Shear Stress Stimulation of p130\textsuperscript{Cas} Tyrosine Phosphorylation Requires Calcium-dependent c-Src Activation\* (Received for publication, January 25, 1999, and in revised form, June 21, 1999) 

Masanori Okuda‡‡§§, Masafumi Takahashi‡‡§§, James Suero‡, Charles E. Murry¶¶, Oren Traub‡, Hisaaki Kawakatsu***, and Bradford C. Berk‡‡ §§

From the §Department of Medicine, Cardiology Division and the ¶Department of Pathology, University of Washington, Seattle, Washington 98195, the **Lung Biology Center, University of San Francisco, San Francisco, California 94110, and the ¶¶Center for Cardiovascular Research, University of Rochester, Rochester, New York 14642

Fluid shear stress (flow) modulates endothelial cell function via specific intracellular signaling events. Previously we showed that flow activated ERK1/2 in an integrin-dependent manner (Takahashi, M., and Berk, B. C. (1996) J. Clin. Invest. 98, 2623–2631). p130 Crk-associated substrate (Cas), a putative c-Src substrate, was originally identified as a highly phosphorylated protein that is localized to focal adhesions and acts as an adapter protein. Recent reports have shown that Cas is important in cardiovascular development and actin filament assembly. Flow (shear stress = 12 dynes/cm\(^2\)) stimulated Cas tyrosine phosphorylation within 1 min in human umbilical vein endothelial cells. Phosphorylation peaked at 5 min (3.5 \pm 0.7-fold) and was sustained to 20 min. Tyrosine phosphorylation of Cas was functionally important because flow stimulated association of Cas with Crk in a time- and force-dependent manner. Flow-mediated activation of c-Src, phosphorylation of Cas, and association of Cas with Crk were all inhibited by calcium chelation and pretreatment with the Src family-specific tyrosine kinase inhibitor PPI. To determine the role of c-Src in flow-stimulated phosphorylation of Cas, we transduced cells with adeno-virus encoding kinase-inactive Src. Expression of kinase-inactive Src prevented flow-induced Cas tyrosine phosphorylation but not ERK1/2 activation. Calcium-dependent activation of c-Src and tyrosine phosphorylation of Cas defines a new flow-stimulated signal pathway, different from ERK1/2 activation. This pathway may be involved in focal adhesion remodeling and actin filament assembly.

By virtue of their unique anatomical location in the vascular wall, vascular endothelial cells (ECs)\(^1\) are exposed to the mechanical forces associated with blood flow. Recent data show that fluid shear stress (flow) modulates vascular structure and function and plays an important role in the pathogenesis of vascular diseases, including atherosclerosis, hypertension, and restenosis (1). Steady laminar flow affects EC gene expression with increased mRNA levels for platelet-derived growth factor (2), monocyte chemoattractant protein-1 (3, 4), endotelin-1 (5), intercellular adhesion molecule-1 (6), vascular Mothers Against Decapenta-plegia (7), and lectin-like oxidized low density lipoprotein receptor (8) and with decreased mRNA levels for angiotensin-converting enzyme (9) and vascular cell adhesion molecule-1 (10). Among many signal molecules that are activated by flow, members of the mitogen-activated protein kinase family, including the p44 and p42 extracellular signal-regulated kinases (ERK1/2) (2, 3, 5, 11), c-Jun NH\(_2\)-terminal kinase (12), and big mitogen-activated protein kinase 1/ERK5 (13, 14) are likely to be important for changes in EC gene expression. However, the proximal mechanisms for flow-mediated signal transduction remain unknown.

Previously we showed that flow activated ERK1/2 in an integrin-dependent manner (30). However, neither focal adhesion kinase nor paxillin were rapidly phosphorylated by flow, suggesting that other focal adhesion proteins were involved. p130 Crk-associated substrate (Cas) is a potential adapter protein for integrin-mediated cell adhesion that has an SH3 domain followed by multiple SH2 binding motifs in the substrate domain. This protein localizes to focal adhesions and associates not only with focal adhesion proteins such as focal adhesion kinase, paxillin, and tensin (15, 16) but also with other signal molecules such as Crk (17, 18), Nck (19), and protein-tyrosine phosphatase 1B (20). Cas was originally identified as a major tyrosine-phosphorylated protein in v-Crk and v-Src-transformed cells. Cas is also tyrosine-phosphorylated during cell adhesion (21) and after stimulation by hormones such as angiotensin II (22, 23), bombesin, lysophosphatidic acid, platelet-derived growth factor, phorbol esters, vasopressin, endothelin, bradykinin (24), and epidermal growth factor (25). Recently, Cas has been shown to be essential for cell migration (26, 27) and actin filament reorganization (28, 29).

In a previous report, we showed that c-Src was activated within 2 min by shear stress in HUVEC (30). We therefore hypothesized that flow would stimulate c-Src-dependent tyrosine phosphorylation of Cas based on several findings. 1) Cas is a major tyrosine-phosphorylated protein in cells transformed by v-Src (31). 2) Cas is tyrosine-phosphorylated upon cell adhesion in a Src-dependent manner (19). 3) Tyrosine phosphorylation of Cas is decreased in Src\(^-\) mouse fibroblasts and Rous sarcoma virus; SH, Src homology region; HBSS, HEPES-buffered saline solution.

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\§§ These authors contributed equally to this study.

† Present address: Dept. of Cardiology, Jichi Medical School, Tochigi 329-04, Japan.

§§ To whom correspondence should be addressed: Center for Cardiovascular Research, Box 679, 601 Elmwood Ave., University of Rochester School of Medicine and Dentistry, Rochester, NY 14642 Tel.: 716-273-1946; Fax: 716-473-1573; E-mail: bradford_berk@urmc.rochester.edu.

\# The abbreviations used are: EC, endothelial cell; BAEC, bovine aortic endothelial cells; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N, N',N''-tetraacetic acid/acetoxymethyl ester; Crk, CT-10 regulated kinase; Cas, Crk-associated substrate; Csk, C-terminal Src kinase; ERK1/2, extracellular signal-regulated kinases 1 and 2; HUVEC, human umbilical vein endothelial cells; Ki-Src, kinase-inactive Src; m.o.i., multiplicity of infection; PYR2, proline-rich tyrosine kinase 2; RSV, Rous sarcoma virus; SH, Src homology region; HBSS, HEPES-buffered saline solution.
increased in Csk−/− cells in parallel with Src activity (17). In this study, we show that shear stress stimulates tyrosine phosphorylation of Cas and association of Cas with Crk in ECs. Further, our results show that calcium-dependent activation of c-Src is required for the shear stress-induced Cas tyrosine phosphorylation.

EXPERIMENTAL PROCEDURES

Cell Culture and Materials—HUVEC were obtained as described previously (32). HUVEC at passages between 2 and 3 were grown to confluence in RPMI 1640 supplemented with 20% fetal bovine serum, heparin, and endothelial cell growth factor. Bovine aortic ECs (BAEC) were isolated from adult aortas, maintained in M199 supplemented with 10% fetal calf serum as described previously (33), and used at passages between 2 and 8. A23187 and a- thrombin were obtained from Sigma. 1,2-bis(2-aminophenoxy)ethane-N,N',N'-tetraacetic acid/acetoxymethyl ester (BAPTA-AM) and herbimycin A were purchased from Calbiochem. PPI (CP-118, 556) was from Pfizer. Monoclonal anti-Crk antibody and polyclonal anti-mouse IgG antibody were obtained from Transduction Laboratory. Monoclonal anti-phosphotyrosine antibody (4G10) and monoclonal anti-Src antibody (GD11) were purchased from Upstate Biotechnology Inc. Monoclonal anti-v-Src antibody (OP-97) was obtained from Oncogene Research Products. Monoclonal antibody specific for the active form of c-Src (clone 28) was generated as described previously (34). Polyclonal anti-phospho-specific ERK1/2 antibody was obtained from New England Biolabs. Polyclonal anti-Cas antibody was purchased from Santa Cruz. Protein A-agarose, cell culture media, and heparin were obtained from Life Technologies, Inc. cDNA for a chicken c-Src that is kinase-inactive (Lys-295 to Met) and acts as a dominant negative Src (KI-Src) was a kind gift of Dr. Sara A. Courtenidge (Sugen Inc., Redwood City, CA (35)).

Shear Stress Protocol—HUVEC or BAEC grown to confluence on gelatin-coated 60- or 100-mm tissue culture dishes were serum-deprived for 3–6 h. Prior to the experiment, cells were rinsed free of culture media with HEPES-buffered saline solution (HBSS; 130 mM NaCl, 5 mM KCl, 1.5 mM CaCl2, 50 mM HEPES, pH 7.4) with 10 mM glucose and either maintained in static condition or exposed to fluid shear stress in a cone and plate viscometer at 37 °C as described previously (36). A polycrystalline resin (Delrin) cone was milled with precise angle measurements and attached to a stainless steel shaft that was in turn attached to a BC215GD-AF model motor and 2GD10K gear head purchased from Oriental Motor Co. The motor was wired to an external controller (Model BLD15-AF) with adjustable potentiometer. A step angle measurements and attached to a stainless steel shaft that was in turn attached to a BC215GD-AF model motor and 2GD10K gear head purchased from Oriental Motor Co. The motor was wired to an external controller (Model BLD15-AF) with adjustable potentiometer. A step down transformer (120 V to 100 V; Sanyo Model TSD-N0GU) was placed in an air incubator (Robbins Scientific hybridization chamber, Model 1000) at 37 °C for experiments.

Immunoprecipitation and Western Blotting of Proteins—After treatment, cells were washed with cold phosphate-buffered saline and lysed in buffer A (25 mM Tris-HCl, pH 7.4, 50 mM NaF, 10 mM Na2PO4, 137 mM NaCl, 1% Triton X-100, 10% glycerol and fresh 2 mM benzamidine, 1 mM Na3VO4, and 10 μg/ml leupeptin). Buffer B (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100 and fresh 2 mM benzamidine, 1 mM Na3VO4, and 10 μg/ml leupeptin) was used for coimmunoprecipitation experiments. Cell lysates were prepared by scraping and centrifugation for 10 min at 14,000 rpm in a microcentrifuge at 4 °C. Prior to the experiment, cells were rinsed free of culture media with HEPES-buffered saline solution (HBSS; 130 mM NaCl, 5 mM KCl, 1.5 mM CaCl2, 50 mM HEPES, pH 7.4) with 10 mM glucose and either maintained in static condition or exposed to fluid shear stress in a cone and plate viscometer at 37 °C as described previously (36). A polycrystalline resin (Delrin) cone was milled with precise angle measurements and attached to a stainless steel shaft that was in turn attached to a BC215GD-AF model motor and 2GD10K gear head purchased from Oriental Motor Co. The motor was wired to an external controller (Model BLD15-AF) with adjustable potentiometer. A step down transformer (120 V to 100 V; Sanyo Model TSD-N0GU) was added, and the motor and gear head were placed on an adjustable platform so that the cone could be lowered onto the base containing the cell culture dish. Adjustable set screws were used to position the cone at a reproducible height related to the cell culture dish. The apparatus (not illustrated) in pJM17. The expression cassette (pAd1.RSV), derived from a parent vector, pXCLL.1, contains 16% of the adenoviral genome cloned into pBR322. The start codon (ATG) of the KI-Src gene is indicated. B, HUVEC transfection efficiency with Ad.LacZ. Cells were seeded on gelatin-coated 60-mm tissue culture dishes. Upon reaching 75–85% confluence, cells were infected with the indicated concentrations of Ad.LacZ for 1 h at 37 °C and then incubated with 5 ml of RPMI 1640 supplemented with 20% fetal bovine serum for the indicated times. Transfection efficiency was determined by 5-bromo-4-chloro-3-indolyl-β-D-galactoside staining in 4% paraformaldehyde-fixed monolayer. C, cells at 75–85% confluence were infected with the indicated concentrations of Ad.KI-Src or Ad.LacZ and incubated for 48 h. Cell lysates were prepared and analyzed by Western blot with anti-c-Src antibody.

Src Kinase Assay—Cell lysates (1.5–1.7 mg of protein) were incubated with antibody (4 μg) against v-Src (OP-07) and immunoprecipitated as described above. Src activity was measured with a Src kinase assay kit (Upstate Biotechnology Inc.). Briefly, 20 μl of kinase buffer (100 mM Tris-HCl, pH 7.2, 125 mM MgCl2, 2 mM EGTA, 0.25 mM sodium orthovanadate, 2 mM dithiothreitol, and 0.5 μg/ml of a specific substrate peptide KVERIGETTGVVVK) and 10 μl of [γ-32P]ATP diluted to 1 μCi/μl with Mn/ATP mixture (75 mM MnCl2, 500 μM ATP) were added to 5 μl of the washed beads. The reaction mixture was incubated for 10 min at 30 °C and then the reaction was stopped by adding 20 μl of 40% trichloroacetic acid. The phosphorylated substrate was then separated from the residual [γ-32P]ATP using P81 phosphocellulose paper and quantified with a scintillation counter.

Adeno viral Construction, Preparation, and Transfection (Fig. 1)—cDNA for a kinase inactive form of chicken c-Src (KI-Src gene) was cloned into the expression vector pAd1.RSV according to the general protocol of Graham and Prevec (37). This vector, derived from the parent vector pXCLL.1, contains 16% of the adenoviral genome cloned into pBR322. The critical E1A region has been deleted and replaced by the Rous sarcoma virus (RSV) long terminal repeat, a multiple cloning site, and the bovine growth hormone polyadenylation sequence. KI-Src-containing pAd1.RSV was cotransfected using Lipofectamine into 293
cells with pJM17, a second plasmid containing the majority of the adenoviral genome (38). After homologous recombination in vivo, plaques resulting from viral cytopathic infection were selected and expanded in 293 cells. 293 cells have been transformed with adenoviral E1A and therefore provide this viral transcription factor in trans. The KI-Src adenovirus (Ad.KI-Src) and a control adenovirus (Ad.LacZ) encoding nuclear-targeted β-galactosidase (39) were propagated in 293 cells as described (37). The virus preparation was purified and concentrated from cell lysates by ultracentrifugation in a CsCl gradient followed by dialysis. Viral titer was determined by optical density at 260 nm and by plaque formation assay using 293 cells and was expressed as plaque-forming units. To optimize the protocol for HUVEC transfection, HUVEC were transfected with Ad.LacZ, and expression was measured by β-galactosidase (40). The frequency of transfection increased linearly with increasing virus concentrations of Ad.LacZ (Fig. 1B). There was no significant change in Cas protein levels during these experiments (Fig. 2A, lower).

**RESULTS**

**Time-dependent Phosphorylation of Cas by Shear Stress in HUVEC**—To gain insight into the role of c-Src in shear stress-mediated signal events, we studied tyrosine phosphorylation of Cas. HUVEC were exposed to flow (shear stress = 12 dynes/cm²) for varying times and harvested for analysis of Cas phosphorylation. Tyrosine phosphorylation of Cas occurred within 1 min, peaked at 5 min (3.5-fold increase), was sustained for 20 min (Fig. 2, A, upper and C), and returned to near baseline by 120 min after stimulation (not shown). There was no significant change in Cas protein levels during these experiments (Fig. 2A, lower).

**Shear Stress-induced Cas Tyrosine Phosphorylation Is Dependent on Calcium in HUVEC**—We (41, 42) and other groups (43, 44) have shown that shear stress stimulates a rapid increase in EC intracellular calcium that is dependent on the magnitude of shear stress. Therefore, we studied the dependence of flow-stimulated Cas phosphorylation on changes in intracellular calcium. Chelation of intracellular calcium with 50 μM BAPTA-AM in the presence of 10 μM EGTA abolished...
flow-induced Cas tyrosine phosphorylation (Fig. 3). Increasing intracellular calcium by treating HUVEC with 10 μM A23187 for 10 min stimulated Cas tyrosine phosphorylation 3.7 ± 0.5-fold. Stimulation by A23187 was completely inhibited by calcium chelation. Finally, α-thrombin (3 units/ml), which also elevates EC intracellular calcium, increased Cas tyrosine phosphorylation 3.1 ± 0.5-fold, and α-thrombin stimulation was completely inhibited by calcium chelation.

Shear Stress-Induced Src Activation Is Calcium-dependent in HUVEC—Previously, we showed that shear stress increased c-Src activity measured by phosphorylation of enolase in HUVEC (30). Therefore we examined whether the shear stress-induced activation of c-Src was inhibited by calcium chelation. The activity of c-Src was analyzed by Western blot (Fig. 4A) and by Src kinase activity (Fig. 4B). Western blot analysis with an antibody that selectively recognizes the active form of c-Src (34) showed a rapid increase in activity (within 0.5 min), which peaked at 2 min (1.8 ± 0.2-fold) and was sustained for 10 min after stimulation (Fig. 4A, left), consistent with previous results (30). Shear stress-induced c-Src activation was completely abolished by chelation of intracellular calcium (Fig. 4, A, right and B). Treatment with a Src family-specific tyrosine kinase inhibitor, PP1 (50 μM), also inhibited shear stress-mediated c-Src activation (Fig. 4B).

Effect of Tyrosine Kinase Inhibitors, Herbimycin A and PP1, on Shear Stress-Induced Phosphorylation of Cas and ERK1/2 in HUVEC—To characterize the tyrosine kinase responsible for Cas phosphorylation by shear stress, we utilized the tyrosine kinase inhibitors, herbimycin A and PP1. PP1 (CP-118, 556), a pyrazolopyrimidine, is a Src kinase family inhibitor (45). Treatment with PP1 inhibited shear stress-mediated Cas tyrosine phosphorylation in a concentration-dependent manner (10 μM, 31.3 ± 14.6%; 50 μM, 83.6 ± 6.8%, Fig. 5A). PP1 did not inhibit ERK1/2 activation significantly (15.6 ± 11.9% inhibition, Fig. 5B). Herbimycin A, a benzoquinone ansamycin antibiotic, inhibits Src family kinases by covalent interactions with sulfhydryl groups (46) and by disrupting Src interactions with heat shock proteins (especially HSP90) (47). Treatment with 1 μM herbimycin A significantly inhibited shear stress-mediated Cas tyrosine phosphorylation (88.0 ± 6.0%, Fig. 5C). In contrast to PP1, treatment with herbimycin A significantly inhibited ERK1/2 activation by shear stress (80% ± 5.9%, Fig. 5D). These
results strongly suggested that Src family tyrosine kinases play an important role in shear stress-induced Cas tyrosine phosphorylation. ERK1/2 activation is dependent on tyrosine kinase activation, but the requirement for Src kinases is unclear.

**KI-Src Overexpression Inhibits Shear Stress-induced Tyrosine Phosphorylation of Cas in HUVEC**—To evaluate the role of c-Src activity in flow-induced Cas and ERK1/2 phosphorylation more specifically, KI-Src was overexpressed in HUVEC. Flow (shear stress = 12 dynes/cm² for 10 min) increased tyrosine phosphorylation of Cas by 3.1 ± 1.0-fold in uninfected cells (Fig. 6A). Expression of β-galactosidase with Ad.LacZ had no significant effect on either basal levels of Cas phosphorylation or shear stress-increased Cas phosphorylation at 1000 m.o.i. (Fig. 6, A and B). Expression of KI-Src inhibited Cas tyrosine phosphorylation in a concentration-dependent manner (Fig. 6). There was a significant decrease in the basal level of Cas phosphorylation to 17 ± 3% control, similar to treatment with 1 μM herbimycin A. There was also complete inhibition of shear stress-stimulated Cas tyrosine phosphorylation at concentrations of Ad.KI-Src ≥ 500 m.o.i. (Fig. 6).

**KI-Src Overexpression Does Not Inhibit Shear Stress-induced ERK1/2 Activation in HUVEC**—To determine whether c-Src activity is required for flow-induced ERK1/2 activation, we examined the effect of KI-Src overexpression on ERK1/2 phosphorylation by shear stress. Flow (shear stress = 12 dynes/cm² for 10 min) increased ERK1/2 phosphorylation by 7.0 ± 1.2-fold (Fig. 7A). Treatment with concentrations of Ad.KI-Src (300 or 1000 m.o.i.) that inhibited Cas phosphorylation had no significant effect on flow-stimulated ERK1/2 phosphorylation (Fig. 7, A and B). Overexpression of β-galactosidase also had no effect on flow-stimulated ERK1/2 phosphorylation. There was a small increase in basal levels of ERK1/2 phosphorylation 48 h after infection with Ad.KI-Src and Ad.LacZ.

**Association between Cas and Crk in ECs Stimulated by Shear Stress in BAEC**—To gain insight into the functional significance of Cas phosphorylation, we determined whether Cas associates with Crk in response to shear stress. BAEC were exposed to either 6 or 12 dynes/cm² for various times (Fig. 8). Lysates were immunoprecipitated with anti-Cas antibody and immunoblotted with anti-Cas antibody or anti-phosphotyrosine antibody. Immunoprecipitation of Crk after exposure of BAEC to shear stress showed a significant increase in co-immunoprecipitated Cas (Fig. 8, A and B) that was dependent on shear stress (Fig. 8C), increased at 12 (lane 3) compared with 6 (lane 2) dynes/cm² and was maximal at 10 min (Fig. 8, A and B). The magnitude of this effect was similar to that observed with 10 units/ml α-thrombin (not shown). The interaction of Crk and Cas was dependent on both Src kinases (inhibition by 25 and 50 μM PP1 and calcium (inhibition by BAPTA-AM + EGTA, Fig. 8C). We also determined whether Sos interacted with Cas as reported by other investigators (17). However, we were unable to detect association of these proteins by immunoprecipitation of either Cas or Sos (not shown) suggesting that shear stress does not promote an interaction between Cas and Sos.

**DISCUSSION**

The major findings of this study are that shear stress rapidly stimulates Cas tyrosine phosphorylation and association of Cas with Crk via a pathway dependent on intracellular calcium and c-Src activity. The conclusion that Cas tyrosine phosphorylation requires c-Src is supported by three experimental results. First, shear stress-induced Cas tyrosine phosphorylation was completely inhibited by adenoviral-mediated gene transfer of KI-Src. Second, shear stress-induced Cas tyrosine phosphorylation was prevented by pretreatment with the Src family tyrosine kinase inhibitors PP1 and herbimycin A. Third, the association between Cas and Crk stimulated by shear stress was inhibited by treatment with PP1.

The two kinases best identified as Cas tyrosine kinases are c-Src (17, 19, 31) and proline-rich tyrosine kinase-2 (PYK2) (48, 49). An important role for c-Src in Cas tyrosine phosphorylation is based on the findings that 1) Cas is significantly tyrosine-phosphorylated in cells transformed by v-Src (31); 2) Cas...
is tyrosine-phosphorylated upon cell adhesion in a Src-dependent manner (19); 3) tyrosine phosphorylation of Cas is decreased in Src−/− mouse fibroblasts and increased in Csk−/− cells in parallel with Src activity (17); and 4) focal adhesion kinase-induced Cas phosphorylation is dependent on the Src-binding site (Tyr-397) of focal adhesion kinase (17). In this study, we demonstrated that c-Src activity is required for shear stress-induced Cas tyrosine phosphorylation. A cooperative interaction between PYK2 and c-Src has been shown to be required for Cas tyrosine phosphorylation in COS cells (48). Therefore, PYK2 may be important in shear stress-mediated Cas tyrosine phosphorylation. In fact, we have found that PYK2 is present in BAEC and is tyrosine-phosphorylated in response to shear stress.2 It has also been shown that PYK2 is involved in ERK1/2 activation by G-protein-coupled receptors (50, 51). Future studies will be required to elucidate the role of PYK2 in shear stress signaling.

In support of a functional role for the c-Src/Cas pathway in shear stress signaling, we demonstrated the association of Cas with Crk in ECs. Crk is an adapter protein composed of SH2 and SH3 domains and has been shown to interact with several signaling molecules, including two GTP exchange factors (Sos and C3G) (52). The interaction of Crk and C3G may regulate focal adhesion rearrangement and cell morphology in response to flow through the small G proteins. Recently, Altun-Gultekin et al. (53) showed that v-Crk activated the Rho signaling pathway and served as a scaffolding protein during the assembly of focal adhesions in PC12 cells. Other recent studies support an important role for Cas in the cardiovascular system. Honda et al. (29) reported that the Cas knockout mouse caused embryonic death because of marked systemic congestion and growth retardation. The heart was poorly developed, and blood vessels were prominently dilated in these mice. The findings of the present study and those of Honda et al. (29) suggest that Cas tyrosine phosphorylation may be important in endothelial responses to flow such as alignment and stress fiber formation (54).

The present study clearly demonstrates the merits of adenoviral-mediated gene transfer to study signal transduction. A variety of nonviral approaches have been used for transfer including liposomes, molecular conjugates, and electroporation. Compared with other vectors, transfection efficiency of adenovirus vector is much higher in both growing and quiescent cells. Whereas it has been difficult to transfect genes into ECs with high efficiency by nonviral approaches, transfection of HUVEC with Ad.LacZ showed 100% efficiency assessed by β-galactosidase assay. Most important, dominant negative gene transfection with adenovirus appears to be a more specific way to inhibit the function of targeted kinases than pharmacologic kinase inhibitors. Data that support a specific mechanism of inhibition for KI-Src include the concentration dependence, ability to use relatively low m.o.i. to achieve inhibition, and specificity as shown by inhibition of Cas phosphorylation but not ERK1/2 activation. The results for ERK1/2 with both KI-Src and PP1 differ from those reported by Jalali et al. (55) who found that flow-induced ERK activation required Src. This difference may be because of the greater specificity of adenoviral dominant negative Src or to cell culture differences.
Flow-induced, Src-dependent p130cas Phosphorylation

In summary, the present study is the first to demonstrate that shear stress stimulates Cas tyrosine phosphorylation through calcium-dependent activation of c-Src in ECs (Fig. 9). This is a novel shear stress-mediated pathway different from ERK1/2 activation. Specifically, Cas tyrosine phosphorylation is not required for ERK1/2 activation by shear stress because Ki-Src overexpression inhibited Cas tyrosine phosphorylation but not ERK1/2 activation. We suggest that the Src/Cas pathway described here is likely to be functionally important in shear stress-mediated EC functions such as regulation of cytoskeleton and cell movement.

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