This article is the introduction of a new thematic series on Wnts in Cardiovascular Development and Disease, which includes the following articles:

An Updated Overview on Wnt Signaling Pathways: A Prelude for More [Circ Res. 2010;106:1798–1806]
The Multiple Phases and Faces of Wnt Signaling During Cardiac Differentiation and Development [Circ Res. 2010;107:186–199]

The Role of Wnt Signaling in Physiological and Pathological Angiogenesis

Wnt Signaling in Cardiac Hypertrophy and Remodeling
Wnt Signaling and Aging-Related Heart Disorders
Wnt Signaling and Stem Cells

Michael Kühl, Guest Editor

The Role of Wnt Signaling in Physiological and Pathological Angiogenesis

Elisabetta Dejana

Abstract: Early stages of vascular development include endothelial cell differentiation in a network of arteries, veins, and lymphatics. Subsequently, to respond to the specific needs of the organs, endothelial cells acquire specialized properties such as permeability control, expression of specific transcellular transport systems, membrane adhesive molecules, and others. Endothelial cell differentiation depends on communication between the surrounding tissues, which is mediated by growth and differentiation factors able to activate specific gene expression programs. Recent reports underline the important role of the Wnt system in vascular morphogenesis in the embryo and in organ-specific endothelial differentiation. Wnt signaling regulates fundamental aspects of development, including cell fate specification, proliferation, and survival, and may use different receptors and signaling pathways. Both loss- and gain-of-function experiments of members of the Wnt signaling pathway were found to cause marked alterations of vascular development and endothelial cell specification. Furthermore, altered Wnt signaling in the endothelium may contribute to pathological conditions such as retinopathies, pulmonary arterial hypertension, stroke, and others. Continued progress in this field holds the potential to identify novel therapeutics for the treatment of these diseases. (Circ Res. 2010;107:943-952.)

Key Words: Wnt β-catenin angiogenesis endothelial cells transcription factors

Blood vessels form a large network of arteries, capillaries, and veins, which provide transport of fluids, gases, macromolecules, and cells within the body. During vertebrate development, blood vessels arise through differentiation of mesodermal progenitor cells called angioblasts into endothelial cells. Endothelial progenitors first assemble into a primitive vascular plexus of the extra embryonic yolk sac and into the aortic primordial of the embryo proper. This process, which includes endothelial cell differentiation, proliferation, and migration, has been defined vasculogenesis. At subsequent steps of development, the vascular plexus is remodeled into a complex system that includes arteries, veins, capillaries, and, to a later stage, lymphatics. The process includes sprouting and expansion of the preexisting vasculature, endothelial cell expression of vascular and organ-specific characteristics, and recruitment of mural cells. Vascular remodeling is needed for the development of an efficient system able to sustain shear stress and transport nutrients and oxygen to the different regions of the body.
organism. Subsequently, to respond to the specific needs of the organs, endothelial cells acquire specialized properties, such as permeability control, expression of specific transcellular transport systems, expression of membrane adhesive molecules, production and binding of chemokines, control of leukocyte traffic, regulation of blood flow, and others. This further level of endothelial cell specialization is induced by the crosstalk with the cells of the surrounding tissues that produce growth and differentiation factors able to activate specific gene expression programs in endothelial cells (see Figure 1). The failure of an appropriate response of endothelial cells to organ-specific requirements may contribute to the pathogenesis of several diseases.4

Transcriptional Regulation of Endothelial Cell Differentiation

All the steps of endothelial cell differentiation are orchestrated by specific transcription factors (Figure 1). Some of the transcriptional mechanisms that determine endothelial identity and then arterial, venous, or lymphatic differentiation have been partially identified (reviewed elsewhere22–28). However, very little is known about the transcription factors that induce organ-specific differentiation of the endothelium.

During the last decade, several groups dedicated a considerable effort in trying to decode the transcriptional mechanisms directing cells of mesodermal origin toward a specific endothelial identity. In contrast to other tissues, endothelial cell differentiation is mediated by several transcription factors that are not cell-specific but act in concert to modulate expression of vascular markers. Through the analysis of the promoter regions of endothelial-specific genes, different transcription factors have been identified as putative inducers of a “common” endothelial cell identity8: these include members of the Ets family, several homeobox (Hox) transcription factors, and members of Sox5 (Sry-related high-mobility group box), in particular, the F subgroup (Sox18, Sox17, and Sox7). However, the identification of the specific transcription factors within each class able to transactivate one or another gene has been only partially accomplished.

After acquisition of “panendothelial” characteristics, endothelial cells undergo further differentiation. It was believed that arterial and venous determination was mostly attributable to endothelial exposure to differing blood flow rates and pressure. However, several studies on different animal models suggest that venous and arterial determination precedes the onset of the circulation (reviewed elsewhere2,3,7–9). Notch signaling and activation of downstream transcription factors (Hey1 and Hey2 [Hes-related proteins]) are required to promote arterial differentiation and to suppress venous fate choices.10,11 In contrast, the transcription factor Coup-TfII is required for venous identity. This factor acts by inhibiting Notch signaling and other effectors of arterial specification.12 Lymphatic endothelial cells differentiate from venous endothelium. The transcriptional factor Sox18, in cooperation with Coup-TfII, promotes expression of Prox-1, which plays a crucial role in final lymphatic determination and maintenance.13 Other transcription factors such as FOXC1 and FOXC2 act in lymphatic remodeling and valve formation.5,14–16

Wnt Signaling in Endothelial Cells

Recent reports underline the important role of the Wnt system in vascular morphogenesis in the embryo and in organ-specific endothelial cell differentiation.4,17–21

Wnt signaling regulates fundamental aspects of development including cell fate specification, proliferation, survival, and overall organogenesis (reviewed elsewhere17,22–26). For an updated and comprehensive review of these aspects in the cardiovascular system, see the article by Rao and Kühl27 in this same series of reviews. Wnt factors use different receptors and signaling pathways. The most-studied and best-understood Wnt signaling pathway is mediated by the transcriptional activity of β-catenin (Figure 2). In absence of Wnt, cytoplasmic β-catenin is degraded by the action of a multiprotein complex (reviewed elsewhere22–28), and Wnt target genes are repressed. The binding of Wnt to the Frizzled/Lrp (low-density lipoprotein receptor–related protein) receptor complex inhibits β-catenin degradation complex and stabilizes β-catenin in the cytoplasm, promoting its translocation to the nucleus. In the nucleus, β-catenin interacts with T-cell factor (TCF)/Lef transcription factors and activates cell transcription.22,29–33

Of note, β-catenin also binds to cadherins (VE- and N-cadherin in endothelial cells) at cell-to-cell adhesions and stabilizes their interaction with the cytoskeleton (Figure 2). In this way, β-catenin acts by stabilizing cell-to-cell adhesion and tissue integrity.34,35 However, when cells are migrating or in sparse conditions and junctions are partially dismantled, a pool of β-catenin36,37 dissociates from junctions and translocates to the nucleus. In nonvascular cells, recent evidence suggests that there are 2 pools of β-catenin at the membrane.38 One is linked to the cadherin complex, which is protected from degradation. A second pool of β-catenin associates to a membrane bound phosphodestruction complex formed by adenomatous polyposis coli (APC) and GSK3, which promotes its degradation. Cadherins can promote the binding of β-catenin to the complex and limits in this way Wnt signaling. Thus, Wnt signaling is indirectly modulated by intercellular junctions.

The Wnt factors can signal also through noncanonical β-catenin–independent pathways.24–28

The Ca2+ pathway is induced by the binding of some members of the Wnt family to specific Frizzled receptors,
which, in turn, activate the phospholipase C and increase intracellular Ca\(^{2+}\).

Another \(\beta\)-catenin–independent Wnt signaling pathway is the planar cell polarity (PCP) pathway, which activates the small GTPases RhoA and Rac and modulates cytoskeletal rearrangements and PCP.

Which Wnt ligand binds and activates which Frizzled receptor and whether all Frizzled receptors trigger \(\beta\)-catenin–dependent or independent signaling remains an open issue. Different Wnt receptors are responsible for inducing one or another signaling pathway, although some receptors\(^{40}\) may activate both canonical and noncanonical pathways. It is likely that the final response is cell context–dependent, ie, related to the type of receptors expressed and the presence or absence of signaling partners in a given cell type at a given moment.

**Noncanonical \(\beta\)-Catenin–Independent Wnt Signaling Pathways in Endothelial Cells**

Some recent publications underline the importance of noncanonical Wnt pathways in regulating endothelial cell growth and angiogenesis.\(^{20,40–44}\) Wnt activation of the Ca\(^{2+}\) pathway induces cell proliferation and inhibits apoptosis in cultured endothelial cells, suggesting a proangiogenic activity in vivo.\(^{41,42}\) PCP also plays a role in vascular morphology, vessel sprouting, and elongation, suggesting a morphogenetic role for this Wnt signaling pathway in vascular development.\(^{44}\) Recently, a member of a subgroup of formins (DAAM1 [Dishevelled-associated activator of morphogenesis]) was found to act downstream of the Wnt/PCP pathway in inhibiting endothelial cell proliferation and angiogenesis.\(^{45}\)

The mechanism of action of DAAM1 is likely to coordinate microtubule assembly and stabilization in the endothelium.

Although the molecular basis of \(\beta\)-catenin–independent signaling pathways is still poorly understood, there is a general consensus that \(\beta\)-catenin–independent signaling antagonizes \(\beta\)-catenin pathway.\(^{24}\) Crosstalk between the different Wnt signaling cascades is possible, and it is also possible that all Frizzled receptors may couple to \(\beta\)-catenin–dependent and –independent pathways. Samarzija et al\(^{46}\) observed...
that Wnt3a induces proliferation and migration of endothelial cells via canonical and noncanonical Wnt signaling pathways. Using an embryonic stem cells (ES) differentiation system in vitro, Yamashita et al.\cite{Yamashita2010} reported that activation of the cells with Wnt3a, which mostly signals through β-catenin, preferentially augments endothelial differentiation while inhibiting cardiomyocyte differentiation. However, Hwang et al.\cite{Hwang2009}, using a similar system, found that Wnt5a, which most frequently signals through noncanonical pathways, directs endothelial differentiation. These observations underline the complexity of the system and how cell responses may be determined by the cellular context, number, and types of Wnt receptors which are activated.

Another β-catenin–independent pathway is mediated by phosphorylation of APC (a member of the destruction complex of β-catenin).\cite{Lambert2002} It has been reported that phosphorylated APC localizes at the tips of cellular extension during cell migration and is required for cell migration and cytoskeletal organization.\cite{Lambert2002} These effects are independent of β-catenin transcriptional activity.

Overall, data available on noncanonical Wnt signaling in vascular development are still scarce, and additional investigation is required to define which functions and at which steps of embryo development noncanonical Wnt pathways may act. The following part of the review is mostly focused on β-catenin–dependent signaling on which more data are currently available.

**Canonical Wnt Signaling in the Embryo Vasculature**

By a systematic RT-PCR analysis, it was found that cultured endothelial cells express quite a large array of Frizzled receptors and Wnt factors, together with Lrp5/6.\cite{Franco2011} This suggests that endothelial cells may be particularly sensitive to Wnt signaling and possibly undergo autocrine stimulation. However, reporter transgenic mouse lines in which Lac-Z expression is under the control of β-catenin–TCF/Lef complex showed relatively low activation of β-catenin transcriptional signaling in the resting vasculature of adult mice. In contrast, detectable activation of Wnt signaling was observed during embryonic development\cite{Lambert2002} in many types of vessels and in the brain microvasculature (see below). Thus, Wnt signaling is important at early steps of vasculogenesis and angiogenesis but is likely silenced in healthy, resting vasculature.

This possibility is confirmed by gene inactivation experiments of different members of the Wnt signaling pathway (for a comprehensive review of these mouse embryo phenotypes, see Franco et al.\cite{Franco2011}). Ablation of Frizzled-5\cite{Huang2015} and Wnt 2\cite{Huang2015} leads to a defective placenta vascularization and, in the case of Frizzled-5, also to defective remodeling of the yolk sac vasculature. Similarly, both loss-of-function and gain-of-function mutations of Frizzled-4 and Lrp5 resulted in early lethality of the mouse embryo resulting from defects of vascular organization, remodeling, and mesenchymal cell coverage.\cite{Lambert2002}

Inactivation of R-spondin, a coactivator of Wnt/β-catenin,\cite{Lambert2002} caused defective placenta and vascular patterning. It was suggested that these defects were attributable to lack of correct angiogenesis and altered angioblast and blood cell specification.

Endothelial-specific deletion of β-catenin affects the development of the embryonic vasculature and results in early lethality in utero.\cite{Lambert2002} The embryos present defective vascular remodeling and diffuse hemorrhages. In some regions, such as the intersomitic vessels or umbilical veins and arteries, the vessels show hyperbranching.\cite{Lambert2002} These alterations were accompanied by changes in the molecular architecture of endothelial adherens junctions, explaining, in part, the pres-
ence of hemorrhages, but defects in the transcriptional activity of β-catenin can also contribute to the development of this pathological phenotype.

Furthermore, loss of β-catenin impairs the development of the endocardial cushion and cardiac valves because of altered endothelial mesenchymal transition.57

An even more dramatic phenotype was observed by inducing endothelial-specific stabilization (gain of function) of β-catenin.51,58 The phenotype was characterized by lack of vascular remodeling, altered elongation of the intersomitic vessels, defects in branching, and loss of venous identity. Consistently, endothelial-specific gain-of-function mutation of Frizzled-4 caused similar alterations in the development and specification of the vasculature.54 Taken together, these data indicate that β-catenin/Wnt signaling must be strictly controlled for harmonic vascular development. Too much or too little signaling may prevent a correct vascular organization and lead to early fetal lethality.

The Role of Wnt in Eye and Brain Vasculature

β-Catenin–dependent Wnt signaling is strongly implicated in the development and differentiation of retina and brain microvasculature. These particular vessels express many common functional and morphological characteristics, such as well-developed tight junctions and a specialized and selective system of membrane transporters.59 This vasculature forms the so-called blood–brain barrier (BBB), which is characterized by a strict control of permeability between blood and the central nervous system. In reporter mice, Wnt signaling was particularly strong during early stages of both retina and brain vasculogenesis.60–62

In the retina, different strategies such as null mutations of Frizzled-4, Lrp5, Lef-1,54,60,63,64 and Norrin54 (see below) were used to abrogate canonical Wnt signaling. All of these conditions induced similar vascular alterations, such as absence of intraretinal capillaries, defects in vascular patterning, arteriovenous anastomosis, and intraocular hemorrhages. Inactivation of the Wnt cofactor Nrarp also caused defects of retina vasculature, with decrease in branching and radial expansion.60

In the embryonic brain, Wnt signaling was quite strong in vessels penetrating into the brain stroma and was still detectable during early postnatal period but declined in the adult.61,65 Wnt signaling followed the same kinetics of brain angiogenesis and BBB development, supporting the idea that Wnts are involved in brain vascularization and induction of BBB properties. Further support of this concept derives from inactivation of Wnt signaling through different strategies, such as ablation of endothelial β-catenin51,62 or of Wnt 7a and -b66 or administration of the soluble inhibitor Frizzled-8 receptor construct.62 Without canonical Wnt signaling, the vessels in the brain parenchyma look enlarged and hemorrhagic. In some regions, endothelial cells grow in multilayers into the vascular lumen, forming multicellular structures.

When β-catenin was ablated postnatally (from postnatal day 1 to 11) vascular morphology was not significantly modified, but vessels presented lack of BBB differentiation and control of permeability.61 These data suggest that β-catenin signaling is important for a correct brain and retina angiogenesis during development and, at later stages, for inducing BBB properties in the brain microvasculature.

Wnt signaling plays opposite effects in the hyaloid vessels. These vessels form a capillary network within the developing eye, which must regress at late stages of development. Regression is mediated by Wnt 7b released by adjacent macrophages.67 Consistently, abrogation of Frizzled-5 also inhibits regression of hyaloid vessels.68

Wnt Mechanism of Action

An important issue is the definition of the mechanism of action of canonical Wnt signaling in modulating vascular morphogenesis. Several downstream cofactors and synergistic pathways have been described in endothelial cells of developing vessels.

Corada et al63 found that during vascular development in the embryo, an endothelial-specific gain-of-function mutation of β-catenin induces activation of the Notch pathway. More specifically, β-catenin increases the transcription of the Notch ligand Delta-like (DII)4, which, in turn, activates Notch-1 and -4. Overyexpression of β-catenin leads to alterations of vascular morphology comparable to those described by overexpression of DII4,69 which include a strong induction of endothelial cell arterIALIZation and lack of venous specification. These effects of β-catenin were detectable only during embryonic vasculogenesis and angiogenesis but were lost in the adult. It appears, therefore, that Wnt and Notch pathways act synergistically in modulating vascular differentiation (Figure 3). On this line, a recent publication70 showed that, using an embryonic stem cell differentiation system in vitro, endothelial cell expression of arterial markers is induced by the synergistic activity of Notch and β-catenin. β-Catenin forms a transcriptional complex with RBP-J transcription factor and the intracellular domain of Notch (NICD), which modulates the transcriptional upregulation of arterial genes. Furthermore, it was found60 that in the retina vasculature, Notch increases the expression of Nrarp, a transcription factor that, from one side, inhibits Notch activation and, on the other, increases β-catenin signaling by activating Lef-1.60 Overall, these data determine a sequential and direct link between β-catenin and Notch signaling systems to tune endothelial cell differentiation and vascular morphogenesis (Figure 3).

Ye et al54 found that, in endothelial cells, a gain-of-function mutation of Frizzled-4, increases the level of the transcription factor Sox 17. Through in vitro assays, these authors observed that Sox 17 is required for Norrin/Frizzled-4/Lrp–mediated angiogenesis in a 3D matrix gel. Sox17 may bind β-catenin and influence its transcriptional activity,71,72 but how and at which level these 2 transcription factors act on angiogenesis in vivo is still unknown.

TCF4/β-catenin complex increases von Hippel–Lindau tumor suppressor,73 which is required for inactivation of the transcription factor hypoxia-inducible factor (HIF)1α, which, in turn, is a potent inducer of vascular endothelial growth factor (VEGF). In addition, HIF1α competes with TCF4 for direct binding to β-catenin.74 β-Catenin/HIF1α interaction occurs at the promoter regions of HIF1α target genes and
increases their expression. Taken together, these data suggest that at low levels of oxygen, the interaction of β-catenin with HIF1-α may prevail and sustain the response of this transcriptional factor. When normoxia is restored, HIF1α is decreased and TCF4 effectively competes for binding to β-catenin, increases von Hippel–Lindau tumor suppressor, and further promotes HIF1α degradation.

Other partners of canonic Wnt signaling are the members of the FOXO (FOXO1, -2, and -3) subfamily of transcription factors. These factors are critically involved in the regulation of apoptosis, proliferation, and oxidative stress. Under oxidative stress, FOXOs are increased, and β-catenin binding to them enhances their transcriptional activity. In nonendothelial cells, FOXO competes with TCF4 for interaction with β-catenin, thereby inhibiting TCF4 transcriptional activity.

In endothelial cells, Taddei et al found that FOXO1 and β-catenin complex binds to claudin 5 promoter and reduces its synthesis. Claudin 5 is an endothelial cell–specific member of the claudin family, which is indispensable for a correct organization of tight junctions. This inhibitory activity of the complex can be released by VE-cadherin expression and clustering at adherens junctions. VE-cadherin can activate phosphoinositide 3-kinase and, in this way, inhibit FOXO1. In addition, VE-cadherin can bind β-catenin and, in confluent cells, recruit this protein at the membrane and limits its nuclear translocation. All of this leads to VE-cadherin–mediated increase in claudin 5, which, in turn, promotes tight junction organization and permeability control.

Another important β-catenin interactor is Smad 2. This transcription factor is associated with transforming growth factor (TGF)β receptor and, on receptor activation, is phosphorylated and translocates to the nucleus. Smad 2–induced transcription is further dependent on coactivators that stabilize its association to DNA. TGFβ receptors can associate with α3β1 integrin and cadherins (VE-cadherin in endothelial cells and E-cadherin in the epithelium). This association generates β-catenin and Smad2 complex, which triggers expression of genes involved in cell motility and epithelial–mesenchymal transition.

Prox1, a key transcription factor in lymphatic differentiation (see above), is also a target of β-catenin/TCF5 in colon cancer.

Taken together, these observations indicate that cell responses to canonic Wnt signaling is strongly influenced by the cell context. β-Catenin may interact with different transcription factors depending on the functional condition of the cells (such as hypoxia, TGFβ signaling, etc). The type of transcription complex formed dictates the downstream cellular responses.

**The Complexity of Canonic Wnt Signaling in Endothelial Cells**

As a further level of complexity, endothelial cells not only express different Frizzled receptors and Wnt ligands, but they also respond to non-Wnt types of ligands. The best studied to date is Norrin.

This cysteine knot protein, although structurally unrelated to Wnt factors, is able to bind Frizzled-4 and LRPS/5 complex and potently induce canonical signal (Figure 2). Partial or complete loss-of-function mutations of genes coding for Norrin, Frizzled-4, or Lrp5 induce retina hypovascularization. Importantly, these mutations are responsible for the so-called Norrie disease, familial exudative vitreoretinopathy and osteoporosis-pseudoglioma that in human beings cause important defects in retina vascularization.

The mechanism of action of Norrin has been partially disclosed by the discovery of TSPAN12, a member of the tetraspanin protein family. The members of this family generate specialized tetraspanin-rich microdomains that function as signaling platforms at the cell membrane. It was found that Norrin multimers act together with TSPAN12 to promote Frizzled-4 multimerization and downstream signaling (Figure 2). These data are of particular interest because TSPAN12 is specifically enriched in endothelial cells in the retina but not in other type of vessels or cells. Furthermore, TSPAN12 does not influence Wnt signaling in general but only when is triggered by Norrin. This pathway, therefore, is an example of how β-catenin–dependent signaling may be regulated in a cell- and organ-specific manner.
Another example of a pathway that modulates Wnt signaling in the endothelium is histone deacetylase (HDAC)\textsuperscript{7,86}. It was recently reported that HDAC7 binds and prevents β-catenin nuclear translocation and signaling. VEGF treatment induces HDAC7 degradation and rescues β-catenin nuclear translocation and signaling. Thus, posttranslational modification of β-catenin, such as phosphorylation or acetylation, may strongly influence its signaling.

Other agents known to strongly increase vascular permeability, such as thrombin, were found to induce β-catenin redistribution from cadherins to cytoplasm and nucleus.\textsuperscript{87,88} Lipid phosphate phosphatase 3 increases β-catenin signaling, which, in turn, induces fibronectin synthesis, endothelial cell migration, and vascular branching.\textsuperscript{89} These observations support the concept that stimuli that do not belong to the Wnt family may also act through β-catenin signaling, modulating its nuclear availability.

Finally, Wnt signaling may influence endothelial cell responses indirectly. A typical example is given by Aicher et al.\textsuperscript{90} These authors found that vasculogenic progenitor cell mobilization from the bone marrow is strongly increased by administration of Dkk1, which is an inhibitor of Lrp5/6 (Figure 2). This effect is mediated by osteoclasts, which are induced by Dkk1 to produce cathepsin K, which, in turn, cleaves the adhesive bonds between stem cells and stromal cells in the bone marrow and promotes vasculogenic progenitor cell mobilization. In this case, therefore, the effect of Wnt inhibition is indirect, involving other cell targets that induce endothelial progenitor mobilization and angiogenic activity.

**Wnt and Vascular Pathology**

Taking into consideration the different pathways and conditions that may influence or be influenced by Wnt, an important question is whether vascular pathologies may derive from altered Wnt signaling. Given the many circumstances in which Wnt signaling plays a critical function, it is conceivable that this occurs, and few examples have been reported in the literature.

Canonical Wnt signaling can promote retina vascularization. This effect is particularly important in diabetic retinopathy, where neovascularization is accompanied by increased β-catenin nuclear signaling. Interestingly, SERPINA3K, a serine proteinase inhibitor, was found to inhibit Wnt signaling by binding to Lrp6, thus inhibiting its dimerization with Frizzled receptors (Figure 2).\textsuperscript{91} SERPINA3K was also able to reduce diabetic retinopathy.\textsuperscript{91}

Norrine disease indicates how abrogation of β-catenin signaling may cause profound effects in retinal vascular development (see above). It is tempting to assume that alteration of the Frizzled-4/Norrin system may also play a role in diabetic retinopathy or retinopathy of prematurity\textsuperscript{92} because it was shown in preclinical models.\textsuperscript{93} Furthermore, Frizzled-4 expression is quite ubiquitous in endothelial cells of different types of vessels.\textsuperscript{54} Venous insufficiency\textsuperscript{94,95} or female infertility related to vascular problems\textsuperscript{96,97} have been described in Norrie disease, suggesting a more general role of altered β-catenin signaling also in vascular regions outside brain or retina.

Secreted Frizzled-related protein is an inhibitor of Wnt signaling that acts by binding Wnt and antagonizes both canonical and noncanonical Wnt signaling. This protein inhibits angiogenesis in different settings including tumors, suggesting that Wnt may be implicated in proliferative diseases by inducing vascular proliferation.\textsuperscript{98} Consistently, nuclear localization of β-catenin was observed in coronary endothelial cells in the ischemic heart.\textsuperscript{99} In these conditions, β-catenin may constitute a defense mechanism to promote angiogenesis in case of hypoxia. Wnt4, produced by mesenchymal stem cells,\textsuperscript{100} promotes vascular proliferation in ischemia.

Healing of diabetic ulcers appears to be improved by localization of vasculogenic progenitors able to release Wnt factors and activate endothelial cells.\textsuperscript{101} Canonical Wnt signaling can induce VEGF synthesis in colonic neoplasia, which, in turn, would increase angiogenesis.\textsuperscript{102} In other conditions, however, the Wnt inhibitor Dkk3 promotes angiogenesis in tumors,\textsuperscript{103} underlying the difficulties in defining a unique pattern of responses in the different experimental conditions.

Idiopathic pulmonary arterial hypertension is a rare disease linked to mutated bone morphogenetic protein (BMP) receptor II, which causes pulmonary artery endothelial cell apoptosis and loss of small vessels. It was found that binding of BMP to this receptor activates canonical and noncanonical Wnt pathways, which, in turn, promote angiogenesis and inhibit vascular regression.\textsuperscript{104}

Several reports indicate that Wnt signaling plays a crucial role in brain vascularization and BBB differentiation.\textsuperscript{17,61,62,65,66} Astrocytes produce Wnt, and this factor regulates the architecture and function of the so-called neurovascular unit (see above). It is, therefore, conceivable that any condition that inhibits Wnt signaling during development or after birth may strongly affect brain perfusion and BBB differentiation.\textsuperscript{105} Vice versa, gain-of-function mutation of Wnt may accelerate establishment of BBB and possibly alter permeability properties in the brain and in other vascular regions.\textsuperscript{61,62,65,66,105}

**Conclusions**

The study of the role of the Wnt system in vascular development and angiogenesis had a particular upsurge in the most recent years. However, we have only a partial knowledge of how the system works in vascular cells and how can be affected in pathological conditions. It is conceivable that endothelial cells derived from different types of vessels express distinct types of receptors and respond differently to Wnts. Furthermore, Wnt may act in concert with other signaling pathways such as Notch or TGFβ in modulating vascular development and endothelial specification. The same Wnt factor or receptor may induce distinct and sometimes opposite responses in endothelial cells depending on the context, such as, for instance, activation by growth factors, oxygen levels, induction of permeability, expression of transcription factors, etc. Furthermore, endothelial cells may produce not only Wnt ligands\textsuperscript{106} but also receptor inhibitors such as soluble Frizzled or Dkks,\textsuperscript{50,106} which may further control and balance Wnt signaling.
Taking all of this into account, it is crucial for future studies to define the specific conditions used, define the cell and vessel type, and combine in vitro with in vivo systems to be able to decipher the complexity of the Wnt pathway. These studies also force us to take into account that endothelial cells are not alike but form a very heterogeneous population that reacts differently to physiological and pathologic stimuli.

**Acknowledgments**

I thank Fabrizio Orsenigo for valuable help in preparing the figures for this article.

**Sources of Funding**

This work was supported by the Fondazione Leducq Transatlantic Network of Excellence, Associazione Italiana per la Ricerca sul Cancro, Association for International Cancer Research UK, the European Community (Project Contracts: JUSTBRAIN; EUSTROKE contract 202213, OPTISTEM contract 223098, ANGIOSCAFF NMP3-LA-2008-214402 and ENDOSTEMCELLS Networks), Istituto Superiore di Sanità, Italian Ministry of Health, and CARIPLO Foundation contract 2008.2463.

**Disclosures**

None.

**References**

41. Goodwin AM, Kitajewski J, D’Amore PA. Wnt1 and Wnt5a affect endothelial proliferation and capillary length; Wnt2 does not. Growth Factors. 2007;25:25–32.


The Role of Wnt Signaling in Physiological and Pathological Angiogenesis
Elisabetta Dejana

Circ Res. 2010;107:943-952
doi: 10.1161/CIRCRESAHA.110.223750

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/107/8/943

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/