Leading Edge
Review

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Basic and Therapeutic Aspects of Angiogenesis

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Blood vessels form extensive networks that nurture all tissues in the body. Abnormal vessel growth and function are hallmarks of cancer and ischemic and inflammatory diseases, and they contribute to disease progression. Therapeutic approaches to block vascular supply have reached the clinic, but limited efficacy and resistance pose unresolved challenges. Recent insights establish how endothelial cells communicate with each other and with their environment to form a branched vascular network. The emerging principles of vascular growth provide exciting new perspectives, the translation of which might overcome the current limitations of pro- and antiangiogenic medicine.

Introduction

Blood vessels supply oxygen and nutrients and provide gateways for immune surveillance. Endothelial cells (ECs) line the inner surface of vessels to support tissue growth and repair. As this network nourishes all tissues, it is not surprising that structural or functional vessel abnormalities contribute to many diseases. Inadequate vessel maintenance or growth causes ischemia in diseases such as myocardial infarction, stroke, and neurodegenerative or obesity-associated disorders, whereas excessive vascular growth or abnormal remodeling promotes many ailments including cancer, inflammatory disorders, and eye disease (Carmeliet, 2003; Folkman, 2007). Vessels are also used as routes for tumor cells to metastasize.

Hallmarks of Vessel Growth

In the embryo, new vessels form de novo via the assembly of mesoderm-derived endothelial precursors (angioblasts) that differentiate into a primitive vascular labyrinth (vasculogenesis) (Swift and Weinstein, 2009) (Figure 1A). Subsequent vessel sprouting (angiogenesis) creates a network that remodels into arteries and veins (Adams and Alitalo, 2007) (Figure 1A). Recruitment of pericytes and vascular smooth muscle cells that enwrap nascent EC tubules provides stability and regulates perfusion (arteriogenesis) (Jain, 2003). In the adult, vessels are quiescent and rarely form new branches. However, ECs retain high plasticity to sense and respond to angiogenic signals.

The term "angiogenesis" is commonly used to reference the process of vessel growth but in the strictest sense denotes vessel sprouting from pre-existing ones. Recent studies provided tremendous insights into fundamental aspects of angiogenesis that have led to a mechanistic model of vessel branching (Adams and Alitalo, 2007; Carmeliet and Jain, 2011; Eilken and Adams, 2010; Phng and Gerhardt, 2009). Attracted by proangiogenic signals, ECs become motile and invasive and protrude filopodia (Figure 1B). These so-called tip cells spearhead new sprouts and probe the environment for guidance cues. Following tip cells, stalk cells extend fewer filopodia but establish a lumen and proliferate to support sprout elongation. Tip cells anastomose with cells from neighboring sprouts to build vessel loops. The initiation of blood flow, the establishment of a basement membrane, and the recruitment of mural cells stabilize new connections (Figure 1C). The sprouting process iterates until proangiogenic signals abate, and quiescence is re-established (Figure 1C).

Although vessels can grow via other mechanisms, such as the splitting of pre-existing vessels through intussusception or the stimulation of vessel expansion by circulating precursor cells (Fang and Salven, 2011; Makanya et al., 2009), we will focus here on the latest insights on vessel sprouting, which likely accounts for a substantial fraction of vessel growth.

Therapeutic Expectations and Challenges

The importance of angiogenesis sparked hopes that manipulating this process could offer therapeutic opportunities (Folkman, 1971). Despite efforts to stimulate angiogenesis therapeutically by proangiogenic factors, most trials failed to meet these expectations. Alternative strategies, based on proangiogenic cell therapies or targeting of microRNAs, offer new opportunities but are in (pre)clinical development (Bonauer et al., 2010).

Antiangiogenic approaches aimed at blocking vessel growth in eye disease and cancer led to the approval of therapeutics targeting vascular endothelial growth factor (VEGF) (Crawford and Ferrara, 2009b). Nonetheless, only a fraction of cancer

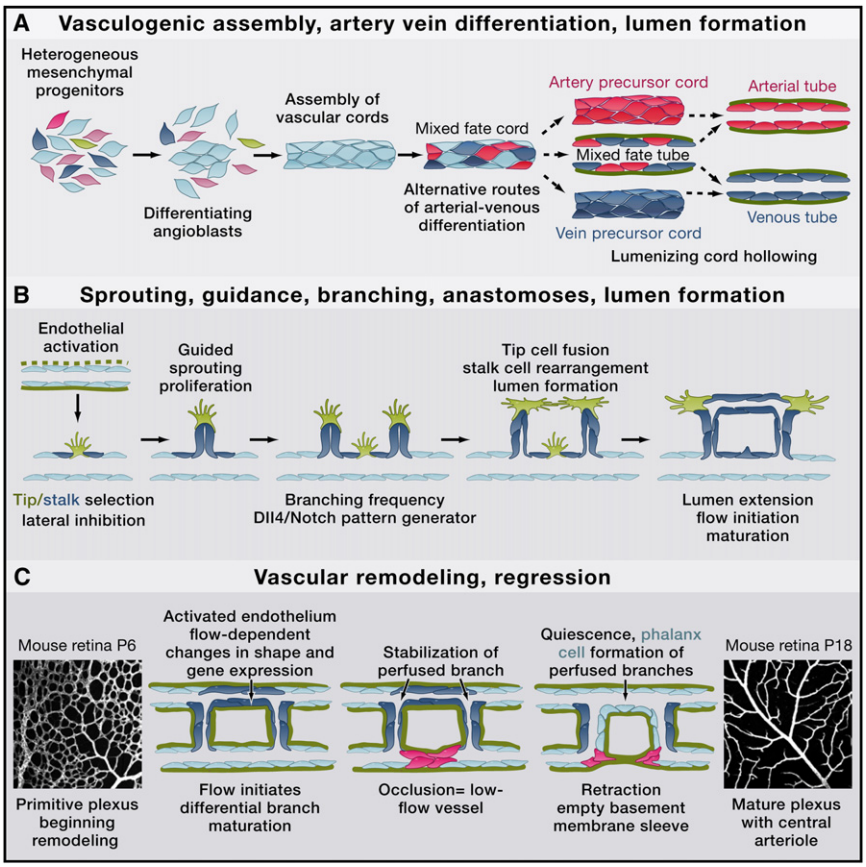

Figure 1. Hallmarks of Vessel Formation

(A) Angioblasts differentiate into endothelial cells (ECs), which form cords, acquire a lumen, and are prespecified to arterial or venous phenotypes.

(B) Steps of vessel sprouting: (1) tip/stalk cell selection; (2) tip cell navigation and stalk cell proliferation; (3) branching coordination; (4) stalk elongation, tip cell fusion, and lumen formation; and (5) perfusion and vessel maturation.

(C) Sequential steps of vascular remodeling from a primitive (left box) towards a stabilized and mature vascular plexus (right box) including adoption of a quiescent endothelial phalanx phenotype, basement membrane deposition, pericyte coverage, and branch regression.

patients show benefit as tumors evolve mechanisms of resistance or are refractory toward VEGF (receptor) inhibitors (Bergers and Hanahan, 2008; Crawford and Ferrara, 2009a). Conflicting results about the benefit of VEGF blockade have kick-started a debate on whether antiangiogenic treatment may trigger more invasive and metastatic tumors (Ebos and Kerbel, 2011). On the upside, "sustained normalization" of abnormal tumor vessels may offer benefit for combating metastasis (Goel et al., 2011).

For antiangiogenic medicine to have an enduring impact on cancer patient survival, an integrated understanding of the molecular principles of vessel growth is needed. Here, we take a cell biological perspective to explore prototypic principles and recently discovered regulatory mechanisms, seeking to develop a framework of the angiogenic process that might provide the basis for novel pro- and antiangiogenic therapies.

Endothelial Differentiation

Arterial and Venous Specification

Following assembly of primitive vessels in the early embryo (such as the dorsal aorta and cardinal vein), remodeling transforms the plexus into a hierarchically organized network of arteries, capillaries, and veins. Arteries form a high-pressure system, enabling transportation of blood to capillaries, whereas veins face low-pressure gradients. The differences in hemodynamic load are reflected in their structures: arteries are supported by layers of vascular smooth muscle cells and a specialized matrix, whereas veins are thinner and surrounded by fewer smooth muscle cells (Gaengel et al., 2009).

Arterial and venous ECs possess specific molecular identities (Adams and Alitalo, 2007; Swift and Weinstein, 2009). For instance, Notch pathway components are highly expressed in arteries but are low in veins. Disruption of Notch signaling causes loss of arterial markers and re-expression of venous signature genes, suggesting that Notch promotes arterial specification by repressing venous identity (Gridley, 2010; Swift and Weinstein, 2009). Notch also controls Eph-Ephrin family members, which configure arterio-venous boundaries. Ephrin-B2 expression in arterial ECs increases in response to Notch, whereas its receptor EphB4 in venous ECs is repressed by Notch. In zebrafish, Sonic Hedgehog acts upstream of Notch, where it triggers arterial differentiation by upregulating VEGF that elevates Notch components. In mice, VEGF secreted by nerves contributes to arterial differentiation of ECs in cotracking vessels (Carmeliet and Tessier-Lavigne, 2005). Neuropilin-1 (NRP1), a VEGF coreceptor, facilitates transduction of arterial effects of VEGF. At the level of gene expression, the transcription factors FOXC1 and FOXC2 drive an arterial gene signature (e.g., DLL4, HEY2, CXCR4) by interacting with VEGF and Notch signaling. Although earlier proposals favored the view that the venous fate is acquired by default, it has become clear that venous identity requires repression of Notch signaling by the vein-specific nuclear receptor COUP-TFII (Swift and Weinstein, 2009). In addition, hemodynamic factors such as blood pressure and flow codetermine arterio-venous differentiation (Jones et al., 2006).

Arterio-Venous Segregation

Zebrafish studies indicate that the cardinal vein does not form by vasculogenesis but instead arises from a common precursor vessel by segregation (Herbert et al., 2009) (Figure 1A). Venous-fated EphB4-positive ECs migrate away from the arterial-fated ephrinB2-positve ECs in the precursor vessel toward the location of the future cardinal vein. VEGF and Notch both restrain ventral sprouting, whereas VEGF-C promotes segregation.

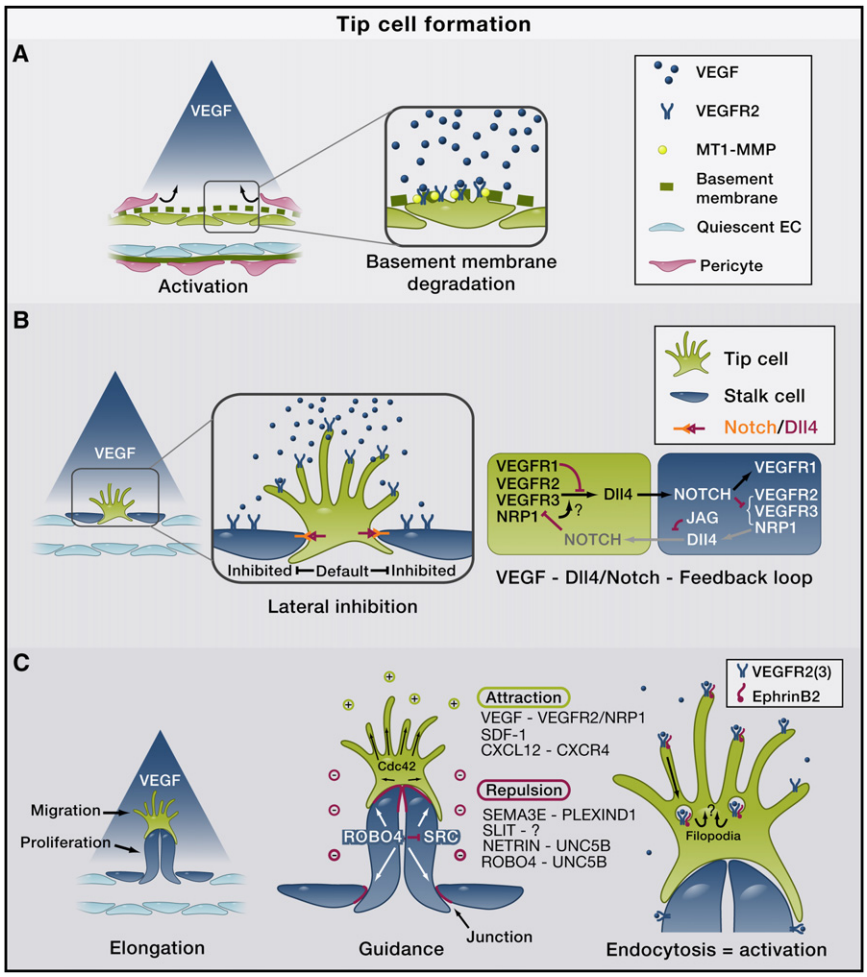

Figure 2. Tip Cell Formation

(A) In response to vascular endothelial growth factor (VEGF) stimulation, endothelial cells (ECs) degrade the basement membrane and pericytes detach, allowing ECs to emigrate.

(B) VEGF/Notch signaling selects tip and stalk cells.

(C) Filopodia guide tip cells by sensing attractive and repulsive cues. Filopodia formation is regulated by CDC42 and endocytosis of the EphrinB2/VEGFR2 receptors. ROBO4/UNC5B signaling promotes stabilization of the endothelial layer through inhibition of SRC.

However, it needs to be determined whether similar events occur in mammals.

Sprouting Angiogenesis

Liberating Endothelial Cells

Endothelial and mural cells share a basement membrane comprised of extracellular matrix proteins that form a sleeve around endothelial tubules (Eble and Niland, 2009). This basement membrane and the coat of mural cells prevent resident ECs from leaving their positions. At the onset of sprouting, ECs therefore must be liberated, a process requiring proteolytic breakdown of the basement membrane and detachment of mural cells (Figure 2A). Basement membrane degradation is mediated by matrix metalloproteases (MMPs) such as MT-MMP1, enriched in tip cells. Control of these proteinases is essential for sprouting, given that excessive degradation of the extracellular matrix, as occurs in plasminogen activator inhibitor 1 (PAI1) deficiency, leaves too little matrix support for the branch to sprout (Blasi and Carmeliet, 2002). MMPs also liberate proangiogenic growth factors that are sequestered in the matrix (Arroyo and Iruela-Arispe, 2010). At the other end, they also generate antiangiogenic molecules by cleaving plasma proteins, matrix molecules, or proteases themselves to prevent inappropriate sprouting and coordinate branching (Nyberg et al., 2005). Detachment of mural cells is stimulated by Angiopoietin-2 (ANG2), a proangiogenic growth factor stored by ECs for rapid release (Augustin et al., 2009; Huang et al., 2010) (Figure 2A).

Lateral Inhibition Selects the Tip Cell

The specification of ECs into tip and stalk cells is controlled by the Notch pathway (Eilken and Adams, 2010; Phng and Gerhardt, 2009) (Figure 2B). Analysis of Notch signaling revealed high Notch activity in stalk cells but low levels of Notch signaling in tip cells. Conversely, tip cells express higher levels of the Notch ligand DLL4. During development or in tumors, blockade of Notch or DLL4 increases filopodia and sprouting as a consequence of excessive tip cell formation (Thurston et al., 2007). Although ECs express several Notch receptors, Notch1 is critical for suppressing tip cell behavior in stalk cells. The hypersprouting phenotype and excessive number of tip cells following Notch inhibition indicate that the tip cell phenotype is the default endothelial response to proangiogenic signals. In contrast to DLL4, the Notch ligand JAGGED1 (JAG1) is expressed primarily by stalk cells. However, JAG1 poorly activates Notch1, as modification of Notch by FRINGE glycosyltransferases favors activation by DLL4 (Eilken and Adams, 2010). Given that some DLL4 protein is detectable in stalk cells, JAG1 helps to maintain differential Notch activity by antagonizing DLL4 that signals back to tip cells (Figure 2B).

VEGF and Dll4/Notch Feedback as a Branching Pattern Generator

VEGF and Notch co-operate in an integrated intercellular feedback that functions as a "branching pattern generator" (Figure 2B). VEGF stimulates tip cell induction and filopodia formation via VEGF receptor-2 (VEGFR2), whereas VEGFR2 blockade causes sprouting defects with blunt-ending channels (Phng and Gerhardt, 2009). VEGFR3 is expressed in the embryonic vasculature but later becomes confined to lymphatics. However, tip cells re-express VEGFR3, and its pharmacological inhibition diminishes sprouting (Tammela et al., 2008). In contrast, loss of

VEGFR1 increases sprouting and vascularization. A soluble variant or a kinase-dead mutant of VEGFR1 rescues vascular defects caused by VEGFR1 deficiency, suggesting that this receptor functions as a VEGF trap. VEGFR1 is predominantly expressed in stalk cells and involved in guidance and limiting tip cell formation (Chappell and Bautch, 2010; Jakobsson et al., 2010).

The feedback loop between VEGF and Notch involves regulation of all VEGFRs by Notch. VEGF/VEGFR2 enhances DLL4 expression in tip cells (Phng and Gerhardt, 2009). DLL4-mediated activation of Notch in neighboring ECs inhibits tip cell behavior in these cells by downregulating VEGFR2, VEGFR3, and NRP1 while upregulating VEGFR1 (Jakobsson et al., 2010; Phng and Gerhardt, 2009). Computational modeling indicates that such an integrated negative feedback loop of VEGF and Notch is sufficient to establish a stable pattern of tip and stalk cells (Bentley et al., 2009). ECs at the angiogenic front dynamically compete for the tip position through DLL4/Notch signaling (Jakobsson et al., 2010). Following VEGF exposure, all cells upregulate DLL4. However, ECs that express DLL4 more quickly or at higher levels have a competitive advantage to become a tip cell as they activate Notch signaling in neighboring cells more effectively. Given the dynamic shuffling of tip-stalk position of ECs during sprouting and the regular exchange of the leading tip cell, DLL4 expression must be dynamically regulated. Precise regulation of DLL4 expression is achieved through a TEL/CtBP repressor complex at the *DLL4* promoter, which is transiently disassembled upon VEGF stimulation, allowing a temporally restricted pulse of DLL4 transcription (Roukens et al., 2010). In line with a central function of DLL4 for vessel patterning dynamics, several other pathways, such as the Wnt/β-catenin pathway, converge on the transcriptional control of DLL4 (Corada et al., 2010).

Tip Cell Guidance

Wiring of the nervous system relies on the formation of correct connections and requires precise guidance of axonal growth cones. The vasculature must also be correctly patterned for optimal oxygen delivery. Emerging vessels use tip cells to guide sprouts properly, and the structure and function of tip cells are reminiscent of axonal growth cones (Adams and Eichmann, 2010; Carmeliet and Tessier-Lavigne, 2005). Little is known regarding the molecular mechanisms regulating tip cell filopodia. Activation of Cdc42 by VEGF triggers filopodia formation, whereas Rac1 regulates lamellipodia formation (De Smet et al., 2009) (Figure 2C). Both the axon growth cone and tip cell use similar attractive and repulsive cues to control guidance. ECs express guidance receptors including ROBO4, UNC5B, PLEXIN-D1, NRPs, and EPH family members, which they use to probe the environment (Figure 2C).

Roundabouts (ROBOs) are guidance receptors. Activation of ROBO1–3 by SLIT ligands (SLIT1–3) provides repulsive signals for axons. ROBO4 is expressed in ECs and maintains vessel integrity, and ROBO4 deficiency induces leakiness and hypervascularization (London et al., 2009). At the molecular level, ROBO4 counteracts the permeability-promoting actions of VEGF by impeding VEGFR2-mediated activation of the kinase SRC. The nature of the ROBO4 ligand remains debated, as ROBO4 lacks SLIT-binding domains. ROBO4 also binds to UNC5B, another guidance receptor, suggesting that ROBO4/UNC5B maintains vessel integrity via UNC5B activation (Koch et al., 2011).

UNC5B is a receptor for Netrins whose expression is enriched in tip cells. Its inactivation results in enhanced sprouting, whereas Netrin1 prompts filopodia retraction of ECs, consistent with a suppressive function of netrins and UNC5B on vessel growth (Adams and Eichmann, 2010). This function of Netrin1 has not been observed by others, suggesting that Netrin1 signaling might involve other yet unidentified receptors (Adams and Eichmann, 2010). Alternatively, UNC5B may function as a dependence receptor that, in the absence of ligand, induces EC apoptosis (Castets and Mehlen, 2010).

Semaphorins are secreted or membrane-bound guidance cues that interact with receptor complexes, formed by NRPs alone or NRP/plexin family proteins (Carmeliet and Tessier-Lavigne, 2005). SEMA3E induces vessel repulsion through interaction with PLEXIN-D1. As ECs express PLEXIN-D1, its loss causes aberrant sprouting into SEMA3E-expressing tissues in zebrafish embryos (Adams and Eichmann, 2010). In the mouse retina, SEMA3E activates PLEXIN-D1 on tip cells to fine-tune the balance of tip and stalk cells necessary for even-growing vascular fronts by coordinating VEGF's activity in a negative feedback (Kim et al., 2011). NRPs bind semaphorins, VEGF, and other ligands, but the vessel abnormalities in NRP1-deficient embryos are related to defective VEGF/NRP1 signaling (Fantin et al., 2009). In fact, most semaphorins suppress angiogenesis (Serini et al., 2009).

EPH receptors and their ephrin ligands are regulators of cell-contact-dependent signaling (Pitulescu and Adams, 2010). Eph-ephrin binding leads to bidirectional signaling in cells expressing the receptor (forward signaling) or ligand (reverse signaling). Eph-ephrins generate mostly repulsive signals. Ephrin-B2 is expressed in arterial ECs, whereas EphB4 marks venous ECs. Both of them regulate vessel morphogenesis, and loss of ephrin-B2 or EphB4 leads to vascular remodeling defects (Pitulescu and Adams, 2010). Intriguingly, ephrin-B2-mediated reverse signaling also controls VEGFR internalization and tip cell behavior (Figure 2C). ECs lacking ephrin-B2 reverse signaling are unable to internalize VEGFR2 and VEGFR3 and cannot transmit VEGF signals properly, together impairing sprouting (Sawamiphak et al., 2010; Wang et al., 2010).

Endothelial Stalk Cell Formation

Control of Stalk Cell Behavior and Elongation

Stalk cells are equipped with the ability to form tubes and branches. Compared to tip cells, stalk cells produce fewer filopodia, are more proliferative, and form a vascular lumen (Figures 3A and 3B). They also establish junctions with neighboring cells and produce basement membrane components to ensure the integrity of the sprout (Phng and Gerhardt, 2009). ECs with excess Notch signaling extend less filopodia and are excluded from the tip position, indicating that Notch activity is dispensable for tip cell formation but required for stalk cell specification (Jakobsson et al., 2010). The importance of a balanced tip/stalk specification by Notch is best illustrated by the paradoxical effects of gene inactivation of DLL4 or Notch1 in the endothelium: although more vessels are formed, they are poorly

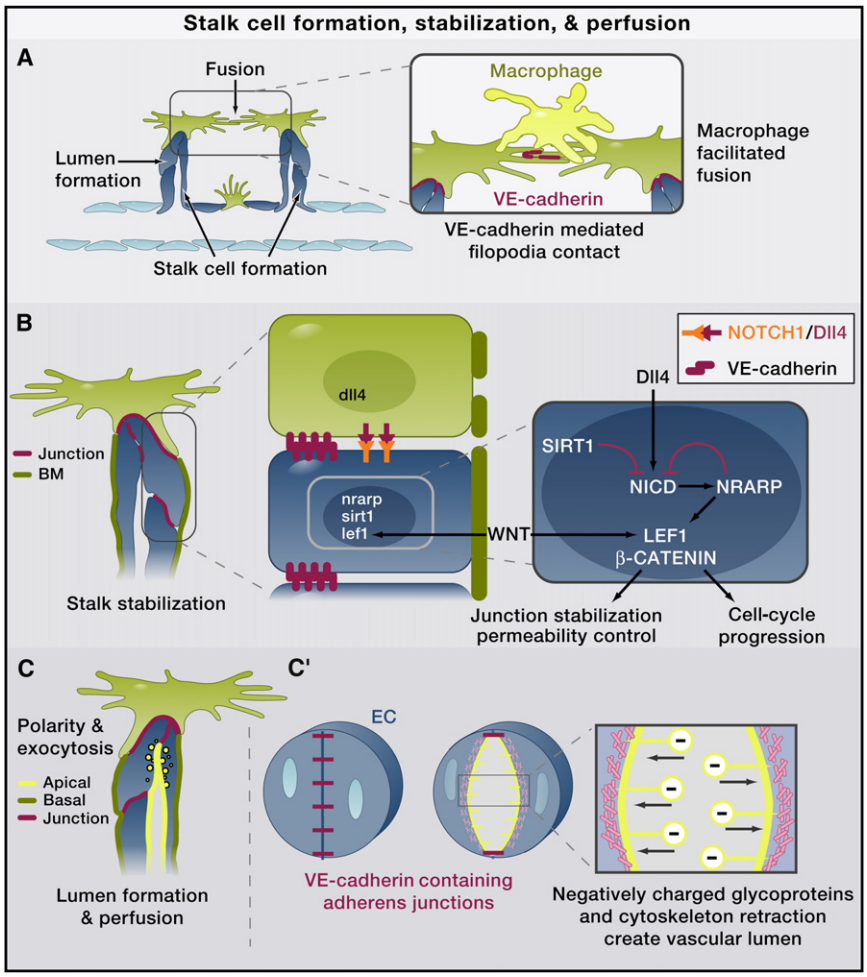

Figure 3. Stalk Cell Formation, Stabilization, and Perfusion

(A) Tip cell fusion and branch anastomosis are facilitated by macrophages; VE-cadherin promotes cell-cell adhesion between tip cells.

(B) Stalk cell stabilization relies on Notch activity that is fine-tuned by NRARP and SIRT1. WNT and Notch intersect via NRARP and LEF1/β-CATENIN to stabilize connections.

(C) Models of lumen formation: fusion of pinocytotic vesicles (left; C), contraction of the cytoskeleton following exposure of negatively charged glycoproteins on the lumenal surface of endothelial cells (ECs) (right; C′).

perfused and dysfunctional (Phng and Gerhardt, 2009; Thurston et al., 2007).

Activation of Notch involves the cleavage of Notch receptors leading to the release of the intracellular domain (NICD), forming a complex with the transcription factor RBPj/CBF1 and Mastermind-like proteins to drive target gene expression. This complex not only activates transcription but also promotes its own turnover to prevent sustained Notch activation. The Notch-regulated ankyrin repeat protein (NRARP) negatively regulates Notch responses by dissembling the Notch coactivator complex and promoting NICD degradation. Modulation of Notch in growing vessels is important, as NRARP allows stalk cells to proliferate. NRARP also augments Lef1/β-catenin signaling to maintain stability of nascent vessel connections (Phng et al., 2009). Control of Notch signaling by reversible acetylation of NICD is another layer of Notch regulation (Guarani et al., 2011). Acetylation enhances Notch responses by interfering with NICD1 turnover, whereas deacetylation by SIRT1 opposes NICD1 stabilization, thereby limiting Notch activity.

Negative regulation of Notch signaling in stalk cells might, at first sight, appear counterintuitive. However, it is important to note that tip and stalk cells are transient phenotypes and not stable cell fates. To expand the vessel network, ECs undergo iterative cycles of sprouting, branching, and tubulogenesis, requiring dynamic transitions between tip and stalk cell phenotypes (Eilken and Adams, 2010; Phng and Gerhardt, 2009). Fine-tuning of the Notch signaling amplitude and duration by NRARP and SIRT1 could serve to dynamically adjust the timing of tip and stalk transitions, thereby adapting vessel branching frequency.

Lumen Formation

Vessels need to establish a lumen, which occurs by different mechanisms (Iruela-Arispe and Davis, 2009; Zeeb et al., 2010) (Figures 3C and 3C′). Observations in intersomitic vessels indicate that ECs form a lumen by coalescence of intracellular (pinocytic) vacuoles, which interconnect with vacuoles from neighboring ECs (cell hollowing) (Figure 3C). Recent studies in large axial vessels suggest that ECs adjust their shape and rearrange their junctions to open up a lumen (cord hollowing) (Figure 3C′). In this model, ECs first define apical-basal polarity. Thereafter, the apical (lumenal) membrane becomes decorated with negatively charged glycoproteins that confer a repulsive signal, opening up the lumen. Subsequent changes in EC shape, driven by VEGF and Rho-associated protein kinase (ROCK), expand the lumen (Strilić et al., 2009; Zeeb et al., 2010). Tube morphogenesis also requires Ras-interacting protein 1 (RASIP1), a regulator of GTPase signaling controlling cytoskeletal rearrangements, adhesion, and EC polarity (Xu et al., 2011). The mechanisms of lumen formation likely depend on the vascular bed or type of vessel formation.

Vessel Branch Fusion and Perfusion

Tip cells contact other tip cells to add new vessel circuits to the existing network. By accumulating at sites of vessel anastomosis and interacting with filopodia of neighboring tip cells during fusion, macrophages can support vessel anastomosis (Fantin et al., 2010) (Figure 3A). However, anastomosis does not require macrophages, suggesting that they only facilitate fusion events,

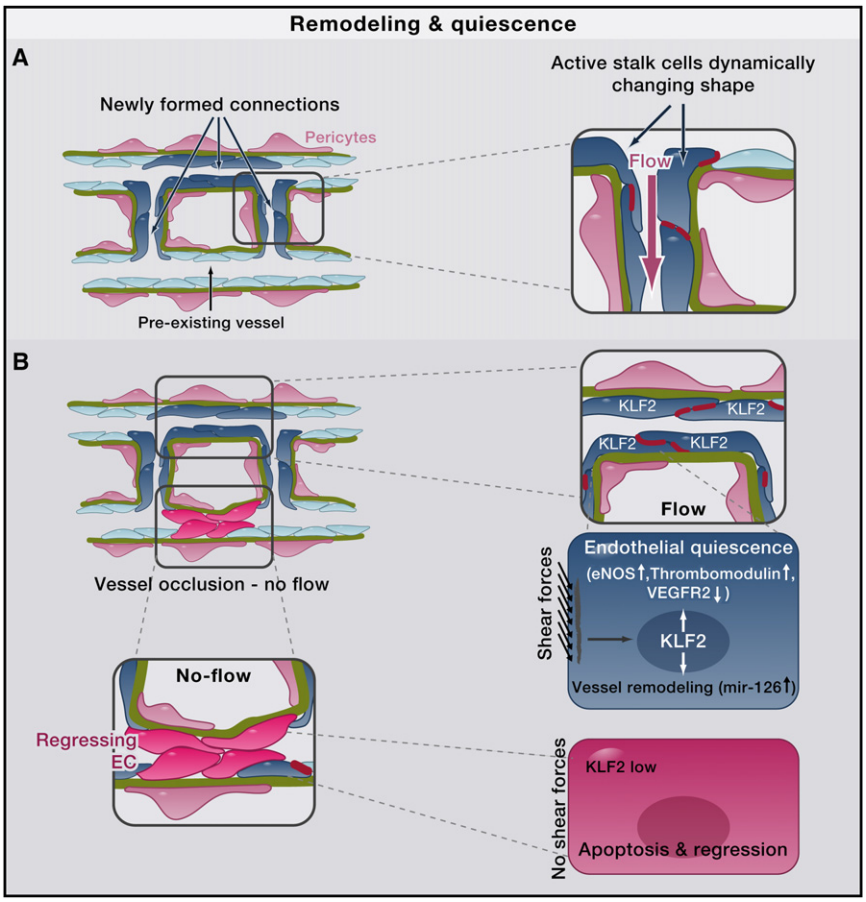

Figure 4. Remodeling and Quiescence

(A) Stalk cells undergo remodeling in response to flow.

(B) Upregulation of the transcription factor KLF2 in response to blood flow ensures remodeling of the vasculature. In consolidated vessels, KLF2 promotes quiescence and the formation of patent vessels with an antithrombogenic endothelial lining. Hypoperfused vessels undergo regression.

possibly via cell-to-cell communication. Once the contact between tip cells is established, VE-cadherin-containing junctions consolidate the connection (Figure 3A).

New vessel connections must become stable to generate an enduring loop. The deposition of extracellular matrix into the basement membrane, the recruitment of supporting pericytes, reduced EC proliferation, and increased formation of cell junctions all contribute to this process. The onset of blood flow in the new lumen shapes and remodels vessel connections and activates the shear stress-responsive transcription factor Krüppel-like factor 2 (KLF2) (Figures 4A and 4B). In zebrafish, KLF2 induces vessel remodeling by upregulating the EC-specific miR-126 that modulates PI3K and MAPK signaling (Nicoli et al., 2010). Hemodynamic forces also remodel large arteries and are important for vessel maintenance and collateral vessel expansion. Upon perfusion, oxygen and nutrient delivery reduces VEGF expression and inactivates endothelial oxygen sensors, together shifting endothelial behavior toward a quiescent phenotype.

Vessel Maturation, Stabilization, and Quiescence

For vessels to become functional, they must mature—at the level of the endothelium and vessel wall and as a network. At the network level, maturation involves remodeling into a hierarchically branched network and adaptation of vascular patterning to local tissue needs. This involves recruitment of mural cells and deposition of extracellular matrix (Jain, 2003). ECs also acquire tissue-specific differentiation adapted to meet local homeostatic demands and thus differ in phenotype (Dyer and Patterson, 2010).

Mural Cell Differentiation

A fundamental feature of vessel maturation is the recruitment of mural cells. Pericytes establish direct cell-cell contact with ECs in capillaries and immature vessels, whereas vascular smooth muscle cells cover arteries and veins and are separated from ECs by a matrix (Gaengel et al., 2009). Vessel maturation relies partly on transforming growth factor β (TGF-β) signaling. TGF-β stimulates mural cell induction, differentiation, proliferation, and migration and promotes production of extracellular matrix (Pardali et al., 2010). Loss of function of TGF-β receptor 2 (TGFBR2), endoglin, or activin receptor-like kinase 1 (*Alk1*) in mice causes vessel fragility in part due to impaired mural cell development (Pardali et al., 2010). In humans, mutations in *ENDOGLIN* and *ALK1* cause hereditary hemorrhagic telangiectasia (HHT), a disease characterized by arteriovenous malformations with abnormally remodeled vessel walls (Pardali et al., 2010). Which of the TGF-β family members' signaling is impaired in HTT and whether smooth muscle cells are affected directly (or rather indirectly through EC effects) require further study. For instance, by activating ALK5 (TGFBR1) in ECs, TGF-β signaling contributes to vessel maturation by secretion of PAI1, preventing degradation of the perivascular matrix.

Pericyte Recruitment

Recruitment of mural cells is controlled by platelet-derived growth factor (PDGF) receptor-β (PDGFR-β) (Gaengel et al., 2009) (Figure 5A). Endothelial PDGFB signals to PDGFR-β expressed by mural cells, stimulating their migration and proliferation. Adequate expression, matrix binding, and spatial presentation of PDGFB to PDGFR-β are essential for vascular maturation, and inactivation of either *Pdgfb* or *Pdgfrb* induces pericyte deficiency, vascular dysfunction, micro-aneurysm formation, and bleeding (Gaengel et al., 2009). *Pdgfb* mouse mutants with insufficient pericyte coverage display blood brain barrier defects, causing neuronal damage (Quaegebeur et al., 2010).

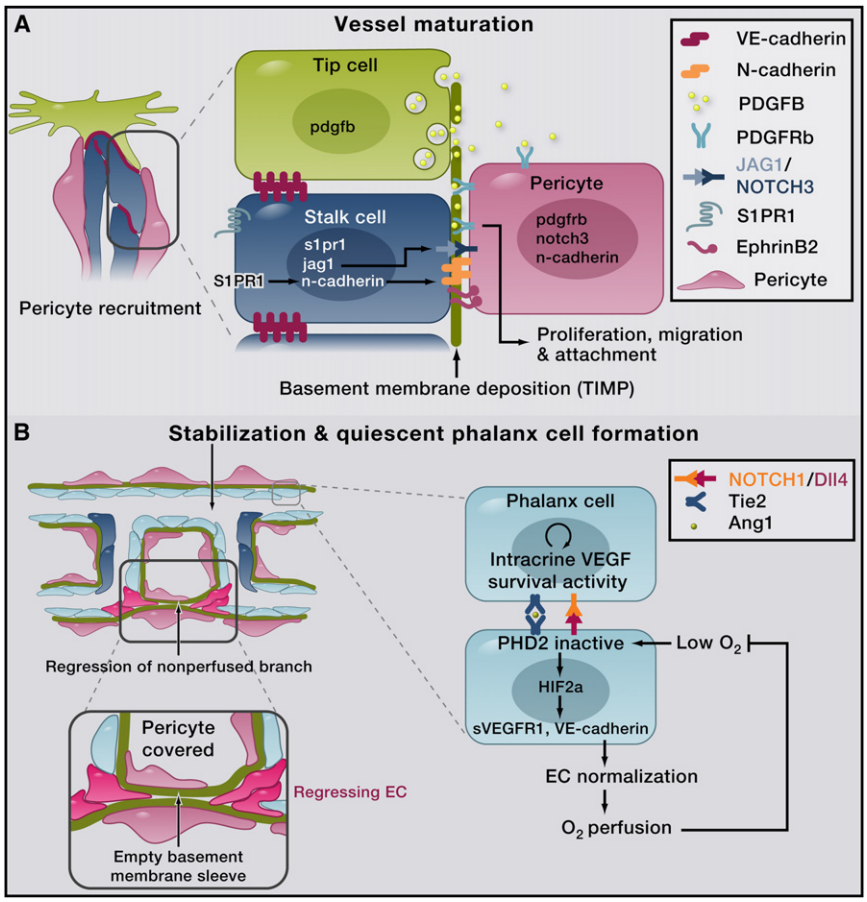

Figure 5. Vessel Maturation, Stabilization, and Quiescent Phalanx Cell Formation

(A) Vessel stabilization relies on the recruitment of pericytes involving PDGFRß, S1PR1, ephrinB2, and Notch3 signaling and the formation of N-cadherin junctions. Basement membrane deposition is favored by protease inhibitors (TIMPs).

(B) Perfused vessels become mature through pericyte coverage and acquisition of an endothelial phalanx phenotype. Right: Inactivation of PHD2 by low oxygen levels, leading to HIF2α-mediated upregulation of sVEGFR1 and VE-cadherin, thereby improving perfusion.

Sphingosine-1-phosphate receptor (S1PR) signaling also controls EC/mural cell interactions. Endothelial-derived S1P binds to G protein-coupled S1PRs (S1PR1–5) (Lucke and Levkau, 2010). S1P triggers cytoskeletal, adhesive, and junctional changes, affecting cell migration, proliferation, and survival. Disruption of S1PR1 or loss of both S1PR2 and S1PR3 in mice causes defective coverage of vascular smooth muscle cells and pericytes, a phenotype reminiscent of *Pdgfb* and *Pdgfrb* mutant mice. However, the primary defect is located in ECs, where S1P1 controls trafficking of N-cadherin to the ablumenal side of ECs in order to strengthen EC-pericyte contacts (Figure 5A).

Angiopoietin-1 (ANG1), produced by mural cells, activates its endothelial receptor TIE2 (Augustin et al., 2009; Huang et al., 2010). ANG1 stabilizes vessels, promotes pericyte adhesion, and makes them leak resistant by tightening endothelial junctions. Contrary to common belief, ANG1 seems less required for mural cell recruitment than originally thought (Jeansson et al., 2011). Mural cells also require ephrinB2 for association around ECs, as mural cell-specific ephrinB2 deficiency causes mural cell migration and vascular defects (Pitulescu and Adams, 2010) (Figure 5A). Notch signaling also controls maturation and arterial differentiation of vascular smooth muscle cells (Gridley, 2010). Mice lacking Notch3 lose arterial characteristics and develop arterial defects, whereas *NOTCH3* mutations in humans cause degeneration of vascular smooth muscle cells in CADASIL, a human stroke and dementia syndrome (Figure 5A).

Phalanx ECs Express Oxygen Sensors to Regulate Vessel Perfusion

Vessels can adjust their shape and function to meet changing tissue oxygen demands. Hypoxia-inducible factors (HIFs) orchestrate adaptive responses of ECs to changes in oxygen tension by controlling gene networks that govern survival, metabolism, and angiogenesis (Fraisl et al., 2009; Majmundar et al., 2010). HIF activity is regulated by oxygen-sensing prolyl hydroxylase domain proteins (PHD1–3). In normoxia, PHDs use oxygen to hydroxylate HIFs, thereby targeting them for proteasomal degradation. Oxygen sensors become inactive in hypoxic conditions, allowing HIFs to escape degradation. PHD2 regulates the endothelial phalanx cell phenotype. In search for a conceptual distinction from angiogenic tip and stalk cells, the cobblestone-like appearance of quiescent ECs prompted the term "phalanx" cells given their resemblance to the ancient Greek military formation (Mazzone et al., 2009). Haplodeficiency of PHD2 counteracts the abnormal vessel shape in tumors, promoting a more streamlined "phalanx-like" phenotype (Mazzone et al., 2009). Reduced PHD2 levels stabilize HIF2α, thereby enhancing levels of soluble VEGFR1 and VE-cadherin, counterbalancing endothelial disorganization (Figure 5B). This oxygen sensor thereby allows ECs to dynamically adapt vessel shape to their primordial function of oxygen delivery.

Quiescent ECs Have Barrier Properties

Resting ECs form barriers between blood and surrounding tissues to control the exchange of fluids and solutes and transmigration of immune cells. Essential for this function is the ability of ECs to regulate cell-cell adhesion between each other and neighboring cells. This relies on transmembrane-adhesive proteins, including VE-cadherin and N-cadherin at adherens junctions, as well as occludins and members of the claudin and junctional adhesion molecule (JAM) family at tight junctions (Cavallaro and Dejana, 2011). Tight junction molecules maintain and regulate paracellular permeability, whereas adherens

junction molecules mediate cell-cell adhesion, cytoskeletal reorganization, and intracellular signaling. VE-cadherin is a key component of EC junctions. In complex with VEGFR2, VE-cadherin maintains EC quiescence through recruitment of phosphatases that dephosphorylate VEGFR2, thus restraining VEGF signaling. Distinct types of VE-cadherin-based adherens junctions establish stable or transitory interactions with the cytoskeleton that either solidify EC adhesion and barrier properties or facilitate EC separation and movement (Falk, 2010). Activation of TIE2 by ANG1 protects vessels from VEGF-induced leakage by inhibiting VEGF's ability to induce endocytosis of VE-cadherin.

Vessels Express Survival Signals

As endothelial proliferation decelerates during maturation, ECs must adopt survival properties to maintain integrity of the vessel lining. Autocrine and paracrine survival signals from endothelial and support cells protect the vessel from environmental stresses. One such survival factor is VEGF, which activates the PI3K/AKT survival pathway. Interestingly, ECs themselves are the pivotal source for VEGF's prosurvival activity. Mice lacking VEGF in ECs suffer bleeding, microinfarcts, and EC rupture (Warren and Iruela-Arispe, 2010). When produced by ECs as "intracrine" factor, VEGF prevents EC apoptosis in nonpathological conditions (Figure 5B). This intracrine activity of VEGF differs from its paracrine function in stimulating angiogenesis, as loss of endothelial VEGF does not cause developmental vascular defects (Warren and Iruela-Arispe, 2010).

Signaling by fibroblast growth factors (FGFs) has also been implicated in maintaining vascular integrity due to their ability to anneal adherens junctions (Beenken and Mohammadi, 2009). Inhibition of FGF signaling results in dissociation of adherens junctions and tight junctions, subsequent loss of ECs, and vessel disintegration (Murakami et al., 2008). Notch signaling is critical for generating and maintaining vascular homeostasis. A consequence of Notch activation is the establishment of mature and patent vessels that promote perfusion and relieve tissue hypoxia. Conversely, blockade of DLL4 or Notch1 in the adult causes vascular tumors and hemorrhage (Liu et al., 2011; Yan et al., 2010). Similarly, endothelial inactivation of RBPj reinitiates vascular growth in adulthood (Figure 5B). Activation of Notch in mural cells by endothelial DLL4 also contributes to vessel stability by stimulating deposition of BM components.

Signaling by TIE2 and ANG1 also controls survival and vessel quiescence (Augustin et al., 2009). ANG1 clusters TIE2 junctionally at inter-EC junctions in *trans* to promote survival and EC quiescence (Figure 5B). Blood flow is another important survival cue for ECs as fluid shear stress potently inhibits EC apoptosis. KLF2 is activated by shear stress and evokes quiescence by upregulating endothelial nitric oxide synthase and the anticoagulant factor thrombomodulin, keeping vessels dilated, perfused, and free of clots, and by downregulating VEGFR2, which prevents tip cell formation (Figure 4B). Other EC quiescence factors include bone morphogenic protein 9 (BMP9) and cerebral cavernous malformation proteins (CCM1–3), whose defective signaling causes vascular malformations (Leblanc et al., 2009). ECs in nonperfused vessels regress from their locations or undergo apoptosis (Figure 4B).

Other Signaling Pathways and Limitations of the Model

Although the described model offers a framework to explain the activity of numerous pro- and antiangiogenic molecules, there are other angiogenic pathways, with documented effects on vessel growth in vivo, whose roles in vessel branching have not or have only incompletely been characterized. Examples include chemokines, integrins (Desgrosellier and Cheresh, 2010), several transcriptional regulators, Wnt ligands and their frizzled receptors (Franco et al., 2009), other members of the FGF, PDGF, and TGF-ß superfamilies, or the VEGF homolog PlGF that transmits angiogenic signals through VEGFR1 (Fischer et al., 2008). Identifying their role in vessel branching or the other types of vessel growth will generate a unifying model that can serve as a source for future drug development.

The Vascular-Metabolic Interface

Blood vessels transport nutrients to energy-utilizing tissues, and hence, vessels as well as proangiogenic signals can affect metabolism (Fraisl et al., 2009) (Figures 6A and 6C). In metabolically active tissues, the uptake of nutrients is linked to energy demand to maintain tissue homeostasis. Interestingly, high levels of VEGF-B, a VEGF member with poor angiogenic activity, are found in metabolically active tissues, where it is coexpressed with genes like VEGF, stimulating mitochondrial biogenesis, and controls *trans*-endothelial uptake of fatty acids into other tissues (Hagberg et al., 2010). Through this mechanism, VEGF-B prepares tissues for fatty acid consumption. Notably, besides their role in supplying nutrients, ECs themselves can also promote growth and repair of metabolically active tissues independent of perfusion by secreting angiocrine factors (Butler et al., 2010; Ding et al., 2010). How vascular growth signals coordinate metabolism is only beginning to become understood.

The converse crosstalk is also true, with metabolism affecting vascular growth (Fraisl et al., 2009) (Figures 6B and 6C). Metabolic sensors and regulators control vessel growth, often stimulating angiogenesis in nutrient-deprived conditions in order to prepare the tissue for oxidative metabolism upon repletion of oxygen and nutrients. Examples include PGC1α, LKB1, AMPK, FOXOs, and SIRT1 (Fraisl et al., 2009). In conditions of oxygen and nutrient scarcity, PGC1α stimulates angiogenesis by upregulating VEGF through interaction with ERRα; this angiogenic burst, coupled to mitochondrial biogenesis, prepares the ischemic tissue for oxidative metabolism upon revascularization (Fraisl et al., 2009). Also, an increase in cellular levels of AMP (reflecting energy deprivation) induces VEGF-driven angiogenesis through activation of AMPK. Vascular growth is similarly controlled by LKB1, an activating kinase of AMPK and regulator of metabolism. The vascular-metabolic interface is further regulated by FOXO transcription factors, which are upregulated during fasting and restrict angiogenic behavior (Fraisl et al., 2009). Interestingly, FOXO1 and Notch1 are controlled by SIRT1, a deacetylase activated by NAD^+ in conditions of energy distress and nutrient deprivation.

Vessel Growth in Disease

Insufficient vessel growth and regression contribute to numerous disorders, ranging from myocardial infarction and stroke to neurodegeneration. Conversely, uncontrolled vessel growth

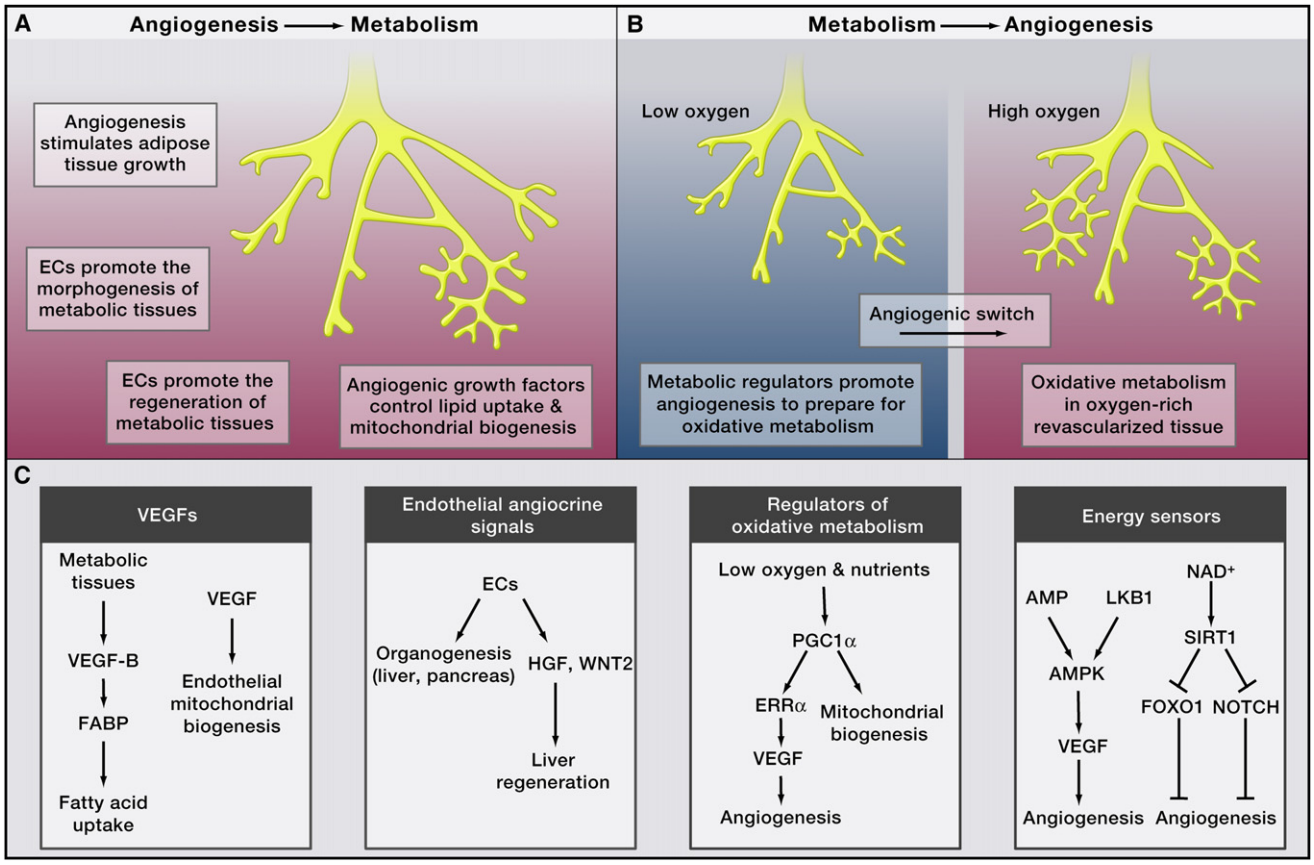

Figure 6. Angiogenesis—Metabolism Crosstalk

(A) Endothelial cells (ECs) promote growth and repair of metabolically active tissues by releasing angiocrine signals, whereas angiogenic molecules stimulate *trans*-endothelial transport of fuel to surrounding tissues.

(B) Metabolic sensors and regulators stimulate angiogenesis and mitochondrial biogenesis in order to prepare the ischemic tissue for oxidative metabolism upon repletion of oxygen and nutrients following revascularization.

(C) Schematic models of the molecular basis of angiogenesis—metabolism crosstalk.

promotes tumorigenesis and ocular disorders such as age-related macular degeneration. Historically, this has led to concepts of pro- and antiangiogenic therapy, aiming to restore adequate vessel densities. However, sprouting angiogenesis alone might be insufficient to fully revascularize ischemic tissues, as also collateral vessels have to enlarge to supply bulk flow (Schaper, 2009). It has become clear that vessel densities can no longer be considered separately from vessel function when designing angiogenic therapeutics. We anticipate that insights into pathological angiogenesis, guiding future diagnostic and therapeutic approaches, will increasingly focus on the functional quality of vessels and their effects on local metabolism rather than on vessel quantity alone.

Tumor Vessels Are Abnormal

Tumor vessels display abnormal structure and function (Goel et al., 2011; Jain, 2005) with seemingly chaotic organization (Figure 7A). Highly dense regions neighbor vessel-poor areas, and vessels vary from abnormally wide, irregular, and tortuous serpentine-like shape to thin channels with small or compressed lumens. Every layer of the tumor vessel wall is abnormal. ECs lack a cobblestone appearance, are poorly interconnected, and are occasionally multilayered. Also, arterio-venous identity is ill defined, and shunting compromises flow. The basement membrane is irregular in thickness and composition, and fewer, more loosely attached hypocontractile mural cells cover tumor vessels, though tumor-type-specific differences exist.

The resulting irregular perfusion impairs oxygen, nutrient, and drug delivery (Goel et al., 2011; Jain, 2005). Vessel leakiness together with growing tumor mass increases the interstitial pressure and thereby impedes nutrient and drug distribution. The loosely assembled vessel wall also facilitates tumor cell intravasation and dissemination. As a consequence of poor oxygen, nutrient, and growth factor supply, tumor cells further stimulate angiogenesis in an effort to compensate for the poor functioning of the existing ones. However, this excess of proangiogenic molecules only leads to additional disorganization as the angiogenic burst is nonproductive, further aggravating tumor hypoperfusion in a vicious cycle. The hypoxic and acidic tumor milieu constitutes a hostile microenvironment that is believed to drive selection of more malignant tumor cell clones and further promotes tumor cell dissemination. The uneven delivery of chemotherapeutics together with a reduced

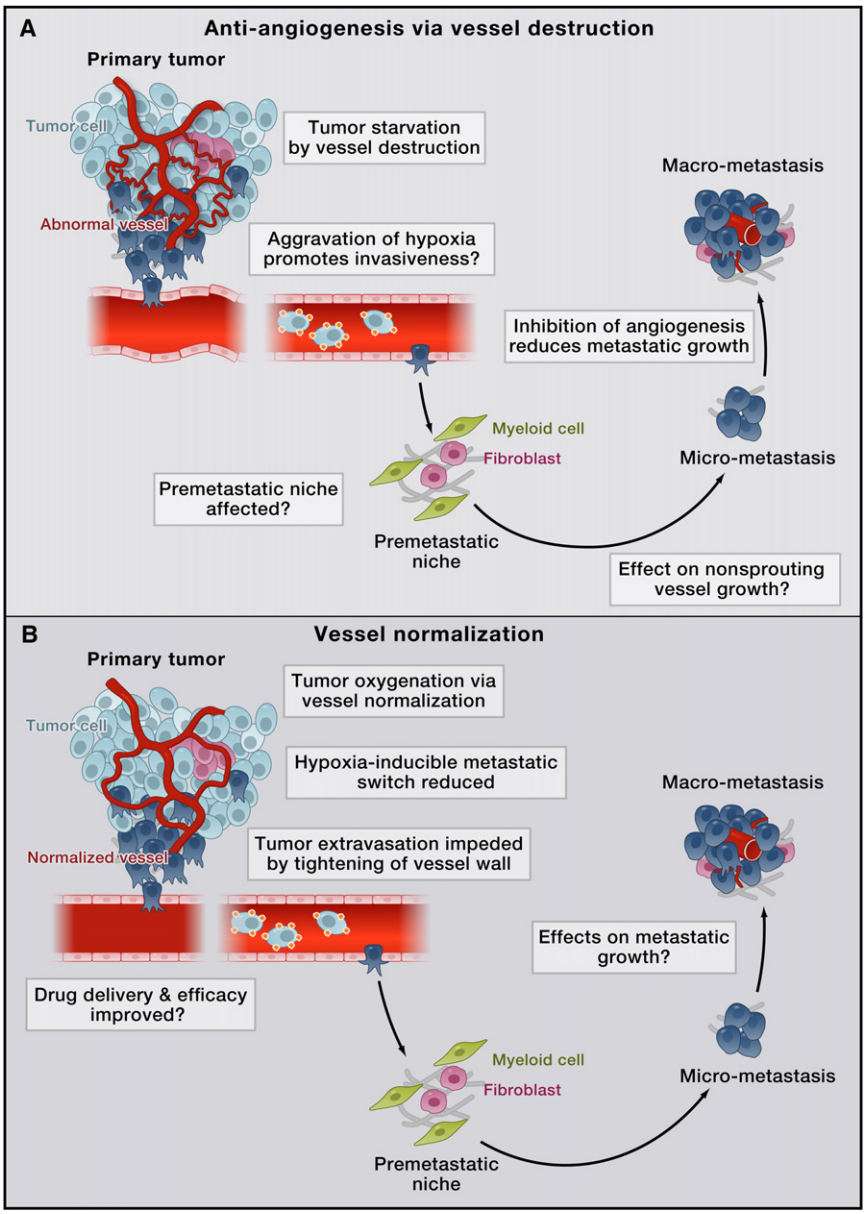

Figure 7. Antiangiogenesis versus Vessel Normalization

(A) Antiangiogenic agents that destroy abnormal tumor vessels and prune the tumor microvasculature can aggravate intratumor hypoxia, which can activate a prometastatic switch; the question mark reflects ongoing debate as to whether this metastatic switch exists in patients treated with VEGF (receptor) inhibitors.

(B) Antivascular targeting strategies that normalize abnormal tumor vessels are believed not to aggravate tumor hypoxia or even to improve oxygen supply, thereby impeding the hypoxia-driven prometastatic switch. Their effect on stabilizing and tightening of the tumor vessel wall makes the vessels less penetrable for disseminating tumor cells. When improving drug delivery and tumor oxygenation, vessel normalization can also enhance conventional chemotherapy and irradiation.

efficacy of radiotherapy, owing to the lower intratumoral oxygen levels, limit the success of conventional anticancer treatment.

Modes of Tumor Vascularization

Besides sprouting, tumors utilize other modes of vessel growth. For example, tumor cells can co-opt pre-existing vasculature without a need to stimulate vessel branching initially. Once the tumor outgrows this supply, hypoxia evokes a secondary angiogenic response. Bone marrow-derived progenitors can also promote tumor vascularization or control the angiogenic switch during metastasis, but their importance is debated and context dependent (Fang and Salven, 2011). If tumors would be able to switch mechanisms of vascular growth and some of these mechanisms rely less on VEGF, they would possess the means to escape from treatment with VEGF (receptor) inhibitors. Identifying the molecular basis of these alternative modes of vessel growth will thus be critical to improve the efficacy of antiangiogenic treatment.

Role of Myeloid Cells in Tumor Vessel Vascularization

Various hematopoietic lineages influence tumor angiogenesis (Kerbel, 2008). $VEGFR1^+$ hematopoietic precursors or TIE2-expressing monocytes (TEMs) are located close to growing tumor vessels and release angiogenic molecules (De Palma and Naldini, 2009). Expression of ANG2 by tumor ECs activates TEMs to stimulate angiogenesis (Mazzieri et al., 2011). Tumor-associated macrophages, especially those polarized to a proangiogenic M2-like phenotype, stimulate angiogenesis by releasing PlGF that also contributes to vessel disorganization (Grivennikov et al., 2010; Qian and Pollard, 2010; Rolny et al., 2011). Mast cells promote tumor angiogenesis by secretion of proteases that liberate proangiogenic factors from the extracellular matrix. Additionally, $CD11B^+Gr1^+$ neutrophils release the proangiogenic factor Bv8, particularly in tumors that are resistant against VEGF blockade (Ferrara, 2010b). Recruitment of other bone marrow-derived cells (BMDCs) can also contribute to tumor vascularization. For instance, $CXCR4^+$ BMDCs are retained inside the cancer via production of SDF1α, the ligand of CXCR4, and boost tumor vascularization by releasing angiogenic factors. An increasing body of evidence implicates myeloid cells in the resistance of tumors against treatment with VEGF (receptor) inhibitors (Ferrara, 2010b).

Role of Cancer-Associated Fibroblasts in Tumor Vessel Vascularization

Another stromal cell type gaining increasing attention is the cancer-associated fibroblast (Crawford and Ferrara, 2009a; Nyberg et al., 2008; Pietras and Ostman, 2010). These cells originate from local mesenchyme in organs where tumors grow or

become recruited from the bone marrow (Wels et al., 2008). Cancer-associated fibroblasts promote tumor vascularization by recruiting endothelial progenitor cells (EPCs) or releasing proangiogenic factors (Crawford and Ferrara, 2009a; Erez et al., 2010). In chronic myeloid leukemia, malignant cells upregulate PlGF in bone marrow stromal cells to create a vascularized soil for leukemia cells (Schmidt et al., 2011).

Clinically Approved Antiangiogenic Therapies

VEGF has become the prime antiangiogenic drug target with approval by the US Food and Drug Administration of several VEGF (receptor)-based inhibitors for clinical use (Crawford and Ferrara, 2009b). The anti-VEGF antibody (bevacizumab [Avastin]) is approved in combination with chemotherapy or cytokine therapy for several advanced metastatic cancers, including non-squamous non-small cell lung cancer, colorectal cancer, renal cell cancer, and metastatic breast cancer. Based on a randomized phase II trial, bevacizumab monotherapy has been approved for recurrent glioblastoma. Additionally, four multitargeted pan-VEGF receptor tyrosine kinase inhibitors (RTKIs) have been approved: Sunitinib [Sutent] and Pazopanib [Votrient] for metastatic RCC, Sorafenib [Nexavar] for metastatic RCC and unresectable hepatocellular carcinoma, and Vandetanib [Zactima] for medullary thyroid cancer. Sunitinib has also been recommended for treatment of advanced pancreatic neuroendocrine tumors. Clinical agents for wet age-related macular degeneration, characterized by neovascularization of leaky vessels, include an anti-VEGF Fab (ranibizumab [Lucentis]) and a VEGF aptamer (pegaptanib [Macugen]), with Avastin being used off-label. VEGF blockade prolongs progression-free survival or overall survival of cancer patients in the range of weeks to months and improves visual acuity in patients with age-related macular degeneration.

The clinical benefit of treatment with VEGF (receptor) inhibitors is attributable to several mechanisms. First, these blockers inhibit tumor vessel expansion by blocking vascular branching or inhibiting homing of BMDCs (Figure 7A). Additionally, these drugs induce regression of pre-existing tumor vessels and sensitize ECs to effects of chemotherapy and irradiation by depriving them of VEGF's survival activity. Normalization of abnormal tumor vessels by pruning immature pericyte-devoid vessels and by promoting maturation into more functional vessels is another mechanism (Goel et al., 2011) (Figure 7B). The resulting sensitization to cytotoxic or radiation therapies relying on conversion of oxygen to radicals in combination with improved chemotherapeutic delivery may explain partly why combination delivery of bevacizumab/cytotoxic agents is often superior (Jain, 2005). However, the importance of vessel normalization versus pruning for the overall anticancer effect of VEGF (receptor) inhibitor treatment requires future study. Furthermore, vessel normalization observed with treatment is transient, as these drugs induce excessive vessel regression, or tumor vascularization escapes VEGF blockade. In conditions where vascular leakage causes life-threatening intracranial edema (e.g., in glioblastoma) or blindness (e.g., in wet age-related macular degeneration), restoration of normal barrier properties by VEGF (receptor) blockade may be a relevant mechanism (Goel et al., 2011). Besides targeting tumor vessels, these inhibitors also target tumor cells expressing VEGF (receptor), whose growth is stimulated by VEGF.

Challenges and Concerns of VEGF (Receptor) Inhibitor Treatment

Contrary to preclinical experiments, where long-term benefit of VEGF (receptor) inhibition can be achieved, the clinical benefit in prolonging cancer patient survival with advanced disease is limited, and a fraction of patients are intrinsically refractory or acquire resistance (Bergers and Hanahan, 2008; Ebos and Kerbel, 2011; Ferrara, 2010a). Recent trials using VEGF (receptor) blockers showed that the benefit, initially reported for progression-free survival, was no longer detected when analyzing overall survival (Ebos and Kerbel, 2011). The first phase III trial evaluating the adjuvant effect of anti-VEGF therapy following surgical tumor resection did not prolong disease-free survival (Van Cutsem et al., 2011). It is also curious why monotherapy with VEGF receptor kinase inhibitors induces benefit in some tumors but is ineffective in others or evokes side effects when combined with chemotherapy. Validated genetic or molecular biomarkers for anti-VEGF (receptor) responsiveness are much needed to identify responsive patients and tailor antiangiogenic therapy but are not yet available (Jain et al., 2009). Mechanism-based side effects of anti-VEGF (receptor) treatment (hypertension) show predictive value for antitumor efficacy.

The relative inefficacy of VEGF (receptor) inhibitors in oncological practice calls for more suitable preclinical cancer models (Bagri et al., 2010; Francia et al., 2011) and has spurred research into mechanisms underlying resistance (Box 1) (Bergers and Hanahan, 2008; Ebos and Kerbel, 2011; Ferrara, 2010a). Certain tumors produce proangiogenic factors besides VEGF, even prior to treatment, and are thus relatively insensitive to VEGF (receptor) inhibition. Others become unresponsive during treatment, when hypoxia upregulates "rescue" angiogenic molecules (e.g., PlGF, FGFs, IL-8). Second, vessel co-option or lining of tumor channels by ECs with cytogenetic abnormalities may not be as sensitive to VEGF (receptor) inhibitors. Also, the precise modes of vascular supply in the pre- and micrometastatic niches remain insufficiently characterized (Figure 7B). Poor vascularization, as in pancreatic cancer, or mature tumor capillaries, as in hepatocellular carcinoma, may reduce sensitivity to VEGF (receptor) inhibitor treatment. Finally, depriving the tumor of its vascular supply may select "hypoxia-resistant" tumor clones (Ebos and Kerbel, 2011).

Recent preclinical data also raised concerns that VEGF (receptor) inhibitors might fuel cancer invasiveness and metastasis by aggravating intratumoral hypoxia and creating a proinflammatory environment (Ebos and Kerbel, 2011) (Figure 7A). These findings are debated, as other preclinical studies have not observed an increase in malignancy (Padera et al., 2008), and large meta-analyses have not shown a worse clinical outcome (Ebos and Kerbel, 2011; Miles et al., 2011). One exception is glioblastoma that exhibits a more invasive phenotype after VEGF (receptor) blockade in preclinical models and patients, possibly as a consequence of a hypoxic cancer stem cell niche that drives recurrence of a more aggressive tumor (Norden et al., 2009). Conflicting reports on whether discontinuation of VEGF (receptor) blockade boosts a tumor (angiogenesis) rebound call for further clarification. Moreover, the most effective dosing and duration of VEGF (receptor) inhibitor treatment remain to be determined.

Box 1. Mechansisms of Resistance against VEGF (receptor) Blockade

VEGF-independent vessel growth: Tumors produce additional proangiogenic molecules besides VEGF, before or after treatment with VEGF (receptor) blockers.
Sprouting-independent vessel growth: Tumors possess/switch to modes of vessel growth (vessel co-option, vascular mimicry, intussusception, etc.) that can be less sensitive to VEGF (receptor) blockade.
Stromal cells: Both myeloid cells and cancer-associated fibroblasts produce other proangiogenic factors besides VEGF or recruit proangiogenic bone marrow-derived cells.
Endothelial cell (EC) instability: Endothelial cells with cytogenetic abnormalities or tumor ECs, which differentiate from cancer stem cell-like cells (as in glioblastoma), may not be as sensitive to VEGF (receptor) blockade as sprouting ECs.
Vascular independence: Mutant tumor clones or inflammatory cells are able to survive in hypoxic tumors; their reduced vascular dependence impairs the antiangiogenic response. Certain tumors have a hypovascular stroma. Tumors can also metastasize via lymphatics; their growth may not be blocked by antiangiogenic therapy.
Mature vessels: Mature supply vessels are covered by vascular smooth muscle cells and not easily pruned by EC-targeted treatment.
EC radioresistance: Hypoxic activation of HIF1α renders ECs resistant to irradiation.
Organ-specific differences: Tumors show opposite invasive behaviors depending on the organ of inoculation.
Gene variations: Gene variations in VEGF receptors determine the responsiveness to VEGF (receptor) blockade.
Vessel normalization: Transient vessel normalization can reduce antiangiogenic drug delivery and efficacy; alternatively, barrier tightening could impede drug penetration.
Primary tumor versus metastasis: Distinct signals regulate angiogenesis in primary versus metatstatic tumors.

Alternative Therapeutic Antitumor Vascularization Strategies

All approved antiangiogenic therapies have been developed to starve tumors by destroying their vascular supply. Approaches with a similar mechanism of action but different targets are under development. However, alternative strategies that are not solely based on vessel destruction are being considered as well. We will highlight a few prototypic examples.

Given that VEGF (receptor) inhibitors are more efficient at destroying capillaries devoid of pericytes, simultaneous targeting of ECs and pericytes might enhance their antiangiogenic efficacy. Preclinical treatment with PDGFRß inhibitors reduces tumor progression by facilitating pericyte detachment, thereby rendering vessels more immature and vulnerable to regression. Also, multitargeted tyrosine kinase inhibitors blocking both PDGFRß and VEGF receptors (besides many other targets) were more efficient than inhibitors of VEGF signaling alone. However, combining selective PDGFRß and VEGF receptor blockers did not meet expectations (Nisancioglu et al., 2010). PDGFRß blocking studies also highlighted the importance of considering not only effects on the primary tumor alone but also on metastasis, as poor pericyte attachment promotes metastasis (Gerhardt and Semb, 2008).

The ''sustained vascular normalization'' concept proposes not to destroy tumor vessels but to restore their structure and function, so that improved perfusion and oxygenation counteract the hypoxia-driven expression of genes controlling epithelial-mesenchymal transition, invasion, and intravasation, which prompt the metastatic switch (Goel et al., 2011; Mazzone et al., 2009; Rolny et al., 2011) (Figure 7B). The normalized vessel wall also restricts tumor cell intravasation (Mazzone et al., 2009), while responses to chemo- or immunotherapy can be improved (Goel et al., 2011; Rolny et al., 2011).

Conclusions and Perspectives

Despite progress in understanding the molecular basis of angiogenesis, and successful translation of VEGF blockade for the treatment of age-related macular degeneration and some cancer patients, challenges must be overcome to improve the overall efficacy of antivascular strategies to combat cancer more efficiently. A question of high priority is whether the approved antiangiogenic regimes are optimally used in terms of dosing, duration, and combination therapy. The role of VEGF (receptor) inhibitors in micrometastatic disease in adjuvant settings (e.g., upon resection of the primary tumor) will require further research given the paucity of available preclinical data and suitable animal models. Another priority is to identify predictive biomarkers, tailored for particular tumors, stages, and treatment. Third, development of additional antiangiogenic drugs, independent of VEGF signaling, and evaluation of their potential in clinical trials, in particular as combination therapy with current VEGF (receptor) inhibitors, is likely to expand the antiangiogenic armamentarium. Fourth, the therapeutic potential of sustained vessel normalization to suppress metastasis and enhance chemotherapy will need to be evaluated clinically, and additional studies are required to establish how it could be combined best with available vessel pruning therapies. Also, antivascular approaches could be beneficial for the treatment of nonsolid malignancies (e.g., leukemias) or for the treatment of children or pregnant women with cancer or individuals with inflammatory disorders (e.g., arthritis) who have not been considered eligible for VEGF blockade because of side effects. Finally, the recent molecular breakthroughs in our understanding of vessel growth should kindle renewed interest in developing strategies to revascularize ischemic tissues.

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