Antitumorigenic effect of plumbagin by induction of SH2-containing protein tyrosine phosphatase 1 in human gastric cancer cells

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Abstract. A recent study reported that plumbagin downregulated the activity of Janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) pathway to show various antitumor effects in multiple myeloma cells. We aimed in this in vitro study to demonstrate the inhibition of JAK2/STAT3 pathway by plumbagin through inducing SHP1 expression in the MKN-28 gastric cancer cell line. We performed western blot analysis to measure SHP1, phospho-JAK2/STAT3 level, and observed that plumbagin induced SHP1 expression and simultaneously downregulated phospho-JAK2/STAT3 in MKN-28 cells, with negative SHP1 expression. This effect was consistent when JAK2/STAT3 signaling was activated by interleukin-6 (IL-6), and ameliorated when cells were treated with prevanadate, a protein tyrosin phosphatase inhibitor. Furthermore, plumbagin significantly reduced gene expression of cyclin D1, vascular endothelial growth factor (VEGF)-1, Bcl-xl, survivin and matrix metalloproteinase-9 (MMP-9), known target products of STAT3 activation in gastric carcinogenesis by reverse transcription-polymerase chain reaction (RT-PCR). Several functional studies such as water soluble tetrazolium salt-1 (WST-1) assay, wound closure assay, Matrigel invasion assay and annexin V assay were also performed, and we validated the functional effect of plumbagin for inhibition of cell proliferation, migration and invasion, and induction of apoptosis. Collectively, our findings suggest that plumbagin is a potential regulator of cellular growth, migration, invasion and apoptosis by inhibiting both constitutive and inducible STAT3 activity through induction of SHP1 in gastric cancer cells.

Introduction

Gastrointestinal cancers account for a large portion of cancer-related death worldwide, including Korea. Among them, gastric cancer is still one of the most common leading causes of death, and if it is diagnosed in advanced or metastatic stage, the overall prognosis is still poor even though there has been rapid progress for therapeutic modalities of gastric cancer, including surgery or chemotherapy. Thus, there is a need to find novel therapeutic agents effective against malignant gastric disease, especially for advanced or metastatic cancer.

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) is a quinonoid constituent extracted from the roots of the medicinal plant Plumbago zeylanica L (1). The roots of Plumbago zeylanica have been used for various treatment aims in Oriental medicine fields. One promising effect of plumbagin is that it shows antitumor potential against various types of cancer. For example, an in vivo study demonstrated that plumbagin significantly inhibited the growth of azoxymethane-induced intestinal tumors in rats (2), and several in vitro studies reported that plumbagin showed anti-carcinogenic effects including cell proliferation or invasion or induced cell cycle arrest or apoptosis in breast cancer (3), melanoma (4), non-small lung cancer (5) or prostate cancer cells (6).

Several pivotal studies have clearly demonstrated the molecular mechanisms of antitumor effects of plumbagin in various types of cancer cells. Hafeez et al reported that plumbagin inhibited constitutive expression of epidermal growth factor receptor (EGFR), phosphorylation and DNA binding activity of signal transducer and activator of transcription 3 (STAT3) and nuclear factor-xB (NF-xB) in pancreas cancer cells (7). Manu et al showed that plumbagin has a potential blocking activity of CXC chemokine receptor 4 (CXCR4) and potential for inhibition of invasion and migration in breast and gastric cancer cells (8). A recent in vitro study investigated the underlying mechanism of plumbagin in gastric cancer...
cells, and demonstrated that plumbagin inhibited NF-κB p65 nuclear translocation and phosphorylation of p65, IκBα and IkBα kinase (IKKα), and downregulated NF-κB-related gene products, such as inhibitor of apoptosis 1 (IAP1), X-linked inhibitor of apoptosis (XIAP), B-cell lymphoma-2 (Bcl-2), Bcl-XL and vascular endothelial growth factor (VEGF) (9). Another well-designed *in vitro* study showed that plumbagin suppresses STAT3 activation pathway through induction of SH2-containing protein tyrosine phosphatase 1 (SHP1), a non-receptor type protein tyrosine phosphatase (PTPase), in multiple myeloma cells (10). However, impact of plumbagin on STAT3 signaling pathway in gastric carcinogenesis has not been reported yet.

Previously, *in vitro*, we observed that SHP1 expression was markedly reduced or negative in various gastric cancer cell lines, which was mainly caused by epigenetic silencing mechanism, and exogenous introduction of SHP1 plasmid significantly downregulated Janus kinase 2 (JAK2)/STAT3 pathway and their target genes (unpublished data). From this background, we aimed in this *in vitro* study to demonstrate the ability of plumbagin to induce SHP1 expression and suppress JAK2/STAT3 signaling pathway in gastric cancer cells.

**Materials and methods**

**Reagents and cell line.** Plumbagin (purity >97%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and, dissolved in dimethyl sulfoxide (DMSO) to a concentration of 100 mmol/l and stored at -20˚C, and diluted to indicated concentration immediately before use. Recombinant human interleukin-6 (IL-6) and broad-acting PTPase inhibitor sodium pervanadate was purchased from Sigma-Aldrich. A rabbit polyclonal IgG antibody against human SHP1 (sc-287) and β-actin (sc-47778) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Mouse monoclonal IgG antibodies against human STAT3 (no. 9139) and phospho-STAT3 (Tyr 705, no. 4113), and rabbit polyclonal antibodies against human JAK2 (no. 3230) and phospho-JAK2 (Tyr 1007/1008, no. 7771) were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA).

The human gastric cancer cell line (MKN-28) was obtained from Korean Cell Line Bank (Seoul National University, Seoul, Korea), and cultured in RPMI supplemented with 10% heat-inactivated FBS and penicillin/streptomycin (1.0%) (all from Gibco, Carlsbad, CA, USA). Cells were incubated in a humidified atmosphere of 5% CO₂ at 37˚C.

**Western blot analysis.** Total 80-100 µg of cytoplasmic proteins were extracted using Celllytic M (C2978; Sigma-Aldrich) with Complete Mini (pretase inhibitor cocktail; Roche Diagnostics GmbH, Mannheim, Germany). Primary antibodies were diluted at 1:1,000 in the blocking buffer (Tris-buffered saline with Tween-20; Biosesang, Gyeonggi, Korea) containing 5% non-fat skim milk (Difco; Becton-Dickinson and Co., Sparks, MD, USA). Probed membranes were incubated for 12 h at 4˚C. The membranes were incubated with goat anti-mouse or anti-rabbit IgG as a secondary antibody for 1 h at room temperature. The protein bands were detected by exposing membrane to enhanced chemiluminescence (Perkin-Elmer, Waltham, MA, USA) for 1 min.

**Reverse transcription-polymerase chain reaction (RT-PCR).** Total RNA was extracted from whole cells using TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA) method, and subsequently complementary DNA (cDNA) was produced by using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) and treatment with 1 unit of DNase (Promega Corp., Fitchburg, WI, USA). We performed RT-PCR by modifying a previously described method. In brief, 20 ng of prepared cDNA was used to make 25 µl of PCR product using EconoTaq™ Plus Green Master Mix (Lucigen Corp., Middleton, WI, USA). PCR was done under the following conditions: initial denaturation at 94˚C (2 min), followed by 30-40 cycles of denaturation at 94˚C (15 sec), annealing at 55˚C (15 sec), extension at 72˚C (15 sec) and final extension at 72˚C (10 min). The oligonucleotide sequences are summarized in Table I. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for each sample. PCR products (5 µl) were loaded on a 2% agarose gel, and positive bands were obtained by staining with ethidium bromide (Amresco LLC, Solon, OH, USA).

**Water soluble tetrazolium salt-1 (WST-1) cell proliferation assay.** To quantify the inhibitory effect of plumbagin on cellular proliferation, we used a commercial WST-1 assay kit (EZ-Cytox; DoGen, Seoul, Korea) as manufacturer's instructions (11). Briefly, 1x10⁴ MKN-28 cells/well were cultured in a 96-well plate at 37˚C for 24 h, and treated with plumbagin at 20 or 40 µM for 3, 6 and 9 h. We also cultured untreated cells for the same time period as a control. After treatment, 10 µl of WST was added in each well for 4 h, and absorbance at 450 nm was measured by an ELISA reader (Epoch; BioTek Instruments, Inc., Seoul, Korea). All the experiments were performed in triplicate.

**Wound healing assay.** After treated with plumbagin at 20 or 40 µM for 6 h, cells were equally seeded on a 6-well plate chamber, and after attachment, a monolayer wound was made using 200 µl pipette tip. The media were changed to remove floating debris, and the vertical distance between the sides of the wound was measured at 24 and 48 h after wound injury using software (12). All the experiments were performed in triplicate.

**Matrigel invasion assay.** Following treatment with plumbagin at 20 or 40 µM for 6 h, 4x10⁴ cells/well were placed into the 24-well Matrigel Invasion Chambers (BD Biosciences, Franklin Lakes, NJ, USA) in 2% FBS medium, and in the lower wells 10% FBS was added. After 24 h of incubation, filter membranes were stained with crystal violet, and the number of positive invading cells which penetrated through membrane pore was counted under x20 magnification in at least five randomly selected separate areas.

**Annexin V assay.** To compare the different percentage of apoptotic cells by plumbagin treatment, we used an Annexin V-FITC assay kit (Anse Technologies Co., Ltd., Seoul, Korea) according to the manufacturer's instructions. Briefly, 1x10⁶ MKN-28 cells/well were treated with 20 or 40 µM of plumbagin for 6 h, washed and resuspended in binding buffer, followed by staining with an Annexin V-FITC.
and propidium iodide (PI) solution for 10 and 30 min, respectively. After staining, samples were analyzed using FACSCalibur Flow Cytometer (Becton-Dickinson and Co., San Jose, CA, USA).

Statistical analysis. The SPSS ver. 19.0 (SPSS, Inc., Chicago, IL, USA) was used for all analyses. Continuous data are presented as mean ± standard deviation. A Student's t-test was performed for continuous data, and p<0.05 was considered as statistically significant.

Results

Plumbagin inhibits phosphorylation of constitutive STAT3 in MKN-28 cells. First, we investigated the effect of plumbagin to modulate the activity of JAK2/Stat3 signaling in the gastric cancer cells. MKN-28 cells were treated with different concentration (10, 20 and 40 µM) of plumbagin for 6 h, and western blot analysis was performed to measure phosphorylation level of JAK2 at tyrosine 1007/1008 and Stat3 at tyrosine 705. Plumbagin significantly inhibited phosphorylation of JAK2 and Stat3 from 20 µM, and in contrast, total JAK2 and Stat3 showed similar level regardless of plumbagin treatment, which supports that plumbagin negatively modulates JAK2/Stat3 activity mainly via dephosphorylation rather than protein degradation. Because SHP1 is one of non-transmembrane PTPase which negatively modulate JAK2/STAT3 signaling in epithelial cells (13), we also observed induction of SHP1 expression by plumbagin treatment starting at 20 µM (Fig. 1A).

Our data suggest that plumbagin might dephosphorylate and downregulate JAK2/STAT3 activity by induction of SHP1 expression in MKN-28 cells. We also observed the time-course to inhibit phosphorylation of JAK2/STAT3 and to induce SHP1 by treatment with 40 µM of plumbagin for indicated time points. Phosphorylation of JAK2/STAT3 was ameliorated at 6 h, whereas SHP1 expression appeared at 3 h, which suggests that a time-lag might exist between induction of SHP1 and downregulation of JAK2/STAT3 activity in MKN-28 cells (Fig. 1B).

Inhibition of SHP1 restores phosphorylation of JAK2 and STAT3 in MKN-28 cells. Because we observed that SHP1 was implicated in the dephosphorylation and inactivation of JAK2/STAT3 signaling, for the next step, we validated this mechanism by using PTPase inhibitor sodium pervanadate (10,16,17). Treatment with indicated concentration of pervanadate and 40 µM of plumbagin for 6 h, phosphorylated STAT3 ameliorated even in 60 min stimulation with 50 ng/ml of IL-6, and SHP1 expression was restored during stimulation (Fig. 3). These findings suggest that plumbagin can also suppress IL-6-induced STAT3 phosphorylation as well as constitutive STAT3 phosphorylation, and SHP1 might play a critical role in this process.

Table I. Characteristics of primers for RT-PCR.

<table>
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<th>Gene</th>
<th>Primers (5’→3’)</th>
<th>GenBank accession no.</th>
<th>Size (bp)</th>
<th>Reference</th>
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<td>SHP1</td>
<td>F: ACTGGGAGCTGCACTCTGAGG</td>
<td>NM_080549.2</td>
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<td>(31)</td>
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<td></td>
<td>R: CCCCCTCTGACGGAGAGC</td>
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<tr>
<td>Cyclin D1</td>
<td>F: TCCGGGATGATTGGAAATGC</td>
<td>XM_006718653.1</td>
<td>150</td>
<td>(44)</td>
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<td></td>
<td>R: TGGTACGGTGTTCTTGGAG</td>
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<td></td>
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<tr>
<td>VEGF-1</td>
<td>F: GGAGTGTGTCGCCCCACGGAGGTCCAC</td>
<td>NM_001287044.1</td>
<td>343</td>
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<tr>
<td>Survivin</td>
<td>F: TTTCTGCCACATCTGTGATCG</td>
<td>NM_001012271.1</td>
<td>391</td>
<td>(46)</td>
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<tr>
<td></td>
<td>R: TGTCGAGGAAGCCTTCTTGT</td>
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<tr>
<td>MMP-9</td>
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<td>NM_004994.2</td>
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<td></td>
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<tr>
<td>GAPDH</td>
<td>F: GGTCTCTCTGACTTCAACA</td>
<td>NM_002046</td>
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<td>(48)</td>
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<td></td>
<td>R: AGCCAAATTCGTTGTCATAC</td>
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Plumbagin inhibits IL-6-induced STAT3 phosphorylation in MKN-28 cells. Previous in vitro studies demonstrated that stimulation with human recombinant IL-6 upregulates phosphorylated STAT3 level to promote invasive activity in gastric cancer cells (14,15). Thus, we investigated whether plumbagin could modulate IL-6-induced phosphorylation of STAT3 in gastric cancer cells. MKN-28 cells were stimulated with 50 ng/ml of IL-6 at indicated time points (30 and 60 min), and we observed that phosphorylated STAT3 was significantly upregulated from 30 min, whereas SHP1 expression continued to be weakly positive. However, treatment with 20 and 40 µM of plumbagin for 6 h, phosphorylated STAT3 ameliorated even in 60 min stimulation with 50 ng/ml of IL-6, and SHP1 expression was restored during stimulation (Fig. 2). These findings suggest that plumbagin can also suppress IL-6-induced STAT3 phosphorylation as well as constitutive STAT3 phosphorylation, and SHP1 might play a critical role in this process.

RT-PCR, reverse transcription-polymerase chain reaction; SHP1, SH2-containing protein tyrosine phosphatase 1; VEGF-1, vascular endothelial growth factor-1; MMP-9, matrix metalloproteinase-9; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. F, forward; R, reverse.
also support our suggestion that SHP1 might be closely related to the mechanism of plumbagin-induced inhibition of JAK2/STAT3 activity in MKN-28 cells.

Plumbagin downregulates STAT3-associated target genes in MKN-28 cells. In various human malignancies including gastric cancer, STAT3 is commonly activated and acts as a pivotal transcription factor to upregulate multiple target genes involving proliferation, invasion/metastasis and anti-apoptosis, such as VEGF-1, matrix metalloproteinase-9 (MMP-9), Bcl-xL, survivin or cyclin D1 (18). To investigate the effect of plumbagin in regulating target gene expression related to STAT3 pathway in gastric cancer cells, treatment with 20 or 40 µM of plumbagin was performed for 6 h with or without stimulation of IL-6, and by RT-PCR. Plumbagin restored SHP1 expression in both constitutive and IL-6-stimulated conditions, and reduced gene expression of VEGF-1, MMP-9, Bcl-xL, survivin and cyclin D1, which were maximally reduced by 40 µM concentration (Fig. 4). These findings suggest that plumbagin modulates mRNA expression of STAT3-related target genes via restoration of SHP1 expression in MKN-28 cells.

Plumbagin inhibits cell proliferation, migration and invasion, and induces apoptosis in MKN-28 cells. To determine functional effects of plumbagin for STAT3-related cellular proliferation, migration, invasion and apoptosis in gastric cancer cells, MKN-28 cells were treated with 20 or 40 µM of plumbagin. By performing WST-1 cell proliferation assay, we observed that plumbagin significantly inhibited cell proliferation in a time- and dose-dependent manner, with maximal inhibitory effect at 40 µM in 6 h treatment (Fig. 5). After treatment with plumbagin for 6 h, we made wound injury in a 6-well plate using a pipette tip, and we observed that plumbagin significantly reduced the relative number of cells after 24 and 48 h after injury and this inhibitory effect was more prominent at 40 than 20 µM of plumbagin (Fig. 6). We treated MKN-28 cells with 20 or 40 µM of plumbagin for 6 h, and cultured for 24 h in Transwell plate with a pore membrane, and then fixed and stained the cells by crystal violet. We also observed that plumbagin significantly reduced the relative number of cells.

Figure 1. Effect of plumbagin on constitutive Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) activity and SH2-containing protein tyrosine phosphatase 1 (SHP1) expression. (A) Reduction of p-JAK2/p-STAT3 and restoration of SHP1 expression by various concentrations of plumbagin. MKN-28 cells (2x10⁶) were treated with 10, 20 and 40 µM of plumbagin for 6 h, and then whole cell extracts were collected and probed for p-JAK2, p-STAT3 and SHP1 antibody. (B) Modulation of p-JAK2/p-STAT3 and SHP1 expression by plumbagin at various time points. MKN-28 cells (2x10⁶) were treated with 40 µM of plumbagin for 3 and 6 h, and then proteins were prepared and probed for p-JAK2, p-STAT3 and SHP1 antibody.

Figure 2. Effects of plumbagin on interleukin-6 (IL-6) inducible signal transducer and activator of transcription 3 (STAT3) activity and SH2-containing protein tyrosine phosphatase 1 (SHP1) expression. (Left panel) MKN-28 cells (2x10⁶) were stimulated with 50 ng/ml of IL-6 for 30 and 60 min, and then p-STAT3 and SHP1 expression levels were analyzed by western blot analysis. (Right panel) MKN-28 cells (2x10⁶) were pre-treated with 20 and 40 µM of plumbagin for 6 h, and stimulated with 50 ng/ml of IL-6 for 60 min. Whole cell extracts were collected and analyzed for p-STAT3 and SHP1 expression.

Figure 3. Reversion of p-JAK2/p-STAT3 and amelioration of SH2-containing protein tyrosine phosphatase 1 (SHP1) by pervanadate. MKN-28 cells (2x10⁶) were pre-treated with 25 and 50 µM of protein tyrosine phosphatase (PTPase) inhibitor sodium pervanadate for 30 min, followed by 40 µM of plumbagin for 6 h. Whole cell extracts were used for analysis of p-JAK2, p-STAT3 and SHP1 expression by western blot analysis.
invading cells, and 40 µM of plumbagin was more effective than 20 µM (Fig. 7). Finally, we investigated pro-apoptotic effect of plumbagin by Annexin V assay, and relative number of apoptotic cells was significantly increased by 40 µM of plumbagin, rather than 20 µM (Fig. 8). Taken together, these findings suggest that plumbagin significantly inhibits cell

Figure 4. Effects of plumbagin in modulation of signal transducer and activator of transcription 3 (STAT3)-related target gene expression. (A) MKN-28 cells were treated with 20 or 40 µM of plumbagin for 6 h with or without stimulation with 50 ng/ml of interleukin-6 (IL-6) for 60 min. Total RNA was extracted from each sample and used for reverse transcription-polymerase chain reaction (RT-PCR) analysis. (B) Densitometric analysis of vascular endothelial growth factor (VEGF)-1, matrix metalloproteinase-9 (MMP-9), survivin and cyclin D1. Data are expressed as mean ± standard deviation, and obtained from triplicate. *P<0.05, compared with control, **p<0.05, compared with plumbagin 20 µM.
**Discussion**

In this *in vitro* study, we showed that plumbagin restored SHP1 expression to downregulated JAK2/STAT3 activity and their target genes, and consequently, led to anti-proliferative, migratory, invasive and pro-apoptotic effects in gastric carcinoma cells. To our knowledge, our study firstly demonstrates that plumbagin inhibits STAT3 pathway through induction of SHP1 activity in stomach cancer cells. Little has been reported about anti-cancer role of plumbagin in gastric cancer, and only few studies focused on its inhibitory effects on NF-κB or CCR4 pathway (8,9), or cytotoxic effect through generation of reactive oxygen species (ROS) (19). Also, recent studies reported several candidate molecules to downregulate JAK2/STAT3 activity and exhibit antitumor effect in gastric cancer cells (20-23). However, none of them showed molecular link between SHP1 and JAK2/STAT3 pathway in gastric cancer cells.

SHP1 is non-receptor-type PTpase, which is encoded by *PTPN6* gene located on human chromosome 12p13 (24), and

Figure 5. Effects of plumbagin for cellular proliferation. MKN-28 cells (1x10^4 cells/well) were treated with plumbagin at 20 or 40 µM for 3, 6 and 9 h. Data are the absorbance at 450 nm by an ELISA reader and expressed as mean ± standard deviation. All experiments were performed in triplicate. *P<0.05, compared with control, †p<0.05, compared with plumbagin 20 µM.

Figure 6. Effects of plumbagin for cell migration. (A) Representative images of wound closure. MKN-28 cells were treated with plumbagin at 20 or 40 µM for 6 h, and then cells were equally seeded on a 6-well plate chamber and a monolayer wound was made using 200 µl pipette tip. (B) Analysis of vertical wound distance. The vertical distance between the sides of the wound was measured at 24 and 48 h after wound injury. Data are presented as mean ± standard deviation. All experiments were performed in triplicate. *P<0.05, compared with control, †p<0.05, compared with plumbagin 20 µM.

Figure 7. Effects of plumbagin for cell invasion. (A) Representative images of Matrigel invasion assay. MKN-28 cells (4x10^4 cells/well) were treated with plumbagin at 20 or 40 µM for 6 h, and placed into the 24-well Matrigel invasion chambers. After 24 h, filter membranes were stained with crystal violet. White bar indicates 50 µm. (B) Analysis of invading cells. The number of positive invading cells was counted under x20 magnification. Relative number of positive cells was calculated by regarding the number of positive cells in control group as 100%. Data are presented as mean ± standard deviation. Cell counting was performed in at least five randomly selected separate areas. *P<0.05, compared with control, †p<0.05, compared with plumbagin 20 µM.
it has been reported as a negative regulator of JAK2/STAT3 activity by dephosphorylation of JAK2 and STAT3 to act as a PtPase (25,26). Previous studies demonstrated that SHP1 is inactivated by aberrant methylation of CpG island promoter in various hematopoietic malignancies, and their functional roles have been extensively investigated in hematopoietic cancer cells (27-30). However, only few studies reported CpG island promoter hypermethylation in epithelial cells such as colon cancer cells (13,31), and little is known about reduced gene expression or promoter hypermethylation of SHP1 in gastric cancer cells except that several studies briefly reported the methylation rate of CpG island promoter of SHP1 in gastric carcinoma tissues (32,33). In colon cancer cells, SHP1 expression was mainly regulated by DNA methylation and upregulated by DNA methyltransferase inhibitors such as 5-aza-2'-deoxycytidine (5-aza-dC). Furthermore, increased SHP1 expression by transfection with SHP1 plasmid vector or treatment with demethylating agent such as 5-aza-dC substantially decreased p-JAK2/p-STAT3 level (13). We observed previously that SHP1 expression was epigenetically regulated and closely related with STAT3 activity in gastric cancer cells (unpublished data). Thus, as the next step, we searched for a candidate molecule which can induce SHP1 to inhibit JAK2/STAT3 pathway.

Previous pivotal studies extensively investigated the crucial role of STAT3 for initiation and progression of gastric cancer. Persistent infection of CagA-positive *Helicobacter pylori* (*H. pylori*) strain activates constitutive STAT3 via chronic JAK2 activity, which in turn promotes target gene transcription associated with proliferation, invasion, metastasis and angiogenesis. In terms of gastric epithelial cells, IL-6 family ligands such as IL-6 and IL-11 is associated with chronic inflammation by CagA-positive *H. pylori* and development of gastric cancer (18,34). The suppressors of cytokine signaling (SOCS) family proteins such as SOCS-1 or -3 have been reported as important regulators of JAK2/STAT3 pathway by negative feedback mechanism (35,36). Numerous PtPases have been presented as promising targets to inhibit STAT3 signaling in various kinds of cancer, including SHP1 (37), SHP2 (38), PTP-1D (39) and PTEN (40). However, little is known about the expression level and roles of PtPase in gastric tumorigenesis, and their inhibitory action on STAT3 signaling is still controversial. Several previous studies have focused on the effect of SHP2, and an in vitro study demonstrated that phosphorylated CagA preferentially activates SHP2/extracellular signal-regulated kinase (ERK) pathway and induces cell growth inhibition (41), whereas immunohistochemical studies using human stomach tissues showed that SHP2 expression was significantly enhanced in *H. pylori*-infected gastric cancer (42,43). Previously, we observed that exogenous expression of SHP1 in gastric cancer cells significantly inhibited cellular proliferation, migration and invasion (unpublished data), however, this phenomenon should be further validated in immunohistochemical studies using human gastric carcinoma tissues. This study might further support the hypothesis that SHPI negatively regulates STAT3 activity because induction of SHP1 by plumbagin inhibited JAK2/STAT3 signaling and inhibition of SHP1 reversed the plumbagin effects on JAK2/STAT3 pathway.

In conclusion, our study suggests that plumbagin might have promising anti-cancer potential via upregulation of SHP1 expression and inhibition of JAK2/STAT3 pathway in gastric cancer cells, and SHP1 might be an alternative target.
to regulate STAT3 activity in gastric carcinogenesis. Further preclinical studies concerning the effects of plumbagin in STAT3 overexpressing gastric cancer should be performed, and also other promising agents which can upregulate SHP1 expression in gastric cancer cells need to be investigated.

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