Formation of the blood–brain barrier: Wnt signaling seals the deal

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Capillaries in the brain are especially selective in determining which blood-borne components gain access to neurons. The structural elements of this blood–brain barrier (BBB) reside at the tight junction, an intercellular protein complex that welds together adjacent endothelial cell membranes in the microvasculature. In this issue, Liebner et al. (Liebner, S., M. Corada, T. Bangsow, J. Babbage, A. Taddei, C.J. Czupalla, M. Reis, A. Felici, H. Wolburg, M. Fruttiger, et al. 2008. *J. Cell Biol.* 183: 409–417) report that Wnt signaling plays an active role in the development of the BBB by regulating expression of key protein constituents of the tight junction. Such mechanistic insight has implications for a variety of neuropathological states in which the BBB is breached.

The brain occupies a privileged compartment in the body. This was first appreciated over a century ago by the demonstration that dyes injected into the blood did not extravasate into the brain. It is now apparent that this gatekeeping is a combination of highly selective active transport and, at the ultrastructural level, a physical barrier localized to the tight junction complex between brain endothelial cell membranes (Fig. 1; Zlokovic, 2008). Many of the proteins comprising the tight junction, such as claudins (Cldns), occludin, and junctional adhesion molecules, have been identified, but the mechanisms governing their expression and assembly into a complex during neurovascular development remain incomplete. Liebner et al. (see p. 409 of this issue) surmised that the Wnt signaling pathway, which is already prominent in brain development, was a good place to start.

As an initial step, they took advantage of a transgenic reporter mouse that monitors Wnt signaling activity via the expression of galactosidase. Reporter activity was readily observed in brain endothelial cells throughout the developing vascular network but dropped off sharply in postnatal animals and was nearly absent in adults. For a functional correlate, the authors used mice expressing both loss and gain of function mutants of β-catenin, a key protein that is stabilized upon propagation of the Wnt signal. A marker of leaky brain vessels, plasmalemmal vesicle–associated protein-1, as well as Cldn3 and Cldn5 staining in their tight junctions responded appropriately to the gain or loss of β-catenin activity in these mice. Enhanced staining of junctional Cldn3 was also observed in cultured primary mouse brain endothelial cells stimulated with Wnt3a ligand. In these cells, total Cldn3 protein and mRNA were increased in response to Wnt3a in a β-catenin–dependent manner. Thus, manipulation of the Wnt pathway, at least at the level of β-catenin stability, clearly impacted vessel integrity.

It is important to recognize that in addition to mediating the transcriptional output from Wnt signaling, β-catenin also functions in cell–cell adhesion through its interaction with cadherins at the adherens junction (Brembeck et al., 2006). Therefore, any resulting alterations to the adherens junction complex could indirectly impact its close neighbor, the tight junction. Moreover, a previous study involving conditional ablation of endothelial β-catenin ascribed increased paracellular permeability to deficient cell–cell contacts (Cattelino et al., 2003). Fortunately, there are ways to distinguish the adhesion from the signaling activities imparted by β-catenin. With this in mind, Liebner et al. (2008) showed that the junctional staining of Cldn3 was greatly diminished in the presence of a dominant interfering mutant of TCF4, a transcription factor that β-catenin associates with to launch gene activation. Conversely, a gain of function mutant transcription factor enhanced staining. Consistent with gene activation, the levels of Cldn3 transcript were inflected by the mutant transcription factors in the expected directions. Whether the Cldn3 gene is a direct target of Wnt signaling was not pursued, but Liebner et al. (2008) strongly implicate Wnt signaling in driving its expression.

This paper has implications for our understanding and treatment of disorders involving the BBB. The study was largely focused on the developing brain, and thus any relationship to genetic vascular disorders, particularly those attributable to defective Wnt pathway genes, would garner attention. Among these, familial exudative vitreoretinopathy (FEVR) stands out prominently. FEVR is characterized by incomplete vascularization of the retina and was independently linked to defective genes coding

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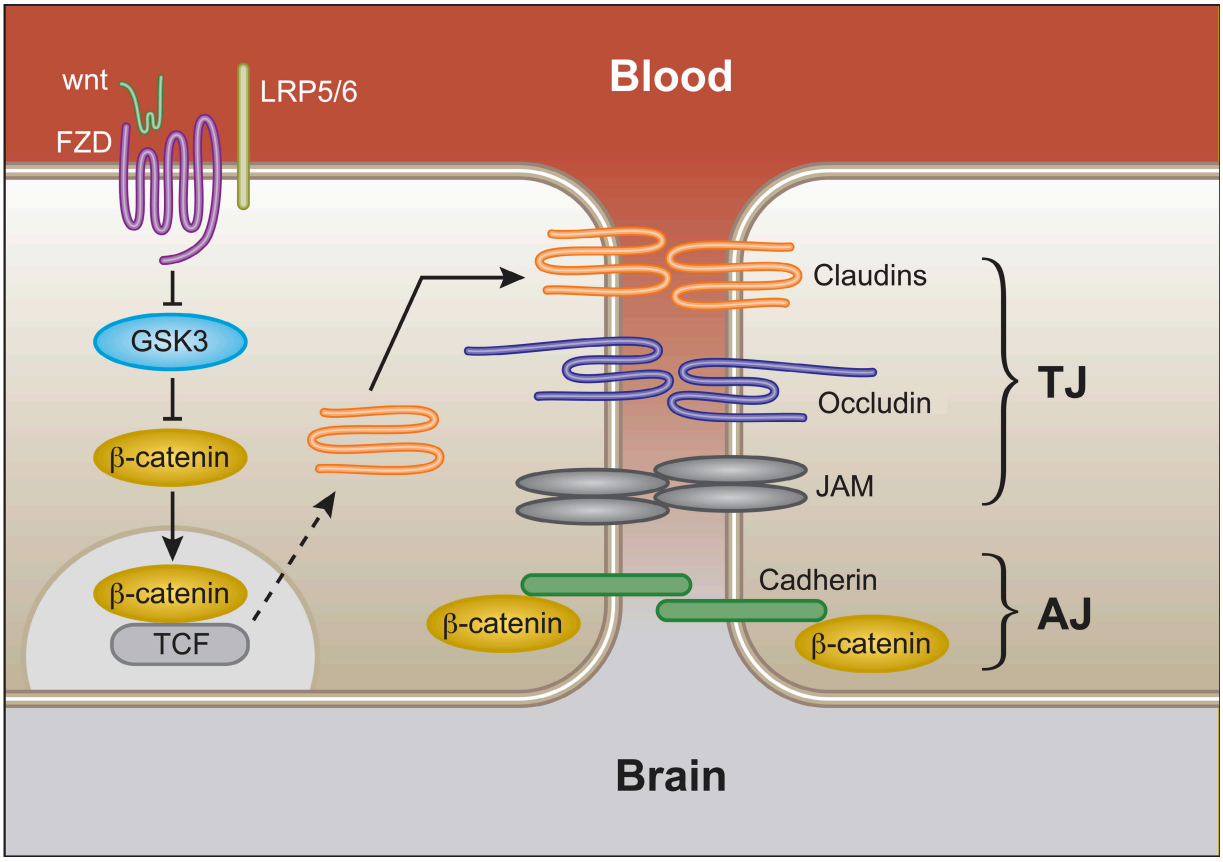

Figure 1. **Wnt signaling and the BBB.** Depiction of the primary constituents of the tight junction (TJ) and the adherens junction (AJ) at the interface between endothelial cell plasma membranes. Activation of Wnt receptors FZD and LRP5/6 inhibits GSK3 to stabilize β-catenin that in turn enters the nucleus to activate T cell factor (TCF)–dependent transcription. This drives Cldn3 gene activation either directly or indirectly (dashed line arrow), and the resulting Cldn protein reinforces the tight junction. JAM, junctional adhesion molecule.

for Wnt ligand receptors Frizzled 4 (FZD4) and LRP5 (Robitaille et al., 2002; Jiao et al., 2004). Norrie disease, also characterized by abnormal retinal vasculature, was linked to mutations affecting the secreted protein norrin, which was later identified as a ligand for FZD4 (Xu et al., 2004). Although Wnt signaling is clearly implicated in these disorders, the mechanism downstream of the ligand–receptor interaction is unknown. Considering the new findings by Liebner et al. (2008), it is conceivable that the impairment in Wnt signaling linked to FVER and Norrie disease could lead to inadequate reinforcement of retinal endothelial tight junctions. Interestingly, small hemorrhages were noted in the retina and cerebellum of FZD4$^{-/-}$ mice, which also exhibited high background staining with anti–mouse IgGs, indicative of leaky vasculature (Xu et al., 2004). Accordingly, Liebner et al. (2008) noted a decrease in retinal vascular permeability induced by ischemia when β-catenin was conditionally activated in postnatal mice.

Breakdown of both the functional and physical properties of the BBB has been implicated in the initiation or exacerbation of a host of adult central nervous system (CNS) disorders, including multiple sclerosis, Alzheimer's disease, Parkinson's disease, cancer, and stroke (Zlokovic, 2008). The physical property of the BBB resides at the tight junction complex, but the mechanisms underlying its loss of integrity in disease are poorly understood. Calcium, G-protein signaling, RhoGTPases, and various kinases have all surfaced as regulators of tight junction proteins, including the Cldns (Hawkins and Davis, 2005; Persidsky et al., 2006). Liebner et al. (2008) now add a well-defined transcriptionally active signaling pathway to this understanding. Pathways modulating Cldns are particularly attractive candidates, as enhanced paracellular permeability of the BBB has been reported in Cldn5-deficient mice (Nitta et al., 2003). Notably, a selective loss of Cldn3 at the tight junction has been associated with experimental autoimmune encephalomyelitis in mice, a model of multiple sclerosis (Wolburg et al., 2003). It should now be of interest to reexamine the pathological models involving the BBB in the context of Wnt signaling and its manipulation therein.

The BBB is of particular interest in the development of new therapeutics for degenerative and inflammatory diseases of the CNS (Persidsky et al., 2006). Retarding the unwanted passage of leukocytes and water-soluble plasma components into the brain will likely require a multifaceted approach, including reparation or reinforcement of the tight junction fence. The new findings by Liebner et al. (2008) suggest that this might be accomplished by therapeutic activation of Wnt signaling in the brain. Possible approaches could include activation of the Wnt coreceptors LRP5 and LRP6 by R-spondins or by agonistic monoclonal antibodies (Kim et al., 2005). Activation of Wnt signaling with small molecule therapeutics is currently approachable with inhibitors of glycogen synthase kinase 3 (GSK3). In the

Wnt pathway, GSK3 phosphorylates β-catenin, thereby marking it for destruction in the proteosome. Coincidently, GSK3 is already a prime target for Alzheimer's disease, where it hyperphosphorylates the Tau protein (Bhat et al., 2004; Hooper et al., 2008). Strengthening of tight junctions via enhanced Wnt signaling might provide an additional unanticipated benefit with GSK3 inhibitors in neurodegenerative diseases. This mechanism could in part account for the observed neuroprotective effect of a GSK3 inhibitor in a mouse model of hypoxiaischemia brain injury (Cowper-Smith et al., 2008). Conversely, transient inhibition of Wnt signaling and the ensuing breakdown of the tight junction could enable access of therapeutics normally denied by the BBB. Modulation of Cldns in particular might offer a unique opportunity because they play a special sieving role in gating the passage of blood-borne solutes on the basis of size (Nitta et al., 2003).

At one level, the proposal by Liebner et al. (2008) has substantial precedent. The literature is replete with studies purporting a role for Wnt signaling in the development and maintenance of the CNS (De Ferrari and Moon, 2006). However, most of these studies relate to direct effects of Wnts and their receptors on the genesis, survival, and morphology of neurons themselves and not so much to brain endothelium. Although Wnt signaling has also been generously appropriated into vascular biology (Zerlin et al., 2008), there is a dearth of studies specifically linking it to brain vascularization. The findings by Liebner et al. (2008) should now prompt us to consider an endothelial component, and in particular the integrity of the tight junction, when examining developmental, genetic, or pathological outcomes attributable to Wnt signaling in the CNS. FEVR is a pertinent example of this. Age-related macular degeneration, another vascular disorder of the eye, has also been linked to Wnt signaling through polymorphisms in the gene coding for LRP6 (Haines et al., 2006). Alzheimer's disease has been linked to both hypoactive alleles of LRP6 and to overexpression of the secreted Wnt inhibitor dkk1 (De Ferrari et al., 2007; Caraci et al., 2008). Moreover, amyloid-β peptide, considered a culprit in Alzheimer's disease, binds to FZD and blocks its activation by Wnt (Magdesian et al., 2008). Nearly all of the associations of Wnt signaling with neurodegenerative disorders point to a deficiency in signaling, as does its new association with the integrity of the tight junction.

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