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# Wnt/frizzled Signaling in Endothelium: A Major Player in Blood-Retinal- and Blood-Brain-Barrier Integrity

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The Wnt/frizzled signaling pathway is one of the major regulators of endothelial biology, controlling key cellular activities. Many secreted Wnt ligands have been identified and can initiate diverse signaling via binding to a complex set of Frizzled (Fzd) transmembrane receptors and coreceptors. Roughly, Wnt signaling is subdivided into two pathways: the canonical Wnt/ $\beta$ -catenin signaling pathway whose main downstream effector is the transcriptional coactivator  $\beta$ -catenin, and the noncanonical Wnt signaling pathway, which is subdivided into the Wnt/ $\text{Ca}^{2+}$  pathway and the planar cell polarity pathway. Here, we will focus on its cross talk with other angiogenic pathways and on its role in blood-retinal- and blood-brain-barrier formation and its maintenance in a differentiated state. We will unravel how retinal vascular pathologies and neurovascular degenerative diseases result from disruption of the Wnt pathway related to vascular instability, and highlight current research into therapeutic options.

The Wnt signaling pathway is one of the main regulators of endothelial biology. In mammals, 19 different Wnt secreted ligands have been identified to date (Nusse and Clevers 2017); they can initiate distinct signaling upon binding at the cell surface to a combination of multiple transmembrane receptors of the Frizzled (Fzd) family (10 in humans and mice), and low-density lipoprotein receptor-related proteins 5/6 (LRP5/6) coreceptors (Gordon and Nusse 2006). Wnt proteins are highly hydro-

phobic and posttranslationally modified with lipids by the membrane-bound-O-acyltransferase porcupine (PORCN), restricting their diffusion in the aqueous environment primarily toward neighboring cells (Willert et al. 2003; Takada et al. 2006). Studies of their crystal structures show that its lipid tail is required for Wnt interaction within the Fzd extracellular cysteine-rich domain (Janda et al. 2012). Wnt secretion relies on the conserved multipass transmembrane and putative sorting receptor Wntless

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(WIs, also known as Evi/ Sprinter), which shuttles Wnt proteins from the Golgi to the plasma membrane (Bänziger et al. 2006).

On a molecular level, two types of Wnt signaling pathways have been described: the canonical Wnt/ $\beta$ -catenin signaling pathway, which functions to stabilize and induce nuclear translocation of  $\beta$ -catenin, and the noncanonical Wnt signaling pathway, apparently  $\beta$ -catenin independent, which is subdivided into the Wnt/ $\text{Ca}^{2+}$  pathway and the planar cell polarity (PCP) pathway (Gordon and Nusse 2006; Rao and Kühl 2010). Although the Wnt pathways have been separated into two branches for simplicity, the canonical and noncanonical pathways overlap on several levels. It has been shown that the noncanonical Wnt signaling may inhibit the canonical Wnt pathway in receptor- and ligand-dependent manners or reciprocally (Grumolato et al. 2010). The cytoplasmic scaffold protein Dishevelled (Dvl) is then activated and acts as a hub for the transduction of distinct Wnt pathways though different domains (Beitia et al. 2021).

Previous studies have shown that endothelial cells (ECs) express high levels of distinct Fzd receptors, LRP5/6, and Wnt factors (Goodwin et al. 2006). However, the mechanisms underlie the variety of outcomes triggered by Wnt in vascular homeostasis is still under debate. The canonical  $\beta$ -catenin-mediated pathway is the most well-characterized, whereas mechanisms of the downstream noncanonical pathways in vascular development and stabilization are just beginning to be elucidated.

In this paper, we have included a limited discussion on the role of Wnt/Fzd signaling in EC in the development of the blood-retina and blood-brain-barriers (BRB and BBB, respectively) and its involvement in the pathogenesis of retinal and neurodegenerative diseases.

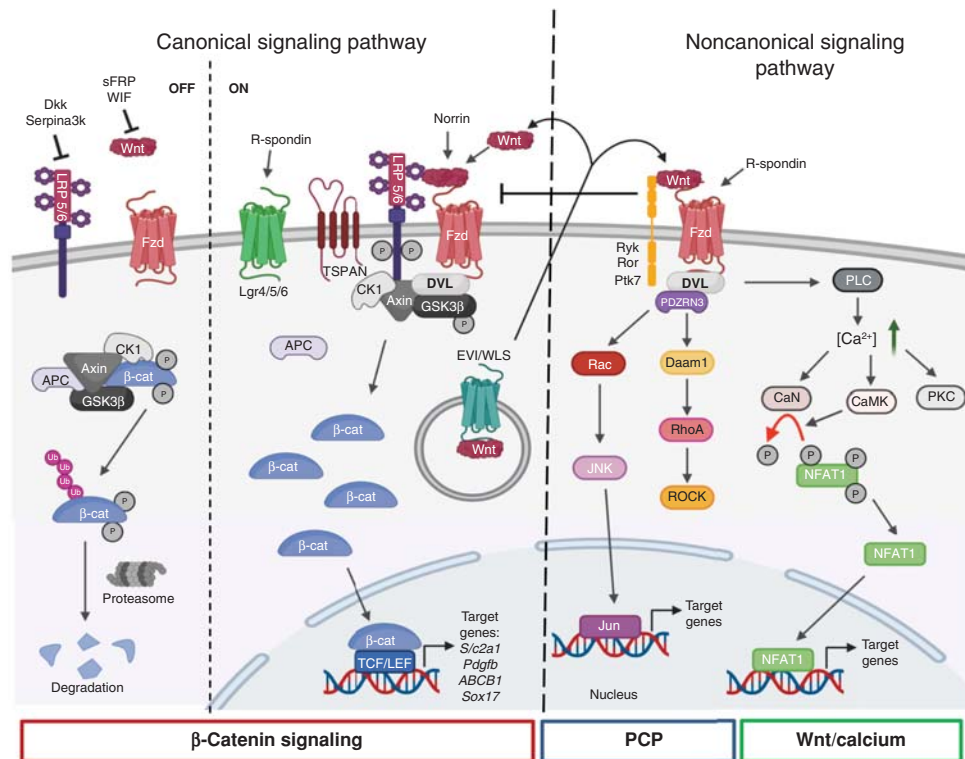
## Wnt SIGNALING PATHWAYS

### Canonical Wnt Signaling

Wnt canonical signaling has been reported to be required for the complex regulation of new vessel development, mainly for angiogenesis of the

BRB and BBB differentiation and maturation (Liebner et al. 2008; Daneman et al. 2009).  $\beta$ -Catenin is reported as the central player of the canonical Wnt pathway. In the presence of Wnt ligands, Wnt/Fzd forms a ternary complex with the coreceptor LRP5/6, which in turn triggers LRP5/6 phosphorylation by casein kinase 1 $\gamma$  (CK1 $\gamma$ ). This signaling cascade results in the formation of large signalosomes with the recruitment of Dvl and Axin proteins (Davidson et al. 2005; Zeng et al. 2005), which are endocytosed (Bilić et al. 2007; Schwarz-Romond et al. 2007). This mechanism leads to disruption of the destruction complex, halting degradation of the Wnt signaling transducer  $\beta$ -catenin in the cytoplasm and ultimately induces its translocation to the nucleus.  $\beta$ -Catenin is then associated with the lymphoid enhancer factor (Lef)/T-cell factor (TCF) transcription factors and other cofactors, resulting in the expression of target genes, such as *Cldn5* (coding for claudin-5) (Liebner et al. 2008), *Slc2a1* (coding for GLUT1) (Daneman et al. 2009), *Pdgfb* (coding for platelet-derived growth factor B [PDGF-B]) (Reis et al. 2012), and *ABCB1* (coding for the efflux transporters of the blood-brain-barrier P-glycoprotein [P-gp]) expression (Lim et al. 2008; Kania et al. 2011). Without Wnt stimulation, cytoplasmic  $\beta$ -catenin is targeted to a complex with the glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), CK1 $\gamma$ , and the tumor suppressor adenomatous polyposis coli (APC) complex linked to the scaffolding protein Axin that induces phosphorylation of  $\beta$ -catenin by GSK3 $\beta$  and its rapid proteasomal degradation (Sakanaka et al. 1998). Target genes are repressed in the absence of nuclear  $\beta$ -catenin (Fig. 1; Cavallo et al. 1998).

Evidence that several inhibitors are present during angiogenesis has further increased the complexity of this pathway. Secreted Fzd-related proteins (sFRP members) (Dufourcq et al. 2008) and Wnt inhibitory factor 1 (WIF-1) (Ramachandran et al. 2012) can directly bind and sequester Wnt ligands, and thereby block Wnt signaling output. For example, sFRP1 (FzdA) expression impairs EC proliferation as a result of reduced expression of cyclin E and cdk2 activity and subsequently reduces neovascularization in a model of mouse hindlimb ischemia (Ezan 2004).



**Figure 1.** Schematic representation of the canonical and noncanonical Wnt signaling pathways. (*Left*) Canonical signaling pathway or Wnt/ $\beta$ -catenin signaling pathway. In the OFF state, Wnt ligands do not bind to Frizzled (Fzd) receptors. Thus, cytoplasmic  $\beta$ -catenin binds to its destruction complex formed by glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), Axin, adenomatous polyposis coli (APC), and casein kinase 1 (CK1), which induces  $\beta$ -catenin phosphorylation (P), thereby targeting it for degradation by the ubiquitin (Ub) proteasome system. Inhibitors of Wnt ligands (secreted Frizzled-related protein [sFRP] or Wnt inhibitory factor [WIF]) or of coreceptors low-density lipoprotein (LDL) receptor-related protein (LRP5/6) (Dickkopf [Dkk] and serine protease inhibitor [Serpina3K]) contribute to blocking of Wnt signaling. In the ON state, Wnt ligands bind to the receptor complex consisting of Fzd and LRP5/6, which then recruits the Dishevelled (DVL) protein to the plasma membrane. The  $\beta$ -catenin destruction complex is recruited to the membrane, which prevents phosphorylation and degradation of  $\beta$ -catenin. This protein can then accumulate in the cytoplasm and translocate to the nucleus to form a complex with the transcription factors T-cell factor (TCF)/lymphoid enhancer factor (LEF) and stimulate the transcription of Wnt/ $\beta$ -catenin target genes. Wnt secretion is a fine process controlled by the evenness interrupted (EVI)/Wntless (WLS) protein. Other coreceptors (tetraspanin [TSPAN] and leucine-rich repeat containing [Lgr4/5/6]) and ligands (Norrin and R-spondin) can activate or enhance the Wnt signaling pathways. (*Right*) Noncanonical signaling pathways. Activation of these pathways involve binding of Wnt ligands to the receptor complex formed by Fzd and other key coreceptors (related to tyrosine [Y] kinase [Ryk], receptor tyrosine kinase-like orphan receptor [Ror], protein tyrosine kinase 7 [Ptk7]). Depending on Wnt/Fzd interactions and coreceptors, distinct downstream effectors are involved to transduce alternative  $\beta$ -catenin-independent signaling pathways, namely, the planar cell polarity (PCP) signaling and Wnt/ $\text{Ca}^{2+}$  signaling pathways. Activation of the Wnt/PCP pathway leads to the formation of a DVL/Dishevelled-associated activator of morphogenesis 1 (Daam1) complex, which activates the small GTPase RhoA and subsequently Rho-associated protein kinase (ROCK) to regulate actin cytoskeleton. The PCP pathway can also activate the small GTPase Ras-related C3 botulinum toxin substrate (Rac) and then c-Jun amino-terminal kinase (JNK), which then phosphorylates and activates Jun and induces transcription of Jun target genes. The Wnt/calcium pathway involves activation of downstream phospholipase C (PLC), which induces increased release of intracellular  $\text{Ca}^{2+}$  levels and then activates protein kinase C (PKC) and  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase (CaMK). This last protein can now dephosphorylate the transcription factor nuclear factor of activated T cells (NFAT) leading to its nuclear import and induction of the transcription of its target genes. The calcium pathway can also promote NFAT dephosphorylation and activation through the calcineurin (CaN) axis. R-spondin ligands (more specifically R-spondin3) can also modulate and activate the Wnt/calcium pathway. Created with BioRender.com. ( $\beta$ -cat)  $\beta$ -Catenin, ( $\text{Ca}^{2+}$ ) calcium.

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Binding of sFRP2 to Fzd receptors on the surface of tumor ECs activates downstream Wnt signaling, enhancing angiogenesis (van Loon et al. 2021). In contrast to sFRPs, another class of inhibitors, the Dickkopf (DKK) protein family, which includes members of DKK1, 2, 3, and 4, inhibits Wnt signaling by preventing the interaction of Wnts with the coreceptors LRP5/6 (Niehrs 2006) and have been shown to be involved in development or tumoral angiogenic events (Pendás-Franco et al. 2008; Min et al. 2011; Choi et al. 2017). For the reader, other  $\beta$ -catenin-independent Wnt/Lrp6 signaling routes have emerged that add a high level of complexity for Wnt signaling (Acebron and Niehrs 2016). Their roles in vascular biology are still unknown.

### The Wnt Noncanonical Pathway

As alluded to above, depending on Wnt/Fzd interaction and coreceptors, distinct Wnt downstream effectors are involved with transducing alternative  $\beta$ -catenin-independent Wnt signaling pathways, such as the Wnt/ $\text{Ca}^{2+}$  and PCP signaling (Fig. 1). Several groups have reported that noncanonical Wnt signaling regulates EC proliferation and vascular network formation (Masckauchán et al. 2006; Cheng et al. 2008; Descamps et al. 2012). Deletion of the Wnt secretion factor Evi (Evi-ECKO) in mouse ECs, uncovered the role of noncanonical Wnt proteins secreted by EC in vascular network stabilization. Evi-ECKO mice showed reduced retinal and tumoral vascularization. This effect was rescued by noncanonical Wnt5a treatment (Korn et al. 2014).

In the Wnt/ $\text{Ca}^{2+}$  cascade, the binding of Wnts to Fzd receptors leads to activation of DVL and downstream phospholipase C (PLC), which allow the release of intracellular  $\text{Ca}^{2+}$  and activation of  $\text{Ca}^{2+}$ -sensitive enzymes, such as  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase (CaMKII) and protein kinase C (PKC). CaMKII activation dephosphorylates the transcription factor nuclear factor of activated T cells (NFAT) leading to its nuclear import. sFRP2 was reported to activate Wnt/ $\text{Ca}^{2+}$  signaling in ECs to induce angiogenesis (Courtwright et al. 2009). Stefaner et al. (2011,

2013) have shown that macrophages secrete non-noncanonical Wnt proteins to control endothelial sFlt1/VEGFR1 expression through an NFAT-calcineurin axis, to limit retinal vessel branching and wound healing. Another study showed that the Wnt signaling enhancer Respondin3 (RSPO3), a secreted protein, highly expressed in endothelium, maintains vascular stability during blood retinal development and vascular remodeling via the activation of Wnt/ $\text{Ca}^{2+}$  pathway in ECs (Scholz et al. 2016).

ECs are characterized by distinct forms of polarity, creating morphological and functional asymmetries that are a prerequisite for their functions. At the tissue level, ECs form a planar polarized monolayer, with a directional cell alignment. It was proposed that this polar tissue pattern along a plane axis (orthogonal to the apico/basal axis) was mediated to some extent by the noncanonical Wnt/PCP pathway. At the cellular level, ECs possess an apicobasal polarity that has been extensively studied in the context of endothelial lumen formation (Iruela-Arispe and Davis 2009; Xu and Cleaver 2011; Lammert and Axnick 2012; Pelton et al. 2014). At the molecular level, both forms of polarity appeared to be intertwined.

First observed and dissected in model organisms as yeast, *Caenorhabditis elegans*, and *Drosophila melanogaster*, where it regulates the orientation of hair on wings and facets in eye, PCP is now known to be involved in mammals in a variety of tissue in regulating gastrulation, sensory cell orientation (Montcouquiol et al. 2003; Wang and Nathans 2007), alveologenesis (Zhang et al. 2020), and in cellular processes such as asymmetric cell division and collective cell migration (Wallingford 2012). Fzd receptors and intracellular Dvl are necessary components. A requirement for Wnt as upstream regulators is still controversial (Ewen-Campen et al. 2020; Yu et al. 2020). A key feature of planar polarity, as reported in *Drosophila* studies, is the asymmetrical localization of PCP core proteins (Simons and Mlodzik 2008). The core PCP proteins are conserved in vertebrate species and include Fzd3/6, Celsr1-3 cadherins, Vangl1/2, Prickle1/2, DVL1-3, and Ankrd6. Their localization in an asymmetric manner, at or near the cell

membrane, is thought to create the polarization of cells. These proteins at the membrane would favor interaction with neighboring cells to spread polarization across tissues. Finally, the PCP pathway activates, via the hub DVL, PCP downstream effector proteins, including DAAM1 and small GTPases such as RhoA, and Rac1 to activate Jun amino-terminal kinase (Wu and Mlodzik 2009). The effector proteins control cytoskeletal rearrangements but are not asymmetrically localized within the cell.

In EC, the identification of molecular PCP effectors is still highly challenging. This is partly due to the nature of this signaling pathway, which typically contributes to tissue organization, and to the limited set of suitable tools to study PCP signaling dynamics. Genetic studies have started to add some pieces to this puzzle, specifically related to the PCP effect on vascular formation. Loss of Wnt5a leads to impaired blood vessel development (Cirone et al. 2008), whereas Wnt5a activation induces the noncanonical Par3/PKC complex and thus reinforces brain EC tight junctions (Artus et al. 2014). The Scribble polarity protein was shown to be required for endothelial-directed migration (Michaelis et al. 2013). In lymphatic ECs, asymmetric localization of CELSR1 and VANGL2, PCP core proteins, was reported during lymphatic valve formation controlling the stabilization of endothelial adherent junctions (Tatin et al. 2013). However, mechanisms by which PCP signaling elicits context-specific processes is still unclear. The study of PCP is hampered by a lack of clarity and consensus on how to (1) define adequate noncanonical EC properties, (2) analyze the regulation of the effectors, and (3) measure signaling in vascular cells. It appears crucial to identify and assess the activity of downstream signaling events in ECs to understand the role of PCP signaling in tissue formation and maintenance. Using genetic screening, our group has identified an actor in the PCP pathway in ECs, the E3 ubiquitin ligase Pdzrn3, and demonstrated its role in vessel network formation (Sewduth et al. 2014). Proteomic screening has shown that Pdzrn3 is an effector in Wnt5a/ROR signal transduction (Konopelski Snavely et al. 2021).

## ROLE OF THE Wnt/PCP SIGNALING IN ECs TO ORCHESTRATE VASCULAR DEVELOPMENT

In this work, we review studies in which Wnt proteins were shown to provide polarity cues at the level of the endothelium during angiogenesis. This research has benefited from an increasing body of genetic tools, sophisticated imaging technology, and computational modeling of blood flow.

Laminar shear flow was shown to drive EC elongation and planar polarization with an asymmetric organization of organelles against the flow axis (Rogers et al. 1985; Tzima et al. 2005). Robust analyses demonstrate that EC dynamically sense the blood flow, migrating against the direction of the blood flow to form a stable vascular network. Consequently, vessel segments under low flow constraint are unstable and regress (Franco et al. 2015). Wnt5a and Wnt11 ligands have been reported to participate functionally in the control of the flow-induced EC polarization response (Franco et al. 2016). In this context, noncanonical Wnt signaling modulates the threshold for flow-dependent EC polarization, inducing premature vessel regression, and leading to a decrease in vessel density (Franco et al. 2016).

Noncanonical Wnt ligands coordinate polarized EC behaviors during vascular morphogenesis (Carvalho et al. 2019). Cell polarization entails highly orchestrated intracellular molecular reorganization events mediated by key regulators of polarity. Wnt5a signaling may activate the Rho family small GTPases Cdc42 reinforcing the link between actin cytoskeleton and intercellular junction proteins during angiogenic collective cell migration (Carvalho et al. 2019). Mouse mutants of PCP signaling effectors, such as the ubiquitin ligase Pdzrn3 (Sewduth et al. 2014) and the polarity protein PAR3 (Hikita et al. 2018), display similar vascular defects with a destabilization of the polarized mouse retinal vascular network. EC polarization may be achieved by the integration of the Par3/aPKC $\zeta$  polarity protein complex-induced signaling and cytoskeleton rearrangement in ECs (Sewduth et al. 2014, 2017; Hikita et al. 2018).

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### CROSS TALK BETWEEN Wnt AND OTHER ANGIOGENIC PATHWAYS

Only a few studies have examined the interplay between the Wnt signaling and other key pathways involved in angiogenesis. One of the most crucial regulators of vascular development is the vascular endothelial growth factor (VEGF) signaling pathway, which includes VEGF ligands (VEGFA, B, C, and D) and receptors (VEGFR1-3) (Ferrara 2009). In EC, VEGF is a key initiator of angiogenesis and sprouting, and a fine regulator of tip cells selection through activation of VEGFR2 (Ruhrberg et al. 2002; Gerhardt et al. 2003; Jakobsson et al. 2010).

An interesting link between the VEGF and Wnt pathways has been described *in vitro* by Skurk and coworkers. The authors propose that the VEGF/PI3-kinase/Akt signaling is downstream of  $\beta$ -catenin and contributes to the pro-angiogenic actions of  $\beta$ -catenin on ECs (Skurk et al. 2005). Subsequently, two studies demonstrated the role of R-spondin (RSPO), a family of secreted proteins that activate Wnt/ $\beta$ -catenin signaling, in regulating developmental vasculogenesis and angiogenesis through the regulation of VEGF (Kazanskaya et al. 2008; Gore et al. 2011). In fact, RSPO3 was identified as a key regulator of VEGF expression via the activation of Wnt/ $\beta$ -catenin signaling in mouse placenta and in a *Xenopus* model (Kazanskaya et al. 2008). In addition, the RSPO3/Wnt signaling pathway has been shown to promote angiogenesis via VEGFC and VEGFR3 in zebrafish (Gore et al. 2011).

In central nervous system (CNS) microvessels, impaired endothelial  $\beta$ -catenin signaling following  $\beta$ -catenin genetic depletion or overexpression of Axin1, a member of the  $\beta$ -catenin protein degradation complex, led to a decreased expression of VEGFR2 and VEGFR3 (Martowicz et al. 2019). Interestingly, the  $\beta$ -catenin-dependent regulation of VEGFR2 and 3 was tissue-specific and loss of  $\beta$ -catenin did not affect the level of VEGF receptors in lung tissues. Since the transcription factor SOX17 has been identified as a  $\beta$ -catenin target in EC, a positive inducer of Wnt/ $\beta$ -catenin signaling and a promoter of both angiogenesis and VEGFR2 expression in the CNS (Corada et al. 2013, 2019; Zhou et al. 2015), the

authors proposed a model where  $\beta$ -catenin drives VEGFR2 expression via the up-regulation of Sox17 (Martowicz et al. 2019). However, how  $\beta$ -catenin precisely regulates VEGFR2 levels during angiogenesis in the CNS is still poorly understood and could involve multiple partners. DKK1 was reported to act via the VEGFR1 and SDF-1 signaling pathway, independent of the Wnt canonical signaling pathway, to regulate angiogenesis (Choi et al. 2017). Additional studies are needed to identify the possible tissue-specific molecular mechanism by which  $\beta$ -catenin drives endothelial VEGFR2/R3 expression to gain a better understanding of the cross talk between these two pathways in this context.

The Notch signaling pathway is an evolutionary highly conserved signaling machinery, which has emerged as an essential regulator of multiple steps involved in vascular development and angiogenesis including endothelial sprouting, tip versus stalk cells selection, and arterial specification (Roca and Adams 2007; Mack and Iruela-Arispe 2018). This pathway involves five ligands, namely, Delta-like (Dll)1, Dll3, Dll4, Jagged1, and Jagged2 that interact with four Notch transmembrane receptors (Notch1–Notch4). Activation of the receptors leads to the proteolytic cleavage of the Notch intracellular domain (NICD) and its translocation to the nucleus where it can regulate gene expression through cooperation with recombinant signal-binding protein for immunoglobulin  $\kappa$  J (RBPJ) (Guruharsha et al. 2012).

Different studies have demonstrated an interplay between the Notch and the Wnt/ $\beta$ -catenin signaling pathways during vascular morphogenesis. Phng et al. reported that the Notch-regulated ankyrin repeat protein (Nrarp), induced by Notch signaling, acts downstream of this pathway to regulate vascular density by controlling stalk cell proliferation and stabilization of new endothelial connections in mouse retina and zebrafish. Nrarp was found to be a negative regulator of Notch signaling by destabilizing NICD, and an activator of the Wnt/ $\beta$ -catenin signaling by binding to lymphoid enhancer factor 1 (Lef1) (Phng et al. 2009). Moreover, in a mouse model overexpressing  $\beta$ -catenin specifically in ECs, treatment with DAPT, an inhibitor of Notch signaling, was able to partially rescue embryonic



vascular defects (Corada et al. 2010). It was shown that in ECs, the transcriptional complex formed by  $\beta$ -catenin/NICD/RBPJ induced by the angiotensin1/Tie2 axis, drove the expression of Dll4 to enhance Notch signaling and promote vascular quiescence (Zhang et al. 2011). Moreover, the endothelial transcription factor ERG has been shown to directly interact with the  $\beta$ -catenin/NICD/RBPJ complex, to control the fine balance between Dll4 and Jagged1 expression in vitro and in mouse retina to maintain vascular homeostasis (Shah et al. 2017). Similarly, our group reported that mice with EC-specific deletion of *Fzd7* displayed reduced  $\beta$ -catenin and Notch signaling. Notably, impairment of the Notch signaling was rescued in vitro and in vivo by activation of  $\beta$ -catenin signaling (Peghaire et al. 2016).

## Wnt SIGNALING AND EYE VASCULATURE

### Wnt Signaling in the Development of Eye Vasculature

The discovery of a genetic link between Wnt signaling and human retinal vascular defects has fostered studies on the role of Wnt signaling in retinal angiogenesis in mice (Xu et al. 2004). Over time, the vascular system of the mouse retina has been used as a robust model for analyzing the molecular and cellular mechanisms regulating angiogenesis (Stahl et al. 2010).

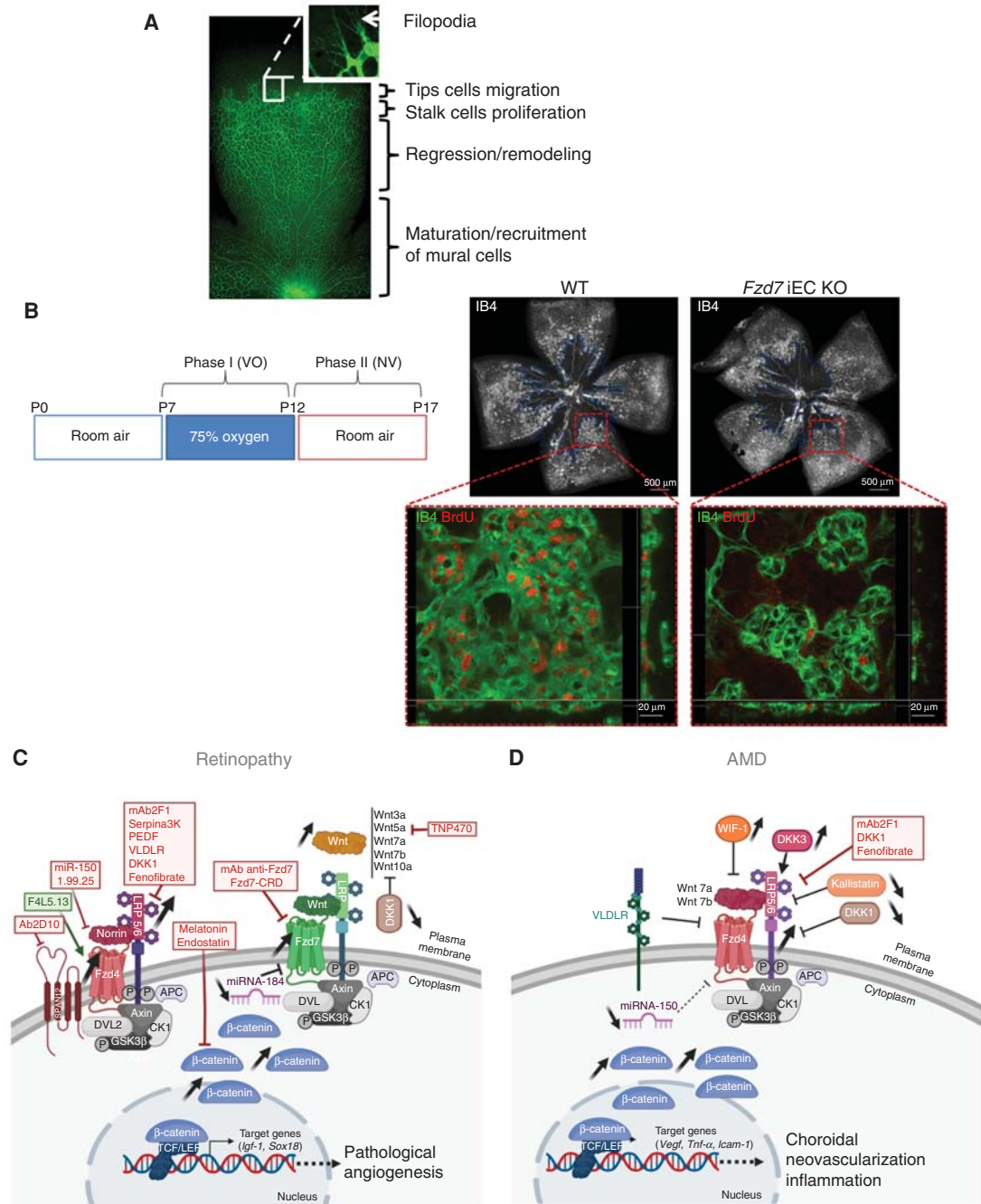
At birth, the mouse retina is avascular, then vascular networks are formed, following a hierarchical pattern under the control of major angiogenic factor such as VEGF (Gerhardt et al. 2003). Deposition of a basement membrane, recruitment of mural cells and dynamic vessel regression contribute to the shaping and maintenance of a functional BRB (Fig. 2A; Korn and Augustin 2015).

Consistent with human disease, mice deficient for *Ndp* (or *Norrie Disease Protein* gene encoding Norrin), *Fzd4*, or *Tspan12* show major defects in retinal vascularization, including loss of BRB integrity, a delayed regression of hyaloid vasculature and absence of the secondary and tertiary plexus (Richter et al. 1998; Ye et al. 2009; Wang et al. 2012; Lai et al. 2017). *LRP5*-

null mice display vascular defects with a persistent hyaloid vasculature, similar to the *Norrin* and *Frizzled4* mutants (Kato et al. 2002). These studies highlight the importance of Norrin ligand-induced signaling through the FZD4/LRP5/TSPAN12 receptor complex, for the development and maintenance of retinal vasculature (Xu et al. 2004; Junge et al. 2009; Ye et al. 2010; Chen et al. 2011). Stabilization of  $\beta$ -catenin by genetic approaches, or promoted by the ETS transcription factor ERG, rescues these phenotypes (Zhou et al. 2014b; Birdsey et al. 2015). Molecular analyses identified claudin-5 and plasmalemma vesicle-associated protein (PLVAP) as downstream targets of Norrin/FZD4/LRP5 signaling (Chen et al. 2012; Wang et al. 2012; Zhang et al. 2017). Finally, the major facilitator superfamily domain-containing protein 2 (MSFD2A), a membrane transport protein, was recently identified as a transcriptional target of the Norrin/LRP5/ $\beta$ -catenin pathway in governing endothelial transcytosis and inner BRB integrity (Wang et al. 2020).

A number of reports highlight the molecular complexity of Wnt signaling in the regulation of ocular angiogenesis (Liu et al. 2003; Lobov et al. 2005; Yi et al. 2007; Liu and Nathans 2008; Franco et al. 2016; Bats et al. 2020). In the developing eye, hyaloid vessel regression is tightly regulated by *Fzd5* and the macrophage-secreted *Wnt7b* (Lobov et al. 2005; Liu and Nathans 2008). Our group demonstrated that endothelial *Fzd7* drives postnatal retinal angiogenesis via the activation of *Dvl*/ $\beta$ -catenin signaling (Peghaire et al. 2016). Disruption of noncanonical *Wnt5a* and *Wnt11* ligands or the endothelial ubiquitin ligase *PDZRN3*, was associated with premature vessel regression in the mouse retina (Sewduth et al. 2014; Franco et al. 2016). A similar phenotype was observed in murine retinal vessels deficient for the Wnt secretion factor *Evi*, or for endothelial *RSPO3*, an enhancer of *Wnt*/ $Ca^{2+}$  signaling (Korn et al. 2014; Scholz et al. 2016; Carvalho et al. 2019). Additionally, the noncanonical *Wnt5a*/*ROR2*/*PCP* signaling contributes to the regulation of retinal vessel sprouting by coordinating the collective polarity of ECs, a process closely related to cell migration (Korn et al. 2014; Franco et al. 2016; Carvalho et al. 2019).





**Figure 2.** Role of Wnt signaling in vascular eye pathology. (A) Representative image of a whole flat-mounted retina at P7 from WT mouse with vessels stained with isolectin (IB4, green). Unlike in humans, mouse intra-retinal vasculature occurs postnatally to be essentially mature at about 3 weeks postnatal. With a highly regulated patterning in the development of eye vasculature, mice retina provides a useful experimental tool to deciphering angiogenic mechanisms such as vessel sprouting corresponding to tip cells migration and stalk cells proliferation, vessel regression, maturation, and remodeling during postnatal period. (B, left) Experimental studies of retinopathy of prematurity (ROP) and proliferative diabetic retinopathy (DR) are enabled by the oxygen-induced retinopathy (OIR) model, a well-established animal model of ischemia-induced retinal neovascularization (NV). (Legend continues on following page.)

### Wnt Signaling in Human and Murine Retinal Vascular Diseases

In humans, Norrie disease is an X-linked recessive disorder, due to mutations in the *NDP* gene, VI, which causes persistent hyaloid vessels, incomplete retinal vascularization, and pathologic neovessel proliferation, often resulting in blindness from birth (Berger et al. 1992).

Familial exudative vitreoretinopathy (FEVR) is another inherited blinding disorder that shares phenotypic characteristics with Norrie disease (Gilmour 2015). While half of all FEVR pathogenic mutations are found within *FZD4*, *LRP5*, *TSPAN12*, and *NDP*, others affect genes functionally related to the Wnt/ $\beta$ -catenin signaling: *CTNNB1*, *ILK*, *KIF11*, *JAG1*, and *DLG1* (Xiao et al. 2019; Jia and Ma 2021; Zhang et al. 2021).

Osteoporosis-pseudoglioma syndrome (OPPG) is an autosomal recessive disorder caused by inactivating mutations in *LRP5*, and also leads to persistent primary vitreous vasculature (Gong et al. 2001).

Coats disease is a single-eye condition, predominantly affecting men, that is characterized by microaneurysms, retinal detachments, and lipid exudates (Sen et al. 2019), as a direct consequence of Wnt pathway alterations in *NDP*, *FZD4*, and *RCBTB1* (Black et al. 1999; Robitaille et al. 2011; Wu et al. 2016).

Ischemic retinopathies, including retinopathy of prematurity (ROP) and diabetic retinopathy (DR), are ocular disorders characterized by an initial phase of ischemia, followed by a second phase of abnormal neovascularization that may culminate into retinal detachment



**Figure 2.** (Continued) Neonatal mice are exposed to 75% oxygen from postnatal day 7 (P7) to P12, corresponding to vasobliterative phase (phase I, VO) inducing a central avascular zone (delimited by blue line, image on the right) and returned to room air from P12 to P17 to induce maximum pathologic NV at P17 (phase II). (Right) Representative images showed areas of VO (indicated by blue line) in P17 OIR retinas from WT mice and from *Fzd7* iEC-KO mice. Screenshot of retinal quadrants focus on proliferation (BrdU incorporation, red) in preretinal tufts (IB4 staining, green) at P17 from WT and *Fzd7* iEC-KO OIR mice. Note that *Fzd7* endothelial deletion limits EC proliferation in pathological neovessels. (Panel B from Bats et al. 2020; reprinted, with permission, from John Wiley and Sons © 2019.) Acquired vascular eye disorders include retinopathies (C) and age-related macular degeneration (AMD) (D) in which the involvement of Wnt signaling is fully recognized. (C) Dysregulation of Wnt signaling, with an emphasis on the canonical Wnt/ $\beta$ -catenin pathway, has been widely studied in retinopathies for its implication in the development of pathological angiogenesis. *Fzd4*, *LRP5/6*, and  $\beta$ -catenin are up-regulated either in retinas from diabetic patients or in animal models of retinopathies. Plasma and vitreous fluid levels of DKK1, a Wnt inhibitor, are lower in patients with DR compared to controls. Experimental genetic loss of *Lrp5*, *Dvl2*, or *Fzd4* significantly attenuates NV in OIR. Increased expression of Wnt3a, Wnt5a, Wnt7a, Wnt7b, and Wnt10a but not Norrin is associated with pathologic angiogenesis in retinopathies. The suggested protective effect of Norrin against OIR is mediated by the induction of insulin-like growth factor (IGF-1), a very potent angiogenic molecule. A negative regulator of Wnt/ $\beta$ -catenin signaling, miR-184, is down-regulated in OIR, while the expression of *Fzd7*, its downstream target, is induced during the NV phase. *Fzd7* endothelial deletion exerts a specific inhibitory effect on pathological angiogenesis after OIR by limiting endothelial cell (EC) proliferation in pathological neovessels. (D) Alteration of Wnt signaling is also associated with abnormal choroidal neovascularization (CNV) and inflammation in AMD. CNV initiation involves activation of Wnt/ $\beta$ -catenin pathway, which in turn stimulates vascular endothelial growth factor (VEGF) to mediate angiogenesis. AMD patients exhibit high levels of phospho-LRP6 and  $\beta$ -catenin, especially in the endothelium of choroidal tissue, low plasma levels of the endogenous Wnt inhibitors, kallistatin or DKK1, and, on the contrary, high levels of other Wnt modulators, WIF-1 and DKK3, in the aqueous humor. In mouse, Wnt7a and Wnt7b deletion decrease severity of laser injury-induced CNV. Deficiency for VLDLR, a negative regulator of Wnt pathway, leads to abnormal intraretinal and choroidal NV and inflammation. Finally, laser-induced CNV is promoted by miR-150 deficiency, a negative regulator of *Fzd4* expression. Antiangiogenic strategies targeting Wnt signaling are of real interest in the treatment of retinopathies (C) and AMD (D). Inhibitors and inducers of the Wnt pathway, already tested in angiogenic models of retinopathy and AMD, are shown in red and green, respectively. Their therapeutic potential is detailed in the Therapies for Vascular Diseases section. Created with BioRender.com.

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and blindness (Rivera et al. 2017). In humans, mutations on *NDP*, *FZD4*, and *LRP5* genes have been identified as risk factors for developing severe ROP (Drenser 2016). Association between the activation of Fzd4/LRP5-6/ $\beta$ -catenin pathway and pathological angiogenesis has been widely documented in both human DR and experimental models of retinopathy (Wang et al. 2019b). Patients with DR also exhibit low plasma and vitreous fluid levels of the Wnt inhibitor DKK1 (Qiu et al. 2014). In mice, genetic loss of *Lrp5*, *Dvl2*, or *Fzd4* significantly attenuates neovascularization in oxygen-induced retinopathy (OIR) (Chen et al. 2011; Ngo et al. 2016). Inactivation of endothelial Wnt ligands secretion does not impact the vasobliterative phase of OIR (Franco et al. 2016), further suggesting that Wnt signaling contributes preferentially to the neovascular process of the disease. Unlike many Wnt ligands, Norrin is not up-regulated during retinopathy (Chen et al. 2011; Franco et al. 2016; Lee et al. 2017). Its protective effect against OIR may be mediated by the induction of insulin-like growth factor (IGF-1), a highly potent angiogenic molecule (Zeilbeck et al. 2016).

A negative regulator of Wnt/ $\beta$ -catenin signaling, miR-184, is down-regulated in OIR, whereas *Fzd7*, its downstream target, is induced during neovascularization (Takahashi et al. 2015). Similarly, *Fzd7* endothelial deletion exerts a specific inhibitory effect on ischemia-induced retinal pathological neovascularization and EC proliferation (Fig. 2B; Bats et al. 2020).

Altered Wnt signaling is also associated with the exudative form of age-related macular degeneration (AMD). The development of abnormal VEGF-mediated choroidal neovascularization (CNV), under the activation of canonical Wnt pathway, leads to deterioration in central vision (Zhou et al. 2010). First associated with experimental laser-induced CNV (Hu et al. 2013), high levels of phospho-LRP6 and  $\beta$ -catenin were also identified in the endothelium of human choroidal tissues from AMD patients (Tuo et al. 2015; Lin et al. 2018). Dysregulation of circulating levels of Wnt modulators such as kallistatin, DKK1/3 and WIF-1, correlates with

the severity of CNV in AMD patients (Park et al. 2014; Tuo et al. 2015; Qiu et al. 2017). Mice deficient for *Wnt7a* and *Wnt7b* exhibit decreased CNV severity (Lin et al. 2018), whereas genetic deletion of *Vldlr*, a negative regulator of the Wnt pathway, recapitulates key features of wet AMD (Chen et al. 2007; Hu et al. 2008). Finally, recent studies have identified a novel causal link between dysregulation of the Wnt pathway, circadian rhythms, and extensive metabolic reprogramming in exudative AMD (Vallée et al. 2020). Altered Wnt signaling in retinopathies and AMD is shown in Figure 2C.

### Wnt AND BLOOD-BRAIN-BARRIER (BBB) VASCULATURE

The BBB structure creates a physical interface between blood and brain tissue (Bundgaard and Abbott 2008), contributing to the maintenance of a safe and specific microenvironment for proper synaptic functioning and neuronal connectivity (Zhao et al. 2015). BBB dysfunction and damage are increasingly recognized as potential contributors to the pathogenesis of a number of neurodegenerative diseases and of age-related cognitive decline including Alzheimer's disease (The Alzheimer's Disease Neuroimaging Initiative et al. 2016; Nation et al. 2019; Sweeney et al. 2019).

The BBB is characterized by specialized brain microvascular ECs that display unique biological characteristics linked to their barrier function, namely, specialized tight junctions (Huber et al. 2001; Wolburg and Lippoldt 2002), a lack of fenestration, selective nutrient and efflux transporters, low rates of transcytosis (Ben-Zvi et al. 2014; Andreone et al. 2017; Yang et al. 2020), and low expression of leukocyte adhesion protein. The interactions between ECs, mural cells, and glial cells, forming the neurovascular unit (NVU) in the CNS (Abbott et al. 2006), are crucial for the formation and maintenance of the BBB (Armulik et al. 2010; Guérit et al. 2021; Heithoff et al. 2021) and the control of blood flow (Mishra et al. 2016).

The canonical Wnt/ $\beta$ -catenin signaling has a major role during development postnatal brain angiogenesis and barrierogenesis (Liebner et al.

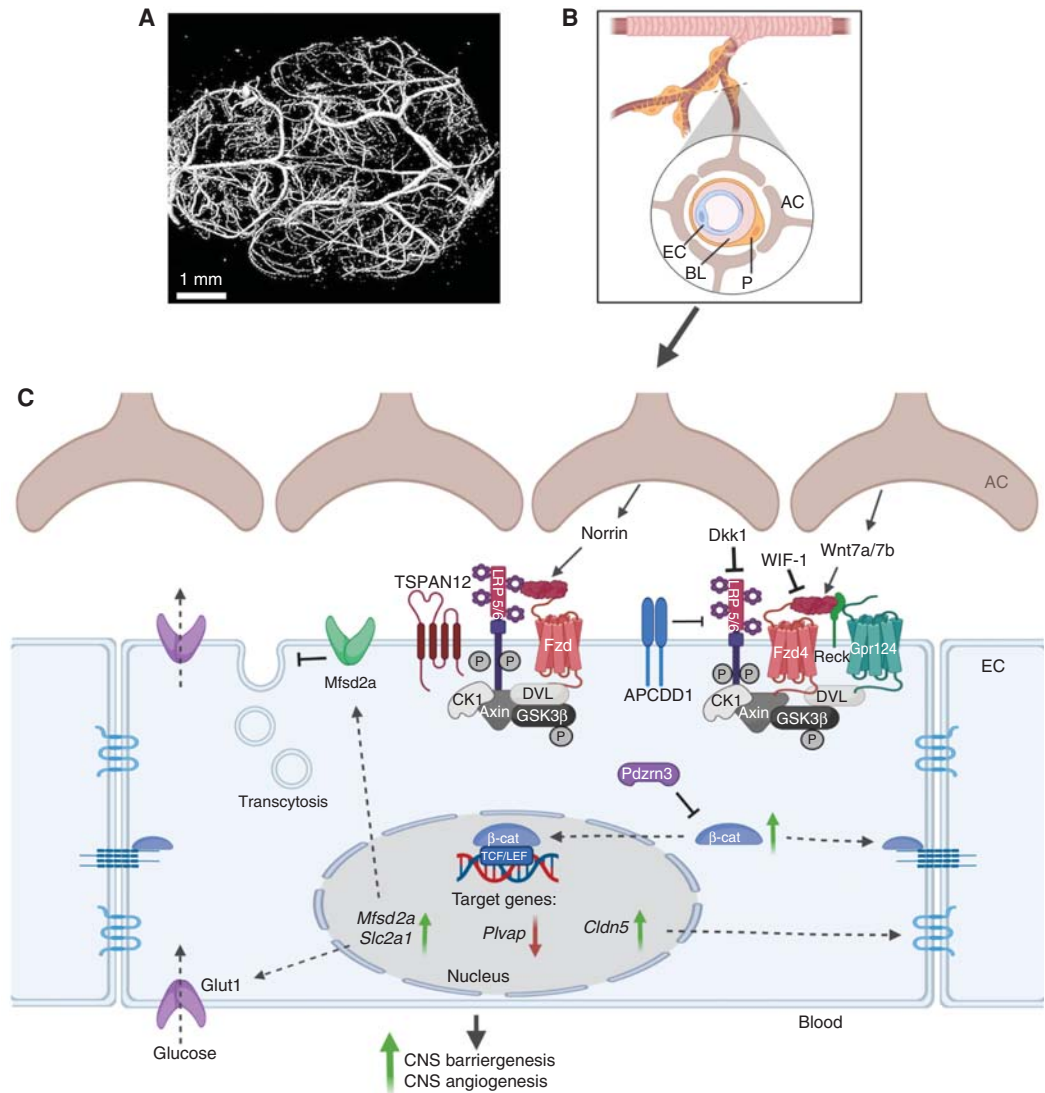
2008; Ye et al. 2009; Wang et al. 2012, 2018; Chang et al. 2017). Consistent with this concept, EC-specific deletion of *Ctnnb1* (gene-encoding  $\beta$ -catenin) leads to embryonic lethality as a result of hemorrhages in the CNS (Daneman et al. 2009). Activation of this pathway requires interaction of the ligands Wnt7a/b to Fzd4 receptors (Liebner et al. 2008; Wang et al. 2018) with the coreceptors LRP5/6 (Zhou et al. 2014b), in concert with the Wnt7-binding glycosylphosphatidylinositol-anchored glycoprotein Reck (Chandana et al. 2010; Vanhollebeke et al. 2015; Eubelen et al. 2018) and the adhesion G-protein-coupled receptor 124 (encoded by *Gpr124*) (Kuhnert et al. 2010; Anderson et al. 2011; Cullen et al. 2011; Eubelen et al. 2018). The receptor complex Fzd4/LRP5/6/Reck/Gpr124 may confer a functional specificity for transducing the Wnt canonical pathway in brain ECs, forming a “Wnt-decoding module” (Eubelen et al. 2018; Vallon et al. 2018; Cho et al. 2019). In addition to Wnt7a/b, Norrin ligand is also involved in Wnt canonical signal induction in brain ECs (Zhou et al. 2014b; Wang et al. 2018). Norrin requires the presence of the coreceptor Tspan-12 (Junge et al. 2009). Both ligands, Wnt7a/b and Norrin, are produced by the neuroepithelium of the developing CNS, concomitant with vessel formation (Stenman et al. 2008; Ye et al. 2011). Their expression is restricted to specific CNS regions, implying local regulation of Wnt pathways. As a result, their combined depletion during development induces a severe leakage throughout the CNS (Zhou et al. 2014b; Wang et al. 2018).

The canonical Wnt/ $\beta$ -catenin signaling plays an essential role in the final differentiation step of brain EC. Its activation up-regulates the expression of genes required to maintain EC barrier features such as *claudin 5* and *Slc2a1* (Glut1) extracellular matrix, whereas *Pvlap*, a component of fenestrated ECs, is repressed (Liebner et al. 2008; Daneman et al. 2009; Zhou et al. 2014b; Jensen et al. 2019). In the adult, understanding the role of endothelial canonical Wnt/ $\beta$ -catenin signaling in CNS under homeostatic and pathologic conditions remains an active field of research. Different groups report that active Wnt- $\beta$ -catenin signaling is detected in the adult

cerebrovasculature (Zhou et al. 2014b) and is required for maintaining BBB integrity (Tran et al. 2016). Mouse mutants with a conditional deletion of *Wls* in astrocytes (to repress Wnt ligand secretion), display edema and increased vascular tracer leakage in adult brain as well as low Wnt pathway activity in brain EC (Guérit et al. 2020; Wang et al. 2020). It is important to note that the regulation of the Wnt/ $\beta$ -catenin signaling pathway has not been determined in the CNS vasculature. However, Wnt modulators such as APCDD1, are expressed throughout the CNS vasculature (Daneman et al. 2010) and have been involved in the maintenance of the retinal barrier (Mazzoni et al. 2017). Wnt antagonists such as *Wif1* and *Dkk1* were shown to disrupt the endothelial BBB phenotype in cerebral tumors (Fig. 3; Phoenix et al. 2016).

There is increasing evidence that the Wnt canonical pathway is essential to regulating the plasticity of brain ECs. For example, activation of the canonical Wnt pathway in choroid plexus ECs (capillaries that do not present BBB properties) partially promotes the acquisition of the EC barrier phenotype (Benz et al. 2019; Wang et al. 2019a). However, activation of Wnt canonical signaling did not appear to be sufficient to maintain the brain EC differentiated phenotype (Sabbagh and Nathans 2020). These data suggest that the maintenance of BBB properties may require activation of additional extrinsic environmental factors and signaling pathways other than Wnt canonical signaling. This concept is supported by a study reporting that activation of Wnt canonical signaling was not sufficient to induce a BBB-specific transcriptional profile in peripheral lung and liver EC (Munji et al. 2019).

While the canonical Wnt signaling pathway's role during BBB formation has been thoroughly investigated, its role in the pathophysiology of vascular cognitive impairment (VCI) remains to be fully elucidated. Dysregulation of Wnt/ $\beta$ -catenin signaling has been linked to vascular disorders in the CNS such as multiple sclerosis (Lengfeld et al. 2017), Alzheimer disease (Liu et al. 2014), and Huntington disease (Lim et al. 2017). Recently, He et al. evaluated the potential effect of Fzd7 in BBB protection



**Figure 3.** The Wnt signaling pathways in central nervous system (CNS) blood vessels. (A) Mouse brain vasculature was imaged with a high-resolution micro-computed tomography (CT) imaging system (Bruker MicroCT). Scale bar, 1 mm. (B) The blood-brain-barrier (BBB) creates a physical interface between blood and brain tissue. The interactions between endothelial cells (ECs), pericytes (Ps), and astrocytes (ACs) form the neurovascular unit in the CNS and are crucial for the formation and maintenance of the BBB. The BBB is characterized by microvascular ECs, which display unique properties linked to their barrier function, including specialized tight junctions, a lack of fenestration, a selective nutrient and efflux transporters system, and a low rate of transcytosis. (C) The canonical Wnt/β-catenin signaling has a major role during CNS barrierogenesis and angiogenesis. Activation of the pathway involves the Norrin ligand produced by astrocytes and the downstream activation of a receptor complex formed by Fzd, LRP5/6, and Tetraspanin12. Alternatively, Wnt ligands (Wnt7a/b) secreted by astrocytes can also bind to the receptor complex consisting of Fzd and LRP5/6, which then recruits the DVL protein to the plasma membrane and induces β-catenin stabilization and its accumulation in the cytoplasm. The ubiquitin ligase PDZRN3 can block this process. Other receptors and coreceptors (probable G-protein-coupled receptor 124 [Gpr124] and reversion-inducing cysteine-rich protein with kazal motifs [Reck]) can enhance this Wnt-dependent signaling pathway, while the Wnt modulator adenomatosis polyposis coli down-regulated 1 protein (APCDD1) can block it. Inhibitors of Wnt ligands (Wnt inhibitory factor 1 [WIF-1]) or of coreceptors LRP5/6 (Dickkopf [Dkk1]) also contribute to the blocking of Wnt signaling. Stabilized β-catenin can then either contribute to the adherens junction at the plasma membrane or translocate to the nucleus and stimulate the transcription of target genes such as claudin 5 (*Cldn5*), which then contributes to the formation of tight junctions. Wnt/β-catenin signaling also increases the expression of solute carrier family 2 member 1 (*Slc2a1*), which is a specialized glucose transporter encoding for Glut1 protein and induces the expression of major facilitator superfamily domain-containing protein 2A (*Mfsd2a*), which inhibits transcytosis. Created with BioRender.com. (BL) Basal lamina, (Plvap) plasmalemma vesicle-associated protein.

**Table 1.** Modulators of Wnt signaling pathways as potential innovative therapies for vascular diseases

Name	Type of compound/ molecule	Target	Tested in	Effects on endothelial cells (ECs) and/or angiogenesis	References
F4L5.13	Tetravalent antibody	Induces Fzd4 and LRP5 proximity	In vitro: in cultured EC In vivo: in postnatal vascularization of mouse retina and in OIR mouse models	Triggers $\beta$ -catenin signaling; promotes EC barrier function in vitro; restores retinal angiogenesis and barrier function in vivo; normalizes NV in OIR models	Chidiac et al. 2021
mAbFzd7	Monoclonal antibody	Specifically recognizes Fzd7 extracellular domain to block Fzd7 signaling	In vitro: in cultured EC In vivo: in OIR mouse models	Normalizes pathological NV in OIR models	Bats et al. 2020
Fzd7CRD	Soluble Fzd7 receptor	Traps Wnt molecules to block Fzd7 signaling	In vitro: in cultured EC In vivo: in OIR mouse models	Normalizes pathological NV in OIR models	Bats et al. 2020
1.99.25	Monoclonal antibody	Specifically recognizes Fzd4-CRD	In vitro: in cultured EC In vivo: in postnatal vascularization of mouse retina and in OIR mouse models; in <i>Vldlr</i> <sup>-/-</sup> KO mouse	Antagonizes Norrin- and WNT3A-induced $\beta$ -catenin signaling; disrupts physiological angiogenesis and induces loss of barrier function; inhibits pathological NV in OIR models; inhibits physiological and retinal pathological angiogenesis in <i>Vldlr</i> <sup>-/-</sup> KO mouse	Paes et al. 2011
miR-184	Liposome-based nanoparticles containing a microRNA-184 mimic	Decreases Fzd7 expression and LRP6 phosphorylation	In vivo: in OIR mouse models	Inhibits Wnt/ $\beta$ -catenin signaling in the retina in OIR mice models	Takahashi et al. 2015
miR-150	MicroRNA-150 mimics	Down-regulates Fzd4 expression	In vitro: in cultured EC In vivo: in OIR mouse models; in CNV mouse models (models of AMD)	Suppresses EC proliferation, migration, and tube formation in vitro; suppresses pathological angiogenesis in OIR models	Liu et al. 2015

*Continued*

**Table 1. Continued**

Name	Type of compound/ molecule	Target	Tested in	Effects on endothelial cells (ECs) and/or angiogenesis	References
Mab2F1	Murine monoclonal antibody	Specifically recognizes E1E2 domain of LRP6	In vitro: in cultured EC In vivo: in rat models of OIR and in streptozotocin-induced diabetes models (models of DR); in CNV (mouse and rat) models	Inhibits $\beta$ -catenin signaling at the Wnt receptor level; inhibits migration and tube formation in vitro; reduces retinal pathological NV in OIR models; reduces retinal vascular leakage and inflammation in OIR and DR models and in <i>Vldlr</i> KO mouse; decreases vascular leakage from CNV lesions and reduces the neovascular area in laser-induced CNV models	Lee et al. 2012; Hu et al. 2013
H1L1	Humanized monoclonal antibody	Specifically recognizes E1E2 domain of LRP6	In vivo: in alkali burn-induced corneal NV rat models	Reduces inflammation and glial stress in corneal NV models	Qiu et al. 2018
Ab 2D10	Antibody	Interacts with human and mouse Tspan12 antigen; blocks interaction between Tspan12 and Fzd4	In vitro: in cultured EC In vivo: in postnatal vascularization of mouse retina; in OIR and <i>Vldlr</i> <sup>-/-</sup> KO mouse	Blocks $\beta$ -catenin signaling; inhibits migration and tube formation in vitro; delays physiological angiogenesis; attenuates retinal pathological NV in retinal vasoproliferative disease	Bucher et al. 2017
Dkk1	Recombinant protein	Binds to LRP5/6	In vivo: in <i>Vldlr</i> <sup>-/-</sup> KO mouse; in OIR mouse models; in streptozotocin-induced DR rat models	Inhibits Wnt/ $\beta$ -catenin signaling, and decreases VEGF in <i>Vldlr</i> <sup>-/-</sup> KO mouse; reduces retinal avascular area and neovascular tufts in OIR mouse models; ameliorates retinal inflammation, vascular leakage, and retinal NV in DR models	Chen et al. 2007, 2009; Tokunaga et al. 2013

*Continued*

Table 1. Continued

Name	Type of compound/ molecule	Target	Tested in	Effects on endothelial cells (ECs) and/or angiogenesis	References
Norrin	Recombinant protein	Activates Wnt/ $\beta$ -catenin signaling	In vitro: in cultured EC In vivo: in OIR models in mouse with ectopic overexpression of Norrin ( $\beta$ B1-Norrin and Rpe65-Norrin); in $Ndp^{y/-}$ mice; in streptozotocin-induced DR rat models	Increases proliferation, migration, and tube formation in vitro; Norrin mutant mice displayed increased physiological angiogenesis and decreased avascular area and pathological NV in OIR models; restores normal retinal angiogenesis in $Ndp^{y/-}$ mice; restores tight junction complex organization and blood-retinal-barrier (BRB) properties	Ohlmann et al. 2005, 2010; Tokunaga et al. 2013; Zeilbeck et al. 2016; Díaz-Coránquez et al. 2020
VLDLR	Nanoparticles containing a plasmid expressing the VLN extracellular domain	Inhibits phosphorylation of LRP6, $\beta$ -catenin accumulation, and Wnt/ $\beta$ -catenin signaling	In vitro: in cultured EC In vivo: in OIR models, alkali burn-induced corneal NV models, and $Vldlr^{-/-}$ mouse models	Inhibits proliferation, migration, and tube formation in vitro; inhibits abnormal neovascularization (NV) in $Vldlr^{-/-}$ mice, in OIR, and corneal NV mouse models	Wang et al. 2015
Curcumin	Organic compound	Attenuates LRP6 phosphorylation, and inhibits $\beta$ -catenin nuclear accumulation	In vitro: in cultured EC In vivo: in a suture-induced corneal NV model ( $Lrp5^{-/-}$ mice)	Attenuates proliferation and disrupts tube formation in vitro; inhibits corneal NV	Zhang et al. 2017
PEDF	Recombinant protein	Binds to LRP6 at the E1E2 domain; blocks Wnt ligand-induced LRP6-Frizzled receptor dimerization	In vitro: in cultured EC In vivo: in OIR mouse models	Blocks Wnt/ $\beta$ -catenin signaling; inhibits migration and increases apoptosis in vitro; reduces ischemia-induced retinal NV in vivo	Longeras et al. 2012

Continued



Table 1. Continued

Name	Type of compound/ molecule	Target	Tested in	Effects on endothelial cells (ECs) and/or angiogenesis	References
PEDF-34	Amino terminal 34 PEDF peptide	Binds to LRP6 at the E1E2 domain; blocks Wnt ligand-induced LRP6- Frizzled receptor dimerization	In vivo: in OIR mouse models	Inhibits endothelial progenitor cells mobilization from bone marrow into blood in vascular retinal NV	Park et al. 2011
SERPINA3K	Adenoviral vectors encoding SERPINA3K	Binds to LRP6 at the extracellular domain	In vivo: in OIR models; in rat streptozotocin-induced DR models; in rat suture-induced corneal NV models	Blocks LRP6 dimerization with Fzd receptor in vitro; reduces NV, inflammation, and vascular leakage in OIR models; displays antifibrotic activity; suppresses corneal NV and inflammation	Zhang et al. 2009, 2010a,b; Zhou et al. 2014a
Lithium	Chemical compound	Inhibits GSK3 $\beta$ ; blocks $\beta$ - catenin degradation	In vitro: in cultured EC In vivo: in <i>Lrp5</i> <sup>-/-</sup> KO mouse; in postnatal vascularization of mouse retina	Activates $\beta$ -catenin signaling; restores tube formation in vitro in LRP5-deficient EC; normalizes deeper layer vessels and ameliorates pathologic glomeruloid vessels in <i>Lrp5</i> <sup>-/-</sup> mice; rescues physiological angiogenesis in mice deleted for Fzd7 in EC	Peghaire et al. 2016; Wang et al. 2016
XAV-939	Chemical compound	Tankyrase 1/2 inhibitor that promotes axin2 stabilization	In vitro: in cultured EC	Promotes $\beta$ -catenin degradation; inhibits $\beta$ -catenin signaling; modest effect on BRB properties	Huang et al. 2009
Melatonin	Chemical compound	Inhibits Wnt/ $\beta$ -catenin signaling	In vitro: in cultured EC in streptozotocin-induced DR rat models	Inhibits high glucose-induced proliferation, migration, invasion, tube formation, and inflammation in vitro; alleviates BRB disruption	Yan et al. 2021

Continued

**Table 1. Continued**

Name	Type of compound/ molecule	Target	Tested in	Effects on endothelial cells (ECs) and/or angiogenesis	References
TNP470	Chemical compound	A noncanonical Wnt5a inhibitor	In vitro: in cultured EC In vivo: in postnatal vascularization of mouse retina; in OIR mouse models	Blocks noncanonical Wnt signaling by targeting methionine aminopeptidase-2; increases vessel regression accompanied by decreased EC proliferation in retinal postnatal angiogenesis; decreases pathological NV in OIR models	Korn et al. 2014

(OIR) Oxygen-induced retinopathy, (DR) diabetic retinopathy, (AMD) age-related macular degeneration, (CNV) choroidal neovascularization.

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after intracerebral hemorrhage. Frizzled-7 activation attenuates BBB permeability and neurological deficits after intracerebral hemorrhage through Dvl/ $\beta$ -catenin/WNT1-inducible signaling pathway protein 1 (WISP) pathway (He et al. 2021). We have proposed that a reactivation of the Wnt/PCP signals in brain EC during pathological ischemic events, such as stroke, would be deleterious in mouse models (Sewduth et al. 2017). Overexpressing *Pdzn3* in EC exacerbates BBB hyperpermeability and accelerates cognitive decline and is correlated with decreased claudin5 expression in brain vessels under chronic cerebral hypoperfusion, whereas depletion of *Pdzn3* is protective for BBB breakdown (Gueniot et al. 2021). Thus, there is great interest in delineating the signaling pathways that regulate the physiological functions of the BBB, and gaining insights into the pathological conditions causing loss or breakdown of the BBB (Fig. 3).

### THERAPIES FOR VASCULAR DISEASES

A multiplicity of studies has recently highlighted the real potential to modulate different component of the Wnt/signaling pathways to treat vascular diseases, particularly ocular vascular disorders (Foulquier et al. 2018; Wang et al. 2019b). For the purpose of this review focusing on the Wnt/signaling pathways in ECs, only studies showing evidence of therapies' efficacy in ECs or in angiogenesis models, have been selected and shown in Table 1 and Figure 2.

### CONCLUSION

Taken together, the data point toward a central role of Wnt/ $\beta$ -catenin signaling during vascular development and homeostasis, where Wnt signaling is a hub integrating other crucial angiogenic intertwined pathways. With the advent of single-cell RNA-sequencing technology and the huge source of available transcriptomic data, one of the next exciting challenges will be to identify the precise combination of Wnt/Fzd partners that interact during physiological or pathological angiogenesis to identify new cell- or tissue-specific pathways.

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## Wnt/frizzled Signaling in Endothelium: A Major Player in Blood-Retinal- and Blood-Brain-Barrier Integrity

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