Editorial

Protein Tyrosine Phosphatases in the Vessel Wall

Counterpoint to the Tyrosine Kinases

Paul E. DiCorleto

A large and growing literature supports the involvement of protein tyrosine kinases in the regulation of vascular cell behavior. Many growth factor receptors are tyrosine kinases, and many cytosolic signaling events are regulated by protein tyrosine phosphorylation. Many of these mitogens, receptors, and intracellular kinases have been studied in vivo in various vascular injury and remodeling models. The biochemical counterpoint to these phosphorylation steps, ie, the reverse reaction of dephosphorylation, has received much less attention in vascular injury studies. This deficiency has begun to be addressed by the rigorous report of Wright et al in this issue.[1] These authors have examined the expression in the artery wall of a family of enzymes, the protein tyrosine phosphatases (PTPases), which dephosphorylate phosphorylated tyrosine residues.

See page 1189

It was originally believed that a small number of PTPases acted broadly to maintain a low basal level of tyrosine phosphorylation against which a large number of specific tyrosine kinases would act to regulate the phosphorylation of specific proteins. This initial concept has turned out to be incorrect. The first PTPase was cloned in 1988.[2,3] Since then, at least 75 distinct PTPases have been identified,[4–6] and it has been estimated that up to 2000 PTPases may be encoded in the mammalian genome.[7] However, until the current report, little was known of the identity of the PTPases expressed by vascular tissue in vivo, their regulation of expression and activity, or their possible role in vascular remodeling.

It is important to note that protein tyrosine kinases and PTPases catalyze opposite biochemical reactions, but they do not always play opposite physiological roles. SHP1 (also known as HCP, hematopoietic cell phosphatase) represents 1 well-characterized example of the multiple ways, both positive and negative, in which a PTPase may act as a regulator of cell function. SHP1 is a cytoplasmic PTPase with 1 catalytic domain and 2 SH_2 domains.[8,9] Disruption of the SHP1 gene in the "moth-eaten" mutation of the mouse results in severe autoimmune and immunodeficiency syndromes.[10,11] Genetic and biochemical analyses support the concept that SHP1 can be a negative regulator of signaling through interactions with the erythropoietin receptor,[12] the T-cell antigen receptor,[13] and c-Kit,[14,15] but it also serves as a positive regulator of Src kinase signaling.[16]

Multiple reports have appeared in recent years on the role of PTPases in various cellular functions and signaling pathways in cultured vascular cells—both endothelial cells (ECs) and smooth muscle cells (SMCs). Many of these studies are based strictly on the use of putatively specific PTPase inhibitors, such as vanadate and phenylarsine oxide; although some investigators have quantified the activity levels of specific PTPases. In both ECs[17,18] and SMCs,[19] specific PTPases have been shown to increase dramatically with increased density and growth arrest. PTPases have been implicated in EC apoptosis and differentiation,[20] as well as in vascular endothelial growth factor–induced migration and proliferation of ECs.[21,22] In addition, these enzymes are involved in the regulation of expression of multiple EC genes, including nitric oxide synthase[23] and leukocyte adhesion molecules.[24,25] In SMCs, PTPases have been implicated in nitric oxide–mediated growth arrest,[26] cell motility,[27,28] and in both angiotensin II and insulin-like growth factor-I signaling cascades.[29]

In vivo studies to extend and confirm, under physiological or pathophysiological situations, the observations made with cultured vascular cells have been very limited. The expression of specific PTPases by the endothelium during mouse development has been reported. Vascular EC PTP has been found to be a Tie-2–specific PTPase that plays a role in vessel morphogenesis and integrity.[30] An EC receptor tyrosine phosphatase (ECRTP/DEP-1) has been reported to play a role in glomerular EC recruitment during kidney development.[31] Finally, the receptor PTPase mu has been found to be expressed almost exclusively in the ECs of multiple tissues, with higher expression in the arterial versus the venous circulation.[32] However, study of the expression of PTPases in vivo during the vascular response to injury has been limited to a recent report, in which the overall level of PTPase activity was found not to have changed after balloon injury to the rat carotid artery.[33] A detailed look at individual PTPases in an arterial injury model is now reported in the present issue.[1]

Wright et al[1] used to advantage the known conserved motifs within the catalytic domain of PTPases to study the expression of this family of enzymes in the vessel wall. The primer pairs used by these investigators to identify PTPases expressed/regulated in the carotid artery amplified a portion of the catalytic domain of all known PTPases. This approach allowed the authors to identify 20 PTPase catalytic domains included in 18 different known PTPases (some PTPases have

Received February 7, 2000; accepted February 7, 2000.

From the Department of Cell Biology, Lerner Research Institute of The Cleveland Clinic Foundation, Cleveland, Ohio.

Correspondence to Paul E. DiCorleto, PhD, Department of Cell Biology—NC10, Cleveland Clinic Lerner Research Institute, 9500 Euclid Ave, Cleveland, OH 44195. E-mail dicorlp@ccf.org

(*Arterioscler Thromb Vasc Biol*. 2000;20:1179-1181.)

***Arterioscler Thromb Vasc Biol.* is available at http://www.atvbaha.org**

2 catalytic domains, and both were found) in the rat carotid artery system. Although no novel PTPases were found in either control or injured carotid arteries, the reverse transcription–polymerase chain reaction approach that was used does not allow one to conclude that all vascular PTPases have been identified. It is worth noting, however, that the same approach did identify a novel PTPase in a rat model of mesangial proliferative glomerulonephritis.[34] The lack of identified novel PTPases in the artery wall may reflect the fact that arteries are often surveyed unintentionally, owing to the presence of blood vessels in the samples used, in gene discovery searches by investigators primarily interested in other organs or tissues.

Of the 18 PTPases that Wright et al identified in carotid tissue, the relative expression of 5 changed significantly after arterial injury: PTPb, SHP1, CD45, PTP1B, and PTPL1. The underlying causes of the changes in expression were of 2 types, which are illustrative of the 2 mechanisms that will lead to positive findings in all gene expression screens of in vivo tissue. The first is that a change in the relative abundance of a PTPase within a tissue sample may be due to an actual increase or decrease in the level of expression of the enzyme per cell. Alternatively, especially in dynamic systems such as the injured vessel wall and in the case of cell type–specific PTPases, changes in the relative abundance of a PTPase may reflect an altered cellular composition of the tissue.

The increased transcript abundance of 3 of the 5 PTPases whose expression was greater after carotid artery injury turned out to reflect changes in the cellular composition of the injured carotid artery. CD45 (also referred to as leukocyte common antigen) is an extensively studied member of the PTPase family whose expression is restricted to leukocytes and erythroid precursor cells.[35,36] It is widely used as a specific marker of leukocytes. Because the number of leukocytes within the vessel wall increases after injury, it is not surprising that the level of CD45 was observed to increase as well. SHP1, or HCP, is another leukocyte PTPase, but unlike CD45, its expression is not entirely restricted to leukocytes. Wright et al[1] detected some SHP1 expression by SMCs after (but not before) injury and by SMCs in culture. PTPb is the most intriguing of the cell type–specific PTPases identified by Wright et al. It is a type III receptor PTPase consisting of a single cytoplasmic catalytic domain, a transmembrane region, and 16 tandem extracellular fibronectin type III–like repeats.[37] The authors' results suggest that PTPb may be a useful marker for ECs (analogous to CD45 for leukocytes), because it was observed to be highly expressed by both arterial and small-vessel ECs and not by other cell types.

Expression by SMCs of 2 PTPases, PTPL1 and PTP1B, was upregulated on injury, though with different patterns/kinetics. PTP1B expression was not detectable before injury but increased in SMCs while the cells were still present in the tunica media. Expression remained elevated after the SMCs had migrated into the intima. By contrast, PTPL1 expression was not detected until the SMCs had entered the intima, and expression was highest by the most luminal SMCs. What might the function be of these PTPases? The morphology and possibly the cell-cell interactions between SMCs in the neointima differ from those of SMCs in the media,[38] and it is possible that PTP1B and/or PTPL1 are involved in creating that difference. PTPL1 is the largest known PTPase; its sequence predicts a 2466–amino acid cytosolic protein. The expression pattern of PTPL1 has suggested that it may function as a structural component or regulator of specialized junctions in epithelial cells.[39]

The PTP1B sequence predicts a 50-kDa cytoplasmic PTPase with a single catalytic domain and no accessory domains. PTP1B was the first PTPase to be cloned and characterized.[3,40] Transfection of PTP1B in cultured cells has revealed roles in downregulating mitogenic signaling in response to activation of many different growth factor receptor tyrosine kinases.[41] Mounting evidence, including that revealed by disrupting the PTP1B gene in mice, has suggested a significant role for PTP1B in insulin receptor signaling.[42–44] In addition, dephosphorylation of β-catenin appears to be accomplished by a PTP1B-like PTPase that exhibits regulated association with cadherin.[45] Efficient (high-avidity) cell-cell binding requires that the cytoplasmic domains of cadherin molecules interact with catenins, which in turn bind to actin microfilaments (reviewed in Reference 46). Thus, PTP1B may regulate SMC homotypic interactions by modulating the phosphorylated state of catenin.

The first step has been taken in our understanding of how PTPases affect vessel wall function, but many important questions remain. Which specific PTPases play a role in EC activation, which is an initiating event in atherogenesis and other inflammatory responses? Would highly specific PTPase inhibitors be useful in blocking the expression of leukocyte adhesion molecules on the EC surface, as is indicated by experiments in cell culture? Would the up- or downregulation of specific PTPases be useful in modulating SMC migration, proliferation, or apoptosis? If a small fraction of the investigators currently focusing on the kinase side of the issue shift their emphasis to the PTPase side, these questions and many others related to PTPase action in the vessel wall will be answered in the near future.

References

1. Wright et al. Protein-tyrosine phosphatases in the vessel wall: differential expression after acute arterial injury. *Arterioscler Thromb Vasc Biol*. 2000;20:1189–1198.
2. Tonks NK, Diltz CD, Fischer EH. Characterization of the major protein-tyrosine-phosphatases of human placenta. *J Biol Chem*. 1988;263:6731–6737.
3. Tonks NK, Diltz CD, Fischer EH. Purification of the major protein-tyrosine-phosphatases of human placenta. *J Biol Chem*. 1988;263:6722–6730.
4. Schaapveld R, Wieringa B, Hendriks W. Receptor-like protein tyrosine phosphatases: alike and yet so different. *Mol Biol Rep*. 1997;24:247–262.
5. Tonks NK. Protein tyrosine phosphatases and the control of cellular signaling responses. *Adv Pharmacol*. 1996;36:91–119.
6. Tonks NK, Neel BG. From form to function: signaling by protein tyrosine phosphatases. *Cell*. 1996;87:365–368.
7. Hooft van Huijsduijnen R. Protein tyrosine phosphatases: counting the trees in the forest. *Gene*. 1998;225:1–8.
8. Matthews R, Bowne D, Flores E, Thomas M. Characterization of hematopoietic intracellular protein tyrosine phosphatases: description of a phosphatase containing an SH2 domain and another enriched in proline-, glutamic acid-, serine-, and threonine-rich sequences. *Mol Cell Biol*. 1992;12:2396–2405.
9. Yi TL, Cleveland JL, Ihle JN. Protein tyrosine phosphatase containing SH2 domains: characterization, preferential expression in hematopoietic

cells, and localization to human chromosome 12p12–p13. *Mol Cell Biol*. 1992;12:836–846.
10. Shultz LD, Schweitzer PA, Rajan TV, Yi T, Ihle JN, Matthews RJ, Thomas ML, Beier DR. Mutations at the murine moth-eaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. *Cell*. 1993;73:1445–1454.
11. Tsui W, Siminovitch K, de Souza L, Tsui F. Motheaten and viable motheaten mice have mutations in the haematopoietic cell phosphatase gene. *Nat Genet*. 1993;4:124–129.
12. Klingmuller U, Lorenz U, Cantley L, Neel B, Lodish H. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. *Cell*. 1995;80:729–738.
13. Pani G, Fischer KD, Mlinaric-Rascan I, Siminovitch KA. Signaling capacity of the T cell antigen receptor is negatively regulated by the PTP1C tyrosine phosphatase. *J Exp Med*. 1996;184:839–852.
14. Paulson RF, Vesely S, Siminovitch KA, Bernstein A. Signalling by the W/Kit receptor tyrosine kinase is negatively regulated in vivo by the protein tyrosine phosphatase Shp1. *Nat Genet*. 1996;13:309–315.
15. Lorenz U, Bergemann AD, Steinberg HN, Flanagan JG, Li X, Galli SJ, Neel BG. Genetic analysis reveals cell type-specific regulation of receptor tyrosine kinase c-Kit by the protein tyrosine phosphatase SHP1. *J Exp Med*. 1996;184:1111–1126.
16. Somani AK, Bignon JS, Mills GB, Siminovitch KA, Branch DR. Src kinase activity is regulated by the SHP-1 protein-tyrosine phosphatase. *J Biol Chem*. 1997;272:21113–21119.
17. Gaits F, Li RY, Ragab A, Ragab-Thomas JM, Chap H. Increase in receptor-like protein tyrosine phosphatase activity and expression level on density-dependent growth arrest of endothelial cells. *Biochem J*. 1995; 311:97–103.
18. Campan M, Yoshizumi M, Seidah NG, Lee ME, Bianchi C, Haber E. Increased proteolytic processing of protein tyrosine phosphatase mu in confluent vascular endothelial cells: the role of PC5, a member of the subtilisin family. *Biochemistry*. 1996;35:3797–3802.
19. Borges LG, Seifert RA, Grant FJ, Hart CE, Disteche CM, Edelhoff S, Solca FF, Lieberman MA, Lindner V, Fischer EH, Lok S, Bowen-Pope DF. Cloning and characterization of rat density-enhanced phosphatase-1, a protein tyrosine phosphatase expressed by vascular cells. *Circ Res*. 1996;79:570–580.
20. Yang C, Chang J, Gorospe M, Passaniti A. Protein tyrosine phosphatase regulation of endothelial cell apoptosis and differentiation. *Cell Growth Differ*. 1996;7:161–171.
21. Kroll J, Waltenberger J. The vascular endothelial growth factor receptor KDR activates multiple signal transduction pathways in porcine aortic endothelial cells. *J Biol Chem*. 1997;272:32521–32527.
22. Huang L, Sankar S, Lin C, Kontos CD, Schroff AD, Cha EH, Feng SM, Li SF, Yu Z, Van Etten RL, Blanar MA, Peters KG. HCPTPA, a protein tyrosine phosphatase that regulates vascular endothelial growth factor receptor-mediated signal transduction and biological activity. *J Biol Chem*. 1999;274:38183–38188.
23. Cieslik K, Lee CM, Tang JL, Wu KK. Transcriptional regulation of endothelial nitric-oxide synthase by an interaction between casein kinase 2 and protein phosphatase 2A. *J Biol Chem*. 1999;274:34669–34675.
24. May MJ, Wheeler-Jones CP, Pearson JD. Effects of protein tyrosine kinase inhibitors on cytokine-induced adhesion molecule expression by human umbilical vein endothelial cells. *Br J Pharmacol*. 1996;118: 1761–1771.
25. Dhawan S, Singh S, Aggarwal BB. Induction of endothelial cell surface adhesion molecules by tumor necrosis factor is blocked by protein tyrosine phosphatase inhibitors: role of the nuclear transcription factor NF-κB. *Eur J Immunol*. 1997;27:2172–2179.
26. Dhaunsi GS, Matthews C, Kaur K, Hassid A. NO increases protein tyrosine phosphatase activity in smooth muscle cells: relationship to antimitogenesis. *Am J Physiol*. 1997;272:H1342–H1349.
27. Hassid A, Yao J, Huang S. NO alters cell shape and motility in aortic smooth muscle cells via protein tyrosine phosphatase 1B activation. *Am J Physiol*. 1999;277:H1014–H1026.
28. Hassid A, Huang S, Yao J. Role of PTP-1B in aortic smooth muscle cell motility and tyrosine phosphorylation of focal adhesion proteins. *Am J Physiol*. 1999;277:H192–H198.
29. Ali MS, Schieffer B, Delafontaine P, Bernstein KE, Ling BN, Marrero MB. Angiotensin II stimulates tyrosine phosphorylation and activation of insulin receptor substrate 1 and protein-tyrosine phosphatase 1D in vascular smooth muscle cells. *J Biol Chem*. 1997;272:12373–12379.
30. Fachinger G, Deutsch U, Risau W. Functional interaction of vascular endothelial-protein-tyrosine phosphatase with the angiopoietin receptor Tie-2. *Oncogene*. 1999;18:5948–5953.
31. Takahashi T, Takahashi K, Mernaugh R, Drozdoff V, Sipe C, Schoecklmann H, Robert B, Abrahamson DR, Daniel TO. Endothelial localization of receptor tyrosine phosphatase, ECRTP/DEP-1, in developing and mature renal vasculature. *J Am Soc Nephrol*. 1999;10: 2135–2145.
32. Bianchi C, Sellke FW, Del Vecchio RL, Tonks NK, Neel BG. Receptor-type protein-tyrosine phosphatase mu is expressed in specific vascular endothelial beds in vivo. *Exp Cell Res*. 1999;248:329–338.
33. Yamashita M, Dimayuga P, Kaul S, Shah PK, Regnstrom J, Nilsson J, Cercek B. Phosphatase activity in the arterial wall after balloon injury: effect of somatostatin analog octreotide. *Lab Invest*. 1999;79:935–944.
34. Wright MB, Hugo C, Seifert R, Disteche CM, Bowen-Pope DF. Proliferating and migrating mesangial cells responding to injury express a novel receptor protein-tyrosine phosphatase in experimental mesangial proliferative glomerulonephritis. *J Biol Chem*. 1998;273:23929–23937.
35. Justement LB. The role of CD45 in signal transduction. *Adv Immunol*. 1997;66:1–65.
36. Altin JG, Sloan EK. The role of CD45 and CD45-associated molecules in T cell activation. *Immunol Cell Biol*. 1997;75:430–445.
37. Krueger NX, Streuli M, Saito H. Structural diversity and evolution of human receptor-like protein tyrosine phosphatases. *EMBO J*. 1990;9: 3241–3252.
38. Schwartz SM. The intima: a new soil. *Circ Res*. 1999;85:877–879.
39. Hendriks W, Schepens J, Bachner D, Rijss J, Zeeuwen P, Zechner U, Hameister H, Wieringa B. Molecular cloning of a mouse epithelial protein-tyrosine phosphatase with similarities to submembranous proteins. *J Cell Biochem*. 1995;59:418–430.
40. Chernoff J, Schievella AR, Jost CA, Erikson RL, Neel BG. Cloning of a cDNA for a major human protein-tyrosine-phosphatase. *Proc Natl Acad Sci U S A*. 1990;87:2735–2739.
41. Lammers R, Bossenmaier B, Cool DE, Tonks NK, Schlessinger J, Fischer EH, Ullrich A. Differential activities of protein tyrosine phosphatases in intact cells. *J Biol Chem*. 1993;268:22456–22462.
42. Ahmad F, Li PM, Meyerovitch J, Goldstein BJ. Osmotic loading of neutralizing antibodies demonstrates a role for protein-tyrosine phosphatase 1B in negative regulation of the insulin action pathway. *J Biol Chem*. 1995;270:20503–20508.
43. Cicirelli MF, Tonks NK, Diltz CD, Weiel JE, Fischer EH, Krebs EG. Microinjection of a protein-tyrosine-phosphatase inhibits insulin action in *Xenopus* oocytes. *Proc Natl Acad Sci U S A*. 1990;87:5514–5518.
44. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science*. 1999;283:1544–1548.
45. Balsamo J, Leung T, Ernst H, Zanin MK, Hoffman S, Lilien J.. Regulated binding of PTP1B-like phosphatase to N-cadherin: control of cadherin-mediated adhesion by dephosphorylation of β-catenin. *J Cell Biol*. 1996; 134:801–813.
46. Kemler R. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet*. 1993;9:317–321.

KEY WORDS: atherosclerosis ■ smooth muscle cells ■ endothelial cells ■ protein tyrosine phosphatases ■ kinase

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Protein Tyrosine Phosphatases in the Vessel Wall: Counterpoint to the Tyrosine Kinases

Paul E. DiCorleto

Arterioscler Thromb Vasc Biol. 2000;20:1179-1181
doi: 10.1161/01.ATV.20.5.1179
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636