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Holey barrier: claudins and the regulation of brain endothelial permeability

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Endothelial tight junctions (TJs)* are an important functional part of the blood-brain barrier (BBB). In this issue, Nitta et al. (2003) demonstrate that claudin-5, a transmembrane protein of TJs, is a critical determinant of BBB permeability in mice. Unexpectedly, knockout of claudin-5 did not result in a general breakdown of TJs but in a selective increase in paracellular permeability of small molecules. This suggests that the BBB can be manipulated to allow selective diffusion of small molecules and makes claudin-5 a possible target for the development of drugs for this purpose.

The BBB maintains the neural microenvironment by forming a tight barrier between the blood and the brain. The BBB is primarily formed by brain endothelial cells (BECs) lining the microvascular system (Wolburg and Lippoldt, 2002). BECs form a tight seal but also mediate selective transcellular transport of nutrients and other essential components into the brain, and efflux of potentially toxic metabolites from the brain (Abbott, 2002). Nonspecific transendothelial transport of soluble components (i.e., transport that is not mediated by specific transporters) across endothelia can occur along two routes: a transcellular route, which may be mediated by vesicular transport, and a paracellular route along a pathway between neighboring cells. Brain capillaries allow little nonspecific transendothelial transport since they exhibit only very low rates of fluid phase transcytosis, and the paracellular route between individual BECs is sealed by TJs that are much tighter than in peripheral microvessels. Hence, TJs are a critical determinant of the permeability properties of the BBB. Since many potentially beneficial drugs are inactive because they are unable to cross the BBB, much effort has been directed toward the understanding of TJs of BECs to identify molecular mechanisms that could be manipulated to enhance drug delivery across the BBB.

In this issue, Shoichiro Tsukita's group (Nitta et al., 2003) reports the identification of claudin-5 as a critical regulator of BEC permeability. They show that knockout of claudin-5 in mice resulted in leakage of tracers from the blood into the brain, although based on electron micrographs of ultrathin sections and immunofluorescence TJs still formed and appeared to be largely intact. Such mutant mice developed normally but died a few hours after birth, which may or may not have been due to the BBB defect. The absence of claudin-5 altered the junctional properties so that they functioned like a molecular sieve, allowing tracer diffusion in a size-selective manner, but it did not cause nonspecific leakage of plasma components into the brain. This was surprising for two reasons. First, claudin-5 had been believed to be the main structural transmembrane component of BEC TJs; hence, its absence was widely expected to result in evident defects in TJ assembly and a general breakdown of the junctional barrier. Second, the size-selective permeability properties in the absence of claudin-5 suggest that BECs have the capability to allow size-selective diffusion of tracers across TJs similar to many epithelial cells. This is an exciting observation since it suggests that it might be possible to manipulate BEC TJs in a manner that results in a transient selective increase in the permeability for small molecules, permitting small drugs to cross the BBB without complete disruption of the junctional barrier.

How might selective permeability be mediated and regulated? In principal, BEC TJs have a similar architecture to those of epithelial cells (Dejana et al., 2000; Tsukita et al., 2001). TJs contain different types of transmembrane proteins, such as junctional adhesion molecules and the polytopic proteins occludin and claudins. These transmembrane components are linked via multiple adaptor proteins (i.e., proteins with multiple protein–protein interaction motifs such as the ZO or MAGI proteins) to the actin cytoskeleton and various signaling components, such as protein kinases and GTP-binding proteins. Claudins appear to be the main structural transmembrane proteins of TJs and are major components of the typical TJ-associated intramembrane strands seen in freeze-fracture replicas (Tsukita et al., 2001). Since only two claudins are known to be expressed by BECs, claudin-5 and -12, it was unexpected that knockout of claudin-5 did not have a major effect on TJ morphology. However, it could be that the absence of claudin-5 was compensated by up-regula-

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*Abbreviations used in this paper: BBB, blood-brain barrier; BEC, brain endothelial cell; TJ, tight junction.

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tion of the expression of another claudin. Additionally, claudin-5 deficiency might cause minor morphological changes that can only be visualized by freeze-fracture EM. Nevertheless, removal or addition of a claudin in cells that express multiple claudin family members does not generally have drastic morphological consequences on TJs, suggesting that the absolute amount of available claudin protein is not a critical determinant of junction biogenesis.

Removal or addition of claudins generally does affect the permeability properties of TJs. Claudins have mainly been linked to selective ion permeability. A prominent example is claudin-16/paracellin-1, which is responsible for paracellular flux of Mg and Ca in the kidney and is mutated in patients with familial hypomagnesemia-hypercalciuria (i.e., renal Mg wasting) (Simon et al., 1999). Therefore, it is thought that claudins form paracellular pores or channels that mediate selective ion permeability (Anderson, 2001; Tsukita et al., 2001). According to this model, the types of claudins that constitute these channels determine their ion selectivity. On the other hand, removal or addition of a claudin in the skin resulted in dehydration and disruption of the epidermal barrier, suggesting that claudins do not only affect ion permeability (Furuse et al., 2002; Turksen and Troy, 2002).

Since removal of claudin-5 did not seem to inhibit junction formation and since it resulted in a size-selective increase in permeability, it is unlikely that this phenotype was caused by gaps in the BBB, but rather likely it was caused by the activation of a mechanism that mediates size-selective paracellular diffusion. Presently, we do not know how this works, but as proposed for epithelia, such a mechanism may involve a series of diffusion barriers that contain fluctuating pores or channels that are formed by other claudin family members and/or occludin (Claude, 1978; Cereijido et al., 1989; Balda and Matter, 2000). The latter protein, occludin, is also a polytopic transmembrane protein like the claudins and is associated with epithelial and endothelial TJs (Furuse et al., 1993). Experiments with cultured epithelial cells demonstrated that occludin regulates size-selective paracellular diffusion of hydrophilic molecules (Balda and Matter, 2000). In BECs, claudin-5 might normally associate with and inhibit such a diffusion system, and therefore its removal results in increased size-selective paracellular permeability.

How could such a permeation mechanism be exploited for the development of therapies to manipulate the BBB? The possibility suggested by Nitta et al. (2003) is to target claudin-5. It might be possible to remove or inactivate claudin-5 temporarily from TJs of BECs using, for example, peptides that compete with its extracellular interactions. A similar approach was used to remove occludin from TJs in cultured epithelia (Wong and Gumbiner, 1997). An alternative tool is *Clostridium* perfringens enterotoxin, which binds and removes claudin-4 from epithelial TJs (Sonoda et al., 1999). One could engineer derivatives of the enterotoxin that are specific for claudin-5 and could therefore be used to permeabilize endothelial TJs. It could be, however, that in vivo removal of a transmembrane protein has too drastic an effect on permeability to be used for drug delivery; therefore, one may have to think of ways to modulate the activity of junctional components involved in the regulation of permeability in a more subtle manner. One possibility is to target occludin: it has been proposed that phosphorylation of its regulatory COOH-terminal cytoplasmic domain regulates paracellular permeability of endothelia (Hirase et al., 2001). Alternatively, it might be simpler to manipulate one of the intracellular signaling components that regulate paracellular diffusion, such as the guanine nucleotide exchange factor GEF-H1, a recently identified TJ-associated activator of RhoA (Benais-Pont et al., 2003; Matter and Balda, 2003). Although more research is needed to develop and validate such reagents and therapies, the study by Nitta et al. (2003) provides a significant advance toward a molecular understanding of the BBB and its regulation, and demonstrates that BBB permeability can be manipulated in a selective manner.

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References

Abbott, N.J. 2002. Astrocyte-endothelial interactions and blood-brain barrier permeability. *J. Anat.* 200:629–638.

Anderson, J.M. 2001. Molecular structure of tight junctions and their role in epithelial transport. *News Physiol. Sci.* 16:126–130.

Balda, M.S., and K. Matter. 2000. Transmembrane proteins of tight junctions. *Semin. Cell Dev. Biol.* 11:281–289.

Benais-Pont, G., A. Punn, C. Flores-Maldonado, J. Eckert, G. Raposo, T.P. Fleming, M. Cereijido, M.S. Balda, and K. Matter. 2003. Identification of a tight junction–associated guanine nucleotide exchange factor that activates Rho and regulates paracellular permeability. *J. Cell Biol.* 160:729–740.

Cereijido, M., L. González-Mariscal, and G. Contreras. 1989. Tight junction: barrier between higher organisms and environment. *News Physiol. Sci.* 4:72–74.

Claude, P. 1978. Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens. *J. Membr. Biol.* 39:219–232.

Dejana, E., M.G. Lampugnani, O. Martinez-Estrada, and G. Bazzoni. 2000. The molecular organization of endothelial junctions and their functional role in vascular morphogenesis and permeability. *Int. J. Dev. Biol.* 44:743–748.

Furuse, M., T. Hirase, M. Itoh, A. Nagafuchi, S. Yonemura, S. Tsukita, and S. Tsukita. 1993. Occludin: a novel integral membrane protein localizing at tight junctions. *J. Cell Biol.* 123:1777–1788.

Furuse, M., M. Hata, K. Furuse, Y. Yoshida, A. Haratake, Y. Sugitani, T. Noda, A. Kubo, and S. Tsukita. 2002. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1–deficient mice. *J. Cell Biol.* 156:1099–1111.

Hirase, T., S. Kwashima, E.Y. Wong, T. Ueyama, Y. Rikitake, S. Tsukita, M. Yokoyama, and J.M. Staddon. 2001. Regulation of tight junction permeability and occludin phosphorylation by RhoA-p160ROCK-dependent and -independent mechanisms. *J. Biol. Chem.* 276:10423–10431.

Matter, K., and M.S. Balda. 2003. Signalling to and from tight junctions. *Nat. Rev. Mol. Cell Biol.* 4:225–236.

Nitta, T., M. Hata, S. Gotoh, Y. Seo, H. Sasaki, N. Hashimoto, M. Furuse, and S. Tsukita. 2003. Size-selective loosening of the blood-brain barrier in claudin-5–deficient mice. *J. Cell Biol.* 161:653–660.

Simon, D.B., Y. Lu, K.A. Choate, H. Velazquez, E. Al-Sabban, M. Praga, G. Casari, A. Bettinelli, G. Colussi, J. Rodriguez-Soriano, et al. 1999. Paracellin-1, a renal tight junction protein required for paracellular Mg2+ resorption. *Science.* 285:103–106.

Sonoda, N., M. Furuse, H. Sasaki, S. Yonemura, J. Katahira, Y. Horiguchi, and S. Tsukita. 1999. *Clostridium* perfringens enterotoxin fragment removes specific claudins from tight junction strands: evidence for direct involvement of claudins in tight junction barrier. *J. Cell Biol.* 147:195–204.

Tsukita, S., M. Furuse, and M. Itoh. 2001. Multifunctional strands in tight junctions. *Nat. Rev. Mol. Cell Biol.* 2:285–293.

Turksen, K., and T.-C. Troy. 2002. Permeability barrier dysfunction in transgenic mice overexpressing claudin 6. *Development.* 129:1775–1784.

Wolburg, H., and A. Lippoldt. 2002. Tight junctions of the blood-brain barrier: development, composition and regulation. *Vascul. Pharmacol.* 38:323–337.

Wong, V., and B.M. Gumbiner. 1997. A synthetic peptide corresponding to the extracellular domain of occludin perturbs the tight junction permeability barrier. *J. Cell Biol.* 136:399–409.