SH2 Domain Containing Protein Tyrosine Phosphatase 2 Regulates Concanavalin A-dependent Secretion and Activation of Matrix Metalloproteinase 2 via the Extracellular Signal-regulated Kinase and p38 Pathways

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ABSTRACT

We investigated the role of SH2 domain containing protein tyrosine phosphatase (SHP) 2 in Concanavalin A (Con A)-dependent signaling that leads to the augmented secretion and activation of matrix metalloproteinase (MMP) 2. In cells expressing mutant SHP-2 in which 65 amino acids in the SH2-N domain were deleted, we found that production, secretion, and proteolytic activation of MMP-2 in response to Con A treatment was severely impaired. Under Con A stimulation, complex formation of SHP-2 with SOS-1 and Grb-2 together with the activation of Ras signaling was clearly observed in wild-type cells, but not in SHP-2 mutant cells. In wild-type cells, Con A-treatment activated dual signaling pathways, extracellular signal-regulated kinase (Erk) and p38, in a Ras-dependent manner, whereas Con A-dependent activation of these signaling pathways was absent in SHP-2 mutant cells. In addition, pretreatment of wild-type cells with U0126, a potent inhibitor for mitogen-activated protein kinase (ERK) kinase 1, or with SB203580, a specific inhibitor for p38, significantly inhibited the Con A-dependent secretion and activation of MMP-2. However, overexpression of active mitogen-activated protein/ERK kinase 1 in SHP-2 mutant cells could not induce clear activation of MMP-2 secretion, although these cells responded well to the Con A treatment in a p38-dependent manner. Finally, reintroduction of wild-type SHP-2 into SHP-2 mutant cells rescued Erk and p38 activation, and also MMP-2 secretion, whereas dominant-negative SHP-2 could block the Con A-dependent activation of Erk and p38. Taken together, our results strongly suggest that SHP-2 plays a critical role as a positive mediator for Con A-dependent activation of MMP-2 secretion via Ras-Erk and Ras-p38 signalings.

INTRODUCTION

The MMPs such as MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are a family of neutral proteases that catalyzes the destruction of extracellular matrix, and consequently promotes tumor invasion and metastasis (1, 2). Both MMP-2 and MMP-9 are secreted from cells as inactive zymogens and subsequently activated by proteolytic cleavage (3). A membrane-type MMP, MT1-MMP, catalyzes the proteolytic activation of MMP-2 (4). In human cancer tissues, accumulated evidence suggests that MMPs are secreted from the stromal fibroblasts; hence, it is widely used for the study of MMPs activation (6–8). We have reported previously that Ras-MAP kinase signaling played a critical role for the activation of MMP-2 secretion by various stimulants including Con A (7, 9–12). However, how Con A activates Ras-MAP kinase signaling to induce MMP-2 secretion remains to be clarified.

To obtain more clues, we investigated the role of SHP-2 in Con A-dependent MMP-2 secretion. SHP-2 is a widely expressed cytoplasmic tyrosine phosphatase with two SH2 domains (13, 14). SHP-2 is implicated in a wide variety of signaling elicited by growth factors, cytokines, hormones, antigens, and extracellular matrices (15), and activates Erk and Akt signaling (16–19). Although these studies showed the importance of SHP-2 signaling in MAP kinase and Akt regulation, all of them focused on the function of SHP-2 in cell growth, differentiation, and cell migration, and its role in tissue remodeling via the activation of MMP secretion remains to be explored.

In this study, we present evidence for the first time that SHP-2 is required for the augmented production, secretion, and proteolytic activation of MMP-2 by Con A-stimulation. In addition, we show that SHP-2-dependent activation of Ras signaling, as well as the dual MAP kinase signalings, Erk and p38, are required for the activation of MMP-2 secretion by Con A.

MATERIALS AND METHODS

Cell Culture, Plasmids Preparation, and Cell Transfection. Wild-type and SHP-2 mutant cells were maintained as described previously (20). COS7 cells were maintained in DMEM containing 5% FBS in a CO2 incubator at 37°C. The c-Myc epitope-tagged active mutant of MEK1 (MEK1EE: substituted Ser218 and Ser222 to Glu; Ref. 11) was ligated into pBabepuro vector (9). HA-tagged wild-type and dominant-negative SHP-2 plasmids were a gift from Akito Maeda (Institute for Liver Research, Kansai Medical University, Moriguchi, Japan). Transfection of the plasmids was performed as described previously (7). Clones were selected by puromycin.

Materials and Chemicals. Con A was purchased from HONEN (Tokyo, Japan); U0126 from Promega Corporation (Madison, WI); SB203580 from Sigma Chemicals (St. Louis, MO); FTI-277 from CALBIOCHEM Biosciences, Inc. (La Jolla, CA); Phospho p42/44, phospho p38, p38, phospho ATF, and ATF antibodies were purchased from Cell Signaling (Beverly, MA); anti-Erk2, anti-Grb-2, anti-SOS-1, and anti-SHP-2 (C-18) from Santa Cruz Biotechnologies (Santa Cruz, CA); anti-MMP-2 from CHEMICON International, Inc. (Temecula, CA); and anti Ras and anti SHP-2 mAb from Transduction Laboratories, Inc. Anti-Myc (9E10) was prepared as described (21).

Assay of MMP-2 Activity by Gelatin Zymography. The activities of MMP-2 in the conditioned media were assayed by zymography as described previously (22). Briefly, media were subjected to gel electrophoresis containing 0.03% gelatin. Gels were then washed twice and incubated overnight at 37°C in the reaction buffer [50 mM Tris-HCl (pH 7.4) and 10 mM CaCl2], stained with Coomassie brilliant blue, and destained.

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**RESULTS**

**Impairment of Con A-dependent MMP-2 Secretion and Activation in SHP-2 Mutant Cells.** We first examined the secretion and activation of MMP-2 by Con A stimulation in wild-type cells (SHP-2/+ +) and cells expressing mutant SHP-2 in which 65 amino acids in the SH2-N domain were deleted (SHP-2/−/−; Ref. 26). Serum-starved cells were incubated with the indicated concentrations of Con A for the indicated time periods. Conditioned media were collected to assay MMP-2 activity by gelatin zymography. As shown in Fig. 1, A and B, top panels, Con A treatment of cells induced a drastic increase in MMP-2 secretion and activation in wild-type cells in a time- and dose-dependent manner. In contrast, secretion and proteolytic activation of MMP-2 by Con A was severely impaired in SHP-2 mutant cells. In wild-type cells, increase in MMP-2 secretion was detected after 1 h of Con A stimulation, and additional increase was observed up to 96 h of incubation. Active form of MMP-2 appeared at 2 h of incubation, and approximately half of the secreted MMP-2 was in its active form after 24 h of incubation. In contrast to wild-type cells, MMP-2 secretion in SHP-2 mutant cells was observed after 6 h of stimulation, and even after 96 h, only a small fraction was in the activated form. The total amounts of secreted MMP-2 in SHP-2 mutant cells were much lower than that of the wild-type cells. It should be noted that, even after 96 h of incubation, the amount of the active MMP-2 secreted from SHP-2 mutant cells could not reach the level similar to that of wild-type cells at 24 h of incubation. These results strongly suggest that synthesis and secretion of MMP-2 in SHP-2 mutant cells is not delayed but suppressed compared with wild-type cells. At the concentration of 15–20 μg/ml of Con A, clear increase in secretion and activation of MMP-2 was observed in wild-type cells, whereas response to Con A in SHP-2 mutant cells was not clear. Immunoblotting of the conditioned media with anti-MMP-2 antibody also confirmed drastic reduction of MMP-2 secretion in SHP-2 mutant cells (Fig. 1, A and B, bottom panels).

To clarify whether the Con A-dependent secretion of MMP-2 was associated with increased intracellular production of MMP-2, we measured the intracellular level of MMP-2 in Con A-treated and untreated cells by immunoblotting with anti MMP-2. As shown in Fig. 1C, increase in intracellular levels of MMP-2 by Con A-stimulation was detected only in wild-type cells, but not in SHP-2 mutant cells. These results indicate that Con A also activates intracellular production of MMP-2 in a mechanism requiring functional SHP-2.

**Con A-dependent Activation of Ras Signaling Is Impaired in SHP-2 Mutant Cells.** We reported previously that expression of a dominant-negative form of Ras inhibited Con A-dependent secretion and activation of MMP-2 (7). To confirm the role of Ras in Con A-dependent signaling, we next examined the effect of FTI-277, a specific farnesyl transfer inhibitor (25), Con A-dependent secretion and activation of MMP-2. After 24-h pretreatment with FTI-277, wild-type cells were treated with 15 μg/ml Con A for an additional 1 h. As shown in Fig. 2A, FTI-277 treatment converted a major fraction of Ras into nonfarnesylated form (27). In addition, pretreatment of wild-type cells with FTI-277 strongly suppressed the Con A-dependent secretion and proteolytic activation of MMP-2, confirming the important role for Ras in this system (Fig. 2B).

To investigate whether Ras is a downstream signaling molecule of MMP-2 under Con A stimulation, we measured the relative amounts of active Ras in wild-type and SHP-2 mutant cells by *in vitro* binding to the GST-Raf fusion protein. As shown in Fig. 2C, Con A-stimulation markedly increased the relative amount of active Ras in wild-type cells. In contrast, the levels of active Ras in SHP-2 mutant cells remained unchanged after Con A-stimulation, although the basal level of active Ras was slightly higher in mutant cells as compared with that of wild-type.

To study the mechanism of SHP-2-dependent activation of Ras under Con A stimulation, we examined whether SHP-2 formed a complex with Grb-2 and SOS-1. Wild-type and SHP-2 mutant cells were stimulated with Con A for the indicated times. After stimulation, cell lysates were immunoprecipitated with anti-Grb-2 or anti-SHP-2. Immunoprecipitates were probed with anti-SHP-2, anti-SOS-1, or with anti-Grb-2. As shown in Fig. 3A, Con A-treatment of wild-type cells induced complex formation of Grb-2 with SHP-2 and SOS-1 in a time-dependent manner (Fig. 3A). In contrast, these complex formations in Con A-treated SHP-2 mutant cells were undetectable, even...
after longer exposure of the film (Fig. 3B). In untreated wild-type cells, Grb-2 did not show clear complex formation with SHP-2. However, Con A treatment of wild-type cells induced a complex formation of SHP-2 with Grb-2 within 5 min, and relative level of complex became peaked at 15 min and then decreased. In addition to SHP-2, SOS-1 was also coprecipitated with Grb-2 in wild-type cells treated with Con A. Although the Grb-2-SOS-1 complex formation increased with time, there is little binding of Grb-2 with SOS-1 without any stimulation that may be responsible for the base level activation of Ras. To confirm additionally, a similar set of cell lysates were immunoprecipitated with anti-SHP-2. Immunoprecipitates were probed with anti-SOS-1 or anti-SHP-2. As shown in Fig. 3, C and D, complex formation of SHP-2 with SOS-1 was observed in Con A-treated wild-type cells but not in SHP-2 mutant cells. These results suggest that SHP-2 regulates Ras signaling via complex formation with Grb-2 and SOS-1 under Con A treatment.

Activation of Erk and p38 by Con A Is Defective in SHP-2 Mutant Cells. To search the downstream effectors of Ras in Con A-dependent signaling, we next studied the Con A-dependent activation of Erk1/2 and p38 MAP kinases in wild-type and SHP-2 mutant cells by immunoblotting with antibodies specific for the phosphorylated forms of p44/42 Erk1/2 and p38 (Fig. 4). We found that, in wild-type cells, phosphorylation of Erk1/2 and p38 was dramatically activated by Con A in a time- and dose-dependent manner, whereas phosphorylation of these proteins was severely impaired in SHP-2 mutant cells. In wild-type cells, phosphorylation of Erk1/2 became detectable at 5 min of stimulation, and similar levels of phosphorylation were maintained with time up to 60 min (Fig. 4A). A dose of 10 μg/ml was sufficient to induce Erk1/2 phosphorylation in wild-type cells at 1 h of stimulation (Fig. 4B). Phosphorylation of p38 also became detectable after 5 min of stimulation, but increased with time (Fig. 4C). In the case of p38, a relatively lower dose was sufficient to induce the activation (Fig. 4D). These results indicate that SHP-2 is a positive mediator for Con A-dependent activation of Erk1/2 and p38.

To confirm the role of Ras in Con A-dependent signaling, we next examined the effect of FTI-277 on Erk and p38 signaling. After 24 h of pretreatment with FTI-277, wild-type cells were treated with 15 μg/ml Con A for additional 1 h. As shown in Fig. 2A, FTI-277 pretreatment with 1/ H9262 with 15/ H9262 C, untreated cells were collected after 24 h of Con A stimulation and subjected to zymography. C, wild-type and SHP-2 mutant cells were serum-starved overnight and stimulated with 15 μg/ml Con A for 1 h. The relative amounts of active Ras in the cell lysates were measured by GST pull-down assay using GST-Raf-1 fusion protein, followed by immunoblotting with anti-Ras as described in "Materials and Methods."
treatment converted a major fraction of Ras into nonfarnesylated form (27). Under the same condition, FTI-277 pretreatment substantially inhibited Con A-induced Erk and p38 phosphorylation (Fig. 5, A and B). These results indicate that SHP-2 regulates Con A-dependent Erk and p38 activation via the Ras pathway.

Requirement of Erk1/2 and p38 for Con A-induced Secretion and Activation of MMP-2. We next examined the role of Erk1/2 and p38 in Con A-dependent secretion and activation of MMP-2 by use of specific inhibitors for Erk and p38. Pretreatment of wild-type cells with U0126, a specific inhibitor for MEK1, completely blocked the Con A-dependent phosphorylation of Erk1/2 (Fig. 6A), and also strongly suppressed MMP-2 secretion and activation (Fig. 6C). Similarly, pretreatment of cells with a highly specific inhibitor for p38 kinase, SB203580, substantially suppressed Con A-dependent phosphorylation of ATF, a downstream effector for p38 (Fig. 6B), and, in turn, inhibited secretion and activation of MMP-2 by Con A (Fig. 6D). These results indicate that activation of dual signaling pathways, Erk and p38, are required for Con A-dependent MMP-2 secretion and its proteolytic activation. To additionally confirm the role of the dual signaling pathways in Con A-dependent MMP-2 secretion, we established a SHP-2 mutant cell line in which constitutively active form of MEK1 (MEK1EE) was overexpressed (Fig. 6E). As shown in Fig. 6F, overexpression of MEK1EE in SHP-2 mutant cells was not sufficient to activate MMP-2 secretion as compared with the parental SHP-2 mutant cells (Fig. 6E, Lanes 1 and 3). These results indicate that multiple signaling pathways are involved in MMP-2 secretion and activation by Con A. However, these cells exhibited relatively stronger response to Con A treatment than the parental cells, and both secretion and activation of MMP-2 in response to Con A is more prevalent in these cells. In addition, pretreatment of these cells with SB203580 again suppressed the Con A-dependent secretion and activation of MMP-2 (Fig. 6G). These results again suggest that activation of dual signalings, Erk and p38, is required for the efficient MMP-2 secretion and activation by Con A.

Rescue of the Mutant Phenotype after Reintroduction of Wild-Type SHP-2 in SHP-2 Mutant Cells. To confirm the role of SHP-2 in Con A-induced MMP-2 secretion, we investigated the effect of Con A stimulation on the SHP-2 mutant cells in which wild-type HA-tagged SHP-2 was reintroduced (C-10 and C-15). As shown in Fig. 7, Con A-dependent activation of Erk, p38, and secretion of MMP-2 in SHP-2 mutant cells was restored by the expression of SHP-2, although the activation levels were not as strong as the wild-type cells. This is possibly because of much lower expression of SHP-2 in the transfected clones as compared with the parental cells (Fig. 7A). In addition, mutant SHP-2 may also interfere with wild-type SHP-2.

To obtain more clues, effect of a dominant negative (CS) mutant of SHP-2 on Con A-dependent signaling was examined. Cos cells were transiently transfected with vector alone or with CS-SHP-2 (Fig. 8A). As shown in Fig. 8, B and C, expression of CS mutant substantially inhibited the Con A-dependent activation of Erk and p38. Collectively, our results strongly suggest that SHP-2 regulates Con A-dependent MMP-2 secretion and activation via the Erk and p38 pathways.

Fig. 6. Requirement of the Erk and p38 pathways for Con A-dependent secretion and activation of MMP-2. A–D, wild-type cells were serum-starved overnight and pretreated with 25 μM U0126 (U0126 +) or 20 μM SB203580 (SB +) for 1 h. Cells indicated as Con A (+) were stimulated with 15 μg/ml of Con A for 1 h in case of immunoblotting and for 24 h for zymography in the presence of the inhibitor. A, after 1 h of stimulation with 15 μg/ml of Con A in the presence or absence of U0126, cell lysates were collected and probed with anti-phospho Erk (top panel) or anti-Erk-2 (bottom panel). B, after stimulation with Con A in the presence or absence of SB203580, cell lysates were collected and probed with anti-phospho ATF (top panel) or anti-ATF (bottom panel). C and D, conditioned media were collected after 24 h of stimulation with 15 μg/ml of Con A in the presence and absence of inhibitors and subjected to zymography for MMP-2 activity. E–G, SHP-2 mutant cells stably expressing myc-tagged active MEK1 (MEK1EE) were prepared as described in “Materials and Methods.” E, cell lysates from parental SHP-2 mutant cells and SHP-2 mutant cells expressing MEK1EE were probed with anti-myc. F, SHP-2 mutant and SHP-2 cells expressing MEK1EE were stimulated with 15 μg/ml Con A (Con A +) or left untreated (Con A −), conditioned media were collected after 24 h and subjected to zymography. G, conditioned media were collected as in D and subjected to zymography.
DISCUSSION

Con A stimulation of cells can induce a drastic increase in the secretion and proteolytic activation of MMP-2; hence, it has been used as a model to study the critical signaling pathway. In this study, we demonstrated for the first time a critical role of SHP-2 in Con A-mediated secretion and activation of MMP-2. We found that Con A stimulation tremendously increased the secretion and activation of MMP-2 along with its intracellular production in wild-type cells, whereas this effect of Con A was severely impaired in the SHP-2 mutant cells, suggesting the critical role for SHP-2 in Con A-mediated MMP-2 secretion and activation. In exploring the molecular mechanism by which SHP-2 mediates Con A-dependent secretion and activation of MMP-2, we have shown that SHP-2-Ras-dependent activation of both Erk and p38 is required for increased secretion and proteolytic activation of MMP-2 by Con A stimulation. Suppression of both Erk and p38 activation by their specific inhibitors substantially inhibited MMP-2 secretion and activation. Moreover, expression of constitutively active MEK1 in SHP-2 mutant cells was not sufficient to induce MMP-2 secretion as compared with the parental control. In addition, although SHP-2 mutant cells showed very little or no response to MMP-2 secretion by Con A stimulation, these cells, when transfected with active MEK1, showed relatively stronger MMP-2 secretion and activation after Con A-treatment, and SB203580 again suppressed this effect of Con A stimulation. These results additionally confirm that activation of both Erk and p38 is required for increased MMP-2 secretion and activation by Con A-stimulation. SHP-2 mutant cells expressing active MEK1 showed relatively stronger response to Con A-dependent MMP-2 secretion because, in these cells, Erk signaling was constitutively activated, and Con A treatment activated some residual p38 activation, which was sufficient to induce secretion and activation of MMP-2. Regulation of MMP-2 secretion and activation seems to involve a complex process. Most studies with oncogenic transformation or chemical stimulation suggested that activation of Ras-MAP kinase was critical (7, 9, 10, 12), whereas Callejas et al. (28) suggested the involvement of multiple signaling pathways like protein kinase A, p39, Akt, and nuclear factor kB. Denkert et al. (29) reported that activation of p38, but not Erk, was required, whereas Smolian et al. (30) suggested that neither Erk nor p38 was critical. In contrast, Park et al. (31) described a critical role of Akt activation in regulating MMP-2 secretion in cancer cells. Our results suggest that simultaneous activation of both Erk and p38 via SHP-2-Ras signaling is critical for increased secretion and activation of MMP-2 by Con A. Moreover, we did not find the involvement of Akt in Con A-dependent MMP-2 secretion and activation (data not shown).

We examined the activation of Erk and p38 in wild-type and SHP-2 mutant cells. Activation of both Erk and p38 were defective in SHP-2 mutant cells, although Con A induced significant activation of these pathways in wild-type cells. The role of SHP-2 in signal transduction is highly complicated. This phosphatase acts to promote mitogenic stimulation of Erk activity (32). It is also a positive regulator for Akt (16, 18) or c-Jun NH2-terminal kinase activation (33), whereas being a negative regulator for c-Jun NH2-terminal kinase activation by cellular stress (32) or IFN-stimulated Janus-activated kinase/signal transducers and activators of transcription pathway (34). Moreover, it mediates cytokine-induced nuclear factor kB activation independently of all of these pathways (20). Our results suggest SHP-2 as a positive regulator for Con A-induced activation of Erk and p38 MAP kinases.
The most novel part of this report is that SHP-2 is a positive regulator for p38 activation. Some previous studies suggest that p38 is required for the activation of SHP-2, whereas requirement of SHP-2 in p38 activation has not been reported (35, 36). To our knowledge, this is the first report that SHP-2 is a positive regulator for p38 activation. Accumulated evidences suggest that SHP-2 plays an important role in connecting growth factor receptors or other tyrosine kinases to Ras-dependent signaling pathways (37–40). Our results are also consistent with these studies and suggest that SHP-2 might act to connect Con A-dependent Ras signaling to its receptor. Indeed, Zhao et al. (41) reported that SHP-2 binds directly with FZ receptors, a major receptor for Con A, supporting our observation. We found that SHP-2 forms a complex with Grb-2 and SOS-1, suggesting the involvement of Grb-2 and SOS-1 in SHP-2-dependent signaling. Although tyrosine phosphorylation of SHP-2 is observed frequently inactivates the complex involving Grb-2, we could not detect phosphorylation of SHP-2 under Con A stimulation. A third molecule might be required for the complex formation of SHP-2 with Grb-2 by Con A activation.

Taken together, our studies suggest an important role of SHP-2 in regulating secretion and activation of MMP-2 with Con A. Pharmacological interference of SHP-2 activity might be clinically useful in regulating certain types of cancers, although additional studies with tumor cell as well as cells transformed with oncogenes are required.

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REFERENCES

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