ and $CD8^+$ cells compared to wild-type controls, leading to tumor destruction and prolonged survival of the tumor-bearing RGS5-deficient mice. These results point to the possibility that pericytes might influence endothelial/leukocyte crosstalk, perhaps mediating immune cell attachment and transmigration into the tumor parenchyma.

In conclusion, mural cell biology and endothelial-mural cell signaling are areas of vascular biology that have developed extensively over the past few years. A number of signaling pathways that play pivotal roles in endothelial-mural cell interactions and vascular development have been identified. Some of these, ie, TGF-β and Notch, play a role in human monogenic vascular disorders, whereas others, such as PDGF and angiopoietins, are being actively pursued as putative targets for drugs aimed at blocking or stimulating blood vessel growth. While we begin to understand the mechanical basis for the key signaling pathways discussed above, the importance of yet other signaling systems (involving eg, MMPs, Ephrins, and others) are so far indicated primarily through phenotypic analysis of mouse mutants and their exact role in endothelial- mural cell signaling needs yet to be defined. Most certainly we will continue to witness progress in this area, and the molecular details of endothelial-mural signaling will be added at rapid pace.

Disclosures

None.

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