Counter-Regulatory Function of Protein Tyrosine Phosphatase 1B in Platelet-Derived Growth Factor–or Fibroblast Growth Factor–Induced Motility and Proliferation of Cultured Smooth Muscle Cells and in Neointima Formation

Yingzi Chang, Bogdan Ceacareanu, Daming Zhuang, Chunxiang Zhang, Qinghua Pu, Alice C. Ceacareanu, Avi Hassid

Objectives—We have previously reported that vascular injury or treatment of cultured vascular smooth muscle cells with platelet-derived growth factor-BB (PDGF-BB) or fibroblast growth factor-2 (FGF2) increases the levels of protein tyrosine phosphatase (PTP)1B. The current study was designed to test the hypothesis that PTP1B attenuates PDGF- or FGF-induced motility and proliferation of cultured cells, as well as neointima formation in injured rat carotid arteries.

Methods and Results—Treatment of cultured cells with adenovirus expressing PTP1B decreased PDGF-BB– or FGF2-induced cell motility and blocked PDGF-BB– or FGF2–induced proliferation, whereas expression of dominant negative PTP1B (C215S-PTP1B) uncovered the motogenic effect of subthreshold levels of PDGF-BB or FGF2, increased neointimal and medial cell proliferation, and induced neointimal enlargement after balloon injury. The inhibitory effect of PTP1B directed against PDGF in cultured cells was associated with dephosphorylation of the PDGF receptor.

Conclusions—PTP1B suppresses cell proliferation and motility in cultured smooth muscle cells treated with PDGF-BB or FGF2, and the phosphatase plays a counter-regulatory role in vascular injury-induced cell proliferation and neointima formation. Taken together with previous studies indicating increased PTP1B levels in cells treated with growth factors, the current findings are the first to report the existence of an inhibitory feedback loop involving PDGF or FGF, and PTP1B in blood vessels. (Arterioscler Thromb Vasc Biol. 2006;26:501-507.)

Key Words: PTP1B ■ growth factors ■ neointima formation ■ cell motility ■ cell proliferation

Migration and proliferation of smooth muscle cells are of critical importance in neointima formation and remodeling occurring in response to vascular injury.1 Increased release of platelet-derived growth factor (PDGF) and/or fibroblast growth factor-2 (FGF2), followed by activation of PDGF and/or FGF2 receptor tyrosine kinase activities, are thought to be major events contributing to vascular remodeling.2 Several reports indicate that injury-induced movement of smooth muscle cells from media to intima and the proliferation of smooth muscle cells in intima are significantly reduced by pharmacological antagonists of the function or availability of PDGF or FGF2.3–7 Conversely, administration of PDGF-BB or FGF2 has been reported to enhance smooth muscle cell movement from media to intima, followed by cell proliferation in vessels with minimal endothelial damage.7,8 These studies indicate that PDGF and FGF are important mediators of neointima formation in models of vascular injury. Tyrosyl phosphorylation of growth factor receptors via their intrinsic tyrosine kinase activities is a pivotal event for activation of downstream signaling that mediates increased motility and proliferation in cultured cells. Furthermore, balloon injury or treatment in vivo with PDGF also induces PDGF receptor tyrosyl phosphorylation in vascular smooth muscle.6,9 a finding consistent with experiments in vitro.

Protein tyrosine phosphatases (PTPs) are thought to play an important role as counter-regulatory agents that attenuate or terminate signaling induced by activation of receptor tyrosine kinases. PTP1B is a ubiquitously expressed nonreceptor phosphatase targeted to several intracellular domains, including the endoplasmic reticulum and focal adhesions.10,11 PTP1B has been most prominently linked with regulation of insulin or insulin-like growth factor-1 (IGF-1) signaling in vitro and in vivo.12–16 We have recently reported that PTP1B attenuates insulin-induced cultured smooth muscle cell motility by decreasing the levels of phosphotyrosyl in insulin receptors.17 In a separate recent study, we reported that PDGF...
and FGF but not IGF-1 significantly increased the levels of PTP1B protein in cultured rat aortic smooth muscle cells.18

Recent studies indicate that PTP1B may also target the PDGF receptor. One such study reported a biochemical association of the PDGF receptor with PTP1B,19 whereas another study presented evidence consistent with PDGF receptor dephosphorylation by PTP1B, although this effect paradoxically failed to alter signaling downstream of the receptor.20 To our knowledge, there are no reports on the capacity of PTP1B to act as inhibitor of PDGF function in cultured smooth muscle cells or neointima formation in injured blood vessels. Moreover, the potential role of PTP1B as modulator of FGF2 activity in vitro or in vivo appears not to have been investigated. The current study was therefore targeted at testing the hypotheses that PTP1B has the capacity to regulate PDGF- or FGF-induced motility and proliferation of cultured rat aortic smooth muscle cells and that the phosphatase plays a role in attenuating neointima formation occurring in response to vascular injury by decreasing smooth muscle cell motility, proliferation, and/or apoptosis.

Materials and Methods

Materials
Detailed materials information is available in the data supplement at http://atvb.ahajournals.org.

Cell Culture
Rats were purchased from Charles River Laboratories (Wilmington, Mass.) or they were bred in the University of Tennessee vivarium. Smooth muscle cells were isolated from thoracic aortas of 6- to 9-day-old Sprague-Dawley rats and cultured as described in a previous report from our laboratory.21 The choice of cells was made on the basis of studies indicating that primary cultured cells from rat pups have characteristics similar to those of the neointima.22,23 In vitro experiments were carried out using primary cultures to minimize culture-induced phenotypic dedifferentiation of cells. Each experiment was performed using cells isolated from different litters of pups.

Measurement of Cell Motility
Cell motility in cultured cells was measured via a protocol described in a recent publication from our laboratory.24 Details of the procedure are provided in the Data Supplement.

Measurement of Cell Proliferation
Cell proliferation in cultured cells was measured by using the In Situ Cell Death Detection Kit (available online at http://atvb.ahajournals.org), treatment of cells with recombinant adenovirus encoding for PTP1B overexpression of PTP1B is sufficient to decrease cell motility induced by PDGF-BB or FGF2. As shown in Figure 1B, overexpression of PTP1B decreased FGF2-induced cell motility by 10% to 90%. PTP1B was more effective in opposing the motogenic response directed against relatively low than high concentrations of PDGF-BB, and indeed the phosphatase essentially blocked the motogenic effect of the lowest concentration of PDGF-BB (0.5 ng/mL) used in our study. As shown in Figure 1B, overexpression of PTP1B also attenuated FGF2-induced cell motility; however, unlike PDGF-BB–induced motility, PTP1B decreased FGF2-induced motility by at least 30%, even at a relatively high concentration of FGF2. These results indicate that an increase of PTP1B levels is sufficient to attenuate PDGF-BB– as well as FGF2-induced motility in primary cultured rat aortic smooth muscle cells.

Measurement of PDGFβ Receptor Phosphotyrosyl Levels
PDGF receptor tyrosyl phosphorylation was determined by immunoprecipitation of PDGFβ receptor with antibody directed against PDGFβ receptor and blotting with antibody directed against PDGFβ receptor phosphotyrosyl residue 770. The detailed procedure is described in the Data Supplement.

Rat Carotid Artery Injury Model
Rat carotid artery injury was generated via a standard procedure.25 Details are provided in the data supplement.

Morphometric Measurement of Neointima Formation
Rat carotid arteries were collected 3, 7, or 14 days after balloon injury. Carotid arteries were fixed by in situ perfusion through the left ventricle with 10% formalin followed by embedding in paraffin. Cross-sections were stained with hematoxylin and eosin for morphometric analysis. Images were collected by using Spot 3.3.2 software. The cross sectional surface areas of neointima and media were measured by using a computerized image analysis system (NIH v.1.62).

Immunohistochemistry
Paraffin-embedded carotid arteries were sectioned (5 μm), dewaxed, rehydrated, and irradiated in a microwave oven at 94°C in 0.1 mol/L citrate buffer, pH 6.0. Levels of proliferating cell nuclear antigen (PCNA) in tissue sections were measured by incubation with anti-PCNA at a dilution of 1:200. Detection was carried out by using the ABC (Avidin-Biotin Complex) method with DAB (3, 3’-diaminobenzidine) as substrate. Total and PCNA-positive cell numbers were determined from analysis of four microscopic fields from each of the tissue sections, at magnification of ×400. The fraction of proliferating cells was calculated as the mean ratio of the number of positive cells to total cell number in each set of tissue sections.

Statistical Analysis
Results are expressed as mean±SEM and were analyzed by using 2-way ANOVA followed by Fisher’s least significant difference test or unpaired t test. P<0.05 is considered statistically significant.

Results

Overexpression of PTP1B Attenuates PDGF-BB– or FGF2-Induced Motility in Cultured Smooth Muscle Cells
These experiments were performed to test the hypothesis that overexpression of PTP1B is sufficient to decrease cell motility induced by PDGF-BB or FGF2. As shown in Figure 1 (available online at http://atvb.ahajournals.org), treatment of cells with recombinant adenovirus encoding for PTP1B induced a significant increase of PTP1B protein levels. Moreover, as shown in Figure 1A, overexpression of PTP1B decreased PDGF-BB–induced cell motility by 10% to 90%. PTP1B was more effective in opposing the motogenic response directed against relatively low than high concentrations of PDGF-BB, and indeed the phosphatase essentially blocked the motogenic effect of the lowest concentration of PDGF-BB (0.5 ng/mL) used in our study. As shown in Figure 1B, overexpression of PTP1B also attenuated FGF2-induced cell motility; however, unlike PDGF-BB–induced motility, PTP1B decreased FGF2-induced motility by at least 30%, even at a relatively high concentration of FGF2. These results indicate that an increase of PTP1B levels is sufficient to attenuate PDGF-BB– as well as FGF2-induced motility in primary cultured rat aortic smooth muscle cells.
Phosphorylation in Cultured Smooth Muscle Cells that mediates increased motility and proliferation in cultured rat aortic smooth muscle cells. Cells were infected for 24 hours with adenovirus expressing EGFP (enhanced green fluorescent protein, as control virus) or PTP1B at multiplicity-of-infection (MOI) values of 10 to 15. Virus-containing media were removed and cells were cultured for an additional 24 hours to allow for expression of transgenic PTP1B. Cell motility was determined via a wounded culture assay in response to treatment with PDGF-BB (0, 0.5, 1 or 10 ng/mL, A) or FGF2 (0, 2, or 20 ng/mL, B) for 24 hours as described in Materials and Methods. Results are the mean±SEM of 3 independent experiments. A, **P<0.01 compared to treatment category lacking PTP1B. B, Open bars indicate cells infected with control virus; striped bars, cells infected with virus expressing PTP1B. Numerals shown inside bars indicate FGF2 concentration in ng/mL. *P<0.01 compared with control virus; **P<0.01 compared with control virus + FGF2.

Overexpression of PTP1B Blocks PDGF-BB- or FGF2-Induced Proliferation in Cultured Smooth Muscle Cells

Smooth muscle cell proliferation is an independent pivotal event in vascular injury-induced neointima formation. The present study was also designed to test the hypothesis that overexpression of PTP1B is sufficient to decrease cell proliferation induced by PDGF-BB or FGF2. As shown in Figure 2, treatment of cultured cells with adenovirus expressing PTP1B blocked PDGF-BB- or FGF2-induced proliferation, indicating that upregulation of PTP1B is sufficient to decrease PDGF-BB- or FGF2-induced proliferation in primary cultured rat aortic smooth muscle cells.

Overexpression of PTP1B Blocks PDGF-BB-Induced PDGFβ Receptor Phosphorylation in Cultured Smooth Muscle Cells

Tyrosyl phosphorylation of growth factor receptors is of critical importance for activation of downstream signaling that mediates increased motility and proliferation in cultured cells. Moreover, it has been recently reported that dephosphorylation of PDGF receptor by PTP1B fails to alter signaling downstream of the receptor in fibroblasts. These findings prompted us to test the hypothesis that PTP1B-induced inhibition of cell motility and proliferation are associated with phosphotyrosyl dephosphorylation of PDGFβ receptor. In the present experiments, we targeted tyrosyl residue 770 in the PDGFβ receptor for investigation because phosphorylation of this residue has been reported to mediate signaling relevant to cell motility and proliferation, involving the small GTP-binding protein Ras. As shown in Figure II (available online at http://atvb.ahajournals.org), treatment of cells with adenovirus expressing PTP1B blocked PDGF-BB-induced phosphorylation of Y770 in PDGFβ receptor, consistent with the view that the antimotogenic and antimitogenic effects of the phosphatase can be attributed to dephosphorylation of residue Y770.

Expression of Dominant Negative PTP1B (C215S-PTP1B) Uncovers the Motogenic Effect of a Subthreshold Level of PDGF-BB or FGF2 in Cultured Smooth Muscle Cells

The next experiments were performed to test the hypothesis that expression of a catalytically-inactive PTP1B allele, previously shown by us to function in dominant-negative manner against the insulin receptor in cultured vascular smooth muscle cells, would enhance PDGF-BB- or FGF2-induced cell motility. The data shown in Figure III (available online at http://atvb.ahajournals.org) demonstrate effective expression of C215S-PTP1B. It should also be noted that expression of dominant negative PTP1B can, by itself, induce cell motility if it occurs at sufficiently high levels, as demonstrated in our previous studies. Therefore, for the present experiments, we titrated dominant negative PTP1B expression down to the level at which it produced no significant increase in motility to avoid the potential confounding effect of altered baseline motility. As shown in Figure 3A, treatment of cells with dominant negative PTP1B uncovered a motogenic effect of PDGF-BB at a low concentration of PDGF-BB, which, when used alone, failed to induce significant cell motility. Similarly, dominant negative PTP1B uncovered the motility stimulatory effect of a subthreshold level of FGF2 (Figure 3B). Taken together, the results support the hypothesis that PTP1B plays a counter-regulatory role against both PDGF-BB and FGF2-induced cell motility.

Expression of Dominant Negative PTP1B Increases Balloon Injury-Induced Neointima Formation in Rat Carotid Arteries

PDGF and FGF are considered to be the principal growth factors mediating neointima formation in blood vessels injured in relatively robust manner via balloon catheter. On the basis of this finding and the aforementioned observations in cultured cells, we next tested the hypothesis that PTP1B plays a counter-regulatory role in neointima formation. The strategy we implemented for these experiments was the use of dominant negative rather than wild-type PTP1B, based on our earlier experiments indicating that vascular injury increases...
the levels of endogenous PTP1B, theoretically making it more difficult to reveal an effect of overexpressed wild-type PTP1B. Moreover, based on our expectation that dominant negative PTP1B would enhance neointima formation, we subjected rat carotid arteries to a relatively mild degree of injury. As shown in Figure IV (available online at http://atvb.ahajournals.org), treatment with adenovirus expressing dominant negative PTP1B induced significant expression of the mutant protein in medial layers of carotid arteries for at least 14 days, but much less so, if at all, in neointimal or adventitial cells, as determined by Western blot analysis of hemagglutinin (HA)-tagged dominant negative PTP1B. As shown in Figure 4A and 4B, no neointima was evident 3 days after injury; moreover, treatment with dominant negative PTP1B induced a significant increase in neointima formation, both 7 and 14 days after injury.

Expression of Dominant Negative PTP1B Increases Balloon Injury-Induced Cell Proliferation but Fails to Alter Apoptosis

Vascular injury-induced neointima formation is determined in part by the balance between cell proliferation and cell death. It has been reported that vascular injury induces a rapid increase of apoptosis, followed by increased cell proliferation. To determine whether increased neointima formation induced by dominant negative PTP1B occurred via altered cell proliferation and/or apoptosis, we next performed experiments to measure the expression of a protein specifically associated with cell proliferation, namely PCNA, via immunohistochemistry, and apoptosis via the TUNEL method involving staining for DNA fragmentation in injured carotid arteries. As shown in Figure 5, expression of DN-PTP1B markedly increased cell proliferation in neointima and media 7 days after injury and, to a lesser extent in neointima only, 14 days after injury. Although there was a tendency toward increased proliferation 3 days after injury, the difference was not statistically significant. In addition to increased cell proliferation, total cell number in intima but not in media was also increased by expression of DN-PTP1B at 7 and 14 days after injury as shown in Table I (available online at http://atvb.ahajournals.org). However, the levels of apoptosis were not significantly altered at any time point in injured carotid arteries treated with adenovirus expressing DN-PTP1B, compared with arteries treated with adenovirus expressing lac Z (data not shown).

Figure 2. Overexpression of PTP1B blocks PDGF-BB- or FGF2-induced proliferation of cultured rat aortic smooth muscle cells. Cells were infected for 24 hours with "empty" adenovirus (as control, containing no inserted protein) or PTP1B at MOI values of 10 to 15. Virus-containing culture media were removed and cells were cultured for an additional 24 hours to allow for expression of transgenic PTP1B. Cell proliferation was determined by counting the number of BrdU-labeled cells in the microscope view field in response to treatment with PDGF-BB (0.25 ng/mL, A) or FGF2 (1 ng/mL, B) for 24 hours as described in Materials and Methods. Results are mean ± SE of 3 independent experiments. *P < 0.01 compared with control (empty virus); **P < 0.01 compared with PDGF + control virus or FGF2 + control virus (ANOVA and Fisher’s PLSD test).

Figure 3. Expression of dominant negative (DN) PTP1B (C215S-PTP1B) uncovers motility induced by subthreshold levels of PDGF-BB or FGF2 in cultured rat aortic smooth muscle cells. Cells were infected for 24 hours with adenovirus expressing EGFP (as control virus) or dominant negative PTP1B (C215S-PTP1B) at MOI values of 10 to 15. Virus-containing media were removed and cells were cultured for an additional 24 hours to allow for expression of dominant negative PTP1B. Images were taken before and after treatment for 24 hours with PDGF-BB (0.1 ng/mL, A) or FGF2 (20 pg/mL, B), and cell motility was determined via a wounded culture assay as described in Materials and Methods. Results are the mean ± SE of 3 independent experiments. *P < 0.05 compared with control virus, control virus + PDGF, control virus + FGF2, or dominant negative PTP1B.
factor receptor,30 and IGF-1 receptor.15 FGF-induced cell motility and proliferation in vitro and kinases, including the insulin receptor,17 epidermal growth induce dephosphorylation of several other receptor tyrosine targeting the PDGF receptor, PTP1B has been reported to smooth muscle cells and in carotid arteries. In addition to the ubiquitous phosphatase PTP1B in cultured rat aortic gated. Thus, the present study addressed the functional role of counter-regulatory agents have been less extensively investi- the protein tyrosine phosphatases that potentially function as downstream signaling events in the cardiovascular system, mechanisms compensated for the inhibitory effect of PTP1B. In contrast, our data indicate that PTP1B is effective in antagonizing the motogenic and mitogenic effects of PDGF or FGF, suggesting a competitive antagonism of growth factor versus phosphatase activity. Alternatively, the finding that PDGF and FGF2 both increase the levels of endogenous PTP1B in cultured vascular smooth muscle may also explain the relative ineffectiveness of ectopic PTP1B expression, occurring on top of elevated endogenous expression induced by growth factors.

In addition to the above-mentioned findings, indicating that wild-type PTP1B attenuated whereas dominant negative PTP1B increased cultured cell motility and/or proliferation, we observed results consistent with similar effects of PTP1B in injured arteries. Thus, we found that treatment of injured arteries with adenovirus expressing dominant negative PTP1B but not lac Z increased cell proliferation, intimal cell number, and neointima formation without affecting apoptosis. These results support the view that PTP1B decreases cell proliferation and neointima formation in injured carotid arteries. On the basis of our experiments using cultured cells, it is likely that migration of cells from media to neointima may also have been increased by DN-PTP1B, although our in vivo experiments did not directly provide information on this issue. It is interesting to note that adenovirus-mediated expression of PTP1B was mostly confined to the medial layer of arteries, presumably because of dilution of adenovirus after cell division and/or clearance of adenovirus via mechanisms involving the immune system. Given the sequence of events thought to induce neointima formation, which involves migration of cells to the neointima followed by intimal prolif- eration,2 the present results are consistent with the view that expression of dominant negative PTP1B in the vascular media is sufficient to affect relatively late events, such as cell proliferation leading to neointimal enlargement.

Our observations in cultured cells support the notion that PTP1B plays an important role in attenuating growth factor-induced vascular remodeling by inducing dephosphorylation of the PDGF receptor, leading to inhibition of smooth muscle cell proliferation and/or migration. Because of the established enzymatic promiscuity of PTP1B, we do not yet know the identity of all tyrosine kinase receptors that may be targeted in vascular injury. On the basis of the notion that both PDGF and FGF play an important role in mediating neointimal expansion,2 however, it is reasonable to speculate that both PDGF and FGF receptors are prime targets of PTP1B in vivo,
although epidermal growth factor receptor, IGF-1 receptor, and insulin receptor, which were documented to play a role in vascular remodeling, may also be targeted.

We have reported that treatment of cultured cells with PDGF-BB or FGF2, but not IGF-1 or epidermal growth factor, increases the protein levels of PTP1B. In addition, vascular injury is associated with increased levels of PTP1B mRNA and protein. Our previous results, taken together with the current findings, indicate the existence of a feedback mechanism whereby PDGF and FGF2 function is not only mediated by the intrinsic activity of cognate receptor tyrosine kinases but also modulated in negative feedback fashion by PTP1B. Moreover, because PTP1B targets IGF-1, insulin, and PDGF receptors, the present studies raise the possibility of PTP1B-mediated negative cross-talk between PDGF or FGF and IGF-1 or insulin activity. On the basis of these results, we believe that further studies into the details of the vascular effects of PTP1B are warranted.

Acknowledgments

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Online figure I: Representative Western blot, showing overexpression of PTP1B via adenoviral infection in vascular smooth muscle cells.
Online figure II. Overexpression of PTP1B blocks PDGF-induced PDGF receptor phosphorylation at tyrosyl residue 770. Cells, at 80-90% confluence, were incubated with adenovirus encoding EGFP (as control) or wild-type PTP1B (at MOI values of 10-15) for 24 h. Virus-containing media were removed and cells were cultured for an additional 24 hours to allow for expression of ectopic PTP1B. Cells were then treated with 0.5 ng/ml PDGF for 10 min, lysed with RIPA buffer, and immunoprecipitated with anti-goat-IgG (as control) or anti-PDGFRβ. Phospho-PDGFRβ levels were measured by probing with antibody directed against phosphoryosyl residue 770 in PDGFRβ. Total PDGFRβ protein levels were determined by reprobing blots with anti-PDGFRβ. Panel A. Uppermost blot shows levels of PTP1B. Middle blot shows levels of phospho-PDGFR. Lower blot shows levels of total PDGFR. Panel B, summary of three independent experiments. PDGFR phosphotyrosyl levels were normalized to total protein levels. Results are mean+SE from 3 independent experiments.
Online figure III. Representative Western blot, showing expression of dominant negative, HA-tagged, PTP1B in vascular smooth muscle cells.
Online figure IV. Representative Western blot, showing expression of dominant negative PTP1B via adenoviral infection, using antibody directed against HA.

Adenoviruses expressing Lac Z or HA-tagged C215S-PTP1B were diluted in 15% poloxamer 407 containing 3% sucrose/PBS and incubated in carotid arteries for 30 min after balloon catheter-induced injury. Carotid arteries were removed 3, 7 or 14 days after injury. Vessel walls were dissected into neointima (injured), media and adventitia (control and injured). Dominant negative PTP1B expression was determined by Western blot analysis using antibody directed against HA. Panel A, 3 days after injury. Panel B, 7 days after injury. Panel C, 14 days after injury.
Table. Effect of expression DN-PTP1B on cell number in medial and neointimal layers after injury.

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Results represent total cell number in a microscopic view field at x400 magnification. Data are expressed as mean ± SE. *p<0.05; **p<0.01 compared with lac Z.
ONLINE MATERIALS AND METHODS

Materials. Adult male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA). Rat pups were purchased from Charles River Laboratories, or they were bred in the University of Tennessee vivarium. Rats were maintained at constant humidity (30-70%), temperature (70°F) and light cycle (6 am to 6 pm) and were fed standard rat chow (Harlan, IN). Cell culture medium (DMEM/F12, 1:1) was from GIBCO-BRL (Grand Island, NY). Fetal bovine serum was obtained from CellGro (Herndon, VA). Porcine pancreatic elastase was purchased from Calbiochem (San Diego, CA). Monoclonal antibody against PTP1B was from Oncogene (San Diego, CA). Polyclonal antibody against PDGF receptor β and phospho-specific anti-PDGF receptor antibody (Tyr770) were from Santa Cruz Biotechnology (Santa Cruz, CA). Protein G-Sepharose beads were from Amersham Pharmacia Biotechnology (Piscataway, NJ). Monoclonal antibody against hemagglutinin antigen (HA) was obtained from Covance (Berkeley, CA). Antibody against proliferating cell nuclear antigen (PCNA) was from Lab Vision (Fremont, CA). ABC immunostaining kit and alkaline phosphatase substrate kit were from Vector Laboratory (Burlingame, CA). In Situ Cell Death Detection and In Situ Cell Proliferation Kits, were from Roche (Indianapolis, IN). All other reagents were of the highest quality available and were purchased from Sigma (St. Louis, MO). Replication-deficient (E-1-deleted) recombinant type 5 adenoviruses expressing enhanced green fluorescent protein (EGFP), viral proteins only (“empty virus”), wild-type PTP1B or dominant negative PTP1B (C215S-PTP1B) were prepared as described in a previous publication from our laboratory.¹
**Measurement of cell motility.** Briefly, confluent cells were infected for 24 h with adenovirus expressing EGFP (as control), wild type PTP1B or dominant negative (DN) PTP1B (C215S-PTP1B) at multiplicity of infection values of 10-15 in DMEM/F12 medium. Following change of culture medium, cells were incubated for an additional 24 h, to provide time for virus-encoded protein expression. Cell movement was measured via a wounded-culture assay by using NIH v.1.62 Image software. Experiments of cell motility were performed in the presence of 5 mmol/L hydroxyurea to prevent cell proliferation, as described previously. Motility was expressed as distance migrated during a 24 h period.

**Measurement of cell proliferation.** Briefly, cultures at 50-60% confluence were infected for 24 h with adenovirus expressing adenoviral proteins only (as control) or wild type PTP1B, at multiplicity of infection values of 10-15 in DMEM/F12 medium. Following virus removal, cells were incubated for an additional 24 h to allow for virus-encoded protein expression, followed by treatment with PDGF-BB or FGF2 for 24 h. Cell proliferation was measured by labeling with BrdU and probing with anti-BrdU conjugated with fluorescein, according to the kit manufacturer’s instructions. Images were obtained via the MetaMorph software (Universal Imaging, West Chester, PA) by using a Nikon microscope equipped with an FITC filter (excitation wavelength 488 nm, emission wavelength 515-540 nm) and a Coolsnap digital camera (Photometrics, Tucson, AZ). BrdU-labeled cells were counted by using NIH image software.

**Measurement of PDGF receptor tyrosine phosphorylation.** Cells, at 80-90 % confluence, were infected for 24 h with adenovirus expressing EGFP (as control) or wild type
PTP1B, at multiplicity of infection values of 10-15 in DMEM/F12 medium. Following removal of virus-containing culture media, cells were incubated for an additional 24 h to allow for virus-encoded protein expression, followed by treatment with PDGF (0.5 ng/ml) for 10 min. Cells were lysed with RIPA buffer (150 mmol/L NaCl, 1% sodium deoxycholate, 1% SDS, 1% Triton X-100, 50 mmol/L Tris, pH 7.2) containing 2 mmol/L sodium vanadate, 1 mmol/L PMSF, 5 µg/ml aprotinin, 1 µg/ml pepstatin, and 5 µg/ml leupeptin. PDGF receptor tyrosine phosphorylation was determined by immunoprecipitation with antibody directed against the PDGFβ receptor, followed by Western blot analysis using phospho-specific antibodies directed against phosphotyrosyl residue 770 in the PDGFβ receptor. We used immunoprecipitation instead of direct Western blotting because direct Western blotting with phospho-specific antibody against tyrosine phosphorylated-PDGF receptor was insufficiently sensitive to detect PTP1B-induced reduction of tyrosine phosphorylation levels in a small fraction of phosphotyrosyl residues. After blotting for Y770 phosphotyrosyl levels, blots were stripped and reprobed with antibody directed against PDGF receptor protein. Band densities were measured using NIH Image software.

*Rat carotid artery injury model.* Male Sprague-Dawley rats, weighing 300 to 350 g, were anesthetized via an intraperitoneal injection of xylazine (5 mg/kg) plus ketamine (60 mg/kg). Right carotid artery injury was induced using a 2-F Fogarty catheter (from Baxter, Irvine, CA) via a rotating motion toward the arteriotomy 3× to gently denude the endothelium. The left carotid artery was used as uninjured control. Following balloon injury, the artery was flushed with saline and a 50 µl solution of 15% poloxamer 407 in 3% sucrose/PBS containing adenovirus expressing lac Z or dominant negative PTP1B
(C215S-PTP1B; at a final concentration of $10^{10}$ pfu/ml) was instilled into the injured area. The virus solutions were kept in the arteries for 30 min, followed by removal via aspiration and closure of the surgical incision. Animals were sacrificed 3, 7 or 14 days after injury. For Western blot analysis of ectopic protein expression, carotid arteries were homogenized in buffer containing 50 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, 1 mmol/L EDTA, 0.1% Nonidet P-40, 0.25% Na-deoxycholate, 0.1% SDS, 2 mmol/L sodium orthovanadate, 1 mmol/L sodium fluoride, 2 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin, and 50 µg/ml PMSF. Immunoblotting was performed according to a published protocol. All experimental protocols were approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center, in accordance with the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, NIH publication No. 86-23).

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