junctions may be differentially regulated during angiogenesis, and that this may be required for proper new vessel formation.

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A11.05

Diapedesis of monocytes is associated with MMP-mediated occludin disappearance in brain endothelium

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The blood-brain barrier controls the entry of circulating molecules and cells into the brain and is characterized by the presence of endothelial cells that are connected by tight junctions. Inflammatory cell trafficking into the brain complicates several neurological disorders and conceivably depends on mechanisms that regulate tight junction opening. A detailed study of tight junction dynamics during diapedesis has been lacking. To examine underlying mechanisms of diapedesis and in particular events that occur at the tight junction, we established a well-defined rat brain endothelial cell line expressing green fluorescent protein fused to the tight junction protein occludin. Live cell imaging studies show that monocytes scroll over the brain endothelial cell surface towards cell–cell contacts, induce gap formation which is associated with local disappearance of occludin and subsequently traverse the endothelium in a paracellular fashion. Western blot analyses indicated that loss of occludin was due to protein degradation. A broad spectrum MMP-inhibitor significantly impaired endothelial gap formation, occludin loss and the ability of monocytes to pass the endothelium. Our results illustrate that therapeutics aimed at the stabilization of the tight junction may be beneficial in neurological disorders to reduce cellular infiltration and resist an inflammatory attack.

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A11.06

Limited contribution of claudin-5 dependent tight junction strands to endothelial barrier function

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Introduction: Related to specific local requirements, barrier properties of the endothelium show a wide variation along the vascular tree, ranging from highly restrictive in the BBB to highly permissive in postcapillary venules. In analogy, the well-studied epithelial monolayers normally form barriers that regulate access of water and solutes to the paracellular space through a complex junction system. Members of the claudin-family were shown to be crucially involved in both structure and function of these tight junction strands. Likewise, the endothelium-specific claudin-5 was shown to be involved in the function of the blood-brain-barrier (BBB).
Aim: To investigate the role of claudin-5 in non-BBB endothelial barriers.
Methods: Highly efficient lentiviral-driven overexpression and silencing of claudin-5 was applied in a native environment of primary vascular endothelial cells (EC). Immuno-fluorescence was used to show targeting and absence of claudin-5 in the lateral membrane, respectively. Permeability of the resulting monolayers for both inert tracers between 342 Da (glucose) and 40 Kda (HRP) and inflammatory cells (monocytes, granulocytes) was measured using a two-compartment system. Junctional structure was visualized using freeze-fracture electron microscopy.
Results: Overexpression of claudin-5 leads to formation of elaborate networks of junction strands, which are absent in non-transduced cultured endothelial cells. Concomitantly, a modest (25%, $p<0.01$) and non-size selective enhancement of the barrier function was observed for macromolecular permeability tracers. In contrast, silencing of endogenous claudin-5 does not affect basal endothelial barrier function in the absence of TJ strands. Diapedesis of monocytes or granulocytes is neither affected by claudin-5 silencing nor by claudin-5 overexpression-induced TJ-strands. The increased barrier for macromolecular tracers that results from claudin-5 overexpression was not affected during diapedesis.
Conclusion: Our data show that in non-BBB endothelium the paracellular barrier function of EC is only limitedly affected by claudin-5-dependent TJs, indicating that claudin-5 independent junctional structures determine the properties of the barrier.

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A11.07

Nuclear targeting of beta-catenin by thrombin during endothelial barrier dysfunction

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Nuclear localization of beta-catenin is a remarkable feature of vascular pathologies. In healthy endothelial cells, beta-catenin is mainly located in adherens junctions, where it complexes to VE-