Growth factor signaling pathways in vascular development and disease

Zoe. L. Grant[1,2] and Leigh Coultas[1,2,3].

[1]The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia

[2]Department of Medical Biology, University of Melbourne, 1G Royal Parade, VIC 3052 Australia

[3]Corresponding author

Author ORCiD

ZLG: 0000-0003-0580-5222

LC: 0000-0001-6466-0890

Address correspondence to:

Leigh Coultas, Development and Cancer Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Vic, 3052 Australia

Ph (+61) 3 9345 2860, Email: lcoultas@wehi.edu.au

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Abstract

Angiogenic blood vessel growth is essential to ensure organs receive adequate blood supply to support normal organ function and homeostasis. Angiogenesis involves a complex series of cellular events through which new vessels grow out from existing vasculature. Growth factor signaling, layered over a range of other signaling inputs, orchestrates this process. The response of endothelial cells to growth factor signals must be carefully controlled through feedback mechanisms to prevent excessive vessel growth, remodeling or destabilization. In this review, we summarize recent findings describing how endothelial cells respond to growth factor signals during blood vessel development and homeostasis and how perturbation of these responses can lead to disease.

Introduction

Blood vessels form a tubular network that facilitates the distribution of oxygen, nutrients hormones and cells throughout the body, along with the removal of waste and metabolic by-products. The inner surface of blood vessels is lined by endothelial cells (ECs) that regulate the trafficking of molecules and cells into tissues. Blood vessel supply must be tailored to match a tissue's metabolic needs and vessel networks expand to meet this requirement through angiogenesis – the coordinated growth of new vessels from pre-existing ones. The establishment of a hierarchical structure of arteries, arterioles, veins, venules and capillaries during the angiogenic process ensures that blood and its nutrients are efficiently delivered. Once established, blood vessels must return to a quiescent state and maintain a stable network so as not to result in activation or dysfunction leading to disease. Excessive blood vessel growth, maladaptive vessel remodeling or loss of barrier integrity can all cause disease.

Blood vessel growth and maintenance is coordinated by diverse growth factor inputs. Prominent growth factor families that regulate EC behavior during vascular development are the vascular endothelial growth factor (VEGF) (Simons et al., 2016), fibroblast growth factor (FGF) (Yang et al., 2015), angiopoietin (ANGPT) (Saharinen et al., 2017) and transforming growth factor beta (TGFβ)/bone morphogenetic protein (BMP) families (Goumans and Ten Dijke, 2018; Goumans et al., 2018). In addition, platelet derived growth factor □ (PDGF□) regulates pericytes and smooth muscle cells – perivascular cells that support the vasculature by maintaining vessel quiescence and stability (Armulik et al., 2011). A parallel vascular system, the lymphatics, is lined by its own specialized type of ECs and has roles in fatty acid absorption, immune cell trafficking and returning interstitial fluid to the blood. Lymphatic networks also develop under the control of the same growth factors that regulate blood vascular development but will not be covered in this review (Vaahtomeri et al., 2017). In addition to growth factors, vascular development and homeostasis are regulated by many other signaling inputs including: ligand-receptor signaling pathways such as Wnt and Notch (Korn and Augustin, 2015; Kume, 2012); cell-cell interactions mediated particularly through VE-cadherin (Giannotta et al., 2013); cell-ECM interactions via integrins (Avraamides et al., 2008) and blood flow mechanosensing (Kutys and Chen, 2016). Here we will focus primarily on recent progress in our understanding of how ECs respond to growth factors during blood vessel development and homeostasis, particularly the VEGF, angiopoietin and BMP pathways. We touch on other signaling inputs only as they relate to growth factor signaling in ECs.

Production of new blood vessels by angiogenesis

Angiogenesis is the process by which new vessels sprout from pre-existing ones and is the predominant method for new vessel growth. Angiogenesis is associated with growing tissues, particularly during development. While generally absent from adults, it can be reactivated in response to tissue-specific metabolic demand or as part of disease (Carmeliet, 2003). During angiogenesis, ECs must go through a series of highly coordinated events. These facilitate the initiation and extension of new vessel sprouts, their fusion with other vessels and the subsequent remodeling of newly formed vessel segments into a mature, hierarchical network (Betz et al., 2016). This process requires extensive EC proliferation and migration. In response to pro-angiogenic stimuli, ECs must modify and adapt their gene expression, metabolism, polarity, shape, and interaction with their external environment. This must all be achieved while maintaining intact barrier integrity to prevent leakage.

Sprouting growth of new blood vessels

Vessel sprouting requires ECs to adopt migratory and proliferative behaviors not normally present in resting vessels. In response to pro-angiogenic growth factors, new vessel sprouts emerge from the wall of existing vessels. These sprouts consist of ECs with specific behavioral properties. “Tip cells”, located at the tip of growing vessel sprouts, are highly migratory and respond to growth factor and guidance cues to navigate newly forming sprouts toward avascular, hypoxic tissue (Gerhardt et al., 2003; Ruhrberg et al., 2002; Siekmann et al., 2013). Tip cells have little lumen and display a morphology and gene expression profile that is consistent with their migratory, invasive behavior (del Toro et al., 2010; Strasser et al., 2010). Trailing behind are the “stalk cells” that make up the body of the sprout. Stalk cells have a lumen and are highly proliferative, contributing new ECs to the growing vessel. Tip and stalk cells are therefore distinct from the quiescent “phalanx cells” that line mature, well perfused vessels (Potente and Carmeliet, 2017). While tip and stalk cell identities are molecularly and functionally distinct, they are temporary and reversible. During angiogenesis, ECs continuously switch between tip and stalk identities, competing with each other for the tip cell position (Jakobsson et al., 2010). Tip/stalk identity is determined in response to several growth factor inputs and subject to feed-back mechanisms that ensure the appropriate balance of each cell type is specified to ensure optimal vessel growth.

VEGFA in sprouting angiogenesis

VEGF signaling is the key growth factor that induces sprouting angiogenesis (Siekmann et al., 2013). The VEGF family consists of VEGFA, VEGFB, VEGFC, VEGFD and placental growth factor (PlGF) (Achen et al., 1998; Joukov et al., 1996; Leung et al., 1989; Maglione et al., 1991; Olofsson et al., 1996). These ligands bind with differing affinity to the tyrosine kinase receptors VEGFR1, VEGFR2 and VEGFR3 (Pajusola et al., 1992; Shibuya et al., 1990; Terman et al., 1991). VEGFA can also bind the co-receptors neuropilin 1 (NRP1) and NRP2, which modulate VEGFR signaling (Parker et al., 2012; Soker et al., 1998). VEGF binding activates receptor hetero- and homodimers, leading to trans-phosphorylation of tyrosines in the intracellular portion of the receptors. This recruits SH2 containing adaptor proteins and subsequent signaling pathway activation (Guo et al., 1995; Huang et al., 2001). VEGFR2 is the main signaling receptor for VEGFA. While VEGFA binds with higher affinity to VEGFR1, it has only weak kinase activity and is produced in both membrane-bound and soluble forms. As such, it is considered a 'decoy' receptor that reduces the amount of free VEGFA available to bind VEGFR2 (Hiratsuka et al., 1998; Kendall and Thomas, 1993; Simons et al., 2016). Mice lacking either VEGFA or VEGFR2 die in utero due to complete failure to develop blood vessels, highlighting their central role in the early stages of blood vessel development (Carmeliet et al., 1996; Ferrara et al., 1996; Shalaby et al., 1995). Indeed, clinically approved anti-angiogenic agents selectively inhibit the activity of VEGFA (Ferrara and Adamis, 2016).

Sprouting angiogenesis is severely impaired in the absence of endothelial *Vegfr2* (Benedito et al., 2012; Zarkada et al., 2015) and gradients of VEGFA initiate and direct angiogenic vessel sprouting while promoting stalk cell proliferation (Gerhardt et al., 2003; Ruhrberg et al., 2002). To prevent over-production of tip cells and chaotic sprouting, feedback mechanisms ensure only those cells with the highest sensitivity for VEGF ligands acquire tip cell identity (Jakobsson et al., 2010). This sensitivity is determined by differential VEGF receptor expression and is regulated through a feedback mechanism involving Notch (Blanco and Gerhardt, 2013). Tip cells express high levels of VEGFR2, activation of which induces expression of the Notch ligand DLL4 (Gerhardt et al., 2003; Liu et al., 2003; Lobov et al., 2007) (Figure 1). This activates Notch on adjacent ECs, preventing them from becoming tip cells through lateral inhibition (Hellstrom et al., 2007; Suchting et al., 2007). Notch represses VEGFR2 and NRP1 while also promoting expression of the decoy receptor VEGFR1 (Aspalter et al., 2015; Chappell et al., 2009; Harrington et al., 2008). VEGFR2 activation also induces the expression of VEGFR3 (Benedito et al., 2012; Tammela et al., 2008) and ligand-

independent VEGFR3 signaling further activates Notch, repressing VEGFR2 (Tammela et al., 2011; Zarkada et al., 2015).

Junctional destabilization through turnover of VE-cadherin is necessary to allow EC migration and competition for the tip cell position in response to VEGFA (Bentley et al., 2014). VEGFR2 activation weakens adherens junctions through Src-dependent VE-cadherin phosphorylation. Src is recruited to activated VEGFR2 via TSAd, where it phosphorylates VE-cadherin leading to its internalization and the consequent destabilization of adherens junctions (Sun et al., 2012). While TSAd and Src promote junctional VE-cadherin turnover during angiogenesis, this only appears to be essential for sprouting angiogenesis in certain types of vessels (Gordon et al., 2016). Furthermore, TSAd/Src regulation of VE-cadherin turnover is dispensable for tip cell selection, suggesting other mechanisms feed into this process (Gordon et al., 2016).

VEGFA activation of Src family kinases further promotes sprouting angiogenesis through activation of the transcriptional co-activators YAP and TAZ (Wang et al., 2017). Typically associated with Hippo signaling, YAP and TAZ act as a nexus for biomechanical signaling inputs that regulate organ size and growth (Totaro et al., 2018). Src-dependent cytoskeletal rearrangements induced by VEGFA activate YAP and TAZ, which promote the expression of genes involved in further cytoskeletal remodeling and adhesion (Kim et al., 2017a; Wang et al., 2017). YAP and TAZ are also regulated by EC junction stability and promote the turnover of VE-cadherin at cell-cell junctions in a manner that permits cell movement and EC proliferation without compromising barrier integrity (Neto et al., 2018). Consistent with these roles, loss of endothelial YAP and TAZ prevents sprouting angiogenesis and results in the formation of leaky vessels (Kim et al., 2017a; Neto et al., 2018; Sakabe et al., 2017; Wang et al., 2017). YAP and TAZ therefore enable coordinated EC movement during VEGFA-induced vessel growth, but also guard against excessive junction remodeling that compromises barrier integrity.

VEGFA also promotes sprouting angiogenesis through modulation of EC metabolism. ECs in growing vascular networks increase their rates of glycolysis to meet the energy needs required for migration and proliferation (De Bock et al., 2013a). VEGFA induces the expression of glycolytic pathway genes including the glucose transporter GLUT1 and the glycolytic enzymes phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFKFB3) and lactate dehydrogenase A (LDHA) (De Bock et al., 2013b; Parra-Bonilla et al., 2010; Yeh et al., 2008). FGF2 signaling via FGFR1 and FGFR3 also promotes glycolysis during angiogenesis. FGF2 signaling increases the expression of Myc, a driver of cell growth and proliferation, which in turn upregulates another glycolytic pathway enzyme, hexokinase 2 (HK2) (Yu et al., 2017).

Mice lacking endothelial PFKFB3 or HK2 have reduced angiogenic vessel growth due to defects in EC proliferation and motility (De Bock et al., 2013b; Yu et al., 2017). Lowering both glycolysis and mitochondrial respiration is necessary to re-establish EC quiescence (Wilhelm et al., 2016) demonstrating that metabolic control is therefore a key mechanism by which growth factors regulate sprouting angiogenesis.

BMP regulation of sprouting angiogenesis

BMP signaling has a critical role in modulating tip and stalk cell fates during sprouting angiogenesis. BMPs are members of the transforming growth factor beta (TGFβ) superfamily that signal upon binding to hetero-tetrameric complexes consisting of two type I and two type II serine/threonine-protein kinase receptors. On ECs, BMPs engage the type I receptors activin receptor-like kinase (ALK) 1, 2, 3 and 6 and the type II receptors BMP type II receptor (BMPRII) and activin type II receptor (ActRII) (Goumans et al., 2009). Upon ligand binding, type II receptors trans-phosphorylate and activate type I receptors. These then recruit and phosphorylate distinct receptor-activated (R-) SMAD transcription factors. Typically, BMPs activate R-SMADs 1, 5 and 8, whereas TGFβ ligands activate R-SMADs 2 and 3. R-SMADs bind to the common mediator SMAD4 and translocate to the nucleus to regulate target gene expression (Atri et al., 2013).

Different BMPs display pro- or anti-angiogenic functions. BMP2, 6 & 7, produced locally by hypoxic or injured tissue signal through ALK2 and 3 to promote angiogenesis (Beets et al., 2013). BMP2 has been shown to promote vein-specific sprouting in zebrafish independent of VEGFA (Wiley et al., 2011), while BMP 6 & 7 activation of ALK3 is necessary for vessel sprouting in the developing mouse retina (Lee et al., 2017). TGFβ and BMP9 & 10 by contrast circulate systemically and promote quiescence and vessel stability. BMP9/10 and TGFβ signaling through ALK1 and ALK5, respectively, promotes stalk cell identity (Baeyens et al., 2016b; Castanares et al., 2007; Tual-Chalot et al., 2014). Differential activation of type II receptors and downstream SMADs may explain why different BMP ligands can have either pro- or anti-angiogenic effects (Aspalter et al., 2015; Lee et al., 2017; Upton et al., 2009). SMAD activation downstream of BMP9 & 10 promotes stalk cell gene expression both directly and through cross-talk with Notch by activating HES/HEY (Larrivee et al., 2012; Moya et al., 2012) (Figure 1). NRP1 expressed on tip cells suppresses SMAD activation downstream of TGFβ and BMP9/10 and hence stalk cell identity (Aspalter et al., 2015). As such, the tip cell fate must be actively suppressed in order for tip cell identity to arise (Aspalter et al., 2015).

Endoglin (ENG) is a co-receptor that promotes TGFβ/BMP signaling via ALK1 and is expressed on ECs (Lebrin et al., 2004). However, mosaic analysis has shown that ALK1 and ENG appear to have opposing roles in tip cell specification. *Alk1* loss-of-function confers a tip cell advantage to ECs (Aspalter et al., 2015), whereas *Eng* loss-of-function reduces tip cell potential (Jin et al., 2017). Despite this, *Eng* deficiency results in a hypersprouting phenotype, however this appears to be secondary to tissue hypoxia caused by arteriovenous malformations that arise as a result of *Eng* disruption (discussed in detail later) (Jin et al., 2017).

Angiopoietin signaling in sprouting angiogenesis

Angiopoietin signaling has complex, context-specific roles in regulating blood vessel growth and has a central role in maintaining vessel stability (Saharinen et al., 2017). The ligands angiopoietin 1 (ANGPT1) and ANGPT2 signal through two tyrosine kinase receptors: tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (TIE1) and TIE2 (TEK) (Saharinen et al., 2017). TIE1 is an orphan receptor normally required for full activation of TIE2 (Korhonen et al., 2016). ANGPT1 is a constitutive agonist of TIE2 and is largely responsible for basal TIE2 activation, promoting EC quiescence and vessel stabilization. In part, ANGPT1/TIE2 achieves this by activating PI3K/AKT signaling, which phosphorylates and expels the Forkhead box O (FOXO) transcription factor FOXO1 from the nucleus. This prevents FOXO1 from promoting the expression of vascular destabilizing genes, which includes *ANGPT2* (Daly et al., 2004) (Figure 2). ANGPT2 acts predominantly as a competitive inhibitor of ANGPT1, preventing TIE2 activation (Maisonpierre et al., 1997). In some contexts ANGPT2 can be a weak agonist (Saharinen et al., 2017) but the physiological relevance of this is not always clear (Mueller and Kontos, 2016). ANGPT2 inhibition of TIE2 weakens EC-EC junctions, causing vascular destabilization (Saharinen et al., 2008).

Pro-angiogenic signals including VEGFA and hypoxia induce ANGPT2 expression (Mandriota and Pepper, 1998; Oh et al., 1999) and ANGPT2 is expressed specifically in tip cells during angiogenesis (del Toro et al., 2010). Tip cells growing into avascular tissue are exposed to hypoxia, which promotes nuclear import of FOXO1 and upregulation of ANGPT2 in tip cells via activation of the kinase Mammalian sterile 20-like kinase 1 (MST1) (Kim et al., 2019) (Figure 1). *ANGPT2* is also induced by YAP, and restoration of ANGPT2 levels can rescue sprouting defects caused by YAP loss (Choi et al., 2015; Wang et al., 2017). Destabilization of adherens junctions promotes relocation of YAP to the nucleus, which occurs in ECs located at the sprouting front where adherens junctions are less stable and VE-cadherin turnover is highest (Choi et al., 2015; Giampietro et al., 2015; Neto et al., 2018; Wang et al.,

2017) (Figure 1). Tip cells not only express high ANGPT2, but are also characterized by low TIE2 expression, which facilitates direct binding of ANGPT2 to □1 integrin (Felcht et al., 2012; Savant et al., 2015). Integrin activation by ANGPT2 promotes EC migration and sprouting angiogenesis by reducing cortical actin and weakening VE-cadherin-containing adherens junctions (Felcht et al., 2012; Hakanpaa et al., 2015). Indeed, genetic inactivation or pharmacologic inhibition of ANGPT2 prevents sprouting angiogenesis (Felcht et al., 2012; Gale et al., 2002; Hackett et al., 2002). In addition to the destabilizing effects of ANGPT2, effective vessel sprouting also requires intact TIE2 signaling as sprouting angiogenesis is impeded in the absence of TIE2 (Park et al., 2017). Furthermore, TIE2 is needed during sprouting angiogenesis to maintain vessel integrity as those vessels that do form in the absence of TIE2 are leaky, consistent with the role for TIE2 in maintaining vessel barrier integrity (Park et al., 2017).

Vessel remodeling, maturation and stability

Under normal circumstances, the growth of new vessels in response to increased oxygen demand is self-limiting, with demand being alleviated once the new vessels have formed. Nonetheless, negative regulators and feedback mechanisms are necessary to ensure that the angiogenic response is proportionate and is resolved rapidly as the angiogenic stimuli abate allowing the vasculature to return to a stable, quiescent state. Failure of these mechanisms results in vessel overgrowth and associated disease. Furthermore, sprouting angiogenesis initially lays down an immature, uniform plexus of vessels without higher order structure. This must be extensively remodeled to create a mature, hierarchic network capable of efficient blood flow. This requires the removal of some vessel segments and the stabilization and maturation of others (Korn and Augustin, 2015). Once the new vessels are established, they need to be maintained. EC loss or damage can cause vessel regression and hypoxia. Excessive vessel permeability can increases interstitial pressure, exacerbating inflammatory responses, impairing the delivery of therapeutics, and distorting tissues affecting their function (Claesson-Welsh, 2015).

Regulation of VEGFA in vessel quiescence and stability

VEGFA is a potent stimulator of vessel growth and permeability and a causative factor in abnormal vessel growth and leakage in disease (Ferrara and Adamis, 2016; Miller et al., 2013). As such, vessel responses to VEGFA must be tightly controlled. Lowering EC sensitivity to VEGFA is essential for ECs to resume quiescence. At high cell density, VE-cadherin

clustering at cell-cell junctions brings the protein tyrosine phosphatases DEP1 and VE-PTP into proximity with VEGFR2, which dephosphorylate and inactivate it (Grazia Lampugnani et al., 2003; Hayashi et al., 2013). VE-cadherin clustering at junctions also prevents the nuclear accumulation of FOXO1, □-catenin and the core polycomb repressor complex 2 (PRC2) protein EZH2. This results in de-repression of genes necessary for junctional integrity, including *PTPRB*, which encodes VE-PTP (Morini et al., 2018). In addition to dephosphorylating and inactivating VEGFR2, VE-PTP can further stabilize junctions by reversing VEGFR2-dependent phosphorylation of VE-cadherin (Simons et al., 2016). VE-PTP is essential to maintain EC quiescence as inactivating mutations in *PTPRB* are commonly found in angiosarcomas, an aggressive, late-onset cancer of endothelial origin (Behjati et al., 2014; Murali et al., 2015).

Notch lowers the sensitivity of ECs to VEGFA by reducing expression of VEGFR2 while promoting expression of VEGFR1 (Figure 2). Notch also reduces glycolysis by suppressing PFKFB3, consistent with it acting as a brake on the VEGFA-induced sprouting phenotype (De Bock et al., 2013b). Tight control of Notch activity is necessary to maintain EC quiescence and homeostasis in adults. Inhibition of DLL4/Notch signaling in adults also causes hyper-proliferative vascular lesions in the liver and skin (Liu et al., 2011; Yan et al., 2010). Genes normally associated with EC growth and activation were upregulated in these lesions suggesting that DLL4/Notch maintains the quiescent phenotype, at least in these organs (Yan et al., 2010).

As discussed above, VEGFA stimulation (among other signals) can activate YAP and TAZ to drive angiogenesis. Chromosomal translocations involving *WWTR1*, the gene encoding TAZ, account for 90% of epithelioid hemangioendotheliomas (EHE), a rare cancer of endothelial origin (Lamar et al., 2018). The TAZ fusion protein inherits a nuclear localization signal from its fusion partner and localizes to the nucleus where it drives a TAZ-like transcriptional program (Tanas et al., 2016). Those EHE that do not have TAZ fusions instead have chromosomal translocations involving *YAP* (Lamar et al., 2018). This highlights the importance of controlling YAP/TAZ activity in order to maintain EC quiescence.

In addition to promoting vessel growth, VEGFA triggers vessel permeability. Src phosphorylation of VE-cadherin in mature vessels in response to VEGFA weakens cell-cell junctions and increases vessel permeability (Schimmel and Gordon, 2018; Sun et al., 2012) (Figure 2). Dismantling of VE-cadherin and junctions in response to VEGFA occurs in minutes (Honkura et al., 2018). Live-imaging of dermal vessel responses to VEGFA *in vivo* showed that VEGFA-induced permeability only occurred in vessels that were negative for the tight junction

protein claudin 5 (Honkura et al., 2018). These vessels consisted of venules and certain capillaries, but not arterioles. VEGFA induces vasodilation as well as vessel permeability, but whereas vessel permeability caused by VEGFA is rapid and short lived, vasodilation persists well after its permeabilizing effect has resolved (Honkura et al., 2018). Similar to its function during angiogenesis, VEGFR3 determines vessel sensitivity to the permeabilizing effects of VEGFA by lowering VEGFR2 expression, thereby reducing the responsiveness of EC junctions to the destabilizing effects of VEGFA (Heinolainen et al., 2017).

While feedback mechanisms ensure VEGFA signaling is kept in check, basal levels of VEGFA signaling are necessary for vessel maintenance. Blood vessels in many organs are dependent on circulating VEGFA for maintenance early in life, however this dependency declines sharply with age and varies by organ type (Baffert et al., 2004; Gerber et al., 1999). For example, fenestrated endothelium is more dependent on VEGFA for maintenance than ECs in muscle, brain and retina (Kamba et al., 2006; Yang et al., 2013). This selective dependence has implications for anti-VEGFA therapy, particularly in the kidney. Glomerular EC are dependent on VEGFA produced by podocytes and as such, patients receiving anti-VEGFA therapy are prone to proteinurea and in extreme cases, kidney disease (Eremina et al., 2008). Autocrine VEGFA production is also necessary for EC homeostasis through regulation of FOXO1 (Lee et al., 2007). FOXO1 lowers glycolysis and mitochondrial respiration through suppression of Myc (Wilhelm et al., 2016), and autocrine VEGFA keeps FOXO1 at low levels, thereby maintaining a basal level of metabolism necessary to maintain vascular integrity (Domigan et al., 2015). Levels of FOXO1 must be carefully balanced however, as too little FOXO1 results in de-repression of Myc and vascular disruption due to the over-production of ECs (Wilhelm et al., 2016). In adults, the loss of FOXO1 and its related family members FOXO3 and 4 causes endothelial hyperplasia, resulting in hemangioma and angiosarcoma formation (Paik et al., 2007).

While generally considered a vasodilator and maintenance factor, there are contexts in which VEGFA can cause vessel obstruction and regression. Sustained high level VEGFA can causes leukostasis resulting in vessel obstruction and non-perfusion, which may contribute to the progression of ischemic retinal diseases like diabetic retinopathy and retinopathy of prematurity (Liu et al., 2017). Spontaneous occlusions occur in brain capillaries at low frequency. While the majority of these are transient and successfully recanalize, a fraction of occluded will regress, reducing brain capillary density with age (Reeson et al., 2018). Interestingly, ectopic VEGFA reduced recanalization rates in occluded brain vessels, whereas

inhibition of VEGFA signaling enhanced them, resulting in less vessel pruning (Reeson et al., 2018). The mechanisms underlying this effect were not investigated.

BMP signaling in flow-mediated remodeling

Remodeling of newly formed vessel beds leads to the selective removal of some vessel segments and the stabilization and enlargement of others. Cross-talk between hemodynamic forces and ligand-receptor signaling shapes and refines the remodeling vasculature (Korn and Augustin, 2015). Maladaptive vessel responses to remodeling cues, including growth factor inputs can have detrimental consequences (Baeyens et al., 2016a). An example is hereditary hemorrhagic telangiectasia (HHT), a disease characterized by arteriovenous malformations (AVMs) in which an artery connects directly to a vein without an intervening capillary network (Nguyen et al., 2017). These fast-flow lesions have a high risk of rupture as veins become exposed to high-pressure arterial blood flow and short-circuit the vascular network, disrupting blood supply and causing tissue hypoxia (Roman and Hinck, 2017). Cell-autonomous ENG/ALK1 signaling is essential for regulating the adaptive responses of ECs to hemodynamic forces and mutations in ENG and ALK1 account for >80% of HHT cases (Nguyen et al., 2017). Recent studies have shown that perturbation of ENG/ALK1 signaling causes HHT because of defective EC responses to hemodynamic cues. During normal remodeling, ECs become polarized and migrate against the direction of flow (Franco et al., 2015; Rochon et al., 2016; Xu et al., 2014). This directs the movement of ECs out of vessels with low flow and into those with high flow (Chen et al., 2012; Franco et al., 2015; Kochhan et al., 2013; Lenard et al., 2015). This migratory response is reversed in ECs lacking ENG or ALK1, resulting in the expansion of distal arteriolar segments (Jin et al., 2017; Rochon et al., 2016). Sustained increase in blood flow shear also causes vessels to increase in diameter through outward remodeling (Baeyens and Schwartz, 2016). In the absence of ENG, control of this adaptive response fails and vessels become excessively dilated (Sugden et al., 2017). Blood flow induced AVMs also occur in the absence of *Smad4* (Ola et al., 2018). Coronary arteries in mice lacking SMAD4 become dilated and ECs within them fail to orient correctly in response to flow (Poduri et al., 2017). Mechanistically, endothelial BMP signaling is likely linked to the hemodynamic response because blood flow potentiates BMP9 signaling by inducing complex formation between ENG and ALK1 (Baeyens et al., 2016b). AVMs in BMP9, ENG, ALK1 and SMAD4 mutants arise due largely to over-activation of PI3K/AKT signaling downstream of blood flow and VEGFA signaling (Jin et al., 2017; Ola et al., 2016; Ola et al., 2018). BMP9/ALK1/SMAD4 were shown to repress PI3K/AKT through casein kinase 2 (CK2)-dependent regulation of PTEN (Ola et al.,

2016; Ola et al., 2018). ENG was shown to further regulate PI3K/AKT through modulation of VEGFR2 trafficking that normally curtails VEGFR2 signaling output (Jin et al., 2017). VEGFR2 activation in the context of AVMs is further exacerbated by increased expression of VEGFA ligand due to tissue hypoxia downstream of flow disruptions (Jin et al., 2017). As such, reducing VEGFR2 activity or PI3K/AKT largely normalized the AVM phenotype caused by disrupting BMP9/ENG/ALK1/SMAD4 function (Jin et al., 2017; Ola et al., 2016; Ola et al., 2018). These studies highlight how BMP signaling integrates the response of ECs to both angiogenic regulatory growth factor signals and mechanosensory signals to prevent runaway vessel growth and remodeling.

Angiopoietin and pericytes in vessel remodeling and stability

Angiopoietin/TIE2 signaling regulates vessel remodeling, stability and quiescence (Augustin et al., 2009; Korn and Augustin, 2015; Saharinen et al., 2017). Although only weakly mitogenic, high levels of ANGPT1 promote EC proliferation resulting in circumferential vessel expansion and the conversion of capillaries into larger venules (Baffert et al., 2004; Cho et al., 2005; Thurston et al., 2005). Similarly, mutations resulting in ligand-independent TIE2 activation in humans cause sporadic and familial venous malformations (Nguyen et al., 2017). Paradoxically, TIE2 inactivation causes similar vessel remodeling to TIE2 over-activation. The main difference is that whereas TIE2 inactivation is associated with vessel leakage, vascular integrity is maintained when remodeling is driven by TIE2 activation (Kim et al., 2016). TIE2 stabilizes cell-cell junctions through VE-cadherin-dependent and -independent mechanisms (Frye et al., 2015), as well as by dephosphorylating VEGFR2 via VE-PTP (Hayashi et al., 2013). Activation of TIE2 further maintains vessel stability by repressing FOXO1-driven ANGPT2 expression (Figure 2). While ANGPT2 can promote leak-free vessel remodeling as a weak TIE2 agonist in healthy vessels (Kim et al., 2016), it only does so when present at high levels. It also becomes a TIE2 antagonist in inflamed vessels (Kim et al., 2016; Korhonen et al., 2016). This is because TIE1, which promotes the agonistic activity of ANGPT2 and ANGPT1, is cleaved from the surface of inflamed ECs (Korhonen et al., 2016). This reduces TIE2 activation and creates a feedback loop in which FOXO1-driven ANGPT2 expression leads to further TIE2 antagonism, resulting in vessel remodeling, destabilization and leakage (Kim et al., 2016; Korhonen et al., 2016). Consistent with this, increased ANGPT2 serum levels are correlated with poor prognosis in a number of inflammatory conditions and treatments that reduce ANGPT2 or activate TIE2 signaling are protective in animal models (Saharinen et al., 2017).

Pericytes have important roles in establishing and maintaining vessel stability through cross-talk with angiopoietin signaling. Compromised pericyte support can have detrimental effects on the vasculature. For example, the progressive loss of pericytes and their support function is believed to trigger the retinal vascular anomalies associated with diabetic retinopathy (Stitt et al., 2016). ANGPT2 can cause pericyte dropout from retinal vessels and high levels of ANGPT2 are found in the ocular fluid of patients with diabetic retinopathy (Hammes et al., 2004). Indeed, failure to recruit pericytes to growing retinal vessels results in capillary regression, microaneurysms, vessel leakage and inflammation – all hallmarks of diabetic retinopathy (Enge et al., 2002; Park et al., 2017). In these growing vessels, pericytes promote vessel stability through TIE2 activation, repressing FOXO1 and ANGPT2 (Park et al., 2017). In line with this, activation of TIE2 by ectopic ANGPT1 can compensate for the absence of pericytes (Uemura et al., 2002). While pericytes in some organs like the lungs are a source of ANGPT1 (Kato et al., 2018), this is not the case in the retina (Kato et al., 2018; Park et al., 2017). Instead, in the absence of pericytes, retinal vessels shed TIE1, which likely causes reduced TIE2 activation followed by FOXO1-driven ANGPT2 expression similar to inflammatory states described above (Park et al., 2017).

Despite their role in the growing retinal vasculature, pericytes were not required for the maintenance of baseline vessel stability in adults (Park et al., 2017). The ablation of pericytes from adult retinal vessels did however sensitize them to the destabilizing effects of VEGFA (Park et al., 2017), which is known to be elevated in eyes with diabetic retinopathy (Miller et al., 2013). While dispensable in the adult retina, pericytes are continuously required for baseline vessel stability in other organs, particularly the skin and lung indicating an organ and context-specific role for pericytes in vessel stabilization (Frye et al., 2015; Park et al., 2017). Similarly, ongoing angiopoietin signaling is needed for the maintenance of some vessels but not others. TIE2 is continuously required for baseline vessel stability in the skin and lung, but not the retina (Frye et al., 2015; Park et al., 2017).

Angiopoietin signaling is also required by the Schlemm's canal, a hybrid blood-lymphatic vessel in the eye necessary for intraocular fluid drainage. Inactivating mutations in TIE2 and ANGPT1 cause primary congenital glaucoma due to under-development of Schlemm's canal (Kim et al., 2017b; Souma et al., 2016; Thomson et al., 2014). ANGPT/TIE2 signaling is not only required for the initial formation of Schlemm's canal, but also for its ongoing maintenance and function (Kim et al., 2017b). Unlike its destabilizing role in the blood vasculature, ANGPT2 can compensate for loss of ANGPT1 during Schlemm's canal development and maintenance (Kim et al., 2017b; Thomson et al., 2014). This may have implications for

therapies under development that are aimed at altering angiopoietin signaling (Saharinen et al., 2017).

Conclusions

Growth factor signaling has a central role in vascular development and maintenance. Modulating the response to these factors is essential in order to ensure the correct development of the network, to maintain homeostasis and to prevent disease. While current inhibitors of angiogenesis that target VEGFA signaling have been ground-breaking therapies for some diseases, particularly in the eye, there is still much room for further clinical benefit. A better understanding of how growth factors regulate vessel growth and homeostasis in diverse contexts will pave the way for rational strategies to improve current therapies, identify new disease treatments and inform on the potential for counterproductive side-effects. This highlights the need for a better understanding of when and how growth factors regulate vessel growth and homeostasis. Given the tissue-specific heterogeneity of the vasculature at the molecular and morphological level (Augustin and Koh, 2017; Potente and Makinen, 2017), it will be particularly important to understand the effect of growth factor pathways on vascular behavior across different organ types to fully understand the consequences of manipulating them.

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No potential conflict of interest was reported by the authors.

References

Achen, M. G., Jeltsch, M., Kukk, E., Makinen, T., Vitali, A., Wilks, A. F., Alitalo, K. and Stacker, S. A. (1998). Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc Natl Acad Sci U S A* **95**, 548-553.

Armulik, A., Genove, G. and Betsholtz, C. (2011). Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Developmental cell* **21**, 193-215.

Aspalter, I. M., Gordon, E., Dubrac, A., Ragab, A., Narloch, J., Vizan, P., Geudens, I., Collins, R. T., Franco, C. A., Abrahams, C. L., et al. (2015). Alk1 and Alk5 inhibition by Nrp1 controls vascular sprouting downstream of Notch. *Nat Commun* **6**, 7264.

Atri, D., Larrivee, B., Eichmann, A. and Simons, M. (2013). Endothelial signaling and the molecular basis of arteriovenous malformation. *Cellular and molecular life sciences : CMLS*.

Augustin, H. G. and Koh, G. Y. (2017). Organotypic vasculature: From descriptive heterogeneity to functional pathophysiology. *Science* **357**.

Augustin, H. G., Koh, G. Y., Thurston, G. and Alitalo, K. (2009). Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. *Nature reviews. Molecular cell biology* **10**, 165-177.

Avraamides, C. J., Garmy-Susini, B. and Varner, J. A. (2008). Integrins in angiogenesis and lymphangiogenesis. *Nat Rev Cancer* **8**, 604-617.

Baeyens, N., Bandyopadhyay, C., Coon, B. G., Yun, S. and Schwartz, M. A. (2016a). Endothelial fluid shear stress sensing in vascular health and disease. *J Clin Invest* **126**, 821-828.

Baeyens, N., Larrivee, B., Ola, R., Hayward-Piatkowskyi, B., Dubrac, A., Huang, B., Ross, T. D., Coon, B. G., Min, E., Tsarfati, M., et al. (2016b). Defective fluid shear stress mechanotransduction mediates hereditary hemorrhagic telangiectasia. *J Cell Biol* **214**, 807-816.

Baeyens, N. and Schwartz, M. A. (2016). Biomechanics of vascular mechanosensation and remodeling. *Mol Biol Cell* **27**, 7-11.

Baffert, F., Thurston, G., Rochon-Duck, M., Le, T., Brekken, R. and McDonald, D. M. (2004). Age-related changes in vascular endothelial growth factor dependency and angiopoietin-1-induced plasticity of adult blood vessels. *Circ Res* **94**, 984-992.

Beets, K., Huylebroeck, D., Moya, I. M., Umans, L. and Zwijsen, A. (2013). Robustness in angiogenesis: notch and BMP shaping waves. *Trends in genetics : TIG* **29**, 140-149.

Behjati, S., Tarpey, P. S., Sheldon, H., Martincorena, I., Van Loo, P., Gundem, G., Wedge, D. C., Ramakrishna, M., Cooke, S. L., Pillay, N., et al. (2014). Recurrent PTPRB and PLCG1 mutations in angiosarcoma. *Nat Genet* **46**, 376-379.

Benedito, R., Rocha, S. F., Woeste, M., Zamykal, M., Radtke, F., Casanovas, O., Duarte, A., Pytowski, B. and Adams, R. H. (2012). Notch-dependent VEGFR3 upregulation allows angiogenesis without VEGF-VEGFR2 signalling. *Nature* **484**, 110-114.

Bentley, K., Franco, C. A., Philippides, A., Blanco, R., Dierkes, M., Gebala, V., Stanchi, F., Jones, M., Aspalter, I. M., Cagna, G., et al. (2014). The role of differential VE-cadherin dynamics in cell rearrangement during angiogenesis. *Nat Cell Biol* **16**, 309-321.

Betz, C., Lenard, A., Belting, H. G. and Affolter, M. (2016). Cell behaviors and dynamics during angiogenesis. *Development* **143**, 2249-2260.

Blanco, R. and Gerhardt, H. (2013). VEGF and Notch in tip and stalk cell selection. *Cold Spring Harb Perspect Med* **3**, a006569.

Carmeliet, P. (2003). Angiogenesis in health and disease. *Nature medicine* **9**, 653-660.

Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M., Fahrig, M., Vandenhoeck, A., Harpal, K., Eberhardt, C., et al. (1996). Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* **380**, 435-439.

Castanares, C., Redondo-Horcajo, M., Magan-Marchal, N., ten Dijke, P., Lamas, S. and Rodriguez-Pascual, F. (2007). Signaling by ALK5 mediates TGF-beta-induced ET-1 expression in endothelial cells: a role for migration and proliferation. *J Cell Sci* **120**, 1256-1266.

Chappell, J. C., Taylor, S. M., Ferrara, N. and Bautch, V. L. (2009). Local guidance of emerging vessel sprouts requires soluble Flt-1. *Dev Cell* **17**, 377-386.

Chen, Q., Jiang, L., Li, C., Hu, D., Bu, J. W., Cai, D. and Du, J. L. (2012). Haemodynamics-driven developmental pruning of brain vasculature in zebrafish. *PLoS Biol* **10**, e1001374.

Cho, C. H., Kim, K. E., Byun, J., Jang, H. S., Kim, D. K., Baluk, P., Baffert, F., Lee, G. M., Mochizuki, N., Kim, J., et al. (2005). Long-term and sustained COMP-Ang1 induces long-lasting vascular enlargement and enhanced blood flow. *Circ Res* **97**, 86-94.

Choi, H. J., Zhang, H., Park, H., Choi, K. S., Lee, H. W., Agrawal, V., Kim, Y. M. and Kwon, Y. G. (2015). Yes-associated protein regulates endothelial cell contact-mediated expression of angiopoietin-2. *Nat Commun* **6**, 6943.

Claesson-Welsh, L. (2015). Vascular permeability--the essentials. *Ups J Med Sci* **120**, 135-143.

Daly, C., Wong, V., Burova, E., Wei, Y., Zabski, S., Griffiths, J., Lai, K. M., Lin, H. C., Ioffe, E., Yancopoulos, G. D., et al. (2004). Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1). *Genes Dev* **18**, 1060-1071.

De Bock, K., Georgiadou, M. and Carmeliet, P. (2013a). Role of endothelial cell metabolism in vessel sprouting. *Cell Metab* **18**, 634-647.

De Bock, K., Georgiadou, M., Schoors, S., Kuchnio, A., Wong, B. W., Cantelmo, A. R., Quaegebeur, A., Ghesquiere, B., Cauwenberghs, S., Eelen, G., et al. (2013b). Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* **154**, 651-663.

del Toro, R., Prahst, C., Mathivet, T., Siegfried, G., Kaminker, J. S., Larrivee, B., Breant, C., Duarte, A., Takakura, N., Fukamizu, A., et al. (2010). Identification and functional analysis of endothelial tip cell-enriched genes. *Blood* **116**, 4025-4033.

Domigan, C. K., Warren, C. M., Antanesian, V., Happel, K., Ziyad, S., Lee, S., Krall, A., Duan, L., Torres-Collado, A. X., Castellani, L. W., et al. (2015). Autocrine VEGF maintains endothelial survival through regulation of metabolism and autophagy. *J Cell Sci* **128**, 2236-2248.

Enge, M., Bjarnegard, M., Gerhardt, H., Gustafsson, E., Kalen, M., Asker, N., Hammes, H. P., Shani, M., Fassler, R. and Betsholtz, C. (2002). Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. *EMBO J* **21**, 4307-4316.

Eremina, V., Jefferson, J. A., Kowalewska, J., Hochster, H., Haas, M., Weisstuch, J., Richardson, C., Kopp, J. B., Kabir, M. G., Backx, P. H., et al. (2008). VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med* **358**, 1129-1136.

Felcht, M., Luck, R., Schering, A., Seidel, P., Srivastava, K., Hu, J., Bartol, A., Kienast, Y., Vettel, C., Loos, E. K., et al. (2012). Angiopoietin-2 differentially regulates angiogenesis through TIE2 and integrin signaling. *J Clin Invest* **122**, 1991-2005.

Ferrara, N. and Adamis, A. P. (2016). Ten years of anti-vascular endothelial growth factor therapy. *Nat Rev Drug Discov* **15**, 385-403.

Ferrara, N., Carver-Moore, K., Chen, H., Dowd, M., Lu, L., O'Shea, K. S., Powell-Braxton, L., Hillan, K. J. and Moore, M. W. (1996). Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* **380**, 439-442.

Franco, C. A., Jones, M. L., Bernabeu, M. O., Geudens, I., Mathivet, T., Rosa, A., Lopes, F. M., Lima, A. P., Ragab, A., Collins, R. T., et al. (2015). Dynamic endothelial cell rearrangements drive developmental vessel regression. *PLoS Biol* **13**, e1002125.

Frye, M., Dierkes, M., Kuppers, V., Vockel, M., Tomm, J., Zeuschner, D., Rossaint, J., Zarbock, A., Koh, G. Y., Peters, K., et al. (2015). Interfering with VE-PTP stabilizes endothelial junctions in vivo via Tie-2 in the absence of VE-cadherin. *J Exp Med* **212**, 2267-2287.

Gale, N. W., Thurston, G., Hackett, S. F., Renard, R., Wang, Q., McClain, J., Martin, C., Witte, C., Witte, M. H., Jackson, D., et al. (2002). Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1. *Dev Cell* **3**, 411-423.

Gerber, H. P., Hillan, K. J., Ryan, A. M., Kowalski, J., Keller, G. A., Rangell, L., Wright, B. D., Radtke, F., Aguet, M. and Ferrara, N. (1999). VEGF is required for growth and survival in neonatal mice. *Development* **126**, 1149-1159.

Gerhardt, H., Golding, M., Fruttiger, M., Ruhrberg, C., Lundkvist, A., Abramsson, A., Jeltsch, M., Mitchell, C., Alitalo, K., Shima, D., et al. (2003). VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol* **161**, 1163-1177.

Giampietro, C., Disanza, A., Bravi, L., Barrios-Rodiles, M., Corada, M., Frittoli, E., Savorani, C., Lampugnani, M. G., Boggetti, B., Niessen, C., et al. (2015). The actin-binding protein EPS8 binds VE-cadherin and modulates YAP localization and signaling. *J Cell Biol* **211**, 1177-1192.

Giannotta, M., Trani, M. and Dejana, E. (2013). VE-cadherin and endothelial adherens junctions: active guardians of vascular integrity. *Developmental cell* **26**, 441-454.

Gordon, E. J., Fukuhara, D., Westrom, S., Padhan, N., Sjostrom, E. O., van Meeteren, L., He, L., Orsenigo, F., Dejana, E., Bentley, K., et al. (2016). The endothelial adaptor molecule TSAd is required for VEGF-induced angiogenic sprouting through junctional c-Src activation. *Sci Signal* **9**, ra72.

Goumans, M. J., Liu, Z. and ten Dijke, P. (2009). TGF-beta signaling in vascular biology and dysfunction. *Cell Res* **19**, 116-127.

Goumans, M. J. and Ten Dijke, P. (2018). TGF-beta Signaling in Control of Cardiovascular Function. *Cold Spring Harb Perspect Biol* **10**.

Goumans, M. J., Zwijsen, A., Ten Dijke, P. and Bailly, S. (2018). Bone Morphogenetic Proteins in Vascular Homeostasis and Disease. *Cold Spring Harb Perspect Biol* **10**.

Grazia Lampugnani, M., Zanetti, A., Corada, M., Takahashi, T., Balconi, G., Breviario, F., Orsenigo, F., Cattelino, A., Kemler, R., Daniel, T. O., et al. (2003). Contact inhibition of VEGF-induced proliferation requires vascular endothelial cadherin, beta-catenin, and the phosphatase DEP-1/CD148. *J Cell Biol* **161**, 793-804.

Guo, D., Jia, Q., Song, H. Y., Warren, R. S. and Donner, D. B. (1995). Vascular endothelial cell growth factor promotes tyrosine phosphorylation of mediators of signal transduction that contain SH2 domains. Association with endothelial cell proliferation. *J Biol Chem* **270**, 6729-6733.

Hackett, S. F., Wiegand, S., Yancopoulos, G. and Campochiaro, P. A. (2002). Angiopoietin-2 plays an important role in retinal angiogenesis. *J Cell Physiol* **192**, 182-187.

Hakanpaa, L., Sipila, T., Leppanen, V. M., Gautam, P., Nurmi, H., Jacquemet, G., Eklund, L., Ivaska, J., Alitalo, K. and Saharinen, P. (2015). Endothelial destabilization by angiopoietin-2 via integrin beta1 activation. *Nat Commun* **6**, 5962.

Hammes, H. P., Lin, J., Wagner, P., Feng, Y., Vom Hagen, F., Krzizok, T., Renner, O., Breier, G., Brownlee, M. and Deutsch, U. (2004). Angiopoietin-2 causes pericyte dropout in the normal retina: evidence for involvement in diabetic retinopathy. *Diabetes* **53**, 1104-1110.

Harrington, L. S., Sainson, R. C., Williams, C. K., Taylor, J. M., Shi, W., Li, J. L. and Harris, A. L. (2008). Regulation of multiple angiogenic pathways by Dll4 and Notch in human umbilical vein endothelial cells. *Microvasc Res* **75**, 144-154.

Hayashi, M., Majumdar, A., Li, X., Adler, J., Sun, Z., Vertuani, S., Hellberg, C., Mellberg, S., Koch, S., Dimberg, A., et al. (2013). VE-PTP regulates VEGFR2 activity in stalk cells to establish endothelial cell polarity and lumen formation. *Nat Commun* **4**, 1672.

Heinolainen, K., Karaman, S., D'Amico, G., Tammela, T., Sormunen, R., Eklund, L., Alitalo, K. and Zarkada, G. (2017). VEGFR3 Modulates Vascular Permeability by Controlling VEGF/VEGFR2 Signaling. *Circ Res* **120**, 1414-1425.

Hellstrom, M., Phng, L. K., Hofmann, J. J., Wallgard, E., Coultas, L., Lindblom, P., Alva, J., Nilsson, A. K., Karlsson, L., Gaiano, N., et al. (2007). Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature* **445**, 776-780.

Hiratsuka, S., Minowa, O., Kuno, J., Noda, T. and Shibuya, M. (1998). Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice. *Proc Natl Acad Sci U S A* **95**, 9349-9354.

Honkura, N., Richards, M., Lavina, B., Sainz-Jaspeado, M., Betsholtz, C. and Claesson-Welsh, L. (2018). Intravital imaging-based analysis tools for vessel identification and assessment of concurrent dynamic vascular events. *Nature communications* **9**, 2746.

Huang, K., Andersson, C., Roomans, G. M., Ito, N. and Claesson-Welsh, L. (2001). Signaling properties of VEGF receptor-1 and -2 homo- and heterodimers. *Int J Biochem Cell Biol* **33**, 315-324.

Jakobsson, L., Franco, C. A., Bentley, K., Collins, R. T., Ponsioen, B., Aspalter, I. M., Rosewell, I., Busse, M., Thurston, G., Medvinsky, A., et al. (2010). Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat Cell Biol* **12**, 943-953.

Jin, Y., Muhl, L., Burmakin, M., Wang, Y., Duchez, A. C., Betsholtz, C., Arthur, H. M. and Jakobsson, L. (2017). Endoglin prevents vascular malformation by regulating flow-induced cell migration and specification through VEGFR2 signalling. *Nat Cell Biol* **19**, 639-652.

Joukov, V., Pajusola, K., Kaipainen, A., Chilov, D., Lahtinen, I., Kukk, E., Saksela, O., Kalkkinen, N. and Alitalo, K. (1996). A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *EMBO J* **15**, 290-298.

Kamba, T., Tam, B. Y., Hashizume, H., Haskell, A., Sennino, B., Mancuso, M. R., Norberg, S. M., O'Brien, S. M., Davis, R. B., Gowen, L. C., et al. (2006). VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. *Am J Physiol Heart Circ Physiol* **290**, H560-576.

Kato, K., Dieguez-Hurtado, R., Park, D. Y., Hong, S. P., Kato-Azuma, S., Adams, S., Stehling, M., Trappmann, B., Wrana, J. L., Koh, G. Y., et al. (2018). Pulmonary pericytes regulate lung morphogenesis. *Nat Commun* **9**, 2448.

Kendall, R. L. and Thomas, K. A. (1993). Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci U S A* **90**, 10705-10709.

Kim, J., Kim, Y. H., Kim, J., Park, D. Y., Bae, H., Lee, D. H., Kim, K. H., Hong, S. P., Jang, S. P., Kubota, Y., et al. (2017a). YAP/TAZ regulates sprouting angiogenesis and vascular barrier maturation. *J Clin Invest* **127**, 3441-3461.

Kim, J., Park, D. Y., Bae, H., Park, D. Y., Kim, D., Lee, C. K., Song, S., Chung, T. Y., Lim, D. H., Kubota, Y., et al. (2017b). Impaired angiopoietin/Tie2 signaling compromises Schlemm's canal integrity and induces glaucoma. *J Clin Invest* **127**, 3877-3896.

Kim, M., Allen, B., Korhonen, E. A., Nitschke, M., Yang, H. W., Baluk, P., Saharinen, P., Alitalo, K., Daly, C., Thurston, G., et al. (2016). Opposing actions of angiopoietin-2 on Tie2 signaling and FOXO1 activation. *J Clin Invest* **126**, 3511-3525.

Kim, Y. H., Choi, J., Yang, M. J., Hong, S. P., Lee, C. K., Kubota, Y., Lim, D. S. and Koh, G. Y. (2019). A MST1-FOXO1 cascade establishes endothelial tip cell polarity and facilitates sprouting angiogenesis. *Nat Commun* **10**, 838.

Kochhan, E., Lenard, A., Ellertsdottir, E., Herwig, L., Affolter, M., Belting, H. G. and Siekmann, A. F. (2013). Blood flow changes coincide with cellular rearrangements during blood vessel pruning in zebrafish embryos. *PLoS One* **8**, e75060.

Korhonen, E. A., Lampinen, A., Giri, H., Anisimov, A., Kim, M., Allen, B., Fang, S., D'Amico, G., Sipila, T. J., Lohela, M., et al. (2016). Tie1 controls angiopoietin function in vascular remodeling and inflammation. *J Clin Invest* **126**, 3495-3510.

Korn, C. and Augustin, H. G. (2015). Mechanisms of Vessel Pruning and Regression. *Dev Cell* **34**, 5-17.

Kume, T. (2012). Ligand-dependent Notch signaling in vascular formation. *Adv Exp Med Biol* **727**, 210-222.

Kutys, M. L. and Chen, C. S. (2016). Forces and mechanotransduction in 3D vascular biology. *Curr Opin Cell Biol* **42**, 73-79.

Lamar, J. M., Motilal Nehru, V. and Weinberg, G. (2018). Epithelioid Hemangioendothelioma as a Model of YAP/TAZ-Driven Cancer: Insights from a Rare Fusion Sarcoma. *Cancers (Basel)* **10**.

Larrivee, B., Prahst, C., Gordon, E., del Toro, R., Mathivet, T., Duarte, A., Simons, M. and Eichmann, A. (2012). ALK1 signaling inhibits angiogenesis by cooperating with the Notch pathway. *Dev Cell* **22**, 489-500.

Lebrin, F., Goumans, M. J., Jonker, L., Carvalho, R. L., Valdimarsdottir, G., Thorikay, M., Mummery, C., Arthur, H. M. and ten Dijke, P. (2004). Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. *EMBO J* **23**, 4018-4028.

Lee, H. W., Chong, D. C., Ola, R., Dunworth, W. P., Meadows, S., Ka, J., Kaartinen, V. M., Qyang, Y., Cleaver, O., Bautch, V. L., et al. (2017). Alk2/ACVR1 and

Alk3/BMPR1A Provide Essential Function for Bone Morphogenetic Protein-Induced Retinal Angiogenesis. *Arterioscler Thromb Vasc Biol* **37**, 657-663.

Lee, S., Chen, T. T., Barber, C. L., Jordan, M. C., Murdock, J., Desai, S., Ferrara, N., Nagy, A., Roos, K. P. and Iruela-Arispe, M. L. (2007). Autocrine VEGF signaling is required for vascular homeostasis. *Cell* **130**, 691-703.

Lenard, A., Daetwyler, S., Betz, C., Ellertsdottir, E., Belting, H. G., Huisken, J. and Affolter, M. (2015). Endothelial cell self-fusion during vascular pruning. *PLoS Biol* **13**, e1002126.

Leung, D. W., Cachianes, G., Kuang, W. J., Goeddel, D. V. and Ferrara, N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* **246**, 1306-1309.

Liu, Y., Shen, J., Fortmann, S. D., Wang, J., Vestweber, D. and Campochiaro, P. A. (2017). Reversible retinal vessel closure from VEGF-induced leukocyte plugging. *JCI Insight* **2**.

Liu, Z., Turkoz, A., Jackson, E. N., Corbo, J. C., Engelbach, J. A., Garbow, J. R., Piwnica-Worms, D. R. and Kopan, R. (2011). Notch1 loss of heterozygosity causes vascular tumors and lethal hemorrhage in mice. *J Clin Invest* **121**, 800-808.

Liu, Z. J., Shirakawa, T., Li, Y., Soma, A., Oka, M., Dotto, G. P., Fairman, R. M., Velazquez, O. C. and Herlyn, M. (2003). Regulation of Notch1 and Dll4 by vascular endothelial growth factor in arterial endothelial cells: implications for modulating arteriogenesis and angiogenesis. *Mol Cell Biol* **23**, 14-25.

Lobov, I. B., Renard, R. A., Papadopoulos, N., Gale, N. W., Thurston, G., Yancopoulos, G. D. and Wiegand, S. J. (2007). Delta-like ligand 4 (Dll4) is induced by VEGF as a negative regulator of angiogenic sprouting. *Proc Natl Acad Sci U S A* **104**, 3219-3224.

Maglione, D., Guerriero, V., Viglietto, G., Delli-Bovi, P. and Persico, M. G. (1991). Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. *Proc Natl Acad Sci U S A* **88**, 9267-9271.

Maisonpierre, P. C., Suri, C., Jones, P. F., Bartunkova, S., Wiegand, S. J., Radziejewski, C., Compton, D., McClain, J., Aldrich, T. H., Papadopoulos, N., et al. (1997). Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* **277**, 55-60.

Mandriota, S. J. and Pepper, M. S. (1998). Regulation of angiopoietin-2 mRNA levels in bovine microvascular endothelial cells by cytokines and hypoxia. *Circ Res* **83**, 852-859.

Miller, J. W., Le Couter, J., Strauss, E. C. and Ferrara, N. (2013). Vascular endothelial growth factor a in intraocular vascular disease. *Ophthalmology* **120**, 106-114.

Morini, M. F., Giampietro, C., Corada, M., Pisati, F., Lavarone, E., Cunha, S. I., Conze, L. L., O'Reilly, N., Joshi, D., Kjaer, S., et al. (2018). VE-Cadherin-Mediated Epigenetic Regulation of Endothelial Gene Expression. *Circ Res* **122**, 231-245.

Moya, I. M., Umans, L., Maas, E., Pereira, P. N., Beets, K., Francis, A., Sents, W., Robertson, E. J., Mummery, C. L., Huylebroeck, D., et al. (2012). Stalk cell phenotype depends on integration of Notch and Smad1/5 signaling cascades. *Dev Cell* **22**, 501-514.

Mueller, S. B. and Kontos, C. D. (2016). Tie1: an orphan receptor provides context for angiopoietin-2/Tie2 signaling. *J Clin Invest* **126**, 3188-3191.

Murali, R., Chandramohan, R., Moller, I., Scholz, S. L., Berger, M., Huberman, K., Viale, A., Pirun, M., Socci, N. D., Bouvier, N., et al. (2015). Targeted massively parallel sequencing of angiosarcomas reveals frequent activation of the mitogen activated protein kinase pathway. *Oncotarget* **6**, 36041-36052.

Neto, F., Klaus-Bergmann, A., Ong, Y. T., Alt, S., Vion, A. C., Szymborska, A., Carvalho, J. R., Hollfinger, I., Bartels-Klein, E., Franco, C. A., et al. (2018). YAP and TAZ regulate adherens junction dynamics and endothelial cell distribution during vascular development. *Elife* **7**.

Nguyen, H. L., Boon, L. M. and Vikkula, M. (2017). Vascular Anomalies Caused by Abnormal Signaling within Endothelial Cells: Targets for Novel Therapies. *Semin Intervent Radiol* **34**, 233-238.

Oh, H., Takagi, H., Suzuma, K., Otani, A., Matsumura, M. and Honda, Y. (1999). Hypoxia and vascular endothelial growth factor selectively up-regulate angiopoietin-2 in bovine microvascular endothelial cells. *J Biol Chem* **274**, 15732-15739.

Ola, R., Dubrac, A., Han, J., Zhang, F., Fang, J. S., Larrivee, B., Lee, M., Urarte, A. A., Kraehling, J. R., Genet, G., et al. (2016). PI3 kinase inhibition improves vascular malformations in mouse models of hereditary haemorrhagic telangiectasia. *Nat Commun* **7**, 13650.

Ola, R., Kunzel, S. H., Zhang, F., Genet, G., Chakraborty, R., Pibouin-Fragner, L., Martin, K., Sessa, W., Dubrac, A. and Eichmann, A. (2018). SMAD4 Prevents Flow Induced Arteriovenous Malformations by Inhibiting Casein Kinase 2. *Circulation* **138**, 2379-2394.

Olofsson, B., Pajusola, K., Kaipainen, A., von Euler, G., Joukov, V., Saksela, O., Orpana, A., Pettersson, R. F., Alitalo, K. and Eriksson, U. (1996). Vascular endothelial growth factor B, a novel growth factor for endothelial cells. *Proc Natl Acad Sci U S A* **93**, 2576-2581.

Paik, J. H., Kollipara, R., Chu, G., Ji, H., Xiao, Y., Ding, Z., Miao, L., Tothova, Z., Horner, J. W., Carrasco, D. R., et al. (2007). FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. *Cell* **128**, 309-323.

Pajusola, K., Aprelikova, O., Korhonen, J., Kaipainen, A., Pertovaara, L., Alitalo, R. and Alitalo, K. (1992). FLT4 receptor tyrosine kinase contains seven immunoglobulin-like loops and is expressed in multiple human tissues and cell lines. *Cancer Res* **52**, 5738-5743.

Park, D. Y., Lee, J., Kim, J., Kim, K., Hong, S., Han, S., Kubota, Y., Augustin, H. G., Ding, L., Kim, J. W., et al. (2017). Plastic roles of pericytes in the blood-retinal barrier. *Nat Commun* **8**, 15296.

Parker, M. W., Xu, P., Li, X. and Vander Kooi, C. W. (2012). Structural basis for selective vascular endothelial growth factor-A (VEGF-A) binding to neuropilin-1. *J Biol Chem* **287**, 11082-11089.

Parra-Bonilla, G., Alvarez, D. F., Al-Mehdi, A. B., Alexeyev, M. and Stevens, T. (2010). Critical role for lactate dehydrogenase A in aerobic glycolysis that sustains pulmonary microvascular endothelial cell proliferation. *Am J Physiol Lung Cell Mol Physiol* **299**, L513-522.

Poduri, A., Chang, A. H., Raftrey, B., Rhee, S., Van, M. and Red-Horse, K. (2017). Endothelial cells respond to the direction of mechanical stimuli through SMAD signaling to regulate coronary artery size. *Development* **144**, 3241-3252.

Potente, M. and Carmeliet, P. (2017). The Link Between Angiogenesis and Endothelial Metabolism. *Annu Rev Physiol* **79**, 43-66.

Potente, M. and Makinen, T. (2017). Vascular heterogeneity and specialization in development and disease. *Nat Rev Mol Cell Biol* **18**, 477-494.

Reeson, P., Choi, K. and Brown, C. E. (2018). VEGF signaling regulates the fate of obstructed capillaries in mouse cortex. *Elife* **7**.

Rochon, E. R., Menon, P. G. and Roman, B. L. (2016). Alk1 controls arterial endothelial cell migration in lumenized vessels. *Development* **143**, 2593-2602.

Roman, B. L. and Hinck, A. P. (2017). ALK1 signaling in development and disease: new paradigms. *Cellular and molecular life sciences : CMLS* **74**, 4539-4560.

Ruhrberg, C., Gerhardt, H., Golding, M., Watson, R., Ioannidou, S., Fujisawa, H., Betsholtz, C. and Shima, D. T. (2002). Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. *Genes Dev* **16**, 2684-2698.

Saharinen, P., Eklund, L. and Alitalo, K. (2017). Therapeutic targeting of the angiopoietin-TIE pathway. *Nat Rev Drug Discov* **16**, 635-661.

Saharinen, P., Eklund, L., Miettinen, J., Wirkkala, R., Anisimov, A., Winderlich, M., Nottebaum, A., Vestweber, D., Deutsch, U., Koh, G. Y., et al. (2008). Angiopoietins assemble distinct Tie2 signalling complexes in endothelial cell-cell and cell-matrix contacts. *Nat Cell Biol* **10**, 527-537.

Sakabe, M., Fan, J., Odaka, Y., Liu, N., Hassan, A., Duan, X., Stump, P., Byerly, L., Donaldson, M., Hao, J., et al. (2017). YAP/TAZ-CDC42 signaling regulates vascular tip cell migration. *Proc Natl Acad Sci U S A* **114**, 10918-10923.

Savant, S., La Porta, S., Budnik, A., Busch, K., Hu, J., Tisch, N., Korn, C., Valls, A. F., Benest, A. V., Terhardt, D., et al. (2015). The Orphan Receptor Tie1 Controls Angiogenesis and Vascular Remodeling by Differentially Regulating Tie2 in Tip and Stalk Cells. *Cell Rep* **12**, 1761-1773.

Schimmel, L. and Gordon, E. (2018). The precise molecular signals that control endothelial cell-cell adhesion within the vessel wall. *Biochem Soc Trans* **46**, 1673-1680.

Shalaby, F., Rossant, J., Yamaguchi, T. P., Gertsenstein, M., Wu, X. F., Breitman, M. L. and Schuh, A. C. (1995). Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* **376**, 62-66.

Shibuya, M., Yamaguchi, S., Yamane, A., Ikeda, T., Tojo, A., Matsushime, H. and Sato, M. (1990). Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family. *Oncogene* **5**, 519-524.

Siekmann, A. F., Affolter, M. and Belting, H. G. (2013). The tip cell concept 10 years after: new players tune in for a common theme. *Experimental cell research* **319**, 1255-1263.

Simons, M., Gordon, E. and Claesson-Welsh, L. (2016). Mechanisms and regulation of endothelial VEGF receptor signalling. *Nat Rev Mol Cell Biol* **17**, 611-625.

Soker, S., Takashima, S., Miao, H. Q., Neufeld, G. and Klagsbrun, M. (1998). Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. *Cell* **92**, 735-745.

Souma, T., Tompson, S. W., Thomson, B. R., Siggs, O. M., Kizhatil, K., Yamaguchi, S., Feng, L., Limviphuvadh, V., Whisenhunt, K. N., Maurer-Stroh, S., et al. (2016).

Angiopoietin receptor TEK mutations underlie primary congenital glaucoma with variable expressivity. *J Clin Invest* **126**, 2575-2587.

Stitt, A. W., Curtis, T. M., Chen, M., Medina, R. J., McKay, G. J., Jenkins, A., Gardiner, T. A., Lyons, T. J., Hammes, H. P., Simo, R., et al. (2016). The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res* **51**, 156-186.

Strasser, G. A., Kaminker, J. S. and Tessier-Lavigne, M. (2010). Microarray analysis of retinal endothelial tip cells identifies CXCR4 as a mediator of tip cell morphology and branching. *Blood* **115**, 5102-5110.

Suchting, S., Freitas, C., le Noble, F., Benedito, R., Breant, C., Duarte, A. and Eichmann, A. (2007). The Notch ligand Delta-like 4 negatively regulates endothelial tip cell formation and vessel branching. *Proc Natl Acad Sci U S A* **104**, 3225-3230.

Sugden, W. W., Meissner, R., Aegerter-Wilmsen, T., Tsaryk, R., Leonard, E. V., Bussmann, J., Hamm, M. J., Herzog, W., Jin, Y., Jakobsson, L., et al. (2017). Endoglin controls blood vessel diameter through endothelial cell shape changes in response to haemodynamic cues. *Nat Cell Biol* **19**, 653-665.

Sun, Z., Li, X., Massena, S., Kutschera, S., Padhan, N., Gualandi, L., Sundvold-Gjerstad, V., Gustafsson, K., Choy, W. W., Zang, G., et al. (2012). VEGFR2 induces c-Src signaling and vascular permeability in vivo via the adaptor protein TSAd. *J Exp Med* **209**, 1363-1377.

Tammela, T., Zarkada, G., Nurmi, H., Jakobsson, L., Heinolainen, K., Tvorogov, D., Zheng, W., Franco, C. A., Murtomaki, A., Aranda, E., et al. (2011). VEGFR-3 controls tip to stalk conversion at vessel fusion sites by reinforcing Notch signalling. *Nat Cell Biol* **13**, 1202-1213.

Tammela, T., Zarkada, G., Wallgard, E., Murtomaki, A., Suchting, S., Wirzenius, M., Waltari, M., Hellstrom, M., Schomber, T., Peltonen, R., et al. (2008). Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. *Nature* **454**, 656-660.

Tanas, M. R., Ma, S., Jadaan, F. O., Ng, C. K., Weigelt, B., Reis-Filho, J. S. and Rubin, B. P. (2016). Mechanism of action of a WWTR1(TAZ)-CAMTA1 fusion oncoprotein. *Oncogene* **35**, 929-938.

Terman, B. I., Carrion, M. E., Kovacs, E., Rasmussen, B. A., Eddy, R. L. and Shows, T. B. (1991). Identification of a new endothelial cell growth factor receptor tyrosine kinase. *Oncogene* **6**, 1677-1683.

Thomson, B. R., Heinen, S., Jeansson, M., Ghosh, A. K., Fatima, A., Sung, H. K., Onay, T., Chen, H., Yamaguchi, S., Economides, A. N., et al. (2014). A lymphatic defect causes ocular hypertension and glaucoma in mice. *J Clin Invest* **124**, 4320-4324.

Thurston, G., Wang, Q., Baffert, F., Rudge, J., Papadopoulos, N., Jean-Guillaume, D., Wiegand, S., Yancopoulos, G. D. and McDonald, D. M. (2005). Angiopoietin 1 causes vessel enlargement, without angiogenic sprouting, during a critical developmental period. *Development* **132**, 3317-3326.

Totaro, A., Panciera, T. and Piccolo, S. (2018). YAP/TAZ upstream signals and downstream responses. *Nature cell biology* **20**, 888-899.

Tual-Chalot, S., Mahmoud, M., Allinson, K. R., Redgrave, R. E., Zhai, Z., Oh, S. P., Fruttiger, M. and Arthur, H. M. (2014). Endothelial depletion of Acvrl1 in mice leads to arteriovenous malformations associated with reduced endoglin expression. *PLoS One* **9**, e98646.

Uemura, A., Ogawa, M., Hirashima, M., Fujiwara, T., Koyama, S., Takagi, H., Honda, Y., Wiegand, S. J., Yancopoulos, G. D. and Nishikawa, S. (2002). Recombinant angiopoietin-1 restores higher-order architecture of growing blood vessels in mice in the absence of mural cells. *J Clin Invest* **110**, 1619-1628.

Upton, P. D., Davies, R. J., Trembath, R. C. and Morrell, N. W. (2009). Bone morphogenetic protein (BMP) and activin type II receptors balance BMP9 signals mediated by activin receptor-like kinase-1 in human pulmonary artery endothelial cells. *J Biol Chem* **284**, 15794-15804.

Vaahtomeri, K., Karaman, S., Makinen, T. and Alitalo, K. (2017). Lymphangiogenesis guidance by paracrine and pericellular factors. *Genes Dev* **31**, 1615-1634.

Wang, X., Freire Valls, A., Schermann, G., Shen, Y., Moya, I. M., Castro, L., Urban, S., Solecki, G. M., Winkler, F., Riedemann, L., et al. (2017). YAP/TAZ Orchestrate VEGF Signaling during Developmental Angiogenesis. *Dev Cell* **42**, 462-478 e467.

Wiley, D. M., Kim, J. D., Hao, J., Hong, C. C., Bautch, V. L. and Jin, S. W. (2011). Distinct signalling pathways regulate sprouting angiogenesis from the dorsal aorta and the axial vein. *Nat Cell Biol* **13**, 686-692.

Wilhelm, K., Happel, K., Eelen, G., Schoors, S., Oellerich, M. F., Lim, R., Zimmermann, B., Aspalter, I. M., Franco, C. A., Boettger, T., et al. (2016). FOXO1 couples metabolic activity and growth state in the vascular endothelium. *Nature* **529**, 216-220.

Xu, C., Hasan, S. S., Schmidt, I., Rocha, S. F., Pitulescu, M. E., Bussmann, J., Meyen, D., Raz, E., Adams, R. H. and Siekmann, A. F. (2014). Arteries are formed by vein-derived endothelial tip cells. *Nature communications* **5**, 5758.

Yan, M., Callahan, C. A., Beyer, J. C., Allamneni, K. P., Zhang, G., Ridgway, J. B., Niessen, K. and Plowman, G. D. (2010). Chronic DLL4 blockade induces vascular neoplasms. *Nature* **463**, E6-7.

Yang, X., Liaw, L., Prudovsky, I., Brooks, P. C., Vary, C., Oxburgh, L. and Friesel, R. (2015). Fibroblast growth factor signaling in the vasculature. *Curr Atheroscler Rep* **17**, 509.

Yang, Y., Zhang, Y., Cao, Z., Ji, H., Yang, X., Iwamoto, H., Wahlberg, E., Lanne, T., Sun, B. and Cao, Y. (2013). Anti-VEGF- and anti-VEGF receptor-induced vascular alteration in mouse healthy tissues. *Proc Natl Acad Sci U S A* **110**, 12018-12023.

Yeh, W. L., Lin, C. J. and Fu, W. M. (2008). Enhancement of glucose transporter expression of brain endothelial cells by vascular endothelial growth factor derived from glioma exposed to hypoxia. *Mol Pharmacol* **73**, 170-177.

Yu, P., Wilhelm, K., Dubrac, A., Tung, J. K., Alves, T. C., Fang, J. S., Xie, Y., Zhu, J., Chen, Z., De Smet, F., et al. (2017). FGF-dependent metabolic control of vascular development. *Nature* **545**, 224-228.

Zarkada, G., Heinolainen, K., Makinen, T., Kubota, Y. and Alitalo, K. (2015). VEGFR3 does not sustain retinal angiogenesis without VEGFR2. *Proc Natl Acad Sci U S A* **112**, 761-766.

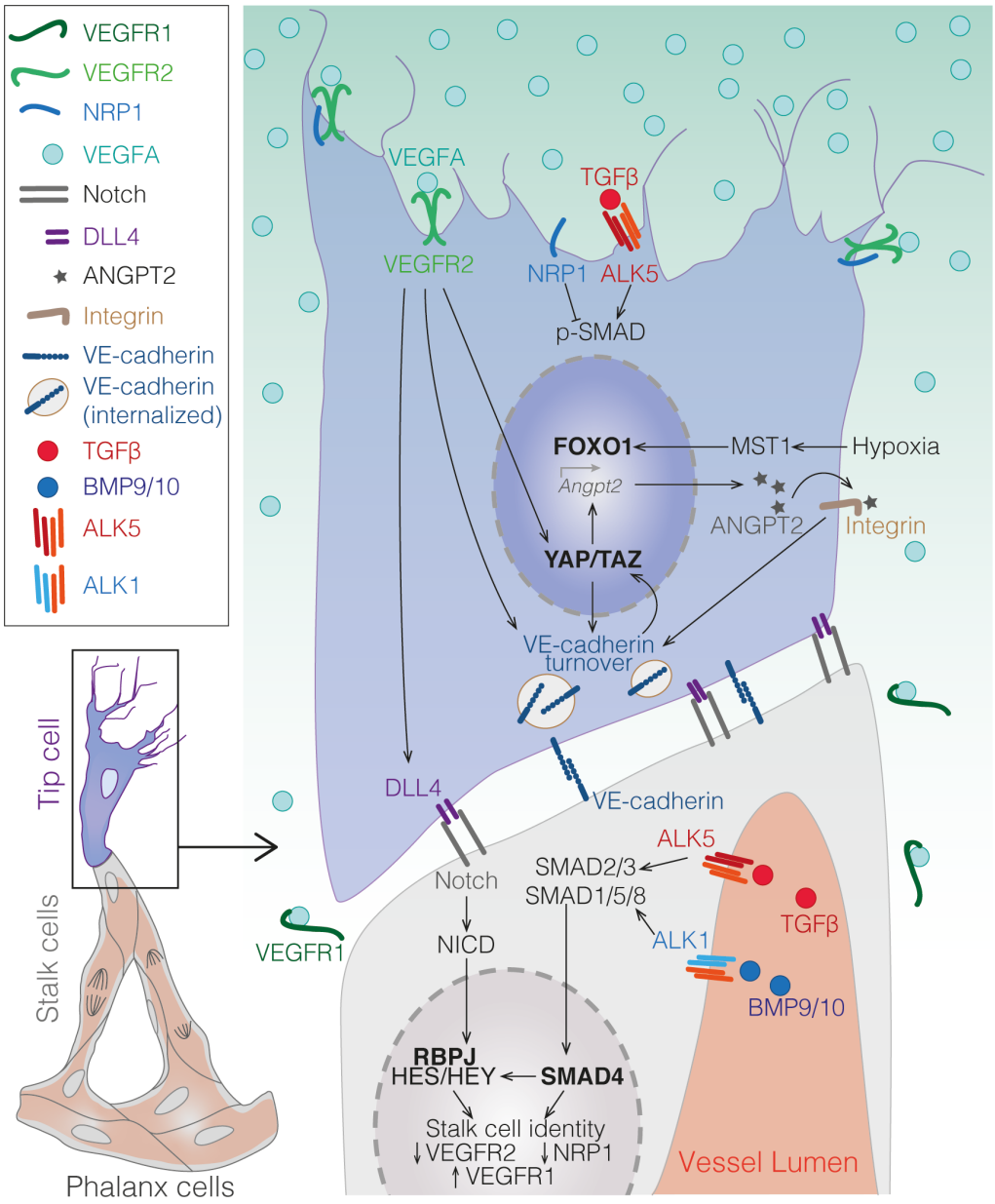

Figure 1. Growth factor signaling pathways specify tip and stalk cell identity during sprouting angiogenesis

VEGFA activates VEGFR2 signaling in tip cells and promotes their migratory behavior by destabilizing VE-cadherin junctions and promoting nuclear localization of YAP/TAZ that further regulates VE-cadherin turnover. Hypoxia triggers the activation of MST1 to induce FOXO1 nuclear localization that leads to expression of ANGPT2. ANGPT2 binds integrins on tip cells that also triggers VE-cadherin junction destabilization. DLL4 is upregulated on tip cells by VEGFR2, which activates Notch receptors on neighboring stalk cells. Notch signaling, together with TGF/BMP/ALK signaling promotes stalk cell identity via RBPJ and SMAD transcriptional regulation. NRP1 on tip cells inhibits phosphorylated-SMAD signaling to prevent stalk cell identity.

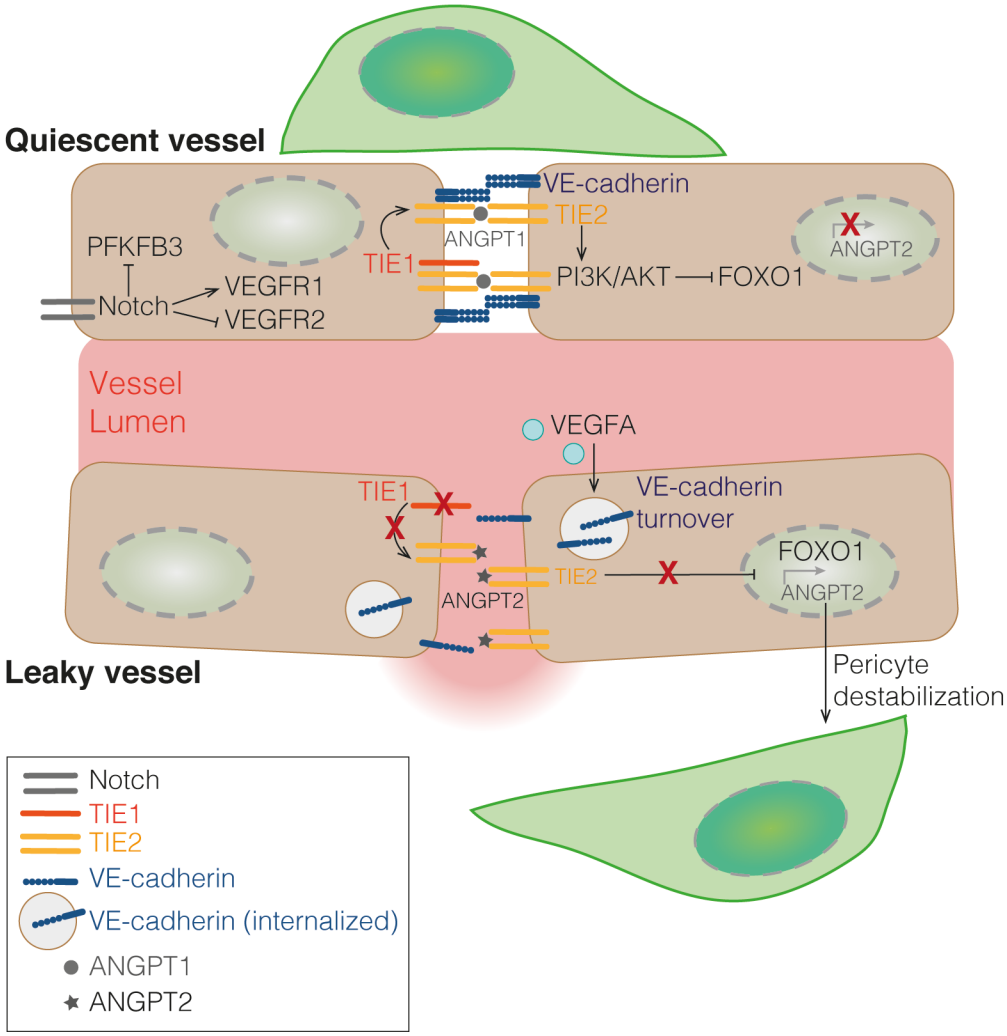

Figure 2. Growth factor signaling in maintaining endothelial quiescence

In quiescent ECs, Notch signaling lowers EC sensitivity to VEGFA by reducing VEGFR2 and increasing VEGFR1 levels as well as reducing glycolysis. ANGPT1/TIE2 signals through PI3K/AKT to prevent FOXO1 nuclear import, preventing expression of vessel destabilizing *Angpt2*. TIE1 reinforces TIE2 signaling. In ECs that become sensitized to VEGFA, VE-cadherin junctions become destabilized causing vessel leakage. ANGPT2 antagonizes TIE2 signaling promoting FOXO1 nuclear import and further upregulating *Angpt2* levels and leading to pericyte destabilization.