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Studies on the regulation of inflammation- and angiogenesis-related vascular endothelial permeability by junctional adhesion molecule-C

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Vascular endothelium forms a semi-permeable barrier that controls passage of macromolecules and fluid between the blood and interstitial space. Endothelial barrier integrity is regulated by cell contractility and integrity of interendothelial junctions, tight junctions (TJ) and adherens junctions (AJ). Changes in endothelial permeability are crucial during both inflammatory responses and neovascularization, and understanding of the signaling mechanisms is extremely important.

Junctional adhesion molecules (JAMs) a subgroup of the Immunoglobulin Super Family including JAM-A, JAM-B, JAM-C. JAM-C is expressed on endothelial cells, where it can be localized at TJ and can regulate leukocyte transendothelial migration.

In this study the homophilic interaction of JAM-C was described for the first time. The homophilic interaction of JAM-C could be attributed to the amino-terminal Ig domain D1 of JAM-C, and particularly to the tripeptide sequence Arg64-Ile65-Glu66 therein. This sequence is conserved in all members of the JAM family.

In order to investigate the role of the homophilic interaction of JAM-C in interendothelial junctions assembly and the regulation of endothelial permeability, we have used microvascular endothelial cells, as an appropriate in vitro model.

Interestingly, we found that in contrast to macrovascular endothelial cells, in quiescent microvascular cells JAM-C was primarily intracellular, whereas stimulation with vasoactive agents triggered its rapid translocation to the interendothelial junctions. Blockage of JAM-C with recombinant extracellular JAM-C or downregulation of its expression with the RNAi approach resulted in the prevention of VEGF- or histamine induced increase in endothelial permeability.

In addition in vivo blocking of JAM-C significantly reduced both inflammation- and angiogenesis- related endothelial permeability.

Signaling mechanisms regulate endothelial barrier integrity by influencing actomyosin driven cell contractility and intercellular junction integrity. These include Ca2+ dependent or independent signaling events which lead to myosin light chain phosphorylation, as well as the cAMP-dependent pathways, PKA and Rap1, that counteract permeability increases .

JAM-C knockdown in microvascular endothelial cells resulted in a decrease in F-actin and prevented stress fibers formation, consistent with a decrease in myosin light chain (MLC) phosphorylation. Futhemore, JAM-C knockdown strengthened VE-cadherin-mediated cell-cell contacts in a Rap1 dependent manner, and independently of the activity of PKA.Knockdown of JAM-C expression also increased the integrity of tight junctions.

In summary, JAM-C has been demonstrated to interact in a homophilic manner, and this interaction is important in the regulation of endothelial permeability both in vitro and in vivo. The loss of JAM-C expression resulted in a decrease in endothelial permeability due to a reduction of actomyosin-driven endothelial cell contractility and an improvement of the integrity of interendothelial junctions, as JAM-C was shown to modulate actin stress fiber formation and myosin light chain phosphorylation, and VE-cadherin adhesiveness in a Rap1 dependent manner. Thus, JAM-C is an important player in the regulation of both inflammation- and angiogenesis-dependent endothelial permeability.