Integrin-Associated Protein Binding Domain of Thrombospondin-1 Enhances Insulin-Like Growth Factor-I Receptor Signaling in Vascular Smooth Muscle Cells

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Abstract—Insulin-like growth factor-I (IGF-I) stimulates vascular smooth muscle cell (SMC) proliferation and migration. The response of smooth muscle cells to IGF-I is determined not only by activation of the IGF-I receptor but also by at least three other transmembrane proteins, \( \alpha_v\beta_3 \), integrin-associated protein (IAP), and SHPS-1. This regulation seems to be attributable to their ability to regulate the transfer of SHP-2 phosphatase, a key component of IGF-I signaling. Ligand occupancy of SHPS-1 with IAP is required for the recruitment and transfer of SHP-2 and subsequent signaling in response to IGF-I. The extracellular matrix protein thrombospondin-1 stimulates an increase in the cell proliferation response to IGF-I. Because thrombospondin-1 is a ligand for IAP, we wished to determine whether the enhancing effect of thrombospondin-1 was mediated through IAP binding. To examine the effect of thrombospondin-1 binding to IAP, we used a peptide termed 4N1K derived from the IAP binding site of thrombospondin-1. Preincubation with 4N1K increased IGF-I–stimulated mitogen-activated protein kinase activation and DNA synthesis. This enhancement seemed to be attributable to its ability to increase the duration of IGF-I–stimulated receptor and insulin receptor substrate-1 (IRS-1) phosphorylation. Preincubation with 4N1K delayed IGF-I stimulation of SHPS-1 phosphorylation (attributable to an alteration in IAP–SHPS-1 interaction), resulting in a delay in SHP-2 recruitment. This delay in SHP-2 transfer seems to account for the increase in the duration of IGF-I receptor phosphorylation and for enhanced downstream signaling. These observations support the conclusion that thrombospondin-1 and IGF-I seem to function coordinately in stimulating smooth muscle proliferation via the thrombospondin-1 interaction with IAP. ([Circ Res. 2003;93:925-931.])

Key Words: insulin-like growth factor-I ▪ thrombospondin-1 ▪ integrin-associated protein ▪ smooth muscle cells ▪ atherosclerosis

A major contributor to the development of atherosclerotic lesions is the migration and proliferation of vascular smooth muscle cells (SMCs). By virtue of its ability to stimulate both SMC migration and proliferation, insulin-like growth factor-I (IGF-I) has been implicated in the development of atherosclerosis. Recent in vivo animal studies have shown that smooth muscle–targeted overexpression of IGF-I stimulates an increase in neointimal formation after carotid artery injury attributable to an increase in the proliferation and migration of SMCs. This change is attributed to a local paracrine effect of IGF-I because serum levels were not affected.

We have previously reported that the extracellular matrix protein thrombospondin-1 (TS-1) can enhance IGF-I–stimulated cell proliferation. TS-1 binds to several cell-surface proteins, including IAP. SMCs express both IAP and \( \alpha_v\beta_3 \), another TS-1 receptor, on their cell surface, both of which have been shown to be involved in the regulation of IGF-I signaling. We therefore wished to determine whether ligand occupancy of IAP by TS-1 accounts for its enhancing effect on IGF-I signaling and whether TS-1 interaction with IAP modified the ability of IAP to regulate IGF-I signaling via an alteration in the SHPS-1/SHP-2 regulatory pathway.
Materials and Methods
The IGF-IR β chain antibody, the insulin receptor substrate-1 (IRS-1) antibody, and the monoclonal phosphotyrosine antibody (PY99) were purchased from Santa Cruz (Santa Cruz, Calif). The polyclonal SHP-2 and SHPS-1 antibodies were purchased from Transduction Laboratories (Lexington, Ky). The antibody against the dual-phosphorylated (active) form of p42/p44 mitogen-activated protein kinase (MAPK) and the antibody against total p42/p44 MAPK protein were obtained from Cell Signaling Technology (Beverley, Mass). The monoclonal antibody against IAP, B6H12, was purified using protein G Sepharose from Pierce according to the manufacturer’s protocol from B cell hybridoma-conditioned media that had been obtained from cells purchased from the American Type Tissue Culture Collection, and a mouse isotype-specific control (IgG1) was purchased from Sigma Chemical Company (St Louis, Mo). Human IGF-1 was a gift from Genentech (South San Francisco, Calif); polyvinyl difluoride membranes (Immobilon P) were purchased from Millipore Corporation. Autoradiographic film was purchased from Eastman Kodak. FBS, DMEM, penicillin, and streptomycin were purchased from Life Technologies. All other reagents were purchased from Sigma Chemical Company unless otherwise stated. Porcine aortic SMCs were isolated and maintained as previously described.13

Synthesis of Cell-Permeable Synthetic Peptides
The peptide derived from the IAP binding domain of thrombospondin-1, termed 4N1K (KRFYVVMWKK), and the control peptide 4NGG (KRKYGGMWKK) were synthesized by step-wise solid-phase peptide synthesis method and purified by C18 reverse-phase high-performance liquid chromatography by the Peptide Synthesis Facility at the University of North Carolina, Chapel Hill.

Measurement of IGF-I–Stimulated DNA Synthesis
IGF-I–stimulated DNA synthesis was assessed as we have described previously.16 Cells were incubated with TS-1 (1 µg/mL), 4N1K (1 µg/mL), or the anti-IAP antibody B6H12 or control IgGl (4 µg/mL). After a 2-hour preincubation, IGF-I was added to each well as appropriate (50 ng/mL), and the incubation continued for an additional 36 hours. Changes in the incorporation of 3H thymidine into DNA were measured using a Beckman Scintillation Counter. Student’s t test was used to compare differences between treatments. The results shown represent the mean (±SEM) from three separate experiments.

Measurement of Cell Proliferation
The effect of 4N1K or 4NGG on IGF-I–stimulated cell proliferation was carried out essentially as we have described previously.4 A 2-hour incubation with 4N1K or 4NGG (both at a concentration of 1.0 µg/mL) was followed by the addition of IGF-I (100 ng/mL). Cells were then incubated for 48 hours, and final cell number in each well was determined. Student’s t test was used to compare differences between treatments. The results shown represent the mean (±SEM) from three separate experiments.

Cell Lysis Immunoprecipitation and Immunoblotting
Cells were incubated overnight in serum-free medium (SFM) with 0.5% BSA and then pretreated with 4N1K or 4NGG (both at a concentration of 1 µg/mL) or TS-1 (1 µg/mL) for 2 hours before adding IGF-I (100 ng/mL) directly to the plate for the length of time indicated in each Figure. Associations between proteins and tyrosine phosphorylation were determined by immunoprecipitation and immunoblotting of cell lysates as we have previously described.2 The lysis buffer contained 50 mmol/L Tris HCL (pH 7.5), 150 mmol/L NaCl, 1% NP40, 0.25% sodium deoxycholate, 1 mmol/L EGTA plus 1 mmol/L sodium orthovanadate, 1 mmol/L sodium fluoride, 1 mmol/L PMSF, 1 µg/mL pepstatin A, 1 µg/mL leupeptin, and 1 µg/mL aprotinin. Cell lysates were incubated overnight at 4°C with the appropriate antibody (IGF-IR, SHPS-1, or IRS-1 using a 1:500 dilution). For immunoblotting, one of six primary antibodies (IGF-IR, SHP-2, SHPS-1, IRS-1, B6H12, or PY99) was used at a dilution of 1:500. Binding of the peroxidase-labeled secondary antibody was visualized using enhanced chemiluminescence following the manufacturer’s instructions (Pierce), and the immune complexes were detected by exposure to autoradiographic film or using the GeneGnome CCD imaging system (Syngene Ltd).

Chemiluminescent images obtained were scanned using a Duoscan T1200 (AGFA), and band intensities of the scanned images were analyzed using NIH Image, version 1.61. The Student’s t test was used to compare differences between treatments. The results shown in each Figure are representative of at least three separate experiments.

Assessment of p42/p44 MAPK Activation
The effect of TS-1, 4N1K, and 4NGG on IGF-I activation of p42/p44 MAPK were determined by immunoblotting with an antibody specific for the dual-phosphorylated (threonine [202] and tyrosine [204]) protein (at a dilution of 1:1000) as we have previously described.16

Results
TS-1 Enhances IGF-I–Stimulated DNA Synthesis via Its Interaction With IAP
We previously reported that TS-1 can enhance IGF-I–stimulated SMC proliferation.9 Because we have also determined that SMCs express both IAP12 and α1β2 on their cell surface, we wished to specifically determine whether TS-1 binding to IAP contributes to this enhancement effect. First, we determined whether the enhancement effect of TS-1 on IGF-I signaling could be inhibited by the anti-IAP antibody, B6H12, and second we determined whether a short (10-amino-acid) peptide (4N1K) containing the C-terminal domain of TS-1 could mimic the enhancing effect of TS-1. 4N1K has been shown to bind specifically to IAP. In Figure 1A, it can be seen that IGF-I stimulated a 1.9±0.3-fold increase (mean±SEM, n=3) in DNA synthesis compared with control cultures. It can also be seen in Figure 1A that TS-1 stimulated an additional significant increase in IGF-I–stimulated DNA synthesis (2.1±0.6-fold [mean±SEM, n=3], P<0.05 compared with cells treated with IGF-I alone). Preincubation with the anti-IAP antibody B6H12 resulted in complete inhibition of the ability of TS-1 to enhance the response to IGF-I, whereas an isotype-specific control antibody had no effect. This supports the conclusion that TS-1 is mediating its effects on IGF-I signaling via its interaction with IAP. In additional support of this conclusion, it can also be seen in Figure 1A that a significant increase in the cellular DNA synthesis response to IGF-1, comparable with that seen with TS-1, was also observed when cells were first incubated with 4N1K (a 1.97±0.6-fold increase [mean±SEM, n=3], P<0.05 compared with cells treated with IGF-I alone). These results suggest that the enhancement effect of TS-1 is mediated primarily through its binding with IAP and that 4N1K can replicate the enhancing effect of TS-1 in this test system. Dose-response experiments (data not shown) determined that at a concentration of 1 µg/mL, 4N1K reproducibly enhanced IGF-I–stimulated DNA synthesis to a similar extent to that observed with TS-1; therefore, this concentration of peptide was used throughout the study.
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Figure 1. TS-1 enhances IGF-I signaling in SMCs. A, DNA synthesis. Porcine aortic SMCs were pretreated with or without B6H12 or control IgG (4 μg/mL) and TS-1 or 4N1K (1 μg/mL) for 2 hours and then incubated with IGF-I (50 ng/mL) for 36 hours. The increase in 3H thymidine incorporation into DNA was then measured. Each data point represents mean±SEM from 3 separate experiments.

**P<0.05 when IGF-I treatment is compared with treatment without IGF-I. ‡P<0.05 when pretreatment with either TS-1 or 4N1K and IGF-I is compared with treatment with IGF-I alone. B, Activation of MAPK. Cells were pretreated as in panel A and then treated with IGF-I (100 ng/mL) for 10 minutes. p42/44 MAPK phosphorylation was determined by immunoblotting with a phosphospecific MAPK antibody. The total amount of MAPK in each sample was determined by immunoblotting with an MAPK antibody. C, IGF-I–stimulated receptor phosphorylation. Cells were pretreated as in panel A and then exposed to IGF-I (100 ng/mL) for 10 minutes. Cell lysates were immunoprecipitated with an anti–IGF-IR antibody and then immunoblotted with an antiphosphotyrosine antibody. To control for loading inequities, the relative amount of IGF-IR protein in each sample is shown in the bottom panel.

**TS-1 Enhances IGF-I–Stimulated MAPK Activation**

To determine whether TS-1 enhanced IGF-I–stimulated DNA synthesis by enhancing its activation of the Ras/Raf/MAPK pathway, we examined the dual phosphorylation of p42/p44 MAPK as an indicator of its activation state. Figure 1B shows that IGF-I stimulated a significant 2.2±0.2-fold (mean±SEM, n=3, P<0.05 compared with cells treated in SFM alone) increase in MAPK phosphorylation. When cells were preincubated with TS-1, the effect of the combination of MAPK activation was substantially greater than IGF-I alone. After 10 minutes of treatment with IGF-I, the cultures that had been exposed to TS-1 showed a 6.9±0.5-fold increase (mean±SEM, n=3) that was significantly greater than the effect of IGF-I alone (P<0.05). Cells preincubated with 4N1K had a 7.4±0.3-fold increase (mean±SEM, n=3) that was also significantly greater than in cells incubated with IGF-I alone (P<0.05).

**TS-1 Enhances IGF-I–Stimulated Receptor Phosphorylation**

Figure 1C shows that IGF-I stimulated a marked increase in receptor phosphorylation, however, if cells were preincubated with TS-1 or 4N1K IGF-I–stimulated receptor phosphorylation was significantly increased (1.7±0.1- and 1.6±0.13-fold increase, respectively [mean±SEM, n=3]; P<0.05 compared with cells treated with IGF-I alone). Because the addition of B6H12 alone alters IGF-IR phosphorylation,9 the effect of B6H12 on the TS-1–stimulated increase in receptor phosphorylation in response to IGF-I could not be tested. These results suggest that the enhancement effect of TS-1 is mediated via its interaction with IAP and that 4N1K can effectively replicate this effect in this system.

**IAP Binding Domain of TS-1, 4N1K, Stimulates an Increase in IGF-I–Stimulated Signaling**

Because 4N1K does not bind to other cell-surface receptors for TS-1, we used this peptide to additionally examine the effect of ligand occupancy of IAP on IGF-I signaling. To confirm that the effect of this peptide was specific, we determined the effect of 4N1K on IGF-I–stimulated cell proliferation. Figure 2A shows that IGF-I stimulates a significant 2.2±0.2-fold increase (mean±SEM, n=3; P<0.05) in cell proliferation after 48 hours. Importantly, this was not significantly different from cells preincubated with control peptide 4NGG (2.0±0.1, mean±SEM, n=3). Furthermore, when cells were preincubated with 4N1K, there was a significant increase in IGF-I–stimulated cell proliferation (an additional 1.7±0.2, mean±SEM, n=3, P<0.05 compared with cells incubated with IGF-I in the absence of 4N1K). These data suggest that the effect of 4N1K is specific and that it provides a useful reagent for examining the role of TS-1 binding to IAP in the absence of its interaction with any other cell-surface receptor.

We next examined the effect of 4N1K compared with 4NGG on both MAPK activation and the intensity and duration of IGF-IR phosphorylation. Figure 2B shows that IGF-I stimulated a 3.1±0.9-fold increase (mean±SEM, n=3; P<0.05 compared with cells treated with SFM alone). When cells were preincubated with 4NGG, IGF-I stimulated a 3.3±1.0-fold increase (mean±SEM, n=3) in MAPK phosphorylation that was not significantly different compared with cells treated with IGF-I alone. When cells were pretreated with 4N1K, IGF-I stimulated a 7.9±0.3-fold increase (mean±SEM, n=3) in MAPK phosphorylation, and this was significantly greater than cells treated with IGF-I alone (P<0.05).

Figure 2C shows that IGF-IR phosphorylation reached maximal phosphorylation at 10 minutes after IGF-I treatment (a 17±1.1-fold increase; mean±SEM, n=3) and that it decreased at 20 minutes after treatment. When cells were preincubated with 4NGG, the tyrosine kinase was activated and reached a level of phosphorylation that was equivalent to IGF-I alone at 10 minutes (16±0.9-fold increase;
Figure 2. IAP binding domain of TS-1 enhances IGF-I action in SMCs. A, Cell proliferation. After a 2-hour incubation with or without 4N1K or control peptide 4NGG (both at a concentration of 1 μg/mL), cells were treated with IGF-I (100 ng/mL) for 48 hours. Cell number in each well was then determined. Each data point represents the mean number of cells (×10⁶) of 3 independent experiments. **P<0.01 when IGF-I treatment is compared with treatment without IGF-I. A mean ± SEM, n=3). The intensity of phosphorylation decreased at 20 minutes to a level that was similar to that seen in the cells treated with IGF-I alone (42.3±3% decrease compared with 45.7±7% decrease when cells were incubated with IGF-I alone; P=NS). When cells were preincubated with 4N1K, the expected increase in IGF-IR phosphorylation at 10 minutes was present but the response was sustained for 20 minutes. In contrast to the 40±10% decrease noted in the presence of IGF-I alone, cells that had been preincubated with 4N1K had only a minimal decrease in IGF-IR phosphorylation (10±7% decrease; mean ± SEM, n=3). Compared with cells incubated with IGF-I alone, the difference in the extent of decrease was significant (P<0.05).

4N1K Reduces Recruitment of SHP-2 to the IGF-IR
We have shown that the duration of IGF-IR phosphorylation in response to IGF-I is determined by the rate of recruitment of SHP-2. Therefore, we next examined whether 4N1K results in an alteration in the time course or amount of SHP-2 recruitment to the IGF-IR. Consistent with our previous results, as shown in Figure 3, SHP-2 was recruited to the receptor after 20 minutes of exposure to IGF-I and there was no significant difference in the level of this recruitment when cells were preincubated with 4N1K. However, when cells were pretreated with 4N1K, the amount of SHP-2 recruited to the receptor at the 20-minute time point was significantly reduced by 51±0.4% (mean ± SEM, n=3, P<0.05). Importantly, SHP-2 recruitment was not completely inhibited. This reduced recruitment of SHP-2 was consistent with the sustained receptor phosphorylation noted in Figure 2C.

4N1K Prolongs IRS-1 Phosphorylation by Inhibiting SHP-2 Recruitment
A major scaffolding protein that is recruited to the activated IGF-IR is IRS-1. Recruitment of IRS-1 and its subsequent phosphorylation by the activated receptor results in recruitment of the Grb2/SOS complex, thereby activating the MAPK pathway.

Figure 4 shows a similar increase in IRS-1 tyrosine phosphorylation in response to IGF-I both in the presence and absence of 4N1K. If the cells were treated with IGF-I alone...
after 10 minutes, IRS-1 phosphorylation was decreased by 41±3.05% compared with the 5-minute time point (mean±SEM, n=3). However, when cells were pretreated with 4N1K, the level of IRS-1 phosphorylation at 10 minutes was not significantly different from that seen at the 5-minute time point (109±4.5%, mean±SEM, n=3). As shown in Figure 4, after 5-minute IGF-I stimulation, there was a 1.95±0.4-fold increase (mean±SEM, n=3) in SHP-2 recruitment to IRS-1; however, when cells were preincubated with 4N1K, there was no increase in SHP-2 recruitment at either 5 or 10 minutes.

**Ligand Binding to IAP Alters SHPS-1 Phosphorylation and SHP-2 Recruitment**

We next examined whether 4N1K binding altered SHP-2 transfer to IGF-IR via an alteration in SHPS-1 phosphorylation and SHP-2 recruitment. Figure 5 shows that SHPS-1 was phosphorylated in response to IGF-I. Maximum phosphorylation occurred after 5 minutes (11±1.6-fold increase; mean±SEM, n=3), and this coincided with the recruitment of SHP-2 to SHPS-1. In contrast to cells incubated in SFM alone, when cells were preincubated with 4N1K in the absence of IGF-I, SHPS-1 phosphorylation was detectable. However, in contrast to control cultures, there was no apparent increase in SHPS-1 phosphorylation after a 5-minute exposure to IGF-I. The level of SHPS-1 phosphorylation at 5 minutes after IGF-I addition in the presence of 4N1K was significantly lower (58.67±8.2% lower, P<0.05) than in the cultures not exposed to 4N1K, and this probably accounted for the lack of SHP-2 recruitment. However, in the presence of 4N1K after 10 minutes of exposure to IGF-I, an increase in SHPS-1 phosphorylation could be detected. This 4.9±1.0-fold increase (mean±SEM, n=3) was sufficient to result in the recruitment of SHP-2 to SHPS-1, because a 2.2±0.5-fold increase (mean±SEM, n=3) in SHP-2 association was detected at 10 minutes. This increase was similar (P=NS) to the 1.7±0.83-fold increase (mean±SEM, n=3) in SHP-2 association that was detected after 5 minutes of IGF-I treatment in the absence of 4N1K. Because the transfer of SHP-2 to IGF-IR was mediated via its recruitment to SHPS-1, the delay in SHPS-1 phosphorylation probably accounted for the delay in SHP-2 recruitment to IRS-1 and IGF-IR.

**TS-1 and 4N1K Reduce the Association of IAP With SHPS-1**

Because we have determined previously that the association of IAP with SHPS-1 is required for IGF-I–stimulated SHPS-1 phosphorylation, we examined whether the alteration in SHPS-1 phosphorylation in response to 4N1K could be attributable to a disruption in the association between the two proteins. In Figure 6, it can be seen that in confluent SMCs, basal association between IAP and SHPS-1 can be detected, as determined by coimmunoprecipitation. However, when cells were preincubated with either TS-1 or 4N1K, there was a marked decrease in the amount of IAP that binds to SHPS-1 (51.6±0.6% and 61±2.0% reductions, respectively; mean±SEM, n=3).

**Discussion**

TS-1 has been shown to act independently and in conjunction with growth factors such as endothelial growth factor and platelet-derived growth factor to stimulate SMC proliferation and chemotaxis. Our prior studies have shown that TS-1 can enhance IGF-I stimulation of SMC proliferation and IRS-1 phosphorylation. This study extends those earlier observations to demonstrate that the effect of TS-1 is mediated, at least in part, by its interaction with IAP. This was shown in two ways, first by blocking the enhancing effect of TS-1 on IGF-I-stimulated DNA synthesis using B6H12, an antibody previously shown to block TS-1 binding to IAP, and second by our results showing that 4N1K, a peptide derived from the IAP binding domain of TS-1, can enhance IGF-I–stimulated SMC DNA synthesis and cell proliferation.

TS-1 has been shown to increase chemotaxis of SMCs in an IAP-dependent manner because its effects could be blocked with an anti-IAP antibody (B6H12) and TS-1–induced cell spreading on vitronectin could be also be...
completely inhibited by B6H12. However, it has also been reported that TS-1–induced DNA synthesis was unaffected by B6H12 and that TS-1–stimulated activation of ERK-1 was inhibited by a β1 integrin antibody and heparin but not B6H12. These observations have led the authors to conclude that TS-1 activates different signaling pathways through distinct cell-surface receptors. Our results show that a domain of TS-1 that interacts with IAP can increase SMC DNA synthesis in response to IGF-I, apparently by increasing MAPK activation. This suggests that the response of the cell to TS-1 depends not only on the specific cell-surface proteins or receptors to which it binds but also on the activation state of other cell-surface molecules.

Our findings suggest that the mechanism accounting for TS-1 enhancement of responses to IGF-I is mediated by altering the rate of SHP-2 transfer. 4N1K binding to IAP resulted in a delay in the SHPS-1 phosphorylation response to IGF-1 and as a result a delay in SHP-2 recruitment. This was associated with a disruption in SHP-2 transfer to both IGF-IR and IRS-1 and was accompanied by prolonged IGF-IR and IRS-1 phosphorylation. After activation of the IGF-IR tyrosine kinase activity, several tyrosines are phosphorylated, thus generating binding sites for signaling intermediates such as IRS-1. After its recruitment to the receptor, IRS-1 is phosphorylated, and this leads to activation of the MAPK pathway. Our previous studies have shown that activation of the MAPK pathway is necessary for DNA synthesis in response to IGF-1. The prolonged phosphorylation of IRS-1 is therefore likely to be directly linked to the enhancement of IGF-IR–stimulated MAPK activation and DNA synthesis. Previous studies have suggested that SHP-2 dephosphorylation of IRS-1 may have a negative effect on insulin signaling. When the SHP-2 binding site of IRS-1 was mutated, it resulted in an increase in IRS-1 phosphorylation and protein synthesis in response to insulin. Similarly, expression of a catalytically inactive mutant of SHP-2 enhanced insulin-stimulated IRS-1 phosphorylation and glycogen synthesis. The negative effect of SHP-2 was attributed to its ability to dephosphorylate the tyrosine residues on IRS-1, to which signaling molecules such as the Grb2/SOS and p85/p110 complexes bind. This suggests that at least some of the positive effects of 4N1K could be mediated via its ability to delay SHP-2 recruitment to IRS-1. This could allow sustained binding of signaling molecules to IRS-1 and thereby enhance MAPK activation.

Our previous studies have shown that the response of SMCs to IGF-1 is regulated by several transmembrane proteins, namely the αβ3 integrin, SHPS-1, and IAP. This seems to be mediated, at least in part, by their ability to regulate the temporal and spatial distribution of SHP-2. After activation of IGF-IR, SHPS-1 is phosphorylated, and this recruits and activates SHP-2, from where it is transferred to the IGF-IR and other downstream signaling molecules. We have shown recently that when IAP binding to SHPS-1 is blocked, IGF-I–stimulated SHPS-1 phosphorylation is inhibited. This inhibition in SHPS-1 phosphorylation is associated with an inhibition in SHP-2 recruitment and transfer to IGF-IR. In this study, we have shown that ligand occupancy of IAP with TS-1 reduces but does not completely block IAP binding to SHPS-1. Given our previous observation that IAP binding to SHPS-1 is necessary for SHPS-1 phosphorylation in response to IGF-I, the partial inhibition of IAP binding to SHPS-1 is the mostly likely explanation for the delay in SHPS-1 phosphorylation after IGF-I treatment in cells that are exposed to TS-1. Because SHPS-1 phosphorylation is necessary for SHP-2 recruitment and transfer, alteration in the time course of SHPS-1 phosphorylation most likely accounts for the delay in SHP-2 transfer. In contrast to our previous study, in which we completely blocked SHPS-1 binding to IAP and thus inhibited IGF-I signaling, ligand occupancy of IAP with TS-1 or 4N1K does not completely inhibit IAP–SHPS-1 interaction, and this presumably accounts for the different outcome.

TS-1 stimulates SMC proliferation and migration, and increases in its expression have been associated with atherosclerotic lesion development, suggesting that TS-1 may play a role in the development of atherosclerosis. However, because TS-1 is a complex protein that interacts with many matrix components, several distinct cell-surface proteins, and receptors, the molecular pathways underlying its effects are likely to be complex. In vitro studies with human SMCs have shown that the proliferative response to TS-1 can be inhibited with an antibody that blocks its binding to IAP and that the effects of TS-1 can be mimicked by 4N1K. Our results additionally support the hypothesis that TS-1 and IGF-I could function coordinately in the progression of atherosclerotic lesion formation via the interaction of TS-1 with IAP. Therefore, an understanding of the molecular mechanism by which the two proteins interact may provide a novel therapeutic target that would allow simultaneous disruption of the actions of both proteins.

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References


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