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Role of ADAMs in Endothelial Cell Permeability. Cadherin Shedding and Leukocyte Rolling

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Endothelial cells constitute a selective barrier that controls the passage of plasma proteins and circulating cells from the blood to tissues. This is achieved by the caveolar-vesicular system and by the dynamic opening and closing of intercellular junctions.[1] Inter-endothelial cell junctions are almost absent in the postcapillary venules, where cellular extravasation and exchange of plasma constituents occur, but are well-organized in the large vessels to ensure strict control of vascular permeability. Cadherins are Ca^{2+}- and protease-sensitive molecules that mediate homotypic cell-to-cell adhesion. Endothelial cells express N-, P- and VE-cadherin. N-cadherin is diffusely spread on the cell surface, while P-cadherin is present in trace amounts. VE-cadherin is a single-chain transmembrane glycoprotein localized at specialized inter-endothelial cell contact regions referred to as adherens junctions.[2]

Vascular inflammation has been implicated in the development and progress of vascular diseases such as hypertension and atherosclerosis.[3] The inflammatory response is initiated by injury of the endothelium inflicted by factors such as oxidized low-density lipoprotein, reactive oxygen species and viruses. Endothelial cell injury prompts the recruitment of circulating leukocytes to the injury site, and the disruption of the endothelial cell barrier allows leukocyte infiltration of the vessel wall (Figure). Leukocyte recruitment and infiltration of the vascular wall is a complex process encompassing a series of adhesion and deadhesion events and distinct adhesion molecules on the activated endothelium and leukocytes.

Cadherin Shedding and Leukocyte Rolling

An important mechanism regulating leukocyte recruitment to the vascular wall involves the proteolytic cleavage of adhesion molecules by cell-associated metalloproteases, a process called “shedding”. The shedding process can be exemplified in the adhesion protein selectin. Leukocyte L-selectin acts as a homing receptor for the targeting and initial interaction between leukocytes and activated endothelium, a process called “rolling”. Endothelial E-selectin and platelet P-selectin are stored intracellulary, but when the cells are activated they translocate to the cell surface, where they participate in the initial rolling of leukocytes over the activated endothelium. Focal shedding of endothelial E-selectin is thought to promote leukocyte infiltration and lesion formation.[4] Also, blood levels of soluble vascular cell and intracellular

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adhesion molecule-1 (VCAM-1 and ICAM-1) are increased in atherosclerosis, suggesting their shedding during lesion development. Additionally, inflammatory mediators such as thrombin cause proteolysis of VE-cadherin, thereby disrupting the organization of endothelial adherens junctions and increasing vascular permeability and edema.[5] The role of VE-cadherin shedding in endothelial cell permeability is further supported by reports that induction of apoptosis in human umbilical vein endothelial cells (HUVEC) is associated with dissolution of adherens junctions, loss of cell-cell contacts, and shedding of VE-cadherin.[6] While factors associated with the expression of transmembrane adhesion molecules and their dysregulation in atherosclerosis have been studied,[7] the mechanisms involved in the release of these adhesion molecules from the cell surface by controlled cleavage or shedding are not well-understood.

Role of ADAMs as Adhesion Molecule Sheddases

Disintegrin and metalloproteases (ADAMs) are membrane-anchored glycoproteins and regulatory enzymes that have been implicated in cell adhesion as well as the proteolytic conversion or shedding of membrane-bound proteins to soluble forms.[8-10] ADAMs, like matrix metalloproteinases (MMPs), are members of the metzincin zinc-dependent metalloprotease superfamily. At least thirty members of the ADAMs protein family share a common structure consisting of a prodomain, a metalloprotease domain, a disintegrin domain, a cysteine-rich domain, an epidermal growth factor (EGF)-like domain, a transmembrane domain, and a cytoplasmic domain.[11,12] Adams are involved in diverse biological functions including fertilization, neurogenesis and angiogenesis as well as disease states such cancer.[13,14]

ADAM-17 (TNF-converting enzyme or TACE) was the first member of the ADAMs family with a defined role as a sheddase that releases TNF-α and its receptors from neutrophils and macrophages during inflammation.[15] ADAM-1, -12, -15 and -17 have been identified in vascular smooth muscle cells, and ADAM-10, -15 and -17 may have potential roles in the regulation of endothelial function via their metalloprotease and proteolytic properties. Also, the adhesive disintegrin domain of ADAMs allows them to interact with integrins. For example, ADAM-15 may be involved in endothelial-leukocyte or endothelial-tumor adhesion by binding to the classic RGD-binding integrins $\alpha_5\beta_1$ and $\alpha_v\beta_3$. The binding of ADAM-12 and -15 disintegrin domains to non-RGD-type integrin $\alpha_9\beta_1$ may also mediate cell-cell interaction.[16]

ADAM-10 and -17 are upregulated in activated endothelium and play a role in ectodomain shedding of adhesion molecules during leukocyte recruitment (Figure).[17] Also, fractalkine (CX_3CL1) and CXCL16 are adhesion molecules that are upregulated in activated endothelium and macrophages, and take part in the initial capture of inflammatory cells. ADAM-10 or -17 cleaves these adhesion molecules to soluble chemoattractant cytokines or chemokines to attract additional inflammatory cells expressing chemokine receptors CX_3CR1 and CXCR6.[18,19] Also, CD44, another adhesion molecule in inflammatory cells, cross-links to endothelial cell hyaluronan and initiates intracellular signaling cascade, leading to activation of ADAM-10 or -17 and cleavage of the soluble ectodomain of CD44. Soluble CD44 fragments in turn compete for uncleaved hyaluronan and promote leukocyte deadhesion and rolling.[20] Thus, ADAMs-induced release of the soluble ectodomain from the adhesion molecules L-selectin, VCAM, ICAM, fractalkine, CXCL16 and CD44 contribute to leukocyte deadhesion and rolling on activated endothelial cells and their migration to the inter-endothelial junction.[4,21,22]

ADAMs and Vascular Permeability

ADAMs-mediated shedding of cell surface molecules is emerging as a critical pathway not only in the regulation of leukocyte recruitment but also in the control of vascular and non-vascular cell-cell interactions. ADAM-15 is upregulated in atherosclerotic lesions suggesting that ADAMs participate in lesion formation. Also, ADAM-10 is localized in the membrane of epithelial cells of benign glands suggesting a role in cell-cell, cell-matrix, and cell-basement

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membrane interactions. Although ADAM-10 does not interact directly with integrins, it may indirectly influence integrin-related adhesion activity by cleaving the L1 membrane adhesion molecule, a type 1 membrane glycoprotein implicated in the migration of neural and tumor cells.[23] Also, in resting cells, calmodulin (CaM) constitutively associates with the pro-ADAM-10 inactive form. An increase in endothelial cell $[Ca^{2+}]_i$ in response to activators such as thrombin or subsequent to leukocyte adhesion would promote the dissociation of CaM and the activation of ADAM-10 (Figure).[20,24] However, the molecular mechanisms via which activated ADAM-10 could affect endothelial cell permeability and leukocyte infiltration are not clear.

In this issue of *Circulation Research* Schulz and colleagues [25] describe the effects of ADAM-10 on permeability of HUVECs and T cell transmigration. They found that ADAM-10 cleaves VE-cadherin ectodomain into a soluble fragment, and the remaining carboxyterminal membrane bound stub is further cleaved by γ-secretase. ADAM-10-mediated cleavage of VE-cadherin is induced by thrombin activation of endothelial cells, Ca^{2+} influx as well as induction of apoptosis by staurosporine treatment. Inhibition of ADAM-10 by GI254023X decreased endothelial cell permeability and transmigration of T cells. Also, transfecting T cells with ADAM-10 siRNA caused a decrease in the rate of transmigration of activated T cells. These elegant studies highlight the importance of ADAM-10 in VE-cadherin removal from endothelial cell surface by controlled cleavage or shedding, a potential regulatory pathway of vascular permeability and the inflammation process associated with atherosclerosis.[26]

Extracellular Proteases and Intracellular Kinases

The identification of the role of ADAM-10 in VE-cadherin shedding poses several challenging questions. An important question is whether the observed effects on endothelial adhesion molecules and cell permeability are unique to ADAM-10 or involve cooperative interactions with other ADAMs. For instance, shedding of VCAM-1 can be mediated by ADAM-17.[21] Also, binding of ADAM-28 to P-selectin glycoprotein ligand-1 enhances P-selectin-mediated leukocyte adhesion to endothelial cells.[27] Additionally, ADAM-15 is an adherens junction molecule whose surface expression can be driven by VE-cadherin.[28]

Because ADAMs have multidomain structure, they are potentially multifunctional with multiple roles depending on their cellular localization. Also, ADAMs may function in concert with other metallorotease superfamilies such as ADAMTS and MMPs. ADAMTS (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs) include at least 19 members evolved as non-integral membrane proteins associated with the cell surface and ECM through specific protein domains. Interestingly, thrombin, an activator of ADAM-10, as well as plasmin promote proteolytic inactivation of ADAMTS-13.[29] Also, ADAMs may have substrate overlap with MMPs and thereby influence the degradation of ECM components. This is supported by reports that purified bovine ADAM-10 cleaves native type IV collagen, a major component of the basement membrane and ECM.[30] MMPs are also known to activate each other.[31] Therefore, the potential interactions between ADAMs and MMPs on endothelial cell permeability need to be examined. In this regard, it is important to investigate the effects of inhibitors of ADAMs and MMPs on leukocyte infiltration. Tissue inhibitors of MMPs (TIMPs) are being considered to target specific MMPs in localized vascular diseases such as abdominal aortic aneurysm. However, the isolated N-terminal domains of TIMP-1 and TIMP-3 may not be sufficient for ADAM-10 inhibition,[32] and specific siRNA may provide a more specific approach.

ADAMs-induced proteolysis can also change the activity of remnant surface molecular complexes which in turn affect signaling pathways inside the cell. While the effects of thrombin can be related to increased Ca^{2+} influx in endothelial cells, the role of localized Ca^{2+} gradients

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and the relation between the intracellular Ca^{2+} stores and other Ca^{2+} regulatory pathways in the surface membrane pumps and exchangers need to be further characterized. Also, while an increase in endothelial cell $[Ca^{2+}]_i$ could activate ADAM-10, the proeolytic fragments generated from this reaction may affect the activity of the same or other membrane channels. For instance, studies have suggested an effect of MMPs on membrane Ca^{2+} and/or K^+ channel activity, possibly through an interaction with membrane $\alpha_v\beta_3$ integrin.[33-36]

An important question also relates to the cellular remnants of cadherin shedding. β-catenin links the cytoplasmic domain of VE-cadherin to the actin cytoskeleton via α-catenin and therefore contributes to establishing VE-cadherin-mediated cell-cell adhesion (Figure). Ca^{2+} influx and ADAMs activation not only induce the proteolysis of extracellular VE-cadherin, and separation of cell-cell adhesion, but also facilitate the degradation of cytoplasmic domain of VE-cadherin by γ-secretase resulting in translocation of β-catenin from the plasma membrane to the cytoplasm where it may alter cell morphology, motility and proliferation.[37]

In addition to Ca^{2+}, the role of Rho kinase and protein kinase C (PKC) in proteolytic dissolution and shedding of adhesion molecules should be considered. Activation of PKC-α may increase endothelial cell permeability by disassembly of VE-cadherin junctions.[38] Also, vascular endothelial growth factor upregulates the expression of ADAMTS1 through PKC signaling.[39] While ADAM-10 is a weak substrate of PKC,[40] phorbol esters activating PKC and the small GTPase Rac can activate ADAM17-dependent shedding of a variety of substrates.[21]

Thus the discovery of the role of specific ADAMs in VE-cadherin shedding paves the way for further investigations to identify the potential interactions of ADAMs with other adhesion molecules, transmembrane and extracellular metalloproteases, as well as intracellular ion and protein kinase-dependent regulatory pathways. The identification of the mechanisms of cleavage of adhesion molecules by various metalloproteases during vascular lesion formation provides novel leads for the development of therapeutic interventions in vascular diseases.

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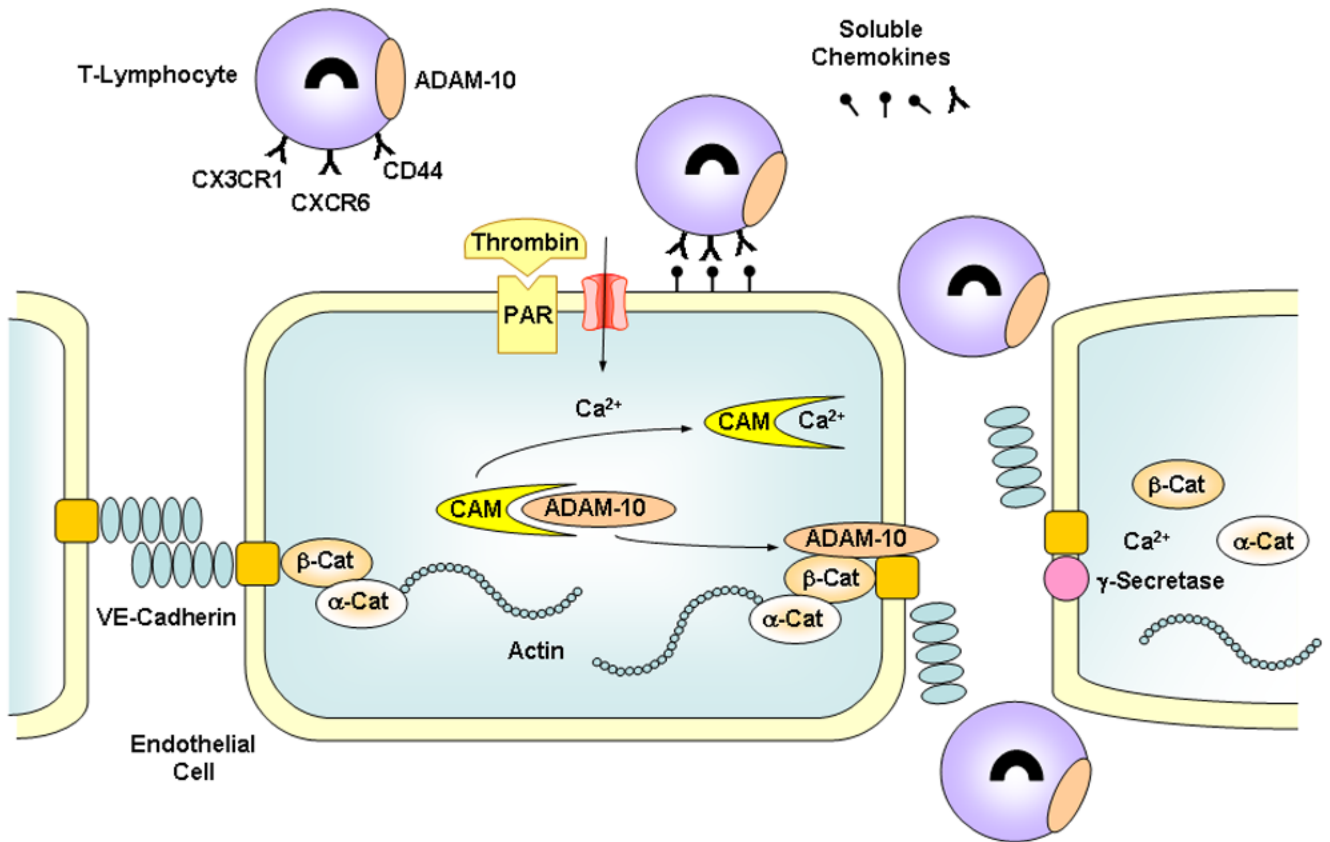

Figure.

Role of ADAM-10 in leukocyte recruitment and transmigration and endothelial cell permeability. In activated endothelium, upregulated cell adhesion molecules such as fractalkine, CXCL16 and hyaluronan ( ) bind their respective receptors CX3CR1, CXCR6 and CD44 (**Y**) resulting in initial capture of inflammatory cells. Inflammatory mediators such as thrombin (acting via protease-activated receptor, PAR), and leukocyte adhesion increase endothelial cell $[Ca^{2+}]_i$, causing the release of pro-ADAM-10 from calmodulin (CAM) and activation of ADAM-10. Activated ADAM-10 cleaves the adhesion molecules resulting in leukocyte deadhesion, initial rolling and subsequent migration to inter-endothelial junction. The released ectodomains act as soluble chemokines to further recruit inflammatory cells to the endothelium. Activated ADAM-10 also cleaves VE-cadherin ectodomain resulting in endothelial cell gap formation and inflammatory cell transmigration. The remaining membrane bound stub of VE-cadherin is cleaved by Ca^{2+}-sependent γ-secretase leading to the release of β-catenin, α-catenin and actin from the plasma membrane and alterations in cell morphology and nuclear signaling.