Novel Role for STAT-5B in the Regulation of Hsp27–FGF-2 Axis Facilitating Thrombin-Induced Vascular Smooth Muscle Cell Growth and Motility

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Abstract—Previously, we have demonstrated that STAT-3 plays a role in thrombin-induced VSMC motility. To learn more about the role of STATs in the mitogenic and chemotactic signaling events of thrombin, here we have studied the role of STAT-5. Thrombin activated STAT-5 as measured by its tyrosine phosphorylation, DNA binding, and reporter gene activity. Inhibition of STAT-5B, but not STAT-5A, by adenovirus-mediated expression of its respective dominant-negative mutants suppressed thrombin-induced VSMC growth and motility. Thrombin induced the expression of Hsp27 and FGF-2 in a time- and STAT-5B–dependent manner in VSMC. In addition, small interfering RNA–directed depletion of Hsp27 levels or adenovirus-mediated expression of its dominant-negative mutant attenuated thrombin-induced Hsp27 and FGF-2 induction, DNA synthesis and motility in VSMC. Together, these results indicate that thrombin-induced VSMC growth and motility require STAT-5B/STAT-3–dependent expression of Hsp27 and FGF-2. These observations also suggest that STAT-5B/STAT-3/Hsp27/FGF-2 signaling via its involvement in the regulation of VSMC growth and motility may play an important role in the pathogenesis of vascular diseases such as restenosis after angioplasty. (Circ. Res. 2006;98:913-922.)

Key Words: fibroblast growth factor-2  ■  heat shock protein 27  ■  G protein–coupled receptor  ■  signal transducer and activator of transcription  ■  vascular smooth muscle cell
thrombin-induced VSMC growth and motility require activation of STAT-5B. In addition, we found that STAT-5B in crosstalk with STAT-3 targets the induction of expression of Hsp27 and FGF-2 in triggering the thrombin-induced VSMC growth and motility. Based on these novel findings, it is likely that the STAT-5B/STAT-3/Hsp27/FGF-2 axis plays a critical role in vessel wall diseases such as restenosis following angioplasty.

Materials and Methods

Reagents
Thrombin was purchased from Sigma-Aldrich (St Louis, Mo). Anti–phospho-Hsp27 (S78/S82) antibodies, anti–STAT-5A antibodies (AF2168), and human FGF-2 ELISA kit (DFB50) were from R&D Systems (Minneapolis, Minn). Anti-Hsp27 (SC-1049) antibodies, anti–STAT-3 antibodies (SC-482), anti–STAT-5B antibodies (SC-836 and SC-1656), anti-PY20 antibodies (SC-508), STAT-5 consensus binding oligonucleotide (5'-AGA TTT CTA GGA ATT CAA TCC-3') (SC-2565) and STAT-5 mutant oligonucleotide (5'-AGA TTT AGT TTA ATT CAA TCC-3') (SC-2566) were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif). Anti–STAT-5A/B antibodies (06–588) were obtained from Upstate Biotechnology (Lake Placid, NY). Phosphospecific anti–STAT-5 (9351S) antibodies were from Cell Signaling Technology (Beverly, Mass). Rat Hsp27 small interfering RNA (siRNA) duplexes (sense, 5'-GCGUGUCCUGGAGCACUAdUAdTdT-3'; antisense, 5'-UGUG- CAGUGGAGGACGCaGdC-3') were made by QIAGEN (Valencia, Calif). Scrambled control siRNA (5'-UAGCGACUAAACACACUCAA-3') was obtained from Dharmaco (Lafayette, Co). T4 polynucleotide kinase was obtained from Promega (Madison, Wis). Super Script First Strand Synthesis System for RT-PCR and TRIzol reagent were bought from Invitrogen (Carlsbad, Calif). [γ-32P]-ATP (3000 Ci/mmol) and [14C]-chloramphenicol (59 mCi/mmol) were from Amersham Biosciences (Piscataway, NJ). All the primers were from Amersham Biosciences (Piscataway, NJ). Rat VSMC were isolated and subcultured as described previously.24

Construction of Recombinant dnSTAT-3, dnSTAT-5A, and dnSTAT-5B Adenoviral Vectors
C-terminally truncated STAT-5A (deletion at C terminus from 713 to 791 amino acid [AA] residues) and STAT-5B (deletion at C terminus from 718 to 793 AA residues) cDNAs26 were released from pmSTAT-5A-713-Prk5 and pmSTAT-5B-718-Prk5 plasmids by digestion with EcoR1 and EcoRV and cloned into the same sites of entry vector pENTR3C. In the case of dnSTAT-3, its cDNA fragment was released from pFS3DM vector18 by digestion with EcoR1 and SalI and cloned into EcoR1 and XhoI sites of entry vector pENTR3C. The final constructs pAd-dnSTAT-5A, pAd-dnSTAT-5B, and pAd-dnSTAT-3 were generated by specific recombination of pbnSTAT-5A-ENTR3C, pbnSTAT-5B-ENTR3C, and pbnSTAT-3-ENTR3C with pAdCMV/V5DEST (Invitrogen). Adenovirus (Ad-dnSTAT-5A, Ad-dnSTAT-5B, and Ad-dnSTAT-3) expressing dnSTAT-5A, dnSTAT-5B, or dnSTAT-3 were produced by transfecting HEK293 cells with pAd-dnSTAT-5A, pAd-dnSTAT-5B, and pAd-dnSTAT-3 plasmids, respectively. Construction of pAd-GFP and pAd-3A (pAd-dnHsp27) have been described previously.27-28 Adenovirus was purified with cesium chloride centrifugation and the titer was determined by plaque assay as described previously.23

Cell Motility
VSMC motility was measured by cell-wounding assay, as described previously.24

Cell Number
VSMC at 72 hours of appropriate treatments were trypsinized, rinsed with and suspended in PBS, and counted using a hemacytometer.

DNA Synthesis
VSMC DNA synthesis was measured by labeling cells with 1 μCi/mL [3H]-thymidine, as described previously.16

Electrophoretic Mobility-Shift Assay
After appropriate treatments, VSMC nuclear extracts were made and analyzed for DNA binding activity, as described previously.24

Enzyme-Linked Immunosorbent Assay
After appropriate treatments, FGF-2 released into the culture medium of VSMC was measured using human FGF-2 ELISA kit following the instructions of the supplier.

Immunoprecipitation
After appropriate treatments, cell extracts were made and an equal amount of protein from each condition was subjected to pull-down assay followed by Western blot analysis.

RNA Isolation, cDNA Synthesis, and RT-PCR
RNA was isolated from cells using TRIzol reagent as per the guidelines of the manufacture. Reverse transcription was performed with Superscript First Strand Synthesis System for RT-PCR based on the protocol of the supplier. The cDNA was then used as template for PCR using specific primers for rat FGF-2 (forward, 5'-CAGCT-CCAAGCAGAAGAG-3'; reverse, 5'-ACAGTATGGCCCTTCTGTCCA-3'), Hsp27 (forward, 5'-TTCGAGATTCGCAAGAGG-3'; reverse, 5'-GACAGGAAGAGGACACCA-3'), and β-actin (forward, 5'-CTTTGACATCGTGAAGACC-3'; reverse 5'-GATAAGAGCACAAATCCCA-3'). The amplification was performed on Gene Amp PCR System 2400 (Applied Biosystems, Calif) using the following amplification systems for the abovementioned genes as follows: for FGF-2, at 94°C for 5 minutes followed by 27 cycles at 94°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute, with final extension at 72°C for 5 minutes; Hsp27, at 94°C for 5 minutes followed by 30 cycles at 94°C for 45 seconds, 56°C for 1 minute and 72°C for 45 seconds, with final extension at 72°C for 5 minutes for β-actin, at 94°C for 5 minutes followed by 25 cycles at 94°C for 45 seconds, 55°C for 1 minute and 72°C for 45 seconds, with final extension at 72°C for 5 minutes. The amplified RT-PCR products were separated on 2% (wt/vol) agarose gel containing ethidium bromide and the band intensities were quantified using NIH ImageJ software.

Western Blot Analysis
After appropriate treatments, VSMC extracts were made and analyzed by Western blotting for the protein of interest using its specific antibodies as described previously.16

CAT Assay
VSMC were transfected with pSIE-CAT plasmid in serum- and antibiotic-free DMEM using FuGENE 6 reagent (Invitrogen). Cells were then quiesced and treated with and without thrombin (0.5 U/mL) for the indicated times, and cell extracts were prepared. In the case of testing the effect of Ad-STAT-5A/B, cells were infected first with the respective virus followed by transfection with pSIE-CAT plasmid DNA. Cell extracts normalized for protein were assayed for CAT activity using [14C]-chloramphenicol and acetyl coenzyme A as substrates as described previously.24

Statistics
All the experiments were repeated three times with similar results. Data are presented as mean±SD. The treatment effects were analyzed by Student’s t test. Values of P<0.05 were considered to be statistically significant. In the case of CAT activity, electrophoretic mobility-shift assay (EMSA), RT-PCR, and Western blotting, 1 representative set of data is shown.
**Results**

Thrombin Activates STAT-5-Dependent Transcription in VSMC

To understand the role of STATs in GPCR agonist–induced VSMC growth and motility, here we have studied the effect of thrombin on STAT-5 activation. Thrombin (0.5 U/mL) stimulated tyrosine phosphorylation of STAT-5 in a time-dependent manner (Figure 1A). On phosphorylation, STATs undergo either homo- or heterodimerization and translocate to the nucleus, where they influence their target gene transcription. To find whether the increases in STAT-5 phosphorylation correlate with its transcriptional transactivation, the STAT-5/DNA-binding activity was measured. Consistent with its effect on phosphorylation, thrombin (0.5 U/mL) also stimulated STAT-5/DNA-binding activity in a time-dependent manner (Figure 1B). No STAT-5/DNA-binding activity was observed with a mutant probe. Furthermore, preincubation of nuclear protein with anti–STAT-5 antibodies (1 μg) that recognize both A and B isoforms reduced the protein/DNA-binding activity, suggesting the presence of STAT-5 in these complexes (Figure 1C). To find whether STAT-5 influences the transcription of genes containing SIE (sis-inducing element) or SIE-like elements in their promoter regions, cells were transfected with pSIE-CAT plasmid DNA, quiesced, and treated with and without thrombin (0.5 U/mL) for the indicated times, and the CAT activity was measured. Consistent with its effect on DNA-binding activity, thrombin induced SIE-dependent CAT activity in a time-dependent manner (Figure 1D). To confirm these results further, we tested the effect of dnSTAT-5A/B. VSMC that were first infected with adenovirus expressing either GFP, dnSTAT-5A, or dnSTAT-5B at a multiplicity of infection (moi) of 80 were transfected with pSIE-CAT plasmid DNA, quiesced, and treated with and without thrombin (0.5 U/mL) for 8 hours, and the CAT activity was measured. As shown in Figure 1E, thrombin induced CAT activity by approximately 3-fold as compared with green fluorescence protein (GFP) control, and this response was blocked by both dnSTAT-5A and dnSTAT-5B. These findings suggest that thrombin activates STAT-5A/B in VSMC.

STAT-5B Mediates Thrombin-Induced VSMC Growth and Motility

To understand the role of STAT-5 in thrombin-induced VSMC growth and motility, we next tested the effect of dnSTAT-5A and dnSTAT-5B. Adenovirus-mediated expression of dnSTAT-5B but not dnSTAT-5A attenuated thrombin-induced VSMC DNA synthesis, cell number, and motility (Figure 2A through 2C). Because only STAT-5B appears to be involved in thrombin-induced VSMC growth and motility, we focused next on identification of its downstream effector molecules.

Thrombin Induces the Expression of Hsp27 via STAT-5B Activation

Some reports have shown that Hsps, particularly Hsp27, play a role in the regulation of cell motility. To understand the mechanisms by which STAT-5B is involved in thrombin-induced VSMC growth and motility, we studied the time-course effect of thrombin on Hsp27 expression. Thrombin (0.5 U/mL) induced the expression of Hsp27 both at mRNA and protein levels in a time-dependent manner, with a maximum of 2-fold increase at 4 hours and sustaining these levels at least for 16 hours (Figure 3A and 3B). DnSTAT-5B inhibited thrombin-induced Hsp27 expression both at mRNA and protein levels (Figure 4A and 4B).

A Requirement for Hsp27 in Thrombin-Induced VSMC Growth and Motility

To understand the functional significance of STAT-5B-Hsp27 signaling, we next studied the role of Hsp27 in thrombin-induced VSMC growth and motility using siRNA approach. Hsp27 siRNA but not control siRNA depleted thrombin-induced Hsp27 levels (Figure 5A). In addition, Hsp27 siRNA blocked thrombin-induced VSMC DNA synthesis, cell number, and motility (Figure 5B through 5D). To confirm these findings, we tested the effect of dnHsp27. DnHsp27 is a phosphorylation-null mutant. Thrombin induced the Ser86 phosphorylation of Hsp27 (equivalent of Ser82 in human Hsp27) in a time-dependent manner (Figure 6A). Adenovirus-mediated expression of dnHsp27 inhibited thrombin-induced Hsp27 Ser86 phosphorylation (Figure 6B). In addition, dnHsp27 inhibited thrombin-induced increases in VSMC DNA synthesis, cell number, and motility (Figure 6C through 6E).

Thrombin Induces FGF-2 Expression via STAT-5B Activation

We have previously shown that thrombin via activation of phosphatidylinositol 3-kinase/Akt/Fra-1 signaling induces the expression of FGF-2 in VSMC and that neutralizing anti–FGF-2 antibodies inhibit thrombin-induced VSMC DNA synthesis and motility. In addition, we found that inhibition of Jak-2 suppresses 5(S)-hydroxyeicosatetraenoic acid–induced FGF-2 expression in human dermal microvascular endothelial cells. Based on these results, we hypothesized that FGF-2 may be an effector molecule of STAT-5B in thrombin-induced VSMC growth and motility. To test this, we studied the effect of dnSTAT-5B on thrombin-induced FGF-2 expression. Adenovirus-mediated expression of dnSTAT-5B completely blocked thrombin-induced FGF-2 expression both at mRNA and protein levels (Figure 7A and 7B).

Thrombin-Induced FGF-2 Expression Requires Hsp27

Because STAT-5B was also involved in thrombin-induced Hsp27 expression, we wanted to learn whether there is any crosstalk between Hsp27 and FGF-2. To address this, we tested the role of Hsp27 in thrombin-induced FGF-2 expression. Hsp27 siRNA inhibited thrombin-induced FGF-2 expression both at mRNA and protein levels (Figure 7C and 7D). To confirm these findings, we also studied the effect of dnHsp27 on thrombin-induced FGF-2 expression. Consistent with the effect of Hsp27 siRNA, dnHsp27 also blocked thrombin-induced FGF-2 expression both at mRNA and protein levels (Figure 7E and 7F). To find whether thrombin-induced increases in the expression of FGF-2, in turn, leads to...
its release, VSMC that were transduced with Ad-GFP (control), Ad-dnSTAT-5A, or Ad-dnSTAT-5B with a moi of 80 were quiesced and treated with and without thrombin (0.5 U/mL) for appropriate time periods, and DNA synthesis, cell number, and motility were measured. A, DNA synthesis was measured by $[^3H]$-thymidine incorporation into TCA-precipitable material. B, Cell number was measured by Trypan Blue dye exclusion assay. C, Cell motility was measured by cell-wounding assay. $^*P<0.01$ vs control, $^{**}P<0.01$ vs thrombin treatment alone.

Figure 2. Thrombin-induced VSMC growth and motility require activation of STAT-5B but not STAT-5A. VSMC that were transduced with Ad-GFP (control), Ad-dnSTAT-5A, or Ad-dnSTAT-5B analyzed by Western blotting using anti-PY20 antibodies. The blot was reprobed with anti–STAT-5A/B antibodies for normalization. B and C, Nuclear extracts containing an equal amount of protein from control and each treatment were assayed for protein/DNA-binding activity using $[^32P]$-labeled STAT-5 consensus and mutant oligonucleotides as probes. Wherever indicated, anti–STAT-5A/B antibodies were added to nuclear extracts 30 minutes before the addition of the $[^32P]$-labeled STAT-5 consensus oligonucleotide probe. D, VSMC were transfected with pSIE-CAT plasmid DNA, quiesced, and treated with and without thrombin (0.5 U/mL) for the indicated times, and cell extracts were prepared. Cell extracts normalized for protein were assayed for CAT activity using $[^3H]$-chloramphenicol and acetyl coenzyme A as substrates. E, VSMC were transduced first with Ad-GFP (control), Ad-dnSTAT-5A, or Ad-dnSTAT-5B at a moi of 80, and, 24 hours later, they were transfected with pSIE-CAT plasmid DNA and quiesced. Cells were then treated with and without thrombin (0.5 U/mL) for 8 hours, and cell extracts were prepared and assayed for CAT activity as described in D. To show the expression of dnSTAT-5A and -B, an equal amount of protein from each sample was analyzed by Western blotting using their specific antibodies. The bar graph represents quantitative analysis of 3 independent experiments. $^*P<0.01$ vs control.

Figure 1. Thrombin activates STAT-5 in VSMC. Quiescent VSMC were treated with and without thrombin (0.5 U/mL) for the indicated times, and either cell or nuclear extracts were prepared. A, Cell extracts containing an equal amount of protein from control and each treatment were immunoprecipitated with anti–STAT-5A/B antibodies, and the immunocomplexes were analyzed by Western blotting using anti-PY20 antibodies. The blot was reprobed with anti–STAT-5A/B antibodies for normalization. B and C, Nuclear extracts containing an equal amount of protein from control and each treatment were assayed for protein/DNA-binding activity using $[^32P]$-labeled STAT-5 consensus and mutant oligonucleotides as probes. Wherever indicated, anti–STAT-5A/B antibodies were added to nuclear extracts 30 minutes before the addition of the $[^32P]$-labeled STAT-5 consensus oligonucleotide probe. D, VSMC were transfected with pSIE-CAT plasmid DNA, quiesced, and treated with and without thrombin (0.5 U/mL) for the indicated times, and cell extracts were prepared. Cell extracts normalized for protein were assayed for CAT activity using $[^3H]$-chloramphenicol and acetyl coenzyme A as substrates. E, VSMC were transduced first with Ad-GFP (control), Ad-dnSTAT-5A, or Ad-dnSTAT-5B at a moi of 80, and, 24 hours later, they were transfected with pSIE-CAT plasmid DNA and quiesced. Cells were then treated with and without thrombin (0.5 U/mL) for 8 hours, and cell extracts were prepared and assayed for CAT activity as described in D. To show the expression of dnSTAT-5A and -B, an equal amount of protein from each sample was analyzed by Western blotting using their specific antibodies. The bar graph represents quantitative analysis of 3 independent experiments. $^*P<0.01$ vs control.
release into the medium was measured by ELISA. As shown in Figure 8A and 8B, thrombin induced the release of FGF-2 by approximately 3-fold as compared with control and this effect was substantially blocked by dnSTAT-5B, dnHsp27, and Hsp27 siRNA. Furthermore, neutralizing anti–FGF-2 antibodies blocked thrombin-induced VSMC growth and motility.32

Thrombin-Induced Hsp27 and FGF-2 Expression Exhibits a Crosstalk Between STAT-5B and STAT-3
Previously, we have reported that STAT-3 mediates thrombin-induced VSMC motility.24 To learn whether there is any crosstalk between STAT-5B and STAT-3 in the induction of expression of Hsp27 and FGF-2 toward mediating the mitogenic and chemotactic effects of thrombin, we first analyzed their interactions by pull-down assay. As shown in Figure 8C, increased association of STAT-5B with STAT-3 was observed in response to thrombin as compared with control. Using a dominant-negative mutant approach, we next tested the role of STAT-3 in thrombin-induced Hsp27 and FGF-2 expression. Adenovirus-mediated expression of dnSTAT-3 suppressed thrombin-induced induction of Hsp27 and FGF-2 (Figure 8D). Adenovirus-mediated expression of dnSTAT-3 also blocked thrombin-induced VSMC DNA synthesis and motility (Figure 8E and 8F).

Discussion
The important findings of the present study are as follows. (1) Thrombin activates STAT-5 in VSMC. (2) Thrombin induces the expression of Hsp27 and FGF-2 in a time- and STAT-5B–depen-
dent manner. (3) Thrombin-induced FGF-2 expression is also dependent on Hsp27. (4) Thrombin-induced VSMC growth and motility exhibited a requirement for STAT-5B–dependent Hsp27 and FGF-2 expression. (5) STAT-5B forms a complex with

Figure 5. Hsp27 siRNA attenuates thrombin-induced VSMC growth and motility. A, VSMC were transfected with scrambled control or Hsp27 siRNA, quiesced, and treated with and without thrombin (0.5 U/mL) for 16 hours, and cell extracts were prepared. An equal amount of protein from control and each treatment was analyzed by Western blotting for Hsp27 using its specific antibodies. The blot was reprobed with anti–STAT-5B antibodies for normalization. B through D, VSMC that were mock or Hsp27 siRNA transfected were quiesced and subjected to thrombin-induced VSMC DNA synthesis, cell number, or motility, as described in the legend of Figure 2. The bar graph represents the quantitative analysis of 3 independent experiments. *P<0.01 vs control, **P<0.01 vs thrombin treatment alone.

Figure 6. DnHsp27 inhibits thrombin-induced VSMC growth and motility. A, Quiescent VSMC were treated with and without thrombin (0.5 U/mL) for the indicated times, and cell extracts were prepared. An equal amount of protein from control and each treatment was analyzed by Western blotting for pHsp27 using its phosphospecific antibodies. B, VSMC were transduced with Ad-GFP (control) or Ad-dnHsp27 at a moi of 80, quiesced, and treated with and without thrombin (0.5 U/mL) for 10 minutes, and cell extracts were prepared and analyzed for pHsp27 as described in A. The blots in A and B were reprobed with anti–STAT-5B antibodies for normalization. C through E, Conditions were the same as in B, except that cells after quiescence were treated with and without thrombin (0.5 U/mL) for appropriate time periods and DNA synthesis, cell number, and motility were measured as described in the legend of Figure 2. *P<0.01 vs control, **P<0.01 vs thrombin treatment alone.
STAT-3 in response to thrombin and blockade of STAT-3 activation via adenovirus-mediated expression of its dominant-negative mutant inhibited thrombin-induced Hsp27 and FGF-2 induction in VSMC and the growth and motility of these cells. Earlier studies have shown that STAT-5 plays a role in the regulation of epithelial cell survival and proliferation. STAT-5 has also been shown to be involved in cytokine-induced hematopoietic cell growth. The findings that STAT-5 regulates the expression of cyclin D1 and Bcl(XL) further support its role in the regulation of cell proliferation and survival. The present results reveal that STAT-5B also targets both Hsp27 and FGF-2 in influencing the cellular growth and motility, at least in VSMC in response to thrombin. It was
demonstrated that thrombin-induced VSMC DNA synthesis and motility require FGF-2 release. However, the mechanism(s) by which thrombin influences FGF-2 expression in VSMC have not been explored previously. In this regard, we have shown earlier that Akt plays a role in thrombin-induced FGF-2 expression in VSMC. In addition, our present results show that FGF-2 is also a target molecule of STAT-5B in thrombin-induced VSMC growth and motility. In view of these results, it appears that several mechanisms are involved in thrombin-induced FGF-2 expression and that FGF-2 may be a converging molecule for signaling events that target cell proliferation and motility in VSMC.

The involvement of Hsp27 in cell motility and tumor progression has been reported previously. Hsp27 interacts with actin and modulates actin cytoskeleton dynamics. However, the present observations reveal that Hsp27 is required for thrombin-induced actin cytoskeleton dynamics. Earlier studies by other investigators have shown that Hsp27 colocalizes with FGF-2 and facilitates its release from endothelial cells in response to estradiol. Based on these results, it is likely that besides its capacity to regulate actin cytoskeleton dynamics, Hsp27 plays a role in the regulation of expression of FGF-2 toward mediating cell proliferation and motility. FGF-2 is a potent mitogen and
cachectrant for many cell types including VSMC. Because both Hsp27 and FGF-2 expression are regulated by STAT-5B and interference with the function of Hsp27 suppresses thrombin-induced FGF-2 induction, it is possible that Hsp27 acts downstream to STAT-5B in the induction of expression of FGF-2. It was demonstrated that Hsp27 via enhancing the degradation of ubiquitinated IκBα is involved in the activation of nuclear factor κB. A mechanism similar to this is quite possible for Hsp27 involvement in thrombin-induced FGF-2 expression as well.

We have previously shown that thrombin-induced VSMC motility requires STAT-3 activation. In addition to these observations, the present results reveal that STAT-3 interacts with STAT-5B in thrombin-induced expression of Hsp27 and FGF-2 and thereby in the regulation of VSMC growth and motility. These observations, along with our previous findings, suggest that FGF-2 is a downstream effector molecule of several signaling events that influence VSMC growth and motility. Based on these findings, we envision a potential role for STAT-5B/STAT-3/Hsp27/FGF-2 signaling in the vessel wall remodeling, particularly in vascular injury settings.

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