Regulation of Myoendothelial Junction Formation
Bridging the Gap

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In this issue of Circulation Research, Heberlein et al1 provide exciting new insight into the actions of plasminogen activator (PA) inhibitor (PAI)-1 by illuminating its role in governing the ability of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) to communicate with each other through specialized contacts defined as myoendothelial junctions (MEJs). In the resistance vasculature, MEJs are cellular extensions through the internal elastic lamina (IEL), whereby ECs make physical contact with adjacent VSMCs. 

At points of cell–cell contact, the presence of gap junction channels enables electrical coupling and intercellular diffusion of small solutes (<1 kDa) that govern vasomotor control. Cell–cell signaling through gap junction channels can be regulated acutely (eg, through phosphorylation of key serine residues on connexin subunits), and their protein complexes undergo hourly turnover in the plasma membrane. Such properties imply that the nature of cell–cell signaling through MEJs is under dynamic regulation. Despite implications that the MEJ is itself a dynamic structure, little is known about how MEJ formation and regression are regulated. New findings presented by Heberlein et al1 illustrate how dynamic MEJ formation and signaling may be enhanced or impaired via PAI-1 regulation of the PA system.

The Plasminogen Activator System and Cell Migration

Plasminogen is synthesized in the liver and released into the circulation as a zymogen of the serine protease, plasmin. The conversion of plasminogen to active plasmin is mediated by tissue (t)-PA and urokinase-type (u)-PA (ie, urokinase). Whereas t-PA is involved primarily in the dissolution of fibrin in the circulation, u-PA binds to the urokinase receptor (uPAR) (ie, CD87) on cell membranes, thereby localizing proteolytic activity to the vicinity of binding. In this manner, uPAR is integral to cell migration and adhesion through breakdown of the extracellular matrix (ECM).3,4 PAI-1, a 45-kDa glycoprotein, is a serine protease inhibitor of both t-PA and u-PA. The availability of PAI-1 can thereby dictate cell migration, as well as fibrinolysis. By interacting with vitronectin, a glycoprotein that anchors cells to the ECM, PAI-1 exerts additional influence on cell motility by sterically interfering with uPAR- and integrin-mediated cell adhesion.5

The net effects of PAI-1 on matrix degradation versus cell adhesion depend on its local availability (Figure, A). PAI-1 is secreted on synthesis from stromal and mesenchymal cells throughout the body, including ECs and VSMCs, with a half-life in the bloodstream of ~10 minutes.6 Expression of PAI-1 is increased dramatically by inflammatory mediators, as well as by metabolic disturbances, including obesity and hyperglycemia. Thus, alterations in PAI-1 levels are implicated in vascular disease associated with atherosclerosis, cancer, diabetes, obesity, and the metabolic syndrome.3,4 Nevertheless, and despite being recognized as a biomarker for vascular disease, little is known about the actions of PAI-1 on the blood vessel wall.

Elucidating a Role for PAI-1 in Governing MEJ Expression

To test the hypothesis that PAI-1 regulates MEJ formation, Heberlein et al1 used a multifaceted approach that evolved from findings in a vascular cell coculture (VCCC) model developed by Isakson and Duling.5 With ECs and VSMCs plated on opposite sides of a Transwell membrane, respective cell layers send projections (designated as MEJs) through the 0.4-μm pores, as defined by immunostaining for actin bridges. Scraping off the respective monolayers left only the projections within the Transwell pores. Digesting each of the respective fractions (VSMCs, ECs, projections), followed by proteomic analyses, revealed PAI-1 to be enriched within projections and associated with actin bridges extending through the pores. Supplementary immunobLOTS illustrated u-PA expression to be greatest within MEJ projections, whereas t-PA expression was greatest in VSMCs. Ensuing experiments investigated the effect of manipulating PAI-1 availability either by absorbing it with an antibody or by the addition of recombinant (r)PAI-1 to respective surfaces of VCCC. Exposure of ECs to a PAI-1 antibody was purported to reduce MEJ formation, whereas addition of rPAI-1 to ECs increased MEJ formation. Respective effects were manifest whether ECs alone were treated with rPAI-1 or in conjunction with VSMC treatment. In striking contrast, if VSMCs alone were treated with the PAI-1 antibody or with rPAI-1, no effect on MEJ formation was apparent. Complementary experiments using biotin-conjugated PAI-1 demonstrated that its trafficking to MEJs was observed only when ECs were treated; similar treatment of VSMCs was not associated with PAI-1 at MEJs. With greater numbers of MEJs in VCCC, selective stimulation of VSMCs with phenylephrine induced...
a more rapid elevation of EC calcium, implying enhanced heterocellular signaling through MEJs.

Collectively, the findings from VCCC of the present study suggested that exposure of the luminal surface of ECs to PAI-1 was essential for trafficking PAI-1 to MEJs and the ensuing regulation of MEJ expression. To support this interpretation using intact microvessels, complementary studies were performed on coronary arterioles of mice based on immunogold labeling of PAI-1. Ultrastructural analyses of electron micrographs illustrated the presence of PAI-1 in MEJs of control mice (C57BL/6). In comparison, there was less PAI-1 in MEJs from PAI-1/H11002 mice and more PAI-1 in MEJs from C57BL/6 mice fed a high-fat diet (associated with greater PAI-1 levels in the bloodstream3,4). Moreover, transplanting the hearts of PAI-1/H11002 mice into C57BL/6 mice increased PAI-1 expression in arterioles of PAI-1/H11002 mice to that seen in arterioles of C57BL/6 mice. The remarkable coherence of findings between results obtained in VCCC with those obtained from intact microvessels provides consistent evidence that PAI-1 availability is a key determinant of the level of MEJ formation (Figure, A).

The VCCC was introduced as a model to investigate the interaction between ECs and VSMCs isolated from the microcirculation of the mouse cremaster muscle.5 In contrast, the present study is based primarily on ECs and VSMCs derived from human umbilical veins. Myoendothelial junctions are associated with the resistance microvasculature to a far greater extent than larger conduit vessels.2 Thus, it is reassuring to have included comprehensive ultrastructural studies of coronary arterioles complemented by similar findings in cremasteric and mesenteric arterioles (see the Online Data Supplement in the article by Heberlein et al1). Furthermore, VCCC findings based on ECs and VSMCs derived from human coronary arteries and microvessels.1 Nevertheless, the functional implication of manipulating PAI-1 expression on heterocellular communication through MEJs is limited to observations of EC calcium responses to VSMC-specific stimulation in VCCC. How altering the expression of MEJs affects vascular function in vivo is a major question to be resolved in light of the actions of PAI-1 presented by Heberlein et al.1 Their

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**Figure.** PAI-1 regulates MEJ formation. According to new findings presented by Heberlein et al,1 MEJ formation is regulated through PAI-1 actions on the PA system. A, Flow diagram indicating how PAI-1 levels determine whether plasminogen activators (t-PA and u-PA) are able to convert plasminogen (inactive zymogen) into plasmin (active protease). A reduction in PAI-1 elevates plasmin concentration (right), thereby increasing matrix degradation, reducing cell adhesion, and diminishing MEJ formation. Conversely, an increase in PAI-1 (left) reduces plasmin production, thereby decreasing matrix degradation, enhancing cell adhesion, and increasing MEJ formation. B, Illustration of a MEJ between an EC and VSMC. Translocation of exogenous PAI-1 (black dots) from the apical surface of ECs to the MEJ (but not from the apical surface of VSMCs) was found to promote MEJ formation in VCCC. These observations raise several new questions about the mechanism of action of PAI-1. (1) What receptor(s) or transporter(s) does PAI-1 act on at the apical membrane of ECs? (2) How is PAI-1 transported from the plasma membrane of ECs to the MEJ? (3) What additional signaling events (eg, transcriptional regulation of proteins localized to MEJs) are activated by PAI-1 within the EC nucleus to promote MEJ formation? (4) How does PAI-1 promote invasion of the ECM and the IEL in forming a new MEJ in vivo? Whereas VSMCs apparently bind PAI-1 at their apical surface, they do not translocate it to the MEJ, leading to the question. (5) What intracellular transport machinery is lacking in VSMCs that prevents these cells from trafficking PAI-1 to the MEJ?
finding of similar effects in VCCC with cells derived from large and small blood vessels has several implications. First, altering PAI-1 levels can exert profound effects on the vasculature throughout the body, which is consistent with PAI-1 being a circulating marker of vascular disease. Second, the ability to send projections and establish heterocellular communication is not unique to VSMCs and ECs of the microcirculation because this phenotype can be readily invoked in VCCC of cells from diverse origins in the vasculature. Whether other cell lineages cocultured under appropriate conditions can develop similar projections and intercellular communication under the regulation of PAI-1 also remains to be determined.

Throughout their study, Heberlein et al interpret the cellular projections formed in VCCC to represent MEJs expressed in the blood vessel wall. This perspective is exemplified in experiments investigating protein levels within Transwell projections. Electric coupling and molecular diffusion through gap junction channels is enabled when neighboring cells each provide complementary connexon hemichannels. In the vessel wall, these specialized regions of contact are defined as electron-dense pentalaminar ultrastructures occurring along the tips of EC and VSMC extensions through the IEL. However, the ultrastructure of MEJs in VCCC has not been resolved, nor has the localization of PAI-1 relative to gap junctions within Transwell projections. Furthermore, because the VCCC model lacks a substantive IEL or ECM, the PAI-1–mediated regulation of proteolysis and of vitronectin binding, which have been attributed to the actions of PAI-1 in vivo, are unlikely to be as effective in VCCC. In turn, this difference in cellular environment implies novel mechanisms by which PAI-1 can govern MEJ formation in VCCC that remain to be elucidated. Applying microvascular ECs and SMCs derived from PAI-1−/− mice to VCCC should help strengthen this novel approach to understanding myoendothelial communication.

Heberlein et al are the first to tackle the exceedingly difficult problem of actually quantifying protein in such minute spatial domains as the MEJ. In the absence of ECM or IEL, the findings that PAI-1 has such remarkable effects on protein expression along actin bridges between ECs and VSMCs and that such effects appear to be unidirectional in nature (mediated through the luminal surface of ECs but not of VSMCs) open new perspectives and multiple questions (Figure, B) with respect to how an accepted marker of vascular disease can affect heterocellular signaling in the vascular wall. The present findings imply further that, through governing MEJ formation and regression, PAI-1 may effect a concerted response that involves localized remodeling of the cytoskeleton and plasma membrane, together with the expression of signaling complexes localized to these specialized regions of cell–cell communication. In turn, PAI-1 availability may also affect the dynamics of intercellular communication in tissues throughout the body. Understanding the nature and expression profile of PAI-1 receptor(s), how PAI-1 is translocated into cells and transported to MEJs, why it is unidirectional from the ECs in affecting MEJs, and the signaling events it triggers in ECs and VSMCs represent key questions that are ripe for future studies.

Sources of Funding
Research performed by the authors is supported by NIH grants R37-HL041026 (to S.S.S.) and F32-HL097463 (to P.B.).

Disclosures
None.

References

Key Words: cell–cell communication, heterocellular signaling, microcirculation, myoendothelial junction, plasminogen activator inhibitor-1
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_Circ Res._ 2010;106:1014-1016
doi: 10.1161/CIRCRESAHA.110.217786
_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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