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Review Article

Molecular Players and Cellular Mechanisms that Govern Cell Behaviour in Adult Angiogenic Sprouting

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ABSTRACT

Blood vessels, guardians of the organism's global homeostasis, are formed achieved by vasculogenesis and angiogenesis. The present review was aimed at molecular players and cellular mechanisms that govern cell behaviour in adult angiogenic sprouting. Studies were accessed through an electronic web-based search strategy from PubMed, Cochrane Library, Google Scholar, Embase, PsycINFO and CINAHL by using a combination of search terms. In adults, angiogenesis is the most common form of blood vessel formation and also under strict control. Although most blood vessels in an adult organism remain quiescent, endothelial cells retain the capability of rapid division in response to physiological stimuli. Tip cell and stalk cell are two principal cells involved in sprouting angiogenesis. A fine-tuned feed-back loop between VEGF and Notch/Dll-4 signaling pathways is established and a cross-talk between these pathways is essential for proper patterning of the vasculature. Once the tip cell has been selected, filopodia guides the growing sprout towards a gradient of VEGF and other attractive guidance cues. An important first step in any anastomosis is the formation of a stable contact between two ECs. The formation of an interconnected luminal space and the formation of multicellular, perfused tubes in different vascular beds-type I and type II anastomosis-appear to occur at different frequencies. Pruning occurs via two different modes-type I and type II-depending on the state of vessel perfusion during the process. Vessels can adjust their shape and function to meet changing tissue oxygen demands. A fundamental feature of vessel maturation is the recruitment of mural cells. The importance of angiogenesis sparked hopes that manipulating this process could offer therapeutic opportunities.

Keywords: Angiogenic Sprouting, Cell Behaviour and Dynamic Mechanisms, Notch/Dll-4 signaling, VEGF.

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INTRODUCTION

The growth, survival and reproduction of living organisms rely on the delivery and recycling of metabolites to and from all cells in the body.[1] In addition to ensuring the delivery and recycling of respiratory gases and nutrients to all tissues in the body, blood vessels are developed as guardians of the organism's global homeostasis by allowing, notably, temperature regulation, hormonal communication and the circulation of immune factors and cells.[2] Formation of blood vessels begins in the early stage of embryonic development and is achieved by two successive processes: vasculogenesis and angiogenesis.[3,4]

The first stage of blood vessel formation is vasculogenesis, which is initiated once the embryo attains a size in which simple diffusion is no longer able to satisfy the nutritional requirements of a rapidly expanding cell population of increasing morphological complexity.[5,6] During this early development, mesodermal cells differentiate into hemangioblasts that produce angioblasts in the course of further differentiation.[7] The aggregation of angioblasts results in formation of blood islands, which then fuse to form primitive capillary plexuses that coalesce into a primitive network.[8,9] The stage of vasculogenesis is completed together with formation of primary vascular plexus and all further transformations of the primary vascular network into a highly branched hierarchical vascular tree, composed of arteries and veins, is through a second mechanism of blood vessel formation known as angiogenesis.[10]

Angiogenesis is the formation of new blood vessel from pre-existing vessels, usually the post-capillary and small terminal venules of the microvascular apparatus.[11] This can be accomplished through two distinct pathways:

The so-called 'sprouting' angiogenesis', which is characterized by proliferation and migration of Endothelial Cells (ECs) into avascular sites and later fusion, of vessel branches from a parent vessel.[12] and The 'intussusceptive or splitting' angiogenesis, which

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occurs by splitting of the existing vasculature by transluminar pillars or transendothelial bridges.[13]

Angiogenesis plays a crucial role in organogenesis and advanced embryonic and fetal development.[5] Most normal angiogenesis occurs in the embryo, where it establishes the primary vascular tree as well as an adequate vasculature for growing and developing organs.[14] It also occurs in adult tissues for wound healing, growth and action of female reproductive organs including ovulation, follicular development, corpus luteum formation, progesterone release, endometrial growth, regression and repair during the menstrual cycle and formation of a fully vascularized tissue for implantation and placentation during pregnancy.[15,16] However, pathological angiogenesis is also observed during conditions like rheumatoid arthritis, psoriasis, diabetic retinopathy, myocardial and limb ischemia and tumor development.[17,18] Although new vessels in the adult arise mainly through angiogenesis, vasculogenesis is not be restricted to early embryogenesis due to the existence of endothelial progenitor stem cells and these bone marrow-derived cells have been found to be incorporated into neovascular foci in the adult, including injured corneas, ischemic hind-limbs and tumor vasculature, suggesting that vasculogenesis can occur in the adult.[19-21] In adults, formation and growth of new vessels are under strict control that is dependent on an intricate balance of both pro-angiogenic (to stimulate) and anti-angiogenic (to negatively regulate the phenomenon) factors. The first molecule definitively identified as a purified angiogenic factor was basic Fibroblast Growth Factor (bFGF or FGF1).[22] This was followed by the identification of numerous 'classic' and 'non-classic' factors.[23-26] Classic stimulators mostly include growth factors and cytokines, among which Vascular Endothelial Growth Factor (VEGF), Placental Growth Factor (PlGF), Platelet-Derived Growth Factor (PDGF), fibroblast growth factor-2 (FGF-2), Transforming Growth Factors (TGFs), angiopoietins (Angs). Non-classic factors include numerous endogenous peptides, among which Erythropoietin (Epo), Granulocyte-Colony Stimulating Factor (G-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), interleukins, angiotensin II (Ang II), Endothelins (ETs), Adrenomedullin (AM), Proadrenomedullin N-terminal 20 Peptide (PAMP), Urotensin-II (U-II), leptin, adiponectin, resistin, neuropeptide-Y, Vasoactive Intestinal Peptide (VIP), Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and substance P.

MATERIALS AND METHODS

PubMed, Cochrane Library, Google Scholar, CINAHL, Embase and PsycINFO database were used for studies reporting the biochemical and physiological counter-regulatory arms of the renin-angiotensin system in the regulation of cardiovascular and renal function from study conception to May 2021. Zotero reference management software for Windows was used to download, organize, review and cite the articles. I also manually searched cross-references in order to identify additional relevant articles. A comprehensive search was performed using the following search terms: "angiogenic sprouting", "cell behaviour in adult angiogenic sprouting", "the molecular players of adult angiogenic sprouting", "cellular mechanisms of angiogenic sprouting" and "factors that govern cell behaviour in adult angiogenic sprouting". Boolean operators like "AND" and "OR" were used to combine search terms.

DISCUSSION

Sprouting angiogenesis is a normal, physiological process in which new blood vessels is formed via sprouting from pre-existing vessels during embryological development as well as in adults in the course of wound healing, reproductive cycling and inflammation.[13,27] It is this, the multi-step process that occurs in an ordered, highly orchestrated series, involving interactions between growth factors, vascular components (such as endothelial cells, vascular pericytes, fibroblasts, smooth muscle cells) and the extracellular matrix.[28,29]

Most blood vessels in an adult organism remain quiescent, however, when nutritional and oxygen demands within a tissue exceed the supply provided by existing blood vessels, the tissue sends out signals that induces endothelial cell activation for the formation of new blood vessels.[30] It is suggested that VEGF is a major player in angiogenesis initiation based on its ability to induce vasodilation via endothelial NO production, which is a prerequisite for endothelial cells to enter the angiogenic cascade.[31,32] It is also a major player because of its endothelial cell permeability increasing effect.[33] This allows plasma proteins to enter the tissue to form a fibrin-rich provisional network, which is mediated by the formation of fenestrations, vesiculo-vacuolar organelles and the redistribution of Platelet Endothelial Cell Adhesion Molecule (PECAM)-1 and Vascular Endothelial (VE)-cadherin and involves Src kinases.[34] VEGF can also induce the expression of proteases and receptors important in cellular invasion and tissue remodeling.[35] In conjunction with other endothelial specific signaling systems such as angiopoietins and Tie receptors, VE-cadherin/β-catenin and $\alpha_v\beta_3$ integrins, VEGFRs relay signals for at least five processes essential in stimulation of vessel growth: vasorelaxation, induction of vascular permeability, endothelial cell migration, proliferation and survival (Figure 1).[18] Thereby the observation that VEGF production is under control of Hypoxia Inducible Factor (HIF) strengthens the suggestion of an early involvement of VEGF in the angiogenic response.[36] Moreover, VEGF Receptor (VEGFR) expression is up-regulated under hypoxic or ischemic conditions as well.[7]

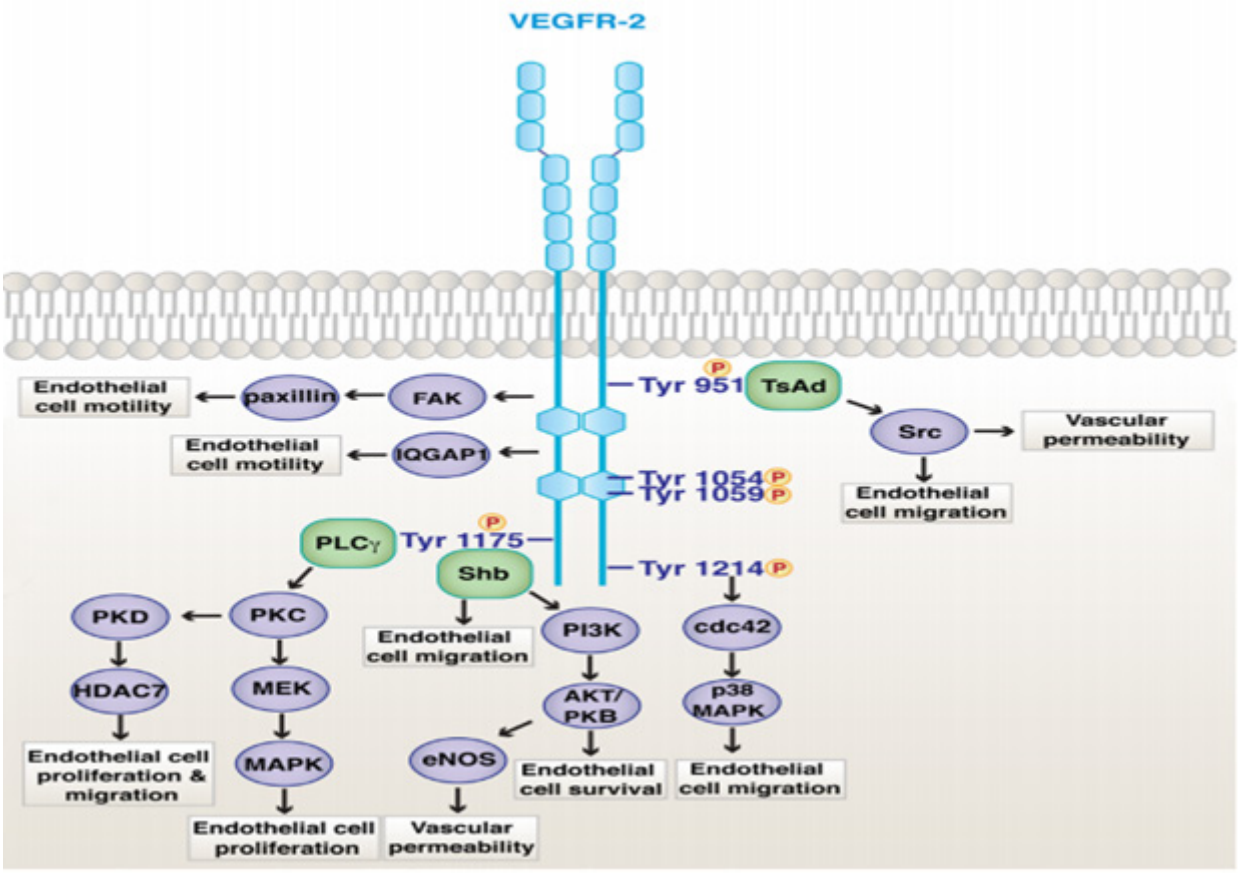

Figure 1: Signaling events initiated upon VEGFR-2 activation by VEGF. The dashed lines indicate potential signaling pathways. Not shown are the interactions of VEGFR-2 with VE-cadherin and β-catenin and with the $\alpha_v\beta_3$ integrin.

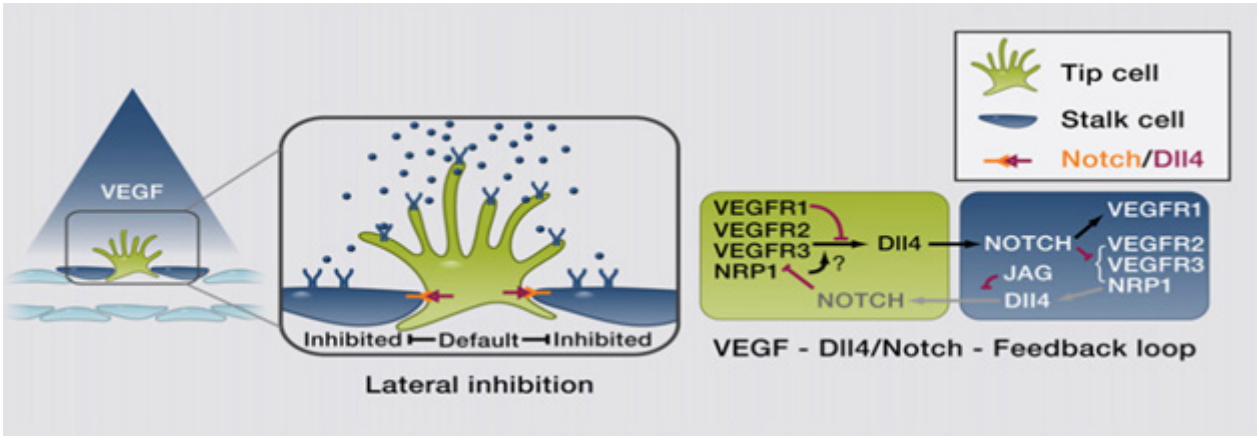

Figure 2: VEGF/Notch signaling selects tip and stalk cells.

Cell Behaviour and Dynamic Mechanisms during Sprouting Angiogenesis

Sprout Initiation

In all types of angiogenesis, either under physiologic or pathologic conditions, endothelial cell activation is the first process to take place.[7] Apparently all ECs exposed to VEGF become activated, but not all ECs respond by directed migration.[37,38] The two principal cells involved in sprouting angiogenesis are "tip cell" and "stalk cell" that bear different morphologies and functional properties (Figure 2). The tip cell extend numerous filopodia, are more migratory and polarized, proliferates minimally and are highly branched structure.[39,40] Adjacent to the tip cell, the following cells, termed stalk cells, produces fewer filopodia, are more proliferative and establish junctions with neighboring cells and synthesize basement membrane components.[41,42] Tip cell migration can occur without stalk proliferation and vice versa.

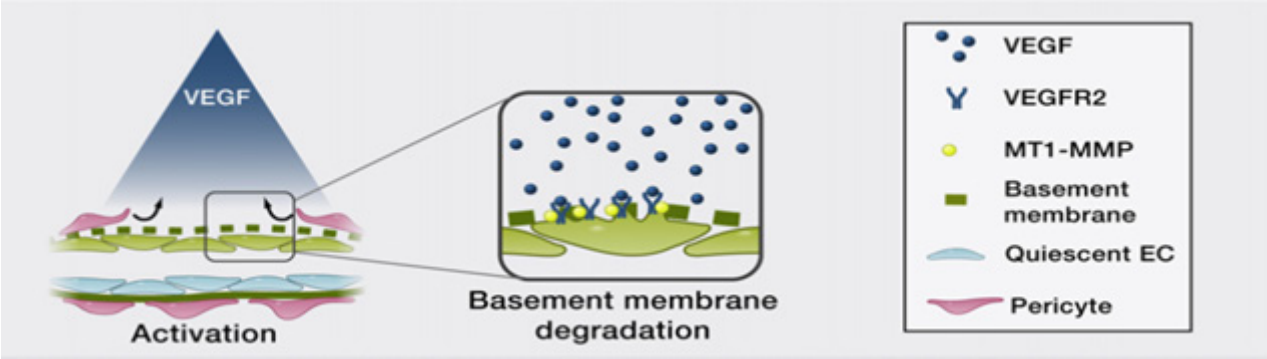

Figure 3: Liberating Endothelial Cells.

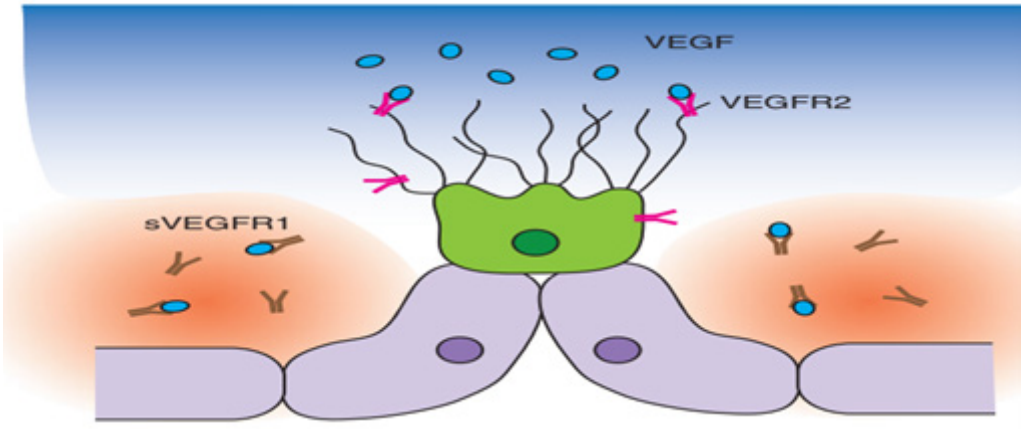

Figure 4: VEGF signaling during sprouting.

However, only a regulated balance between both processes establishes adequately shaped nascent sprouts.[43]

During sprouting angiogenesis, the specification of ECs into tip and stalk cells is controlled by the Notch signaling pathway (Figure 2).[44] The resulting cell-fate specification mechanism is reminiscent of a classical notch-mediated lateral inhibition process,[45,46] in which a cell adopts a particular fate and prevents (by lateral inhibition) its immediate neighbors from acquiring the same fate.[47] In mammals, four Notch receptors and five ligands (Jagged-1, Jagged-2, Dll-1, Dll-3 and Dll-4) are expressed on the surface of cell membranes.[48,49] The Dll-4 and Notch-1 expression is tessellated among endothelial cells in the vessel area, where activation of angiogenesis takes place.[50] ECs that express Dll-4 more quickly or at higher levels have a competitive advantage to become a tip cell by activating Notch-1 receptor in neighboring cells more effectively and suppresses neighboring cells from adopting the same fate through lateral inhibition and thus restricts emergence of an excessive number of tip-cells.[51] These findings imply that Notch-1 is critical for suppressing tip cell behavior in stalk cells. Thereby, ECs with more Dll-4 and less notch activity, will be selected as the tip cell.[52] Analysis of Notch signaling revealed that there is 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 Dll-4.[53]

Endothelial cells dynamically utilizes differential VEGFR levels.[54] ECs with high VEGFR-2 and low VEGFR-1 expression than its neighboring cells produce more Dll-4, which binds to Notch-1 receptors on neighboring ECs and releases the Notch Intracellular Domain (NICD).[55] NICD acts as a transcriptional regulator, decreasing VEGFR-22 and NRP-1 expression while increasing the levels of VEGFR-1, which traps VEGF.[56] and renders neighboring cells less responsive to VEGF. On the other hand, ECs with higher levels of VEGFR-2 increase Dll-4 expression which further increases the cell sensitivity to VEGF and this cell becomes the tip cell selected for outward migration for the parent vessel.[57,58] Beside stimulating tip cell induction, VEGF also stimulates filopodia formation via VEGFR-2 that is abundant on filopodia.[59] This implies that VEGFR-2 internalization and activation of ERK1/2 signaling are important for sprouting, likely because rapid receptor turnover and signaling is essential for ECs at the vascular front to respond strongly and quickly to angiogenic signals.[60] At the forefront, where VEGF levels are highest, VEGF therefore activates VEGFR-2 to stimulate tip cell migration and extension of their filopodia (Figure 2).[61] Alternatively, activation of notch in neighboring cells inhibits VEGFR-2, indirectly inhibiting Dll-4 expression levels, thereby reinforcing the dominance of the selected tip cell and limiting the number of tip cells induced by VEGF.[62] Furthermore, ECs with excess Notch

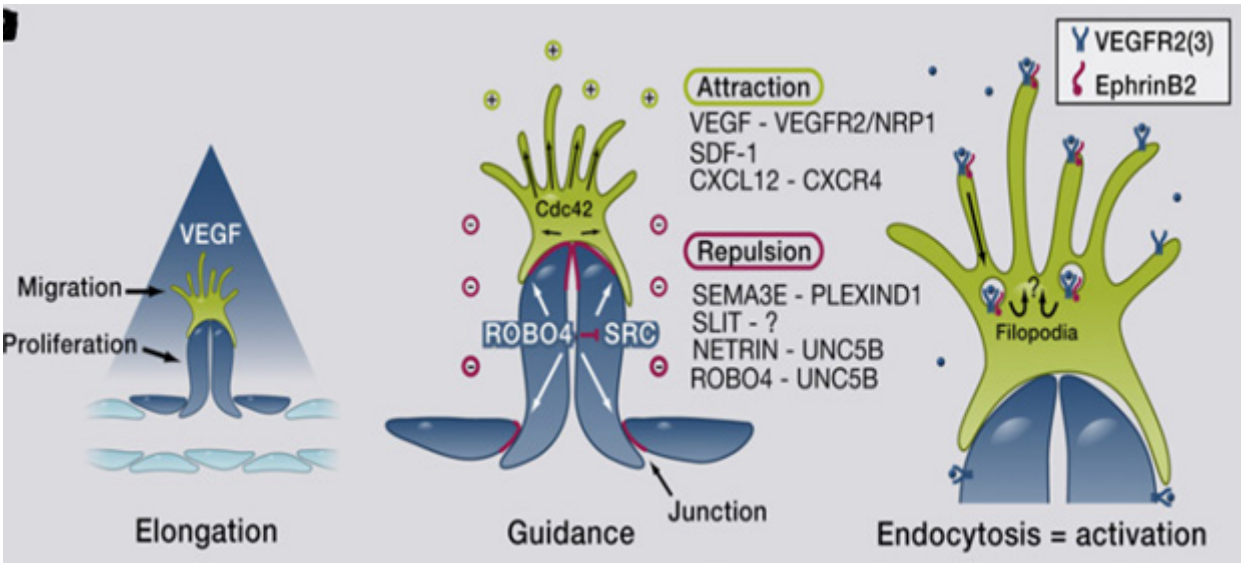

Figure 5: Filopodia guide tip cells by sensing attractive and repulsive cues.

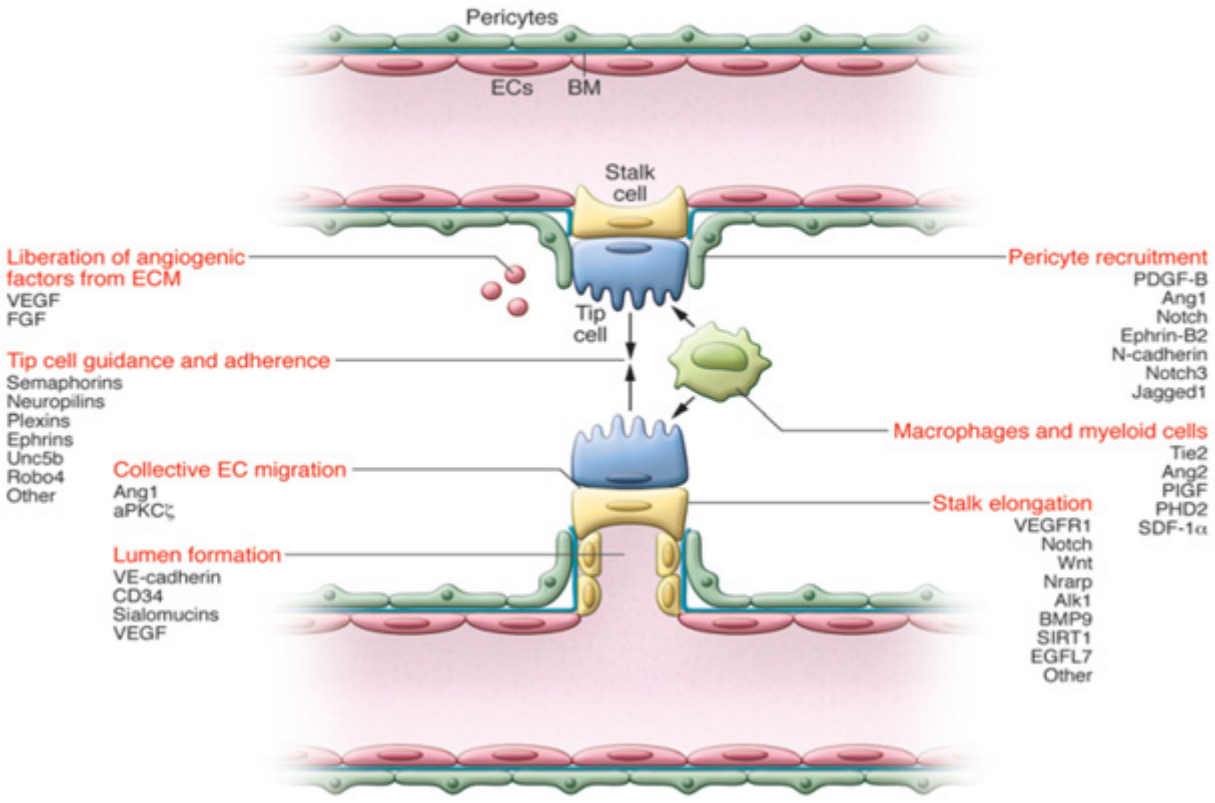

Figure 6: Tip cell guidance and stalk elongation.

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.[55] In contrast to Dll-4, the Notch ligand JAGGED1 (JAG-1) is expressed primarily by stalk cells to antagonizes Dll-4 activity, reducing the induction of Notch signaling in the adjacent tip cell (Figure 2).[63] Thus, Dll-4 and JAG-1 coordinately control the process of angiogenic sprouting and confer robustness to the crucial event of tip-stalk specification.[64,65] In this context, a fine-tuned feed-back loop between VEGF and Notch/Dll-4 signaling pathways is established and a cross-talk between these pathways is essential for proper patterning of the vasculature (Figure 2).[66] Likewise, VEGF and Notch co-operate in an integrated intercellular feedback that functions as a "branching pattern generator". Although activated ECs would be assumed to initially express similar levels of notch and Dll-4 and thus undergo a balanced reciprocal notch activation,

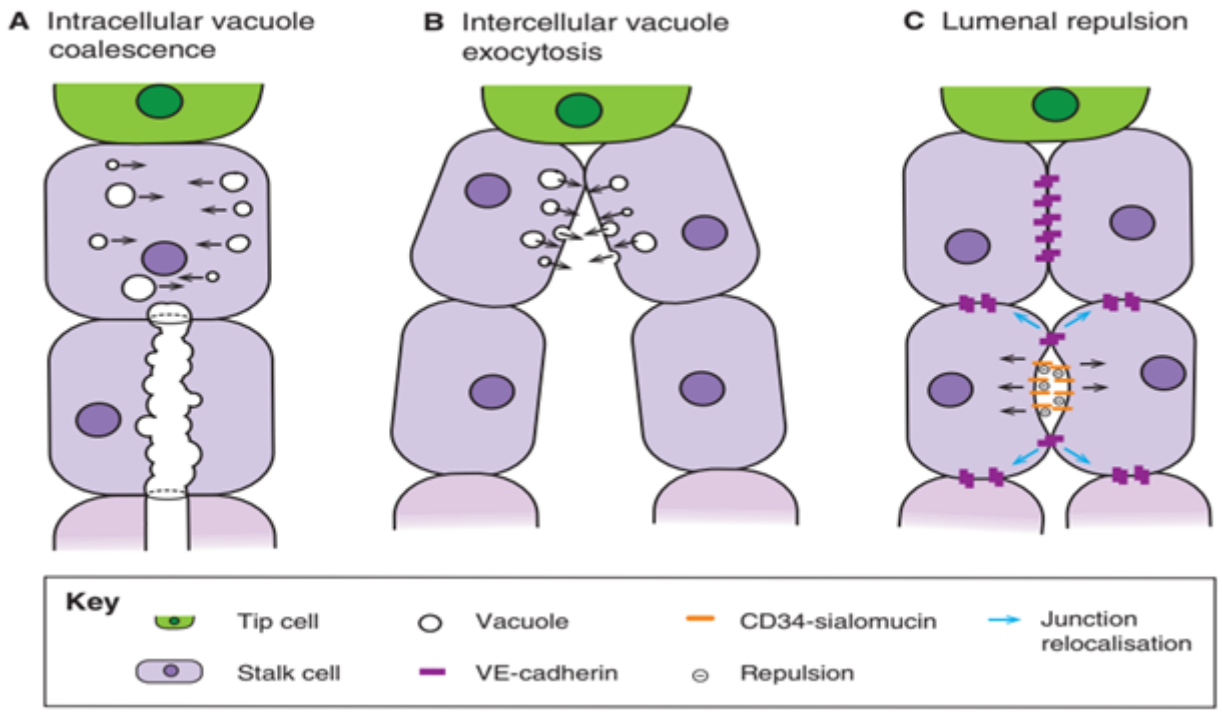

Figure 7: Models of lumen formation during sprout outgrowth.

stochastic differences in local VEGF concentrations, in filopodia elongation (and thus VEGF exposure) or in transcription rate lead to small imbalances: one cell will express slightly higher Dll-4 levels and, thus, will dominate its neighbors by activating more notch signaling.[47]

Extension/Elongation of Sprout

It is the process in which tip cells moves along a chemotactic or physical hindrance-based path, followed by trailing stalk cells that connect the tip cell to the original vessel.[67] For endothelial cells to emigrate from their resident site, they first need to loosen both the basement membrane and the coat of mural cells that prevent resident ECs from leaving their positions.[68] In response to VEGF stimulation, a proangiogenic growth factor stored by ECs-Angiopoietin-2 (ANG2)-is released to degrade the basement membrane and pericytes detach (Figure 3).[69] Likewise, endothelial penetration into new areas of the body is also achieved through a process requiring loosening the matrix by Matrix Metalloproteinases (MMPs), enriched in tip cells.[70] Activation of MMPs by VEGF-VEGFR-2 signaling then liberate proangiogenic Growth Factors (bFGF, VEGF and IGF-1) that are sequestered within the extracellular matrix.[71] Above all, it facilitate the secretion of zymogens (proteinases of the plasminogen activator, chymase or heparanase families) that become activated in the ECM compartment and subsequently selectively plasminogen activators u-PA and t-PA convert the ubiquitous plasma protein plasminogen to plasmin.[72] Plasmin is believed to be the most important protease for the mobilization of fibroblast growth factor-2 from the ECM pool.[73] The displaced FGF-2 molecules were thus released to be involved in the regulation of guided migration[40] and determination the specification of an epithelial tip cell.[74] Control of these all 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.[75] MMP activity and, hence, angiogenesis is counteracted by the family of Tissue Inhibitors of Metalloproteinase (TIMPs).[76] At the other end, MMPs also generate antiangiogenic molecules by cleaving plasma proteins, matrix molecules, or proteases themselves to prevent inappropriate sprouting and coordinate branching.[77]

In response to Vascular Endothelial Growth Factor (VEGF) stimulation, Endothelial Cells (ECs) degrade the basement membrane and pericytes detach, allowing ECs to emigrate.

Once tip-cells are selected and begin to move forward, formation of new capillaries should begin because of the proliferation and migration of endothelial cells.[78] VEGF independently controls the migration and proliferation of endothelial cells forming a new capillary.[79,80] Tip-cells specifically express VEGFR-2 that positively drives the mitogenic and chemotactic responses of endothelial cells to VEGF.[40] In response to the action of VEGF, "tip cells" orient towards the VEGF gradient; also "tip cell" migration is regulated by the interaction of VEGF-VEGFR-2 but do not proliferate in response to VEGF (Figure 4).[38] On the other hand, VEGFR-1 is predominantly expressed in stalk cells and its expression is induced by Notch signaling to reduce VEGF ligand availability.[81] VEGFR-1 is involved in guidance and limiting tip cell formation.[7] Therefore, tip cell migration depends on a

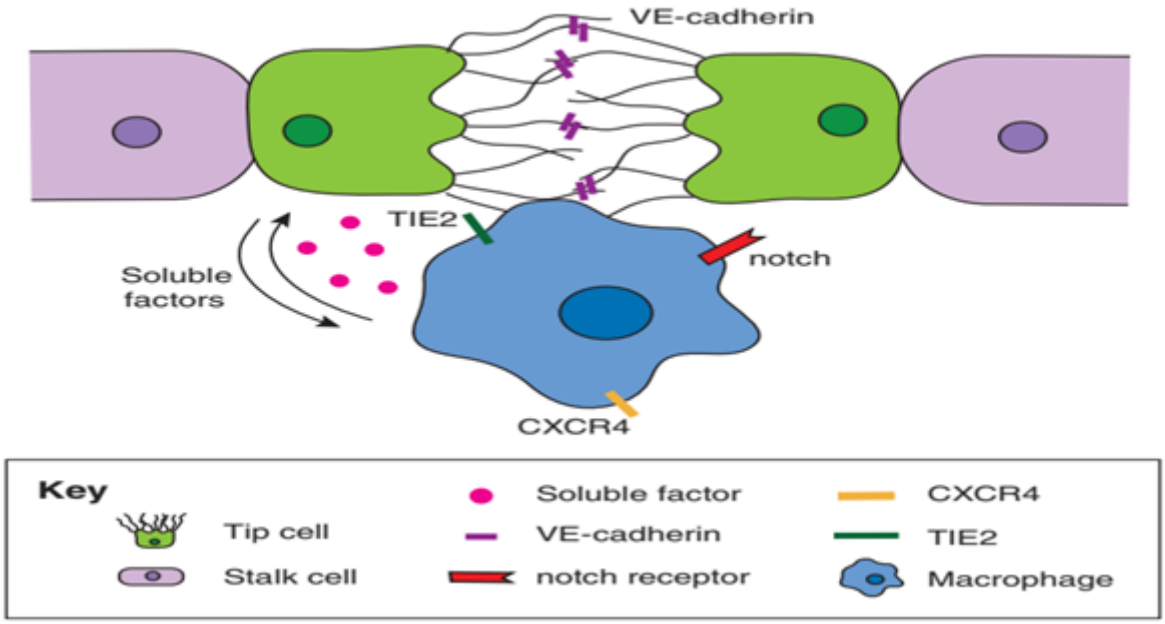

Figure 8: Schematic illustration of tip cell fusion.

gradient of VEGF,[82] whereas stalk cell proliferation is regulated by VEGF concentration (Figure 4).[38]

Soluble VEGFR1 (sVEGFR1; brown) produced by the cells immediately next to the outgrowing vessel branch sequesters VEGF molecules, thereby creating a corridor of higher VEGF levels perpendicular to the parent vessel. This corridor might act to optimize spreading of the vascular network and to avoid contact with nearby emerging sprouts.

EC polarization and hence the directionality of filopodia extension, during migration is dependent on CDC42 activity, a small GTPase of the Rho family (Figure 5).[83] Activation of CDC42 by VEGF triggers filopodia formation.[84] Accordingly the directionality of filopodia extension is regulated by the spatial distribution of this growth factor in the local environment.[82] However, recent work showed that VEGF-induced filopodial extension is more dependent on guidance receptors that are expressed on ECs (Figure 5).[53] Roundabouts 4 (ROBO4) are membrane-bound guidance receptors that increases vessel integrity and reduces angiogenesis by counteracting VEGF/VEGFR-2 signaling.[85] However, VEGF promotes the expression of the transcription factor Hlx1, which increases expression of UNC5B, plexin 5 and SEMA3G, suggesting feedback with ROBO4.[86] Hlx1 maintains the stalk cell phenotype by regulating repulsive signals.[87] Semaphorins (SEMA) are secreted or membrane-bound guidance cues that interact with receptor complexes, formed by NRPs alone or NRP/plexin family proteins.[88] In fact, most semaphorins suppress angiogenesis.[89] SEMA6A regulates VEGFR-2 expression and its downstream signaling,[90] while SEMA3E activates PlexinD1 in tip cells and maintains the tip cell/stalk cell balance by regulating VEGF activity and Dll-4 expression.[91] Netrins are laminin-related secreted bifunctional guidance cues, which bind to extracellular matrix components: attraction and repulsion are mediated by binding to Deleted in Colorectal Cancer (DCC) and UNC5 family receptors, respectively.[92] UNC5B is selectively expressed in the vascular system by arteries, a subset of capillaries and endothelial tip cells.[93] UNC5B inactivation results in enhanced sprouting, whereas netrin-1 prompts filopodia retraction of endothelial cells.[91] Ephrin-B2-expressing ECs, fated to form arterial vessels, segregate from EphB4-expressing ECs, which become venous vessels due to repulsive cues.[94] Intriguingly, ephrin-B2-mediated reverse signaling also controls VEGFR internalization and tip cell behavior (Figure 5). ECs lacking ephrin-B2 reverse signaling are unable to internalize VEGFR-2 and VEGFR-3 and cannot transmit VEGF signals properly, together impairing sprouting.[95]

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.

Once the tip cell has been selected, it guides the growing sprout towards a gradient of VEGF and other attractive guidance cues.[38] The stalk cells that form the body of the sprout then proliferate to deliver the necessary building blocks for the growing sprout and elongate the sprout shaft (Figure 6).[61] *In vitro*, Notch inhibits EC proliferation; however, stalk cells must proliferate to elongate the shaft *in vivo*. To overcome this, stalk cells express the Notch target Notch-Regulated Ankyrin Repeat Protein (NRARP), which limits Notch signaling at branch points while allowing continued WNT signaling to promote EC proliferation and vessel stability.[59] As the vessel elongates, the stalk cells create a lumen, produce a

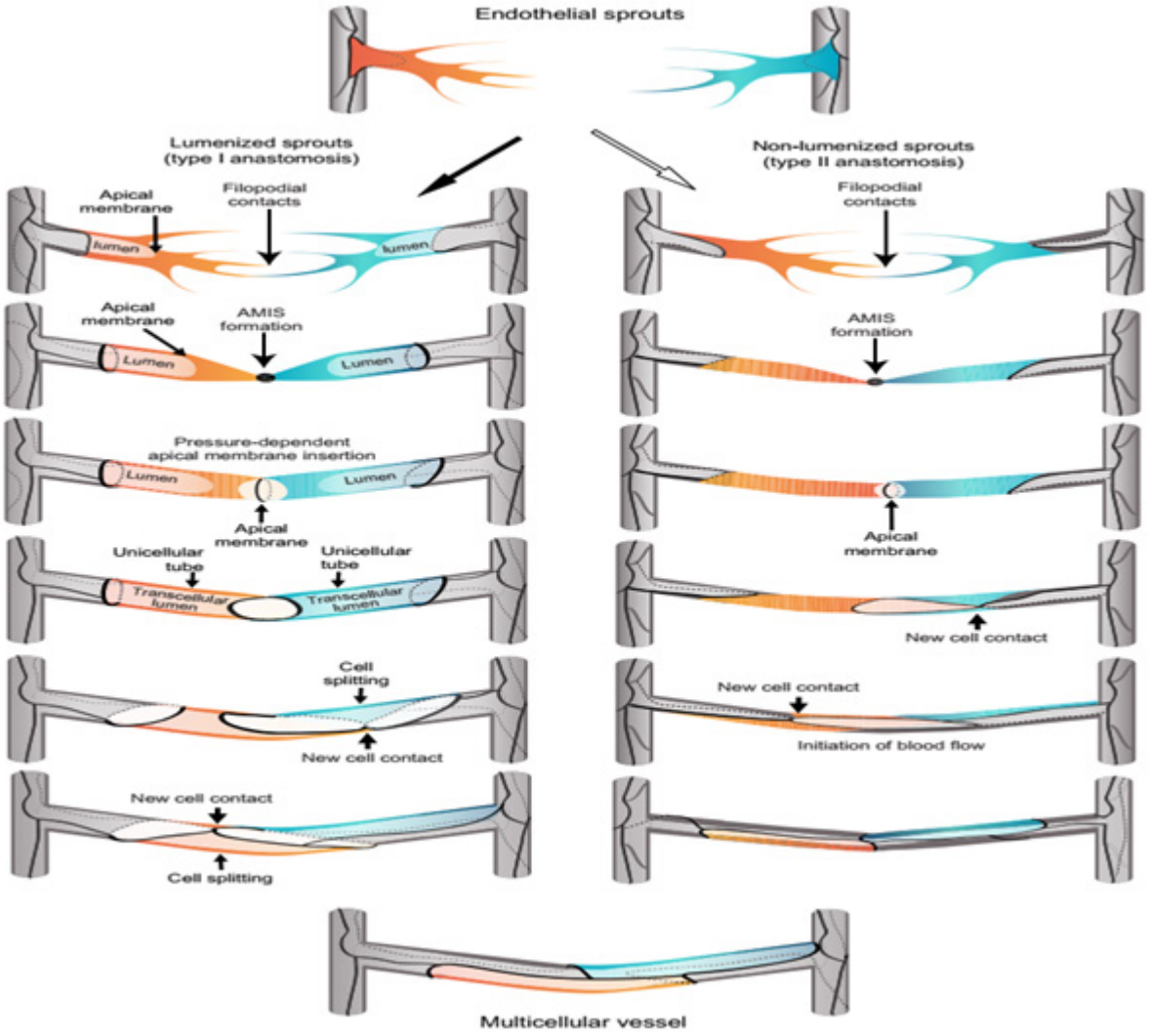

Figure 9: Schematic depicting the different stages of blood vessel fusion in the case of lumenized sprouts (type I anastomosis; left) and non-lumenized sprouts (type II anastomosis; right).

basement membrane and associate with pericytes.[47] However, stalk cell proliferation does not push the tip cell forward, but rather that the tip cells themselves interact with the surrounding substrate to pull the growing sprout further in the direction of growth (Figure 6).[96,97] Furthermore, blood vessel sprouting can initially progress without cell division, indicating that a similar pulling force of the vascular tip cell is likely to be present.[96] However, sustained sprouting and further outgrowth of the vessel branch requires proliferation of the stalk cells and decreased stalk proliferation correlates with branch regression.[59]

The growing sprout moves along a VEGF gradient. Tip cells adhere to the ECM, mediated by integrins and migrate toward guidance signal molecules (e.g., semaphorins and ephrins). Stalk cells trail behind the tip cell and proliferate to allow sprout elongation and lumen formation. While Notch signaling inhibits proliferation, expression of NRARP at branch points allows WNT signaling to maintain stalk cell proliferation. This system allows vascular migration/directionality (by tip cells) and elongation of the shaft (by proliferating stalk cells).

Lumen Formation

After tip cell induction and stalk elongation, a vessel branch needs to generate a lumen via a wide variety of cellular mechanisms that depends on the vascular bed or type of vessel formation.[98-100] In an early study, 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 7A).[101] The intracellular vacuolization is a rapid way to create endothelial cell luminal spaces.[102] Alternatively, it was also argued that ECs are organized in a cord-like fashion,[103] as a result, there is another mechanism of lumen formation in nascent sprouts in which ECs produce and release large exocytotic vacuoles into the intercellular space, which eventually fuse intracellularly to hollow out stalk cells and generate an interconnected luminal space (cord hollowing) (Figure 7B).[104] More recently, aortic lumenization in mouse and

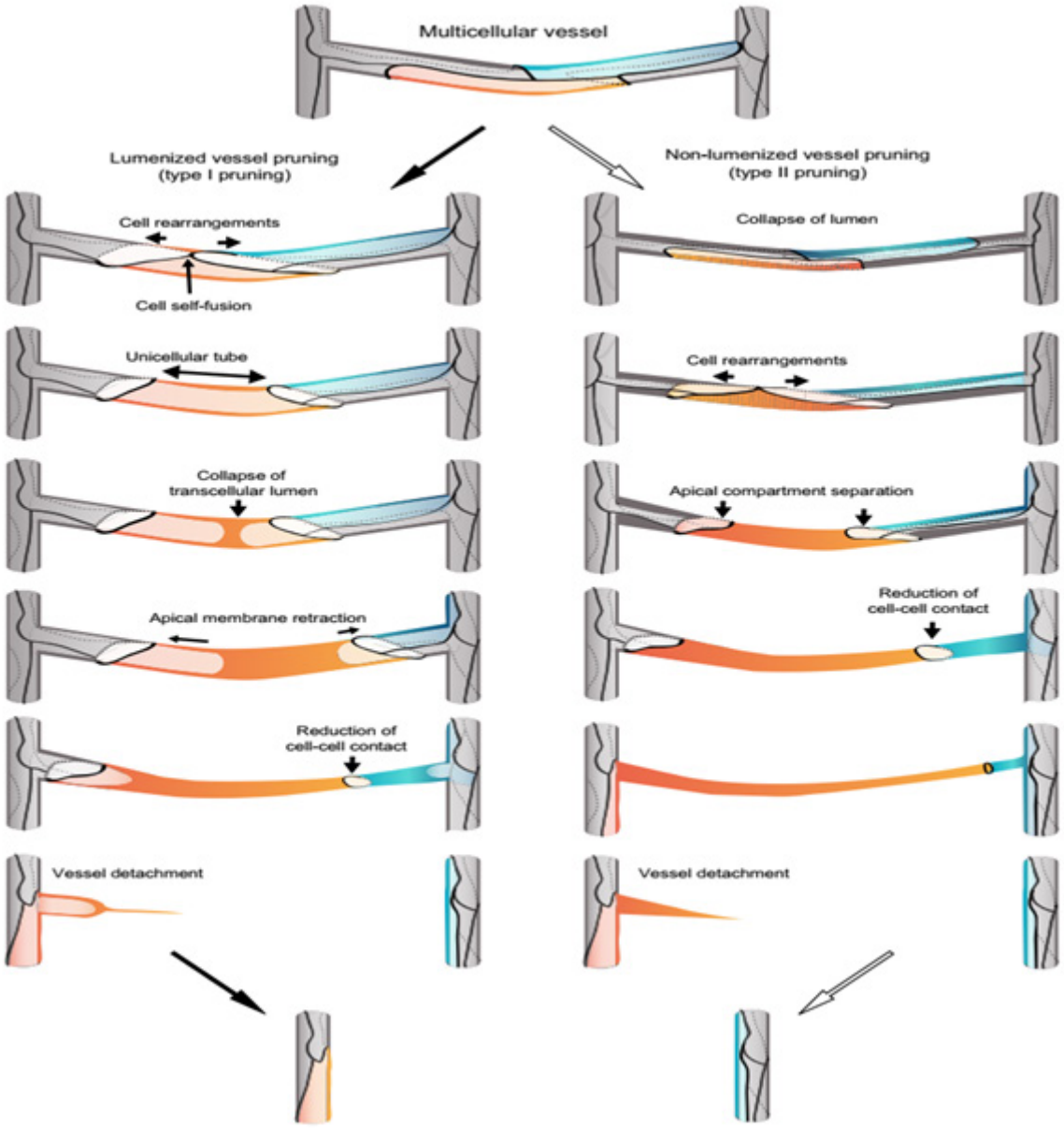

Figure 10: Cell behavior during pruning.

zebrafish was shown to similarly occur extracellularly. However, in these studies, vacuoles were not observed and lumen formation was associated with delocalization of junctional proteins and cell shape changes (luminal repulsion) (Figure 7C).[105]

Cell-cell adhesion between ECs is mediated via tight junctions and adherens junctions that are formed by homophilic interactions of adhesive proteins that further interact with intracellular partners and with the actin cytoskeleton.[106] At tight junctions, adhesion can be mediated by claudins, occludin, members of the Junctional Adhesion Molecule (JAM) family, or by EC Selective Adhesion Molecule (ESAM). Likewise, adherens junction formation was shown to be associated with the inhibition of endothelial cell migration in monolayers.[107,108] Whereas, adherens junctions are predominantly formed by Vascular Endothelial (VE)-cadherin.[109] Thus, VE-cadherin is strictly required for the polarization of endothelial cells *in vitro* and *in vivo*. However, ECs polarization often starts with the delivery via exocytosis of de-adhesive apical glycoproteins, including CD34-sialomucins, such as CD34 and Podocalyxin (PODXL), to the cell-cell contact.[110,111] The negative charge of the sialic acids in CD34-sialomucin in turn induces electrostatic repulsion of the facing EC surfaces, leading to the initiation of lumen formation (Figure 7C).[105] During delivery of apical glycoproteins, the adherens junctions translocate to lateral positions and the new junctions have properties of adherens and tight junctions, as they contain both VE-cadherin (characteristic of adherens junctions) and Zonula Occludens-1 (ZO-1) protein (characteristic of tight junctions).[112] Formation of apical cell surfaces and electrostatic repulsion of negatively charged apical glycoproteins are sufficient

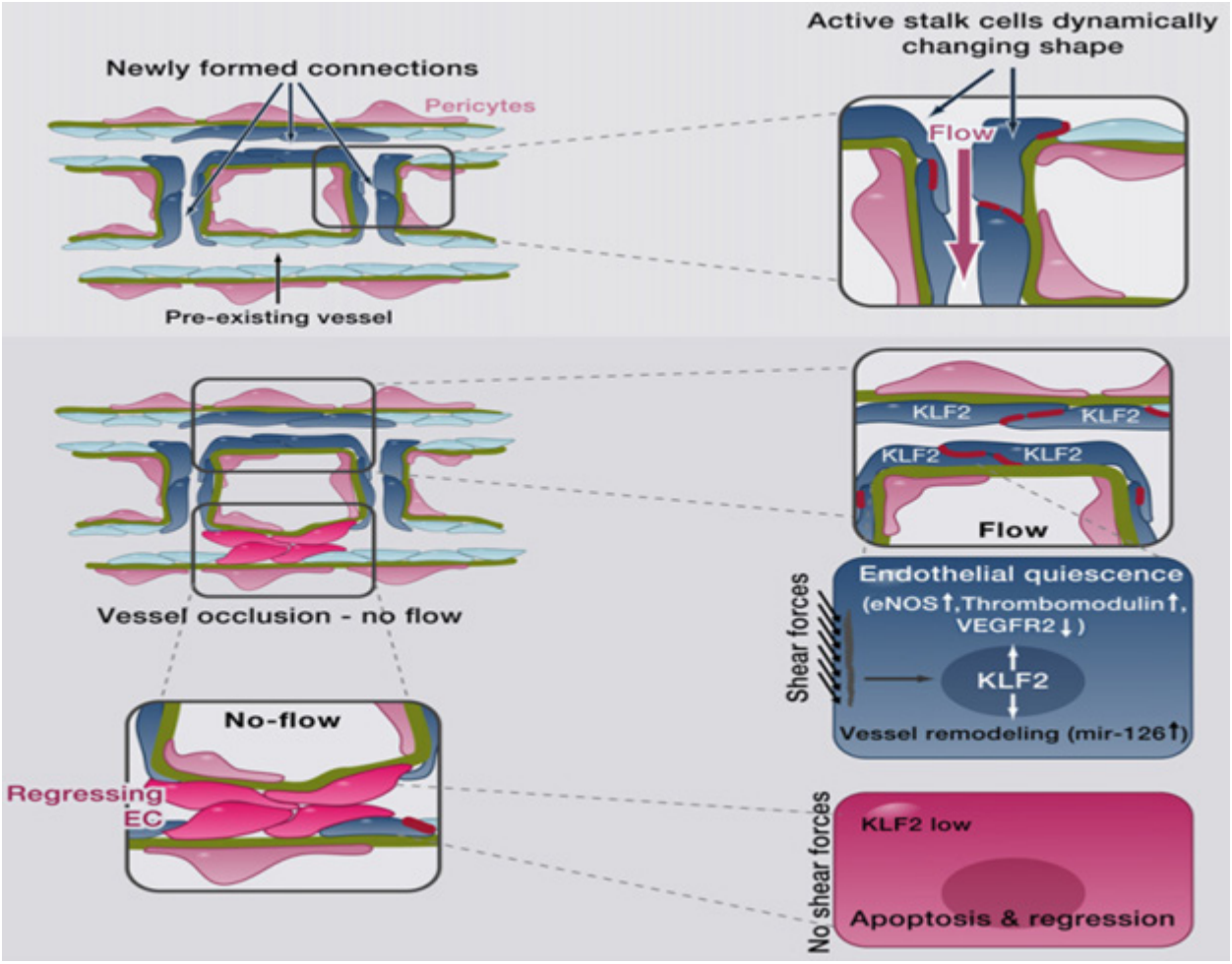

Figure 11: Stalk cells undergo remodeling in response to flow.

for the initial de-adhesion of adjacent endothelial cells and for slit formation, but are not sufficient for the development of a patent vascular lumen because further separation and establishment of the lumen is based on F-actin-mediated cell-shape changes. CD34-sialomucins further recruit F-actin to the lumenal cell surface.[109] VEGF-dependent localization of non-muscle myosin II to this apically enriched F-actin cytoskeleton induces EC shape changes and further separation of the adjacent ECs, fully establishing the aortic lumen.[109] In this model, ECs also define two functionally different phenotypes.[110,111] The first phenotype, represented by endothelial cells in mature blood vessels, is characterized by an apico-basal polarity and junction-mediated contact inhibition. The second phenotype is found in activated tissues and is characterized by the loss of apico-basal polarity and adherent's junctions, a spindle-shaped morphology and the ability for guided migration. Subsequent changes in EC shape, is driven by Rho-associated protein Kinase (ROCK), expand the lumen.[109] Tube morphogenesis also requires Ras-Interacting Protein 1 (RASIP1), a regulator of GTPase signaling controlling cytoskeletal rearrangements, adhesion and EC polarity.[113] In general term, within the vascular system, lumen formation involves a complex molecular mechanism composed of endothelial cell repulsion at the cell-cell contacts within the endothelial cell cords, junctional rearrangement and endothelial cell shape change.[114]

(A) Intracellular vacuole coalescence. Endothelial Cells (ECs) can form a lumen by forming intracellular vacuoles that coalesce and connect with each other and with vacuoles in neighbouring cells. (B) Intercellular vacuole exocytosis. ECs can form a lumen by producing exocytotic vacuoles that are released into the intercellular space. (C) Luminal repulsion. Alternatively, an intercellular lumen can be created by apical membrane (lumenal) repulsion. VE-cadherin (purple) establishes the initial apical-basal polarity in the ECs and localizes CD34-sialomucins (orange) to the cell-cell contact sites. Further separation and establishment of the lumen is based on F-actin-mediated cell-shape changes (not shown).

Sprout Anastomosis: Making New Connections

Vascular anastomosis is the process that generates connections between angiogenic sprouts and blood vessels and hence is fundamental for vascular network formation.[47] Anastomosis can occur between two sprouts and involve two tip cells ('head-to-head' anastomosis), or between sprouts and a functional blood vessel, involving only one tip cell ('head-to-side' anastomosis).[67]

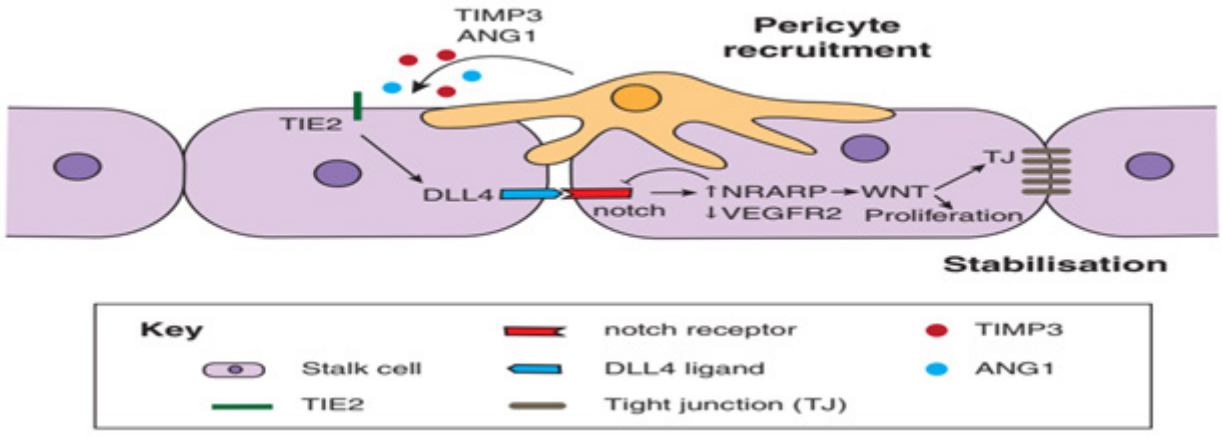

Figure 12: Vessel stabilization.

An important first step in any anastomosis is the formation of a stable contact between two ECs. During initial contact formation, filopodia from neighboring tip cells make and break connections several times before one single connection is stabilized and reinforced by the deposition of Adherens Junction (AJ) proteins at the contact site.[115] Recently, it was found that VE-cadherin is present not only at cell-cell junctions but also at the tips of EC filopodia that shown to localize to tip-tip contact sites immediately upon contact with neighbouring sprouts (Figure 8).[104] As the tip cells of the two sprouts crawled over each other, the VE-cadherin contacts expanded.[47] Although tip cells of adjacent vessels meet via filopodia, how the tip cells meet to establish new contacts is not fully understood. Recent descriptions of macrophage-tip cell interactions, in particular at sites where two tip cells make contact via their filopodia, raised the hypothesis that macrophages might act as 'bridge cells' that facilitate the contact and possibly stabilize nascent connections by releasing soluble pro-angiogenic factors (Figure 8).[116-118] Possible receptor-ligand candidates that mediate this interaction are notch-Dll-4, TIE2 or chemokine receptor CXCR4-Stromal cell-Derived Factor-1 (SDF1). The notch, TIE2 and CXCR4 receptors are expressed in macrophages,[119,120] whereas their respective ligands Dll-4, ANG2 and SDF1 are expressed in tip cells,[121] although CXCR4 has also been described as a tip cell enriched gene.[122] Surprisingly, anastomosis in the complete absence of macrophages is normal, albeit less frequent,[118] illustrating that macrophages might be involved in modulating and refining the connection process.[116]

For simplicity, the vascular lumen is not illustrated. The formation of new connections between growing vessels is facilitated by vessel interactions with macrophages (blue) that can act as bridge cells that promote filopodia contact between tip cells (green). Upon contact, adhesion junctions are formed by VE-cadherin, first at the tips of filopodia and later also along the extending interface of the contacting cells. The precise role of macrophages and the molecular regulation of anastomosis are not understood. Possible candidate pathways involved are the notch, TIE2 and CXCR4 signaling pathways; the notch receptors (red), the TIE2 receptor (green) and the CXCR4 receptor (yellow) are expressed on macrophages and their cognate ligands are expressed on tip cells (not shown).

At adhesion sites, ECs then deposit de novo apical membrane, which eventually leads to the formation of ring-shaped AJs with apical membrane in between; since this process happens in both anastomosing cells, a small luminal pocket is generated at this site.[123] After the initial steps of contact formation and EC polarization, two different cellular mechanisms-type I and type II anastomosis (Figure 9)-lead to the formation of an interconnected luminal space and the formation of multicellular, perfused tubes. Type I anastomosis, which is seen in the case of lumened sprouts, occurs in the presence of blood pressure on the proximal apical membrane of the tip cell and involves the rapid growth and invagination of this apical membrane into the cell body (Figure 9). In this case, blood pressure pushes the luminal space from the connecting stalk cells through the elongated tip cell, while being confined by a growing apical membrane.[124] The hallmark of type I anastomosis is thus the transcellular hollowing of the two interacting tip cells, leading to the formation of two cell segments in the conformation of a unicellular tube. This newly formed unicellular tube is eventually converted into a multicellular tube via complex cellular rearrangements involving cell splitting. In this particular scenario a single cell encompassing a lumen splits on one side, cell splitting does not lead to two cell bodies (one with a nucleus and one without), but rather transforms a doughnut-shaped cell into a flatter endothelial cell.[115]

Type II anastomosis, which is seen in the case of a non-lumened sprout, occurs in the absence of blood pressure (e.g. in a vessel in which the stalk is not lumened up to the tip cell) and also initiates with contact or AMIS formation and the subsequent generation of an apical membrane pocket (Figure 9).[125] Type II anastomosis is characterized by the formation of a multicellular tube omitting a unicellular intermediate stage. Interestingly, a rather similar process of lumen formation has been reported in

Ciona intestinalis during the formation of the notochord,[126,127] suggesting that the connection of serially arranged luminal pockets via cell rearrangements may be a common mechanism of tube formation.

Both processes are initiated by the formation of filopodial contacts between sprouts, which eventually stabilize in one location. An Apical Membrane Initiation Site (AMIS) is formed at this novel contact site and apical membrane is inserted. In type I anastomosis, apical membrane invagination through blood pressure and subsequent apical membrane fusion generates a unicellular tube containing cells with a transcellular lumen. The subsequent transition from a unicellular to a multicellular tube in type I anastomosis involves cell rearrangements and cell splitting. In type II anastomosis, cell rearrangements lead to lumen coalescence and the formation of a multicellular tube.

Network Formation: Remodeling and Maturation

The initial endothelial plexus generated by vascular sprouting consists of a homogenous web of EC tubes and sacs.[40] After the establishment of new connections within the vascular network, significant remodeling occurs; some branches are stabilized whereas others regress (also referred to as pruning).[128,129] Vessel pruning occurs preferentially at loop-forming segments in a process involving lateral migration of ECs from the pruning to the stable vessel.[130] Indeed, both low concentrations of VEGF.[131] and local loss of perfusion.[130] have been shown to trigger regression of vascular segments. Exposure to hyperoxia also leads to excessive regression of capillaries, while arteries become refractory to this insult.[132,133] Hyperoxia also suppresses VEGF production and leads to obliteration by apoptosis of already formed vessels.[134] Furthermore, changes in blood flow can also drive this vessel pruning.[135] was demonstrated that it is not the absence of perfusion per se that leads to pruning, but rather the local difference in blood flow between different branches of the loop drive ECs to orient and migrate against the direction of flow.[136] The strong blood flow acts as an 'attractor' for cells, whereas poorly perfused vessels are less 'attractive' and thus promote regression of less-functional vessel segments.[67] Similar to what has been observed in anastomosis, pruning occurs via two different modes-type I and type II (Figure 10).[137] Type I pruning, which occurs for vessels that maintain a lumen until the later stages of pruning, involves cell self-fusion in which the remaining endothelial cell in a pruning vessel wraps itself around the lumen, fuses with itself and forms a unicellular tube. These cell rearrangements conceptually look like anastomosis in 'reverse mode'.[135] Type II pruning involves the early collapse of the lumen in the multicellular tube, followed by cell rearrangements that lead to a unicellular, non-lumenized bridge.[67] Finally, the remaining cell-cell contact is reduced, culminating in detachment of the vessel. Superficially, pruning resembles anastomosis in reverse.[67] Blood vessel pruning can occur by two different modes, depending on the presence or the absence of a lumen during this process.

In addition to pruning, remodeling also vascular stabilization that includes changes in the diameter of vessel lumens and vascular wall thickening in the vascular walls.[138] It is a complex phenomenon that requires a vast array of molecular signaling. One of the earliest identified events involved in vascular remodeling was the interaction between the receptor tyrosine kinase Tie-2 and its ligand, Angiopoietin (ANG). Mice lacking Tie-2 or ANG die between embryonic days 9.5 and 12.5 and the embryos show a persisting capillary plexus, reminiscent of a defect in vascular remodeling and angiogenesis.[139] In zebrafish, KLF2 induces vessel remodeling by upregulating the EC-specific miR-126 that modulates PI3K and MAPK signaling.[140] Up-regulation 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 (Figure 11).[67]

Blood flow is also critically important for determining the vessel fate [vessels with high flow widen, while vessels with low flow regress], although recent advance of vascular biology strongly argues for the autonomous fate control achieved by blood vessels.[141] Vessels can adjust their shape and function to meet changing tissue oxygen demands (Figure 12). 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.[142] An oxygen-sensing system ensures that phalanx ECs normalize abnormalities in structure and function of ECs to readapt oxygen supply to tissue needs. Hypoxia-Inducible Factors (HIFs) orchestrate adaptive responses of phalanx ECs that line quiescent vessels to changes in oxygen tension.[143] HIF activity is regulated by oxygen-sensing Prolyl Hydroxylase Domain proteins (PHD1-3).[53] 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.[144] Autocrine signals, including VEGF, Ang1, FGF and Notch, maintain ECs in quiescence.[145] Tight junction molecules maintain and regulate paracellular permeability, whereas adherens junction molecules mediate cell-cell adhesion, cytoskeletal reorganization and intracellular signaling.[146] In complex with VEGFR2, VE-cadherin maintains EC quiescence through recruitment of phosphatases that dephosphorylate VEGFR2, thus restraining VEGF signaling.[53] 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.[147] Furthermore, deposition of a BM around quiescent ECs

promotes vessel stabilization, partly because the BM component laminin-α4 in tip cells limits their number by inducing Notch signaling.[148] In addition to the establishment of a quiescent and stable vasculature, Dll-4/notch signaling also appears to be important for further maintenance of the quiescent state (Figure 12).[149]

For vessels to become functional, they must mature-at the level of the endothelium and vessel wall and as a network.[29] A fundamental feature of vessel maturation is the recruitment of mural cells (Figure 12).[150] The factors that are involved in pericyte recruitment include Angiopoietin-1 (ANG1)-TIE2, Platelet-Derived Growth Factor β (PDGFB)-PDBFRB, Transforming Growth Factor-1 (TGFB1)-Activin receptor-Like Kinase 5 (ALK5) and notch signaling components.[29] The current literature collectively suggests that the process of vascular remodeling and maturation is strongly dependent on the ANG ligand-TIE-receptor signaling mechanism. ANG1 induces vessel stabilization via signaling through the TIE2 receptor.[139] ANG2 was initially considered to be an antagonist for ANG1/TIE2 signaling that inhibited vessel stabilization and favoured vascular regression.[151] However, later findings have shown that ANG2 can also activate the TIE2 receptor, albeit weakly and in a context dependent manner.[152,153] Furthermore, ANG1 can have opposing roles either by inducing vascular quiescence or stimulating angiogenesis; differential gene expression profiles are induced in TIE2-expressing ECs depending on whether ANG1 stimulation occurs in the presence or absence of cell-cell contact.[154] Dll-4/notch signaling also plays a crucial role in the vascular stabilization process by affecting both ECs and pericytes via inhibition of angiogenic sprouting.[62] Notch signaling also promotes vascular stabilization more directly through the induction of Notch-Regulated Ankyrin Repeat Protein (NRARP) expression[59] and via the production of ECM components.[149] NRARP limits notch signaling and promotes WNT/CTNNB1 signaling in stalk cells, which supports vascular stability and prevents EC retraction by inducing proliferation and improving intercellular junctions (Figure 12).[59]

Stalk cells (purple) recruit pericytes (orange) to stabilize the vasculature, possibly through the production of stabilizing factors such as TIMP3 and ANG1. ANG1 signalling through the TIE2 receptor stabilizes the vasculature, in part via inducing DLL4 expression in the Endothelial Cells (ECs) and activating notch signalling. Notch activation then plays a dual role in vascular stabilization: first, it downregulates VEGFR2 expression, thereby preventing further sprouting through activation of the VEGF/notch signalling pathway; second, it induces the expression of NRARP, which promotes WNT signalling leading to increased proliferation and Tight Junction (TJ) stabilization.

CONCLUSION

Formation of the vascular system is an essential and rate-limiting step in development and occurs primarily through two main mechanisms, vasculogenesis and angiogenesis. Although most normal angiogenesis occurs in the embryo, it also occurs in adult tissues for wound healing, growth and action of female reproductive organs and during pathological conditions. New vessels in the adult arise through both angiogenesis (mainly) and vasculogenesis. The formation and growth of new vessels within adults depend on an intricate balance of both pro-angiogenic and anti-angiogenic factors. The multi-step nature of sprouting angiogenesis process suggests that the complex and dynamic angiogenic process occurs in an ordered highly orchestrated series. It is suggested that VEGF is a major player in angiogenesis initiation based on its ability to induce vasodilation via endothelial NO production, which is a prerequisite for endothelial cells to enter the angiogenic cascade. During sprouting angiogenesis, the specification of ECs into tip and stalk cells that bear different morphologies and functional properties is controlled by the Notch signaling pathway. For endothelial cells to emigrate from their resident site, they need to loosen both the basement membrane and the coat of mural cells by the action of VEGF. Tip cell migration depends on a gradient of VEGF, whereas stalk cell proliferation is regulated by VEGF concentration. VEGF-induced filopodial extension is more dependent on guidance receptors that are expressed on ECs, including ROBO4, UNC5B, PLEXIN-D1, NRPs and EPH family members that probe the environment. After tip cell induction and stalk elongation, a vessel branch needs to generate a lumen through a wide variety of cellular mechanisms depending on the vascular bed or type of vessel formation. Anastomosis can occur between two sprouts and involve two tip cells, or between sprouts and a functional blood vessel, involving only one tip cell. After the initial steps of contact formation and EC polarization, two different cellular mechanisms-type I and type II anastomosis-lead to the formation of an interconnected luminal space and the formation of multicellular, perfused tubes. After the establishment of new connections within the vascular network, significant remodeling occurs; some branches are stabilized whereas others regress (also referred to as pruning) in order to optimize flow or to adapt to changing demands in blood flow. Pruning occurs via two different modes-type I and type II-depending on the state of vessel perfusion during the process. During maturation, stalk cells transform into phalanx cells, which are so called as they form phalanx. The factors that are involved in pericyte recruitment include Angiopoietin-1 (ANG1)-TIE2, Platelet-Derived Growth Factor B (PDGFB)-PDBFRB, Transforming Growth Factor-1 (TGFB1)-Activin receptor-Like Kinase 5 (ALK5) and notch signaling components. Generally, the importance of angiogenesis sparked hopes that manipulating this process could offer therapeutic opportunities.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

EC: Endothelial Cells; **VEGF:** Vascular Endothelial Growth Factor; **P1GF:** Placental Growth Factor; **PDGF:** Platelet-Derived Growth Factor; **FGF-2:** Fibroblast Growth Factor-2; **TGF:** Transforming Growth Factors; **G-CSF:** Granulocyte-Colony Stimulating Factor; **GM-CSF:** Granulocyte-Macrophage Colony Stimulating Factor; **U-II:** Urotensin-II; **VIP:** Vasoactive Intestinal Peptide; **PACAP:** Pituitary Adenylate Cyclase-Activating Polypeptide; **PECAM:** Platelet Endothelial Cell Adhesion Molecule; **HIF:** Hypoxia Inducible Factor; **NICD:** Notch Intracellular Domain; **ANG:** Angiopoietin; **MMP:** Matrix Metalloproteinases; **PAI:** Plasminogen Activator Inhibitor; **TIMP:** Tissue Inhibitors Of Metalloproteinase; **ROBO:** Roundabouts; **SEMA:** Semaphorins; **NRARP:** Notch Target Notch-Regulated Ankyrin Repeat Protein; **JAM:** Junctional Adhesion Molecule; **PODXL:** Podocalyxin; **ZO:** Zonula Occludens; **ROCK:** Rho-Associated Protein Kinase; **RASP:** Ras-Interacting Protein; **AMIS:** Apical Membrane Initiation Site.

REFERENCES

1. Kim J, Oh WJ, Gaiano N, Yoshida Y, Gu C. Semaphorin 3E-Plexin-D1 signaling regulates VEGF function in developmental angiogenesis via a feedback mechanism. Genes Dev. 2011;25(13):1399-411. doi: 10.1101/gad.2042011, PMID 21724832.
2. Monahan-Earley R, Dvorak AM, Aird WC. Evolutionary origins of the blood vascular system and endothelium. J Thromb Haemost. 2013; 11; Suppl 1: 46-66. doi: 10.1111/jth.12253, PMID 23809110.
3. Saaristo A, Karpanen T, Alitalo K. Mechanisms of angiogenesis and their use in the inhibition of tumor growth and metastasis. Oncogene. 2000;19(53):6122-9. doi: 10.1038/sj.onc.1203969, PMID 11156525.
4. Bridges EM, Harris AL. Angiogenesis-To branch or to expand? Oscillating protein signals control the branching and expansion of blood vessels. eLife. 2016;5:e17079. doi: 10.7554/eLife.17079, PMID 27265819.
5. Drake CJ. Embryonic and adult vasculogenesis. Birth Defects Res C Embryo Today. 2003;69(1):73-82. doi: 10.1002/bdrc.10003, PMID 12768659.
6. Burri PH, Hlushchuk R, Djonov V. Intussusceptive angiogenesis: its emergence, its characteristics and its significance. Dev Dyn. 2004;231(3):474-88. doi: 10.1002/dvdy.20184, PMID 15376313.
7. Karamysheva AF. Mechanisms of angiogenesis. Biochemistry (Mosc). 2008;73(7):751-62. doi: 10.1134/s0006297908070031, PMID 18707583.
8. Swift MR, Weinstein BM. Arterial-venous specification during development. Circ Res. 2009;104(5):576-88. doi: 10.1161/CIRCRESAHA.108.188805, PMID 19286613.
9. Silvestre JS, Smadja DM, Lévy BI. Postischemic revascularization: from cellular and molecular mechanisms to clinical applications. Physiol Rev. 2013;93(4):1743-802. doi: 10.1152/physrev.00006.2013, PMID 24137021.
10. Zygmunt M, Herr F, Münstedt K, Lang U, Liang OD. Angiogenesis and vasculogenesis in pregnancy. Eur J Obstet Gynecol Reprod Biol. 2003; 110; Suppl 1:S10-8. doi: 10.1016/s0301-2115(03)00168-4, PMID 12965086.
11. Folkman J. Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov. 2007;6(4):273-86. doi: 10.1038/nrd2115, PMID 17396134.
12. Risau W. Mechanisms of angiogenesis. Nature. 1997;386(6626):671-4. doi: 10.1038/386671a0, PMID 9109485.
13. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nat Med. 2000;6(4):389-95. doi: 10.1038/74651, PMID 10742145.
14. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995;1(1):27-31. doi: 10.1038/nm0195-27, PMID 7584949.
15. Fraser HM, Duncan WC. Vascular morphogenesis in the primate ovary. Angiogenesis. 2005;8(2):101-16. doi: 10.1007/s10456-005-9004-y, PMID 16240058.
16. Girling JE, Rogers PA. Recent advances in endometrial angiogenesis research. Angiogenesis. 2005;8(2):89-99. doi: 10.1007/s10456-005-9006-9, PMID 16211359.
17. Rivard A, Isner JM. Angiogenesis and vasculogenesis in treatment of cardiovascular disease. Mol Med. 1998;4(7):429-40. doi: 10.1007/BF03401749, PMID 9713822.
18. Karkkainen MJ, Petrova TV. Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. Oncogene. 2000;19(49):5598-605. doi: 10.1038/sj.onc.1203855, PMID 11114740.
19. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, *et al.* Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med. 1999;5(4):434-8. doi: 10.1038/7434, PMID 10202935.
20. Papetti M, Herman IM. Mechanisms of normal and tumor-derived angiogenesis. Am J Physiol Cell Physiol. 2002; 282(5):C947-70. doi: 10.1152/ajpcell.00389.2001, PMID 11940508.
21. Ribatti D, Vacca A, Nico B, Roncali L, Dammacco F. Postnatal vasculogenesis. Mech Dev. 2001;100(2):157-63. doi: 10.1016/s0925-4773(00)00522-0, PMID 11165474.
22. Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, Klagsbrun M. Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. Science. 1984;223(4642):1296-9. doi: 10.1126/science.6199844, PMID 6199844.
23. Folkman J, Klagsbrun M. Angiogenic factors. Science. 1987;235(4787):442-7. doi: 10.1126/science.2432664, PMID 2432664.
24. Meininger CJ, Zetter BR. Mast cells and angiogenesis. Semin Cancer Biol. 1992;3(2):73-9. PMID 1378312.
25. Freeman MR, Schneck FX, Gagnon ML, Corless C, Soker S, Niknejad K, *et al.* Peripheral blood T lymphocytes and lymphocytes infiltrating human cancers express vascular endothelial growth factor: a potential role for T cells in angiogenesis. Cancer Res. 1995;55(18):4140-5. PMID 7545086.
26. Ribatti D, Conconi MT, Nussdorfer GG. Nonclassic endogenous novel regulators of angiogenesis. Pharmacol Rev. 2007;59(2):185-205. doi: 10.1124/pr.59.2.3, PMID 17540906.
27. Brudno Y, Ennett-Shepard AB, Chen RR, Aizenberg M, Mooney DJ. Enhancing microvascular formation and vessel maturation through temporal control over multiple pro-angiogenic and pro-maturation factors. Biomaterials. 2013;34(36):9201-9. doi: 10.1016/j.biomaterials.2013.08.007, PMID 23972477.
28. Conway EM, Collen D, Carmeliet P. Molecular mechanisms of blood vessel growth. Cardiovasc Res. 2001;49(3):507-21. doi: 10.1016/s0008-6363(00)00281-9, PMID 11166264.
29. Jain RK. Molecular regulation of vessel maturation. Nat Med. 2003;9(6):685-93. doi: 10.1038/nm0603-685, PMID 12778167.
30. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011;473(7347):298-307. doi: 10.1038/nature10144, PMID 21593862.
31. Skalak TC, Price RJ. The role of mechanical stresses in microvascular remodeling. Microcirculation. 1996;3(2):143-65. doi: 10.3109/10739689609148284, PMID 8839437.
32. Peirce SM, Skalak TC. Microvascular remodeling: a complex continuum spanning angiogenesis to arteriogenesis. Microcirculation. 2003;10(1):99-111. doi: 10.1038/sj.mn.7800172, PMID 12610666.
33. Ziche M, Morbidelli L, Choudhuri R, Zhang HT, Donnini S, Granger HJ, *et al.* Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. J Clin Invest. 1997;99(11):2625-34. doi: 10.1172/JCI119451, PMID 9169492.
34. Eliceiri BP, Paul R, Schwartzberg PL, Hood JD, Leng J, Cheresh DA. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. Mol Cell. 1999;4(6):915-24. doi: 10.1016/s1097-2765(00)80221-x, PMID 10635317.
35. Gupta K, Kshirsagar S, Li W, Gui L, Ramakrishnan S, Gupta P, *et al.* VEGF prevents apoptosis of human microvascular endothelial cells via opposing effects on MAPK/ERK and SAPK/JNK signaling. Exp Cell Res. 1999;247(2):495-504. doi: 10.1006/excr.1998.4359, PMID 10066377.
36. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, *et al.* Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol. 1996;16(9):4604-13. doi: 10.1128/MCB.16.9.4604, PMID 8756616.
37. Gerhardt H, Ruhrberg C, Abramsson A, Fujisawa H, Shima D, Betsholtz C. Neuropilin-1 is required for endothelial tip cell guidance in the developing central nervous system. Dev Dyn. 2004;231(3):503-9. doi: 10.1002/dvdy.20148, PMID 15376331.
38. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, *et al.* VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol. 2003;161(6):1163-77. doi: 10.1083/jcb.200302047, PMID 12810700.
39. Siekmann AF, Lawson ND. Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries. Nature. 2007;445(7129):781-4. doi: 10.1038/nature05577, PMID 17259972.
40. Ribatti D, Crivellato E. Sprouting angiogenesis, a reappraisal. Dev Biol. 2012;372(2):157-65. doi: 10.1016/j.ydbio.2012.09.018, PMID 23031691.

41. Thurston G, Kitajewski J. VEGF and delta-Notch: interacting signaling pathways in tumor angiogenesis. Br J Cancer. 2008;99(8):1204-9. doi: 10.1038/sj.bjc.6604484, PMID 18827808.
42. Phng LK, Gerhardt H. Angiogenesis: a team effort coordinated by notch. Dev Cell. 2009;16(2):196-208. doi: 10.1016/j.devcel.2009.01.015, PMID 19217422.
43. Ruhrberg C. Growing and shaping the vascular tree: multiple roles for VEGF. BioEssays. 2003;25(11):1052-60. doi: 10.1002/bies.10351, PMID 14579246.
44. Eilken HM, Adams RH. Dynamics of endothelial cell behavior in sprouting angiogenesis. Curr Opin Cell Biol. 2010;22(5):617-25. doi: 10.1016/j.ceb.2010.08.010 , PMID 20817428.
45. Muskavitch MA. Delta-notch signaling and Drosophila cell fate choice. Dev Biol. 1994;166(2):415-30. doi: 10.1006/dbio.1994.1326, PMID 7813766.
46. Lewis J. Notch signalling and the control of cell fate choices in vertebrates. Semin Cell Dev Biol. 1998;9(6):583-9. doi: 10.1006/scdb.1998.0266, PMID 9892564.
47. Geudens I, Gerhardt H. Coordinating cell behavior during blood vessel formation. Development. 2011;138(21):4569-83. doi: 10.1242/dev.062323, PMID 21965610.
48. Benedito R, Duarte A. Expression of Dll4 during mouse embryogenesis suggests multiple developmental roles. Gene Expr Patterns. 2005;5(6):750-5. doi: 10.1016/j.modgep.2005.04.004, PMID 15923152.
49. Radtke F, Schweisguth F, Pear W. The Notch "gospel." EMBO Rep. 2005;6(12):1120-5. doi: 10.1038/sj.embor.7400585, PMID 16299468.
50. Hellström M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, *et al.* Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature. 2007;445(7129):776-80. doi: 10.1038/nature05571, PMID 17259973.
51. Suchting S, Freitas C, le Noble F, Benedito R, Bréant C, Duarte A, *et al.* The Notch ligand Delta-like 4 negatively regulates endothelial tip cell formation and vessel branching. Proc Natl Acad Sci U S A. 2007;104(9):3225-30. doi: 10.1073/pnas.0611177104, PMID 17296941.
52. Williams CK, Li JL, Murga M, Harris AL, Tosato G. Up-regulation of the Notch ligand Delta-like 4 inhibits VEGF-induced endothelial cell function. Blood. 2006;107(3):931-9. doi: 10.1182/blood-2005-03-1000, PMID 16219802.
53. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell. 2011;146(6):873-87. doi: 10.1016/j.cell.2011.08.039, PMID 21925313.
54. Liu ZJ, Shirakawa T, Li Y, Soma A, Oka M, Dotto GP, *et al.* Regulation of Notch1 and Dll4 by vascular endothelial growth factor in arterial endothelial cells: implications for modulating arteriogenesis and angiogenesis. Mol Cell Biol. 2003;23(1):14-25. doi: 10.1128/MCB.23.1.14-25.2003, PMID 12482957.
55. Jakobsson L, Franco CA, Bentley K, Collins RT, Ponsioen B, Aspalter IM, *et al.* Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. Nat Cell Biol. 2010;12(10):943-53. doi: 10.1038/ncb2103, PMID 20871601.
56. Krueger J, Liu D, Scholz K, Zimmer A, Shi Y, Klein C, *et al.* Flt1 acts as a negative regulator of tip cell formation and branching morphogenesis in the zebrafish embryo. Development. 2011;138(10):2111-20. doi: 10.1242/dev.063933, PMID 21521739.
57. Lobov IB, Renard RA, Papadopoulos N, Gale NW, Thurston G, Yancopoulos GD, *et al.* Delta-like ligand 4 (Dll4) is induced by VEGF as a negative regulator of angiogenic sprouting. Proc Natl Acad Sci U S A. 2007;104(9):3219-24. doi: 10.1073/pnas.0611206104, PMID 17296940.
58. Tammela T, Zarkada G, Wallgard E, Murtomäki A, Suchting S, Wirzenius M, *et al.* Blocking VEGFR-3 suppresses angiogenic sprouting and vascular newtwork formation. Nature. 2008;454(7204):656-60. doi: 10.1038/nature07083, PMID 18594512.
59. Phng LK, Potente M, Leslie JD, Babbage J, Nyqvist D, Lobov I, *et al.* NRARP coordinates endothelial Notch and wnt signaling to control vessel density in angiogenesis. Dev Cell. 2009;16(1):70-82. doi: 10.1016/j.devcel.2008.12.009, PMID 19154719.
60. Nakayama M, Nakayama A, van Lessen M, Yamamoto H, Hoffmann S, Drexler HC, *et al.* Spatial regulation of VEGF receptor endocytosis in angiogenesis. Nat Cell Biol. 2013;15(3):249-60. doi: 10.1038/ncb2679, PMID 23354168.
61. Welti J, Loges S, Dimmeler S, Carmeliet P. Recent molecular discoveries in angiogenesis and antiangiogenic therapies in cancer. J Clin Invest. 2013;123(8):3190-200. doi: 10.1172/JCI70212, PMID 23908119.
62. Leslie JD, Ariza-McNaughton L, Bermange AL, McAdow R, Johnson SL, Lewis J. Endothelial signalling by the Notch ligand Delta-like 4 restricts angiogenesis. Development. 2007;134(5):839-44. doi: 10.1242/dev.003244, PMID 17251261.
63. Benedito R, Roca C, Sörensen I, Adams S, Gossler A, Fruttiger M, *et al.* The notch ligand Dll4 and jagged 1 have opposing effects on angiogenesis. Cell. 2009;137(6):1124-35. doi: 10.1016/j.cell.2009.03.025, PMID 19524514.
64. Ridgway J, Zhang G, Wu Y, Stawicki S, Liang WC, Chanthery Y, *et al.* Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. Nature. 2006;444(7122):1083-7. doi: 10.1038/nature05313, PMID 17183323.
65. Noguera-Troise I, Daly C, Papadopoulos NJ, Coetzee S, Boland P, Gale NW, *et al.* Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. Nature. 2006;444(7122):1032-7. doi: 10.1038/nature05355, PMID 17183313.
66. Holderfield MT, Hughes CC. Crosstalk between vascular endothelial growth factor, notch and transforming growth factor-b in vascular morphogenesis. Circ Res. 2008;102(6):637-52. doi: 10.1161/CIRCRESAHA.107.167171, PMID 18369162.
67. Betz C, Lenard A, Belting HG, Affolter M. Cell behaviors and dynamics during angiogenesis. Development. 2016;143(13):2249-60. doi: 10.1242/dev.135616, PMID 27381223.
68. Eble JA, Niland S. The extracellular matrix of blood vessels. Curr Pharm Des. 2009;15(12):1385-400. doi: 10.2174/138161209787846757, PMID 19355976.
69. Huang H, Bhat A, Woodnutt G, Lappe R. Targeting the ANGPT-TIE2 pathway in malignancy. Nat Rev Cancer. 2010;10(8):575-85. doi: 10.1038/nrc2894, PMID 20651738.
70. Augustin HG, Koh GY, Thurston G, Alitalo K. Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nat Rev Mol Cell Biol. 2009;10(3):165-77. doi: 10.1038/nrm2639, PMID 19234476.
71. Arroyo AG, Iruela-Arispe ML. Extracellular matrix, inflammation and the angiogenic response. Cardiovasc Res. 2010;86(2):226-35. doi: 10.1093/cvr/cvq049, PMID 20154066.
72. Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: A moving target for therapeutic intervention. J Clin Invest. 1999;103(9):1237-41. doi: 10.1172/JCI6870, PMID 10225966.
73. Griffioen AW, Molema G. Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases and chronic inflammation. Pharmacol Rev. 52:2. 2000; 52(2): 237-68: 237-68. PMID 10835101.
74. Ghabrial AS, Krasnow MA. Social interactions among epithelial cells during tracheal branching morphogenesis. Nature. 2006;441(7094):746-9. doi: 10.1038/nature04829, PMID 16760977.
75. Blasi F, Carmeliet P. uPAR: a versatile signalling orchestrator. Nat Rev Mol Cell Biol. 2002;3(12):932-43. doi: 10.1038/nrm977, PMID 12461559.
76. Valente P, Fassina G, Melchiori A, Masiello L, Cilli M, Vacca A, *et al.* TIMP-2 over-expression reduces invasion and angiogenesis and protects B16F10 melanoma cells from apoptosis. Int J Cancer. 1998;75(2):246-53. doi: 10.1002/(sici)1097-0215(19980119)75: 2<246::aid-ijc13>3.0.co;2-b, PMID 9462715.
77. Nyberg P, Xie L, Kalluri R. Endogenous inhibitors of angiogenesis. Cancer Res. 2005;65(10):3967-79. doi: 10.1158/0008-5472.CAN-04-2427, PMID 15899784.
78. Tahergorabi Z, Khazaei M. A review on angiogenesis and its assays. Iran J Basic Med Sci. 2012;15(6):1110-26. PMID 23653839.
79. Czubayko F, Liaudet-Coopman ED, Aigner A, Tuveson AT, Berchem GJ, Wellstein A. A secreted FGF-binding protein can serve as the angiogenic switch in human cancer. Nat Med. 1997;3(10):1137-40. doi: 10.1038/nm1097-1137, PMID 9334727.
80. Hansen-Algenstaedt N, Fukumura D, Stoll B, Hicklin D, Jain R. Second wave of angiogenesis during KDR/Flk-1 antibody therapy [abstract]. Proc Am Assoc Canc Res. 1999;40:620.
81. Chappell JC, Bautch VL. Vascular development: genetic mechanisms and links to vascular disease. Curr Top Dev Biol. 2010;90:43-72. doi: 10.1016/S0070-2153(10)90002-1, PMID 20691847.
82. Ruhrberg C, Gerhardt H, Golding M, Watson R, Ioannidou S, Fujisawa H, *et al.* Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. Genes Dev. 2002;16(20):2684-98. doi: 10.1101/gad.242002, PMID 12381667.
83. Etienne-Manneville S. Cdc42-the centre of polarity. J Cell Sci. 2004;117(8):1291-300. doi: 10.1242/jcs.01115, PMID 15020669.
84. De Smet F, Segura I, De Bock K, Hohensinner PJ, Carmeliet P. Mechanisms of vessel branching: filopodia of endothelial tip cells lead the way. Arterioscler Thromb Vasc Biol. 2009;29(5):639-49. doi: 10.1161/ATVBAHA.109.185165, PMID 19265031.
85. Koch M, Hussein F, Woeste A, Gründker C, Frontzek K, Emons G, *et al.* CD36-mediated activation of endothelial cell apoptosis by an N-terminal recombinant fragment of thrombospondin-2 inhibits breast cancer growth and metastasis *in vivo*. Breast Cancer Res Treat. 2011;128(2):337-46. doi: 10.1007/s10549-010-1085-7, PMID 20714802.
86. Testori J, Schweighofer B, Helfrich I, Sturtzel C, Lipnik K, Gesierich S, *et al.* The VEGF-regulated transcription factor HLX controls the expression of guidance cues negatively regulates sprouting of endothelial cells. Blood. 2011;117(9):2735-44. doi: 10.1182/blood-2010-07-293209, PMID 21224470.
87. Herbert SP, Cheung JY, Stainier DY. Determination of endothelial stalk versus tip cell potential during angiogenesis by H2.0-like homeobox-1. Curr Biol. 2012;22(19):1789-94. doi: 10.1016/j.cub.2012.07.037, PMID 22921365.
88. Carmeliet P, Tessier-Lavigne M. Common mechanisms of nerve and blood vessel wiring. Nature. 2005;436(7048):193-200. doi: 10.1038/nature03875, PMID 16015319.
89. Serini G, Maione F, Giraudo E, Bussolino F. Semaphorins and tumor angiogenesis. Angiogenesis. 2009;12(2):187-93. doi: 10.1007/s10456-009-9138-4, PMID 19266292.
90. Segarra M, Ohnuki H, Maric D, Salvucci O, Hou X, Kumar A, *et al.* Semaphorin 6A regulates angiogenesis by modulating VEGF signaling. Blood. 2012;120(19):4104-15. doi: 10.1182/blood-2012-02-410076, PMID 23007403.
91. Adams RH, Eichmann A. Axon guidance molecules in vascular patterning. Cold Spring Harb Perspect Biol. 2010; 2(5):a001875. doi: 10.1101/cshperspect.a001875, PMID 20452960.
92. Dickson BJ, Keleman K. Netrins. Curr Biol. 2002; 12(5):R154-5. doi: 10.1016/s0960-9822(02)00728-5, PMID 11882300.
93. Lu X, le Noble F, Yuan L, Jiang Q, De Lafarge B, Sugiyama D, *et al.* The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system. Nature. 2004;432(7014):179-86. doi: 10.1038/nature03080, PMID 15510105.

94. Salvucci O, Tosato G. Essential roles of EphB receptors and EphrinB ligands in endothelial cell function and angiogenesis. Adv Cancer Res. 2012;114:21-57. doi: 10.1016/B978-0-12-386503-8.00002-8, PMID 22588055.
95. Sawamiphak S, Seidel S, Essmann CL, Wilkinson GA, Pitulescu ME, Acker T, *et al.* Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis. Nature. 2010;465(7297):487-91. doi: 10.1038/nature08995, PMID 20445540.
96. Ausprunk DH, Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. Microvasc Res. 1977;14(1):53-65. doi: 10.1016/0026-2862(77)90141-8, PMID 895546.
97. Caussinus E, Colombelli J, Affolter M. Tip-cell migration controls stalk-cell intercalation during Drosophila tracheal tube elongation. Curr Biol. 2008;18(22):1727-34. doi: 10.1016/j.cub.2008.10.062, PMID 19026547.
98. Wang Y, Kaiser MS, Larson JD, Nasevicius A, Clark KJ, Wadman SA, *et al.* Moesin1 and Ve-cadherin are required in endothelial cells during *in vivo* tubulogenesis. Development. 2010;137(18):3119-28. doi: 10.1242/dev.048785, PMID 20736288.
99. Charpentier MS, Conlon FL. Cellular and molecular mechanisms underlying blood vessel lumen formation. BioEssays. 2014;36(3):251-9. doi: 10.1002/bies.201300133, PMID 24323945.
100. Sigurbjörnsdóttir S, Mathew R, Leptin M. Molecular mechanisms of de novo lumen formation. Nat Rev Mol Cell Biol. 2014;15(10):665-76. doi: 10.1038/nrm3871, PMID 25186133.
101. Zeeb M, Strilic B, Lammert E. Resolving cell-cell junctions: lumen formation in blood vessels. Curr Opin Cell Biol. 2010;22(5):626-32. doi: 10.1016/j.ceb.2010.07.003, PMID 20678912.
102. Koh W, Stratman AN, Sacharidou A, Davis GE. *In vitro* three-dimensional collagen matrix models of endothelial lumen formation during vasculogenesis and angiogenesis. Methods Enzymol. 2008;443:83-101. doi: 10.1016/S0076-6879(08)02005-3, PMID 18772012.
103. Childs S, Chen JN, Garrity DM, Fishman MC. Patterning of angiogenesis in the zebrafish embryo. Development. 2002;129(4):973-82. doi: 10.1242/dev.129.4.973, PMID 11861480.
104. Blum Y, Belting HG, Ellertsdottir E, Herwig L, Lüders F, Affolter M. Complex cell rearrangements during intersegmental vessel sprouting and vessel fusion in zebrafish embryo. Dev Biol. 2008;316(2):312-22. doi: 10.1016/j.ydbio.2008.01.038, PMID 18342303.
105. Strilić B, Eglinger J, Krieg M, Zeeb M, Axnick J, Babál P, *et al.* Electrostatic cell-surface repulsion initiates lumen formation in developing blood vessels. Curr Biol. 2010;20(22):2003-9. doi: 10.1016/j.cub.2010.09.061, PMID 20970336.
106. Dejana E, Orsenigo F, Molendini C, Baluk P, McDonald DM. Organization and signaling of endothelial cell-to-cell junctions in various regions of the blood and lymphatic vascular trees. Cell Tissue Res. 2009;335(1):17-25. doi: 10.1007/s00441-008-0694-5, PMID 18855014.
107. Dejana E. Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis. J Clin Invest. 1996;98(9):1949-53. doi: 10.1172/JCI118997, PMID 8903311.
108. Cavallaro U, Liebner S, Dejana E. Endothelial cadherins and tumor angiogenesis. Exp Cell Res. 2006;312(5):659-67. doi: 10.1016/j.yexcr.2005.09.019, PMID 16256984.
109. Strilić B, Kucera T, Eglinger J, Hughes MR, McNagny KM, Tsukita S, *et al.* The molecular basis of vascular lumen formation in the developing mouse aorta. Dev Cell. 2009;17(4):505-15. doi: 10.1016/j.devcel.2009.08.011, PMID 19853564.
110. Martin-Belmonte F, Gassama A, Datta A, Yu W, Rescher U, Gerke V, *et al.* PTEN-mediated apical segregation of phosphoinositides controls epithelial morphogenesis through Cdc42. Cell. 2007;128(2):383-97. doi: 10.1016/j.cell.2006.11.051, PMID 17254974.
111. Ferrari A, Veligodskiy A, Berge U, Lucas MS, Kroschewski R. ROCKmediated contractility, tight junctions and channels contribute to the conversion of a preapical patch into apical surface during isochoric lumen initiation. J Cell Sci. 2008;121(21):3649-63. doi: 10.1242/jcs.018648, PMID 18946028.
112. Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. Physiol Rev. 2004;84(3):869-901. doi: 10.1152/physrev.00035.2003, PMID 15269339.
113. Xu K, Sacharidou A, Fu S, Chong DC, Skaug B, Chen ZJ, *et al.* Blood vessel tubulogenesis requires Rasip1 regulation of GTPase signaling. Dev Cell. 2011;20(4):526-39. doi: 10.1016/j.devcel.2011.02.010, PMID 21396893.
114. Iruela-Arispe ML, Davis GE. Cellular and molecular mechanisms of vascular lumen formation. Dev Cell. 2009;16(2):222-31. doi: 10.1016/j.devcel.2009.01.013, PMID 19217424.
115. Lenard A, Ellertsdottir E, Herwig L, Krudewig A, Sauteur L, Belting HG, et al. *In vivo* analysis reveals a highly stereotypic morphogenetic pathway of vascular anastomosis. Dev Cell. 2013;25(5):492-506. doi: 10.1016/j.devcel.2013.05.010, PMID 23763948.
116. Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, *et al.* Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood. 2010;116(5):829-40. doi: 10.1182/blood-2009-12-257832, PMID 20404134.
117. Schmidt T, Carmeliet P. Blood-vessel formation: bridges that guide and unite. Nature. 2010;465(7299):697-9. doi: 10.1038/465697a, PMID 20535192.
118. Rymo SF, Gerhardt H, Wolfhagen Sand F, Lang R, Uv A, Betsholtz C. A two-way communication between microglial cells and angiogenic sprouts regulates angiogenesis in aortic ring cultures. PLOS ONE. 2011;6(1):e15846. doi: 10.1371/journal.pone.0015846, PMID 21264342.
119. De Palma M, Murdoch C, Venneri MA, Naldini L, Lewis CE. Tie2-expressing monocytes: regulation of tumor angiogenesis and therapeutic implications. Trends Immunol. 2007;28(12):519-24. doi: 10.1016/j.it.2007.09.004, PMID 17981504.
120. Fung E, Tang SM, Canner JP, Morishige K, Arboleda-Velasquez JF, Cardoso AA, *et al.* Delta-like 4 induces notch signaling in macrophages: implications for inflammation. Circulation. 2007;115(23):2948-56. doi: 10.1161/CIRCULATIONAHA.106.675462, PMID 17533181.
121. Unoki N, Murakami T, Nishijima K, Ogino K, van Rooijen N, Yoshimura N. SDF-1/CXCR4 contributes to the activation of tip cells and microglia in retinal angiogenesis. Invest Ophthalmol Vis Sci. 2010a;51(7):3362-71. doi: 10.1167/iovs.09-4978, PMID 20181837.
122. Strasser GA, Kaminker JS, Tessier-Lavigne M. Microarray analysis of retinal endothelial tip cells identifies CXCR4 as a mediator of tip cell morphology and branching. Blood. 2010;115(24):5102-10. doi: 10.1182/blood-2009-07-230284, PMID 20154215.
123. Bryant DM, Datta A, Rodrıguez-Fraticelli AE, Peränen J, Martín-Belmonte F, Mostov KE. A molecular network for de novo generation of the apical surface and lumen. Nat Cell Biol. 2010;12(11):1035-45. doi: 10.1038/ncb2106, PMID 20890297.
124. Gebala V, Phng L, Gerhardt H. Blood flow drives vascular lumen formation by inverse blebbing. Nat Cell Biol. 2016;18:443-50.
125. Herwig L, Blum Y, Krudewig A, Ellertsdottir E, Lenard A, Belting HG, *et al.* Distinct cellular mechanisms of blood vessel fusion in the zebrafish embryo. Curr Biol. 2011;21(22):1942-8. doi: 10.1016/j.cub.2011.10.016, PMID 22079115.
126. Dong B, Horie T, Denker E, Kusakabe T, Tsuda M, Smith WC, *et al.* Tube formation by complex cellular processes in Ciona intestinalis notochord. Dev Biol. 2009;330(2):237-49. doi: 10.1016/j.ydbio.2009.03.015, PMID 19324030.
127. Denker E, Jiang D. Ciona intestinalis notochord as a new model to investigate the cellular and molecular mechanisms of tubulogenesis. Semin Cell Dev Biol. 2012;23(3):308-19. doi: 10.1016/j.semcdb.2012.03.004, PMID 22465520.
128. Korn C, Augustin HG. Mechanisms of vessel pruning and regression. Dev Cell. 2015;34(1):5-17. doi: 10.1016/j.devcel.2015.06.004, PMID 26151903.
129. Ricard N, Simons M. When it is better to regress: dynamics of vascular pruning. PLOS Biol. 2015;13(5):e1002148. doi: 10.1371/journal.pbio.1002148, PMID 25978860.
130. Chen Q, Jiang L, Li C, Hu D, Bu JW, Cai D, *et al.* Haemodynamics-driven developmental pruning of brain vasculature in zebrafish. PLOS Biol. 2012;10(8):e1001374. doi: 10.1371/journal.pbio.1001374, PMID 22904685.
131. Hlushchuk R, Ehrbar M, Reichmuth P, Heinimann N, Styp-Rekowska B, Escher R, *et al.* Decrease in VEGF expression induces intussusceptive vascular pruning. Arterioscler Thromb Vasc Biol. 2011;31(12):2836-44. doi: 10.1161/ATVBAHA.111.231811, PMID 21921259.
132. Benjamin LE, Hemo I, Keshet E. A plasticity window for blood vessel remodeling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. Development. 1998;125(9):1591-8. doi: 10.1242/dev.125.9.1591, PMID 9521897.
133. Ishida S, Yamashiro K, Usui T, Kaji Y, Ogura Y, Hida T, *et al.* Leukocytes mediate retinal vascular remodeling during development and vaso-obliteration in disease. Nat Med. 2003;9(6):781-8. doi: 10.1038/nm877, PMID 12730690.
134. Stone J, Itin A, Alon T, Pe'er J, Gnessin H, Chan-Ling T, *et al.* Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia. J Neurosci. 1995; 15(7 Pt 1):4738-47. doi: 10.1523/JNEUROSCI.15-07-04738.1995, PMID 7623107.
135. Kochhan E, Lenard A, Ellertsdottir E, Herwig L, Affolter M, Belting HG, *et al.* Blood flow changes coincide with cellular rearrangements during blood vessel pruning in zebrafish embryos. PLOS ONE. 2013;8(10):e75060. doi: 10.1371/journal.pone.0075060, PMID 24146748.
136. Franco CA, Jones ML, Bernabeu MO, Geudens I, Mathivet T, Rosa A, *et al.* Dynamic endothelial cell rearrangements drive developmental vessel regression. PLOS Biol. 2015;13(4):e1002125. doi: 10.1371/journal.pbio.1002125, PMID 25884288.
137. Lenard A, Daetwyler S, Betz C, Ellertsdottir E, Belting HG, Huisken J, *et al.* Endothelial cell self-fusion during vascular pruning. PLOS Biol. 2015;13(4):e1002126. doi: 10.1371/journal.pbio.1002126, PMID 25884426.
138. Howell K, Preston RJ, McLoughlin P. Chronic hypoxia causes angiogenesis in addition to remodeling in the adult pulmonary circulation. J Physiol. 2003;547(1):133-45. doi: 10.1113/jphysiol.2002.030676, PMID 12562951.
139. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, *et al.* Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell. 1996;87(7):1171-80. doi: 10.1016/s0092-8674(00)81813-9, PMID 8980224.
140. Nicoli S, Standley C, Walker P, Hurlstone A, Fogarty KE, Lawson ND. MicroRNA-mediated integration of haemodynamics and VEGF signalling during angiogenesis. Nature. 2010;464(7292):1196-200. doi: 10.1038/nature08889, PMID 20364122.
141. Jones EA. The initiation of blood flow and flow induced events in early vascular development. Semin Cell Dev Biol. 2011;22(9):1028-35. doi: 10.1016/j.semcdb.2011.09.020, PMID 22001248.
142. Mazzone M, Dettori D, de Oliveira RL, Loges S, Schmidt T, Jonckx B, *et al.* Heterozygous deficiency of PHD2 restores tumor oxygenation and inhibits metastasis via endothelial normalization. Cell. 2009;136(5):839-51. doi: 10.1016/j.cell.2009.01.020, PMID 19217150.

143. Majmundar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell. 2010;40(2):294-309. doi: 10.1016/j.molcel.2010.09.022, PMID 20965423.

144. Branco-Price C, Zhang N, Schnelle M, Evans C, Katschinski DM, Liao D, *et al.* Endothelial cell HIF-1alpha HIF-2alpha differentially regulate metastatic success. Cancer Cell. 2012;21(1):52-65. doi: 10.1016/j.ccr.2011.11.017, PMID 22264788.

145. Eichmann A, Simons M. VEGF signaling inside vascular endothelial cells and beyond. Curr Opin Cell Biol. 2012;24(2):188-93. doi: 10.1016/j.ceb.2012.02.002, PMID 22366328.

146. Cavallaro U, Dejana E. Adhesion molecule signalling: not always a sticky business. Nat Rev Mol Cell Biol. 2011;12(3):189-97. doi: 10.1038/nrm3068, PMID 21346732.

147. Falk MM. Adherens junctions remain dynamic. BMC Biol. 2010;8:34. doi: 10.1186/1741-7007-8-34, PMID 20377885.

148. Kitajewski J. Endothelial laminins underlie the tip cell microenvironment. EMBO Rep. 2011;12(11):1087-8. doi: 10.1038/embor.2011.202, PMID 22015689.

149. Zhang J, Fukuhara S, Sako K, Takenouchi T, Kitani H, Kume T, *et al.* Angiopoietin-1/Tie2 signal augments basal Notch signal controlling vascular quiescence by inducing delta-like 4 expression through AKT-mediated activation of β-catenin. J Biol Chem. 2011;286(10):8055-66. doi: 10.1074/jbc.M110.192641, PMID 21212269.

150. Gaengel K, Genové G, Armulik A, Betsholtz C. Endothelialmural cell signaling in vascular development and angiogenesis. Arterioscler Thromb Vasc Biol. 2009;29(5):630-8. doi: 10.1161/ATVBAHA.107.161521, PMID 19164813.

151. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, *et al.* Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. Science. 1997;277(5322):55-60. doi: 10.1126/science.277.5322.55, PMID 9204896.

152. Teichert-Kuliszewska K, Maisonpierre PC, Jones N, Campbell AI, Master Z, Bendeck MP, *et al.* Biological action of angiopoietin-2 in a fibrin matrix model of angiogenesis is associated with activation of Tie2. Cardiovasc Res. 2001;49(3):659-70. doi: 10.1016/s0008-6363(00)00231-5, PMID 11166279.

153. Daly C, Pasnikowski E, Burova E, Wong V, Aldrich TH, Griffiths J, *et al.* Angiopoietin-2 functions as an autocrine protective factor in stressed endothelial cells. Proc Natl Acad Sci U S A. 2006;103(42):15491-6. doi: 10.1073/pnas.0607538103, PMID 17030814.

154. Fukuhara S, Sako K, Minami T, Noda K, Kim HZ, Kodama T, *et al.* Differential function of Tie2 at cell-cell contacts and cell-substratum contacts regulated by angiopoietin-1. Nat Cell Biol. 2008;10(5):513-26. doi: 10.1038/ncb1714, PMID 18425120.

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