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Commentary

Tyrosine Phosphorylation and Endothelial Cell Barrier Regulation

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In this issue of *The American Journal of Pathology*, Sui and co-authors[1] evaluate the involvement of protein Tyr phosphatase (PTPase) μ in the regulation of zonula adherens (ZA) multiprotein complexes and endothelial cell (EC) paracellular permeability. They demonstrate that PTPase μ expression is almost completely restricted to the sites of cell-cell contacts in postconfluent pulmonary ECs, and this expression is required for EC barrier function. They also demonstrate that PTPase μ and VE-cadherin interact at cell borders where PTPase μ decreases tyrosine phosphorylation of VE-cadherin. These studies identify a link between PTPase μ-mediated VE-cadherin phosphorylation and EC permeability. The significance of these findings as they relate to phosphorylation in maintenance of the EC barrier is discussed below.

Inflammatory diseases of the lung are characterized by increases in vascular permeability and enhanced leukocyte infiltration reflecting compromise of the semi-selective EC barrier that controls the movement of fluids, macromolecules, and leukocytes between vascular compartments and the interstitium.[2,3] Paracellular EC permeability is regulated by a balance between contractile (centripetal cytoskeletal tension) and opposing tethering (cell-cell and cell-matrix adhesion) forces and is dependent on multiple signaling events including protein phosphorylation and/or protein translocation to specific intracellular locations.[3,4]

A considerable body of evidence now supports a role for tyrosine phosphorylation in the regulation of vascular permeability.[5] For example, genistein, a general tyrosine kinase inhibitor, significantly decreases basal albumin permeability in cultured pulmonary EC monolayers.[6] In contrast, 2,5 DHC (methyl-2,5-dihydroxycinnamate), a potent inhibitor of receptor tyrosine kinases, produces decreased transendothelial electrical resistance indicative of EC barrier compromise.[6] Some tyrphostins, members of a family of specific tyrosine kinase inhibitors, selectively disrupt either cell-cell junctions or cell-substrate attachments, or both, whereas others have no effect on the EC cytoskeleton.[7] This study also suggests that some basal level of tyrosine phosphorylation is necessary to maintain EC barrier integrity. Collectively, the wide spectrum of the cellular effects of tyrosine kinase inhibitors suggests that specific tyrosine kinases may be involved in the regulation of endothelial barrier properties in a complex manner in defined subcellular compartments.

Several edemagenic agents, including thrombin, vascular endothelial growth factor, and histamine, stimulate protein tyrosine phosphorylation in EC, which correlates with an increase in permeability.[5] It is well established that thrombin-induced EC barrier dysfunction is critically dependent on increased levels of myosin light chain (MLC) phosphorylation, which results in stress fiber formation and increased contraction.[3,8] This process is controlled, at least in part, via activation of calcium/calmodulin-dependent MLC kinase (MLCK).[9,10] At the same time thrombin-induced EC barrier dysfunction occurs in association with thrombin-induced EC protein tyrosine phosphorylation, increased total tyrosine kinase activity in EC homogenates, and rapid translocation of nonreceptor tyrosine kinase, $p60^{src}$ to the cytoskeleton.[6,11] Genistein significantly attenuates $p60^{src}$ tyrosine kinase activity, thrombin-induced MLC phosphorylation, and the associated increase in permeability,[6] suggesting a link between thrombin-induced contraction and permeability and $p60^{src}$ activation.

Recent studies demonstrate that $p60^{src}$ may be involved in the regulation of EC permeability in several distinct ways. Studies of the novel EC MLCK have identified sites of $p60^{src}$ tyrosine phosphorylation in the unique N-terminus of one EC MLCK isoform (EC MLCK-1).[12] Phosphorylation of EC MLCK-1 by purified $p60^{src}$ results in a twofold to threefold increase in MLCK activity.[12] MLCK activation is linked to increased MLCK tyrosine phosphorylation and increased stable association of MLCK with $p60^{src}$[13,14] in pulmonary ECs, implicating MLCK as a cytoskeletal target for $p60^{src}$ *in vivo*. In addi-

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tion, cortactin, a p60^{src} substrate and F-actin binding protein, is present in the p60^{src}-MLCK complex in ECs stimulated with diperoxivanadate, a potent stimulator of tyrosine phosphorylation.[13] Diperoxivanadate-induced cortactin tyrosine phosphorylation is associated with cortactin translocation to the actin cytoskeleton.[13] Translocation of tyrosine-phosphorylated cortactin to the cytoskeletal fraction is also observed in thrombin-activated platelets.[15] Cortactin co-localization with actin peripheral structures, such as membrane ruffles and lamellopodia, is found in several cell types[16] and may be an important regulator of cortical actin assembly/disassembly. A recent study by Dudek and colleagues[17] indicated that p60^{src}-mediated tyrosine phosphorylation of cortactin, as well as binding of this protein to EC MLCK, is important for actin remodeling induced by sphingosine 1-phosphate, a potent EC barrier-enhancing agent.[17] Accordingly, depending on the stimulus and subcellular localization, p60^{src}-mediated tyrosine phosphorylation of cortactin and MLCK may be associated with either increased EC permeability or EC barrier enhancement.

Little is known about the role of tyrosine dephosphorylation in the regulation of EC barrier function. Vanadate, a potent and specific inhibitor of PTPases, increases basal and thrombin-induced MLC phosphorylation in pulmonary EC[18] implicating the involvement of PTPase activity in the regulation of EC contraction. However, PTPases involved in the regulation of MLC phosphorylation have never been identified.

PTPase inhibitors also increase the level of tyrosine phosphorylation of specific target proteins present at cellular boundaries. This increase in tyrosine phosphorylation at the sites of cell-cell contacts correlates with an increase in paracellular permeability,[19,20] implicating the involvement of PTPases in the regulation of cell-cell adhesion. Homophilic endothelial cell-cell adhesion is determined primarily by the function of VE-cadherin, the main component of ZA, a junctional complex, which is tethered to actin microfilaments.[21,22] Some evidence suggests that the phosphorylation state of VE-cadherin may affect EC permeability.[21,23,24] ZA function is also regulated by accessory catenin proteins with junction formation accompanied by tyrosine dephosphorylation of these proteins, correlating with the strength and stability of these junctions.[25] Histamine-induced phosphorylation of VE-cadherin, α/β-catenins, and plakoglobin (γ-catenin) correlates with dissociation of VE-cadherin from the actin cytoskeleton.[26] A time course of diperoxivanadate-induced phosphorylation of β/γ-catenins correlates well with diperoxivanadate-induced decreases in transendothelial electrical resistance.[27] Thrombin-induced tyrosine phosphorylation of β/γ and p120-catenins correlates with the phosphorylation and dissociation of the PTPase SHP2 from cadherin-catenins complexes, where this enzyme is presented in quiescent cells,[28] suggesting that SHP2 could be one of the PTPases regulating levels of phosphorylation of ZA proteins in endothelium.

Several other PTPases associated with ZA and able to dephosphorylate ZA proteins have been identified in various cell types.[29–32] Of particular interest among them is PTPase μ. This receptor PTPase is highly expressed in the lung endothelium and localized almost exclusively at endothelial cell contact sites.[33,34] PTPase μ interacts with several cadherins[30,35] and may potentially be involved in the strengthening of endothelial cell-cell adhesion via maintenance of cadherins in a dephosphorylated state.

In this issue of *The American Journal of Pathology*, Sui and co-authors[1] identify PTPase μ as a regulator of ZA multiprotein complexes and EC paracellular permeability. By immunofluorescence they demonstrate that PTPase μ expression is almost completely restricted to the sites of cell-cell contacts in postconfluent pulmonary EC. Using a siRNA technique, they show convincingly that specific depletion of PTPase μ significantly increases ^{14}C-albumin flux across EC monolayers consistent with EC barrier dysfunction. Moreover, ectopic expression of wild-type PTPase μ, but not catalytically impaired mutants, enhanced EC barrier function in immortalized EC. To examine the molecular mechanisms involved in PTPase μ-mediated EC barrier preservation, the authors examined the interaction of PTPase μ with VE-cadherin *in vivo* and *in vitro*. They demonstrate that PTPase μ co-localized with VE-cadherin at cell borders. These proteins also co-immunoprecipitate under nondenaturing conditions, suggesting that they associated with each other in a functional complex. In addition, *in vitro* studies demonstrate that PTPase μ directly binds to VE-cadherin. Finally, ectopic expression of wild-type PTPase μ decreased tyrosine phosphorylation of VE-cadherin. Collectively, these elegant studies clearly demonstrate that PTPase μ is involved in EC barrier regulation and controls VE-cadherin phosphorylation, suggesting a link between PTPase μ-mediated VE-cadherin phosphorylation and EC permeability.

The current study serves as an important contribution to our understanding of how tyrosine protein phosphorylation is involved in the regulation of EC barrier function. We now know that specific PTPases, such as PTP μ, are intimately involved in the regulation of cell-cell contacts and paracellular EC permeability. However, some important questions remain unanswered. For example, how does tyrosine phosphorylation at the C-terminal cytoplasmic domain of VE-cadherin (and other cadherins) affect homophilic adhesion provided by the extracellular portion of the molecule? How is dephosphorylation of ZA proteins, induced presumably by several PTPases, orchestrated in the cell? How do edemagenic agonists, such as thrombin or barrier-protective substances such as sphingosine 1-phosphate regulate PTPase activity? Further *in vitro* and *in vivo* studies are needed to evaluate the role of tyrosine protein phosphorylation, and specifically PTPase activity, in the regulation of EC permeability and vascular leak in the setting of both normal physiology and pathological conditions.

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