Mechanotransduction in Response to Shear Stress

ROLES OF RECEPTOR TYROSINE KINASES, INTEGRINS, AND Shc*

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Shear stress, the tangential component of hemodynamic forces, activates many signal transduction pathways in vascular endothelial cells. The conversion of mechanical stimulation into chemical signals is still unclear. We report here that shear stress (12 dynes/cm²) induced a rapid and transient tyrosine phosphorylation of Flk-1 and its concomitant association with the adaptor protein Shc; these are accompanied by a concurrent clustering of Flk-1, as demonstrated by confocal microscopy. Our results also show that shear stress induced an association of α,β3 and β1 integrins with Shc, and an attendant association of Shc with Grb2. These associations are sustained, in contrast to the transient Flk-1-Shc association in response to shear stress and the transient association between α,β3 integrin and Shc caused by cell attachment to substratum. Shc-SH2, an expression plasmid encoding the SH2 domain of Shc, attenuated shear stress activation of extracellular signal-regulated kinases and c-Jun N-terminal kinases, and the gene transcription mediated by the activator protein-1/12-O-tetradecanoylphorbol-13-acetate-responsive element complex. Our results indicate that receptor tyrosine kinases and integrins can serve as mechanosensors to transduce mechanical stimuli into chemical signals via their association with Shc.

Cells in the cardiovascular system are exposed to hemodynamic forces as well as chemical factors. Shear stress, the tangential component of hemodynamic forces, acts mainly on vascular endothelial cells (ECs),¹ whereas circumferential stress is borne primarily by vascular smooth muscle cells. The mechanotransduction processes by which these vascular cells convert mechanical stimuli into biochemical signals have gained increasing attention. Several laboratories, including ours, have performed in vitro experiments using flow channels to study the responses of ECs to applied shear stress (see Refs. 1–4 for review). Mitogen-activated protein kinases, including extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), also known as stress-activated protein kinase, are rapidly activated by shear stress (5–7). This results in the transcriptional activation of immediate early genes such as those encoding monocyte chemotactic protein-1 (MCP-1) and c-Fos (8–10). On the upstream side, the shear stress activation of ERK and JNK is modulated by Ras, which in turn is regulated by Son of sevenless (Sos), a guanine nucleotide exchange factor, as evidenced by the findings that negative mutants of Ras and Sos can block the shear stress induction of ERK and JNK (6, 7).

Conceptually, shear stress acts on the EC membrane to activate putative shear stress sensors or receptors which then lead to the activation of the Sos-Ras pathway. To date, several mechanisms of mechanotransduction involving the EC membrane have been suggested. Shear stress activates the seven-span-receptor-coupled G-protein (11), ion channels such as K⁺ channel (12), and the transforming growth factor-β receptor-related Smad6 and Smad7 (13). Several recent studies showed that tyrosine kinases, i.e. focal adhesion kinase (FAK) and c-Src in the focal adhesion site constitute a part of the mechanotransduction in ECs in response to shear stress (10, 14, 15). FAK, by forming a complex with growth factor receptor-binding protein 2 (Grb2), regulates the shear stress induction of ERK and JNK (15). The involvement of these signaling molecules in the focal adhesion sites may be correlated with the dynamic reorientation of focal adhesions in ECs under shear stress (16). Considering the multiplicity of the signaling molecules engaged in the EC responses to shear stress, there is a missing link to integrate the various pathways into an unified theme.

She is an adaptor protein containing a C-terminal Src homology domain-2 (SH2) domain and a central glycine/proline-rich sequence (17). In response to many growth factors such as platelet-derived growth factor and epidermal growth factor (EGF), Shc is tyrosine-phosphorylated and associates with phosphotyrosines of the cognate receptor tyrosine kinases (RTK) through SH2 binding (17–20). Tyrosine-phosphorylated Shc also associates with Grb2 through SH2 interaction (21, 22). The assembly of Shc-Grb2-Sos provides an alternative mechanism in addition to the Grb2-Sos pathway for the activation of Ras. Recently, it has been shown that Shc is involved in the integrin-mediated signal transduction. In A431 cells, Shc is recruited to α1β1, α6β1, and α5β1 when these integrins have been conjugated to their corresponding antibodies (23). In the same study, it was also shown that Shc is necessary and suf-
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Sufficient for the activation of ERK in response to integrin ligation. These results suggest that both growth factors and integrins can regulate the ERK pathway via Shc.

In the current study, we show for the first time that fetal liver kinase 1 (Flik-1), an RTK specific for vascular endothelial growth factor (VEGF), and integrins (αβ3, β1, and β2 integrins) can both function as mechanosensors in ECs, and that shear stress causes both to be associated with Shc. The interaction of Shc with Flik-1 is rapid and transient, whereas its association with the various integrin is sustained. These findings provide new insights into the roles of RTKs and integrins in the transduction of shear stress into chemical signals.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures**—Bovine aortic endothelial cells (BAECs) were isolated from bovine aorta and cultured in a humidified 95% air, 5% CO2 incubator at 37°C. The culture medium was Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 1 mM each of penicillin-streptomycin and sodium pyruvate. All experiments were conducted with cultures prior to passage 10.

**Shear Stress Experiments**—A flow system was used to impose shear stress on cultured ECs as described previously (24). In brief, a 75 × 35-mm glass slide was seeded with BAECs, which were cultured until reaching a confluent monolayer. A silicone gasket was sandwiched between the glass slide and an acrylic plate to create a rectangular flow channel (0.025 cm in height, 2.5 cm in width, and 5.0 cm in length) with inlet and outlet for exposing the cultured BAECs to shear stress. A high reservoir, the flow channel, a low reservoir, and a peristaltic pump were connected to form a circulation loop. Steady, laminar flow across the channel was generated as a result of the height difference between the two reservoirs. During the flow experiments, the system was kept at 37°C in a constant temperature cabinet and equilibrated with 95% humidified air plus 5% CO2.

**Immunoprecipitation and Immunoblotting**—The antibodies used in immunoprecipitation and immunoblotting were PY20 anti-phosphotyrosine monoclonal antibody (mAb) (Transduction Laboratories, Lexington, KY), polyclonal anti-Shc (Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal anti-Gerb2/Sem5 (Santa Cruz Biotechnology), anti-c-Myc mAb (Santa Cruz Biotechnology), polyclonal anti-Flik-1 (Santa Cruz Biotechnology), anti-αβ3 LM609 mAb, polyclonal anti-β3 (Chemicon, Temecula, CA), anti-β1 CD29 mAb (PharMingen, San Diego, CA), and anti-c-Myc (HA) mAb (Roche Molecular Biochemicals). For immunoprecipitation, cells were scraped into a lysis buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1% Triton X-100); the lysate was centrifuged, and the supernatant was immunoprecipitated with the appropriate antibodies and protein A-Sepharose beads (Amersham Pharmacia Biotech) at 4°C overnight. The immunoprecipitated complexes were washed and used for either kinase activity assays or immunoblotting. After SDS-PAGE, proteins in the gel were transferred to a nitrocellulose membrane for immunoblotting. The membrane was blocked with 5% bovine serum albumin followed by incubation with the primary antibody in 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 0.05% Tween 20, containing 0.1% bovine serum albumin. The bound primary antibodies were detected by using a goat anti-mouse or a goat anti-rabbit IgG-horseradish peroxidase conjugate (Santa Cruz Biotechnology) and the ECL detection system (Amersham Pharmacia Biotech).

**Immunostaining and Confocal Microscopy**—Confluent BAEC monolayers were fixed in a phosphate-buffered saline (PBS) containing 3% paraformaldehyde at room temperature for 10 min. The cells were then incubated in PBS containing the polyclonal anti-Flik-1 at a concentration of 1:200 (v/v) for 1 h at room temperature. The specimens were washed in PBS and incubated with a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Molecular Probes, Eugene, OR). The protein complexes were then immunoprecipitated with the anti-c-Myc mAb and protein A-Sepharose beads. To perform immunocomplex kinase assays, the immunoprecipitates were washed twice in the lysis buffer and twice in a kinase assay buffer (25 mM HEPEs, pH 7.4, 0.5 mM NaCl, 1% Triton X-100, 0.1% SDS, 1% deoxycholate, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 50 mM NaF, 2 mM Na3VO4, and 2 mM β-glycerophosphate). Myc-ERK2 was immunoprecipitated with the anti-α-Myc mAb and that glutathione S-transferase (GST)-c-Jun-(1–79) fusion protein was used as the substrate in the immunocomplex kinase assays.

**AP-1/TRE Activation Assays**—pcDNA3 or She-SH2 was co-transfected with either 4xTRE-Pl-Luc or MCP1-Luc-540 into BAECs at 70% confluence by using the transient transfection protocols. The pSV-gal plasmid, which contains a β-galactosidase (β-gal) gene driven by SV40 promoter and enhancer, was included in the co-transfection to monitor the transfection efficiency. The cells were then subjected to shear stress experiments or kept as static controls. The luciferase reporter activities normalized for transfection efficiency were used to assess the effects of She-SH2 on shear stress-induced transcription activation mediated by AP-1/TRE.

**RESULTS**

Shear Stress Increases the Tyrosine Phosphorylation of Flik-1 and Flik-1:She Association in BAECs—The binding of growth factors to their cognate RTKs induces the tyrosine phosphorylation of the cytoplasmic domains of RTKs, leading to the recruitment of the SH2-containing adaptor molecules such as Shc to the phosphorylated tyrosine. To test whether shear stress can activate endothelial RTKs as in the case of growth factor binding, confluent BAEC monolayers were subjected to a shear stress of 12 dynes/cm2 for various lengths of time. In parallel positive control experiments, however, BAECs were stimulated with 10 nM VEGF in the absence of shear. The cell lysates from the various experiments were immunoprecipitated with a polyclonal antibody against Flik-1, a VEGF receptor (27). The immunoprecipitated protein complexes were then immunoblotted with PY20 mAb to detect the change in tyrosine phosphorylation of Flik-1 which has a molecular mass of 210 kDa. As shown in Fig. 1A, shear stress induced the tyrosine phosphorylation of Flik-1 only as early as 1 min, reached a peak level at 5 min, decreased afterward, and returned to the basal level at 30 min. The temporal response of Flik-1 tyrosine phosphorylation induced by shear stress was similar to that found in cells stimulated by VEGF (Fig. 1B). Shear stress induction of Flik-1 tyrosine phosphorylation occurred both in the absence or presence of serum supplements and was not inhibited by pretreating BAEC monolayer with a polyclonal anti-VEGF antibody, indicating that the effect of shear stress was not due to back.
ground growth factors stimulation or to a paracrine or auto-
crine induction of VEGF.

To investigate whether shear stress induction of Flk-1 tyro-
sine phosphorylation was accompanied by an increased associ-
aton of Flk-1 with Shc, the cell lysates were immunoprecipi-
tated with a polyclonal antibody against Shc followed by
immunoblotting with polyclonal anti-Flk-1. As shown in Fig.
2A, shear stress increased the association of Flk-1 and Shc in a
rapid and transient manner with a time course parallel to that
of tyrosine phosphorylation of Flk-1 shown in Fig. 1A. A sa-
control, VEGF treatment also induced the association of Flk-1
and Shc in BAECs (Fig. 2B). In contrast, using anti-rabbit IgG
as a negative control in the immunoblotting, the association of
FlK-1 with Shc was not observed (data not shown). Neither
tyrosine phosphorylation of Flk-1 nor its association with Shc
was due to metabolites released from the shear stress-stimu-
lated ECs, since these responses were not found in ECs incu-
bated with the shearing media (data not shown).

**Shear Stress Increases the Flk-1 Clustering on the Luminal
Membrane**—Binding of the cognate ligands induces the dimer-
ization and thus the activation of various RTKs. To test the
hypothesis that shear stress activates Flk-1 by causing its
clustering, confluent monolayers of BAECs were kept static or
subjected to a shear stress of 12 dynes/cm² followed by anti-
Flk-1 immunostaining. Confocal microscopy revealed that
Flk-1 was mainly distributed on the luminal side of BAECs
(Fig. 3). Quantification of images from static and sheared sam-
ples showed that the application of shear stress for 1 min
enhanced the clustering of Flk-1. This focal pattern of cluster-
ing peaked at 5 min and reduced to the level comparable to that
in the static controls at 30 min after shearing. This time course
is similar to those of Flk-1 tyrosine phosphorylation and Flk-
1/Shc association.

**Shear Stress Increases the Association of Shc with α₅β₃ Inte-
grin and Integrins Containing β₁ and β₅ Subunit**—When ECs
are exposed to shear stress, focal adhesion plaques move dy-
namically on the abluminal membrane (16). We have previ-
ously demonstrated that the α₅β₃ integrin in focal adhesion
sites is involved in the shear stress activation of ERK and JNK
(15). To investigate the role of Shc in the integrin-mediated
signal transduction in response to shear stress, α₅β₃ was im-

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**Fig. 1.** Shear stress, like VEGF, induces tyrosine phosphoryl-
ation of Flk-1 in BAECs. BAEC monolayers were either subjected to
a shear stress of 12 dynes/cm² (A) or treated with VEGF (10 nM) (B) for
various lengths of time as indicated. Five hundred micrograms of the
cell lysate from each sample were subjected to immunoprecipitation
(IP) with a polyclonal anti-Flk-1 antibody and immunoblotting (IB) with
PY20 anti-phosphotyrosine mAb. The bound antibodies were detected
by the ECL system. Shown in the bottom part is densitometry analysis
representing the mean ± S.E. from three separate experiments. Relative
phosphorylation level is defined as the band intensities of the
various samples normalized to that in the peak induction. Asterisks in
A indicate significant difference (p < 0.05) between sheared samples
and static controls (time 0), and those in B indicate significant differ-
ce (p < 0.05) between VEGF-treated samples and untreated controls
(time 0).

**Fig. 2.** Shear stress and VEGF induce the association of Flk-1
with Shc in BAECs. The experimental procedures were essentially
the same as those described in Fig. 1, except that cell lysates from the
various samples were subjected to IP with a polyclonal anti-Shc and IB
with anti-Flk-1. The Flk-1-Shc association is demonstrated by the co-
immunoprecipitated Flk-1 in the anti-Shc immunoprecipitates. Shown
in the bottom part is densitometry analysis representing the mean ±
S.E. from three separate experiments. Relative association level is
defined as the band intensities of the various samples normalized to
that in the peak induction. Asterisks in A indicate significant difference
(p < 0.05) between sheared samples and static controls (time 0), and
those in B indicate significant difference (p < 0.05) between VEGF-
treated samples and untreated controls (time 0).
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Fig. 3. Shear stress increases the Flk-1 clustering in BAECs. A, confluent monolayers of cells were either kept as static controls (represented by 0 min) or subjected to a shear stress of 12 dynes/cm² for 1, 15, or 30 min with the direction of flow from left to right. Cells were fixed and immunostained with a polyclonal anti-Flk-1, which was then detected by a FITC-conjugated goat anti-rabbit antibody. Detection of the FITC staining along the height of the cells was achieved by confocal microscopic scanning. Shown in each panel are combined projections of three sections (0.3 µm for each section) near the luminal membrane. B shows the pixel intensities of the confocal images. The relative intensity level is defined as the pixel intensities relative to the minimum represented by numerical value of 0 and the maximum with numerical value of 1. A curve was plotted for each experiment to show the percentage of pixels at various levels of intensity. The analysis shows that the number of pixels at higher intensity increases in images obtained from sheared for 1 and 5 min, indicating shear stress increased the Flk-1 clustering in these specimens.

Fig. 4. Shear stress induces a sustained association of Shc with α₃β₃ integrin in BAECs, but the association is transient during BAEC adhesion. Confluent monolayers of BAECs were subjected to a shear stress of 12 dynes/cm² (A) or BAECs in suspension allowed to attach to fibrinogen (B) for the time duration as indicated. The cell lysates from the various samples were subjected to IP with anti-α,β₃ LM609 mAb, followed by IB with polyclonal anti-Shc. The Shcα,β₃ association is demonstrated by the co-immunoprecipitation of Shc with α,β₃ in the sheared or attached BAECs. Shown in the bottom part is densitometry analysis representing the mean ± S.E. from three separate experiments. Asterisks indicate significant difference (p < 0.05) between sheared samples and static controls (time 0) or between attached cells and cells in suspension (time 0).

It is possible that mechanotransduction causes the recruitment of Shc to various types of integrins in ECs. Thus, we also investigated whether shear stress increases the association of Shc with integrins containing the β₁ or β₅ subunit by immunoprecipitating the cell lysates with polyclonal anti-Shc followed by immunoblotting with anti-β₁ mAb CD29 or polyclonal anti-β₅. As shown in Fig. 5, β₁- or β₅-containing integrins were not associated with Shc in the static cells. Exposure of BAEC monolayer to shear stress increased the association of Shc with β₁ or β₅, with a time course similar to that for α₃β₃. Together, the data presented in Figs. 2, 4, and 5 demonstrate that Shc associates with both RTKs and integrins in ECs in response to shear stress. In contrast to the transient Shc-Flk-1 association, the Shc-β₁ association is much more sustained.

Shear Stress Induces Shc Tyrosine Phosphorylation and the Association of Shc with Grb2–Wnt in cells are stimulated by growth factors, tyrosine phosphorylation of Shc coincides with its recruitment to RTKs. To investigate whether Shc is tyrosine phosphorylated in response to shear stress, the anti-Shc immunoprecipitates were immunoblotted with PY20 mAb. As shown in Fig. 6A, shear stress caused a sustained increase in tyrosine phosphorylation of Shc, which lasted for at least 6 h after the exposure to shear stress.

FAK regulates Grb2-Sos-Ras pathway in the EC response to...
shear stress, which was demonstrated by the association of FAK with Grb2 (15). To investigate the possible engagement of Shc in the shear stress activation of the Grb2-Sos-Ras pathway, we examined whether Shc associates with Grb2 in the sheared BAECs. As shown in Fig. 6B, there was an increase in the amount of Grb2 co-immunoprecipitated with Shc in ECs subjected to shear stress for 1 min. This increased association of Grb2 with Shc was sustained. In a separate experiment, cell lysates immunoprecipitated with a polyclonal anti-Shc and immunoblotted with the polyclonal anti-Sos revealed that Shc was also associated with Sos in sheared cells (data not shown). The results in Fig. 6 demonstrate that shear stress induces a sustained interaction of integrins with Shc, which not only results in the tyrosine phosphorylation of Shc, but also the association of Shc with the Grb2-Sos complex.

Shc Regulates ERK, JNK, and AP-1/TRE in Response to Shear Stress—Shear stress activates mitogen activated protein kinases, including ERK and JNK (5–7), which in turn cause the transcriptional activation of AP-1 acting on the TRE in the 5′ promoter of some of the shear-inducible genes, e.g. the MCP-1 gene (6, 26). Through its association with Grb2-Sos, Shc can be upstream of these events. We constructed Shc-SH2 that functions as a negative mutant of Shc (28) to investigate its inhibitory effects on the shear stress activation of ERK, JNK, and on the TRE-driven luciferase reporter. Shc-SH2 was co-transfected with either Myc-ERK2 or HA-JNK1 into BAECs, and pcDNA3 parental plasmid was used as parallel controls. The transfected cells were either kept under static condition or subjected to a shear stress of 12 dynes/cm² for 10 min (for Myc-ERK2 assay) or 30 min (for HA-JNK1 assay) followed by immunocomplex kinase assays using MBP or GST-c-Jun-(1–79) fusion protein as the respective substrate. As shown in Fig. 7, shear stress activated Myc-ERK2 and HA-JNK1 in BAECs transfected with pcDNA3 by 2- and 3-fold, respectively. Co-transfection of Shc-SH2 drastically attenuated the shear stress activation of Myc-ERK2 and HA-JNK1. These results indicate that Shc is involved in the upstream signaling for the shear stress induction of ERK and JNK.

BAECs were co-transfected with Shc-SH2 and the chimeric construct 4XTRE-Pi-Luc consisting of luciferase reporter driven by four copies of TRE linked to the rat prolactin mini-
mum promoter. In parallel experiments, cells were co-transfected with pcDNA3 together with 4XTRE-Pi-Luc. A shear stress of 12 dynes/cm² caused 33-fold induction of luciferase activity after 10 min. The cell lysates were immunoprecipitated with anti-Myc mAb for IP kinase assays using MBP and [γ-32P]ATP as substrates. Shown in the bottom panel is IB with anti-Myc mAb, indicating that comparable amounts of Myc-ERK2 were expressed in the various samples. B is the result of a parallel set of experiments in which 3 μg of HA-JNK1 were co-transfected with 9 μg of pcDNA3 or Shc-SH2 into BAEC, followed by the application of shear stress for 30 min. HA-JNK1 was immunoprecipitated for immunocomplex kinase assays using GST-c-Jun-(1-79) and [γ-32P]ATP as substrates. Shown in the bottom panel is IB with anti-HA mAb. Bar graphs, representing mean ± S.E. from three separate experiments, show the kinase activities of the various samples relative to those in the pcDNA3-transfected, static controls.

**FIG. 7. Negative mutant of Shc attenuates shear stress activation of Myc-ERK2 and HA-JNK1 in BAECs.** In A, 3 μg of epitope-tagged Myc-ERK2 were co-transfected with 9 μg of Shc-SH2 or pcDNA3 empty vector into BAECs in 75-cm² tissue culture flasks. The transfected cells were passed onto slides until confluence before subjected to a shear stress of 12 dynes/cm² for 10 min. The cell lysates were immunoprecipitated with anti-Myc mAb for IP kinase assays using MBP and [γ-32P]ATP as substrates. The bands indicated by the arrow represent the phosphorylated MBP after SDS-PAGE and autoradiography. Shown in the bottom panel is IB with anti-Myc mAb, indicating that comparable amounts of Myc-ERK2 were expressed in the various samples. B is the result of a parallel set of experiments in which 3 μg of HA-JNK1 were co-transfected with 9 μg of pcDNA3 or Shc-SH2 into BAEC, followed by the application of shear stress for 30 min. HA-JNK1 was immunoprecipitated for immunocomplex kinase assays using GST-c-Jun-(1-79) and [γ-32P]ATP as substrates. Shown in the bottom panel is IB with anti-HA mAb. Bar graphs, representing mean ± S.E. from three separate experiments, show the kinase activities of the various samples relative to those in the pcDNA3-transfected, static controls.

**DISCUSSION**

This study demonstrates that receptor tyrosine kinases such as Flk-1 and integrins, including α1β3 and those containing β1 or β3 subunit, can serve as mechanosensors in ECs in response to shear stress. Shear stress activates the Shc-dependent pathways through its association with Flk-1 and these mecha

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Mechanism, and then associate with many SH2-containing enzymes and adaptor proteins. As a result, many signal transduction pathways would be activated by shear stress. For example, the PI 3-kinase pathway can lead to the generation of intracellular diacylglycerol and inositol 1,4,5-trisphosphate, and PLC-γ can activate the protein kinase C pathway. Indeed, inositol 1,4,5-trisphosphate and protein kinase C have been shown to be activated by shear stress (37–40). Although other stimuli such as growth factors and environmental stresses also activate RTKs (i.e., aggregation and autophosphorylation), but their effects on cells are different from those by shear stress. VEGF is an EC mitogen (41, 42), UV can lead to apoptosis (43), whereas laminar shear stress is vital for endothelial homeostasis in blood vessels and has been shown to protect ECs from undergoing apoptosis in vitro (44).

Shear stress not only increases the association of Shc with Flk-1, but also its interaction with mechano-sensitive integrins (α5β3 and those containing β1 or β3 subunit). The co-immunoprecipitation of Shc with integrins in the sheared cells suggests an increased association rate or a decreased dissociation rate. We did not find an increased association of Shc with integrins in cells treated with VEGF. This result is consistent with the previous finding that treatment of A431 cells with EGF did not result in the association of α5β3 integrin with Shc or Grb2 (45). Thus, the binding of growth factors to their receptors promote the recruitment of Shc to the RTKs, but not to integrins. In contrast, shear stress increases the association of Shc to both RTKs and integrins.

It has been shown by immunostaining that integrin aggregation caused by beads coated with ligands (e.g., fibronectin, RGD peptide, and anti-integrin antibody) triggers the accumulation of protein-tyrosine kinases (e.g., FAK and c-Src) and signaling molecules (e.g., Grb2, Sos, PLC-γ) in focal adhesion sites (46–48). In addition, biochemical analysis has revealed that Shc is tyrosine-phosphorylated and is associated with α5β3, α1β1, and ααβ3 when these integrins are conjugated to their ligands (23, 49). The similarities in cellular responses to shear stress and to integrin-mediated cell adhesion have led us to propose that integrins serve as mechanosensors (4). Although the Shc-integrin association and Shc tyrosine phosphorylation are responses common to cell adhesion and shear stress, they are transient during cell adhesion (Fig. 4B; Refs. 23 and 49), but sustained with shear stress. The molecular basis underlying the temporal dynamics in the association of Shc with integrins is unknown. Presumably, both shear stress and the integrin-mediated cell adhesion induce conformational changes of integrins to facilitate their association with Shc. However, the events resulting from mechanical stimuli are unique in the sustained nature of the response, which does not occur following stimulation by growth factors or cell adhesion. The dynamic remodeling of the adhesion plaques in ECs exposed to shear stress (16) requires constant association and dissociation of integrins with ECM. It is likely that the enhanced “on-off” rates of integrin/ECM interaction result in a sustained conformational change of integrins, which in turn increases the association of Shc. Under static condition, the anchorage-dependent adhesion of ECs relies on the interaction of integrins with ECM. When cells are exposed to shear stress, such interaction needs to be reinforced to withstand the shearing forces. This reinforcement is probably achieved by moving dynamically the adhesion plaques to the strategic positions while sending signals to the cytoplasm through Shc recruitment.

The activation of Flk-1 leads to the recruitment of Grb2 through SH2 binding (35). Overexpression of Flk-1 or the association of integrins with various ligands causes ERK activation (23, 35, 50). The integrin-mediated ERK activation involves FAK autophosphorylation on Tyr-397, which leads to c-Src recruitment. The association of FAK with Src family protein-tyrosine kinases at focal adhesions further increases the phosphorylation of FAK at Tyr-925, creating a Grb2 binding site (51–54). The data shown in Fig. 7 suggest that Shc regulates not only ERK but also JNK. We have previously shown that shear stress causes Ras activation which regulates both ERK and JNK (6, 10). By interacting with Grb2-Sos, Shc plays a pivotal role in activating Ras, which in turn regulates

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mitogen activated protein kinases and the AP-1 TRE-mediated transcriptional activation. It is to be noted that the shear stress activation of Ras is transient (6), but the association of Shc with Grb2 is sustained (Fig. 6B). Shear stress not only regulates immediate early responses but also other late events such as the formation of stress fibers and the alignment of ECs and their cytoskeletal elements with the direction of flow (see Ref. 1 for review). The binding of integrins to both ECM and actin-associated cytoskeletal proteins (e.g. talin, vinculin, α-actinin, and paxillin) has been suggested to provide a path for mechanical signaling (54, 56) and thus may be important in morphological remodeling.

In addition to RTKs and integrins, other molecules on the membrane and at cell junctions may also be involved in the mechano-chemical transduction processes. Angiotensin II receptor plays an important role in mechanical stress-induced cardiac hypertrophy (57), whereas platelet endothelial cell adhesion molecule-1 is tyrosine-phosphorylated in response to shear stress (58). Some of these molecules may also regulate Shc. For example, the Gαs-coupled angiotensin II receptor activates Ras via the Shc-Grb2-Sos pathway in cardiac myocytes (59) and Gβγ subunits of G proteins mediate the tyrosine phosphorylation of Shc and the formation of Shc-Grb2 complex (60). Thus, while Shc is important in the RTK- and integrin-mediated responses of endothelial cells to mechanical stimuli, it may also be involved in other mechanotransduction pathways, with some of them yet to be identified.

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