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Original Article

Heparanase promotes human gastric cancer cells migration and invasion by increasing Src and p38 phosphorylation expression

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Abstract: Gastric cancer is one of the most common cancers and it remains difficult to cure, primarily because most cancer stem like cells possess higher capability of invasion and metastasis. Heparanase acts as a master regulator of the aggressive tumor phenotype in part by enhancing expression of proteins and activating signaling molecules. There were less associated with heparanase of molecular biology mechanism in human gastric cancer. We first evaluated the endogenous expression of heparanase in human gastric cancer cell lines and found Heparanase expression higher in SGC-7901 than MGC-803. Using the technology of RNAi in SGC-7901 cells down regulated heparanase gene, and reduced SGC-7901 cells migration and invasion. On the other hand, recombinant heparanase protein added in MGC-803 cells enhanced MGC-803 cell migration and invasion. The elevated cell migration and invasion were impaired by treatment of Src inhibitor pp2 or p38 inhibitor SB 203580. We further found that Stable knockdown of heparanase in SGC-7901 cells decreased phosphorylation of Src and p38. The phosphorylation of p38 was inhibited in response to pp2 treatment while the addition of SB 203580 to SGC-7901 cells did not change phosphorylation of Src. These data suggest that heparanase facilitates invasion and migration of human gastric cancer cells probably through elevating phosphorylation of Src and p38.

Keywords: Gastric cancer, heparanase, src kinase, p38 kinase, migration, invasion

Introduction

Gastric cancer is one of the most aggressive tumors and is frequently associated with lymph node metastasis, peritoneal dissemination, and hematogenous metastasis. Heparanase activity plays a decisive role in cell dissemination associated with cancer metastasis. It is an endo-D-glucuronidase that releases 5-7kDa fragments of heparan sulfate from intact heparan sulfate chains of proteoglycans and is known to have multiple important roles in promoting tumor growth, angiogenesis, and metastasis [1-3]. Moreover, up-regulation of heparanase mRNA and protein expression has been documented in a variety of primary human tumor biopsies whereas adjacent normal-looking tissue did not exhibit detectable levels of heparanase [4, 5]. Heparanase up-regulation correlates with increased lymph node and distant metastasis, and with increased microvessel density (MVD) and with reduced postoperative survival of head and neck cancer, colon cancer, pancreatic carcinoma, prostate cancer and cervix cancer patients [6, 7]. Our study demonstrates that Heparanase expression up-regulation is significantly related with increased invasion depth, tumor embolus in vessel, lymphnode metastasis, distant metastasis, clinical stage and with MVD of gastric cancer [8].

Some researchs had suggested that there are two mechanisms that heparanase enhances invasion, angiongenesis and metastasis of tumor: one is that heparanase can cleave heparan sulfate (HS) of heparan sulfate protoglycans (HSPGs), the main polysaccharide constituent of extracellular matrix (ECM) and base mem-

brane (BM) by its enzymatic activity. The cleavage of heparan sulfate by heparanase, results in disassembly of extracellular barriers, promoting cell migration which is also related with inflammation, invasion and metastasis of tumor; releasing heparan sulfate-bound bioactive angiogenic and growth-promoting factors, for example: bFGF, VEGF, KGF, HGF, uPA, and tPA, which can enhance angiogenesis and growth of tumor [8, 9]. The other is that heparanase can elicit signal transduction cascades to induced some factors to promote the invasion, angiogenesis and metastasis of tumor. However, signal transduction pathways regulated by heparanase are not clearly studied, which has been associated with aggressive stages of gastric cancer and is correlated with poor prognosis [10-13]. Therefore, the aim of the present study was to characterize heparanase functions in gastric cancer cells and to explore the possibly carcinogenic mechanism of their invasion and migration with Src kinase and p38 kinase of mitogen activated protein kinases (MAPKs).

Materials and methods

Cell line and regents

Human gastric carcinoma MGC-803 and SGC7901 cells line were obtained from The Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China). The cells were grown in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) without antibiotics at 37°C, 5% CO_2. Human recombinant heparanase protein (San Diego, CA) was diluted by PBS. The selective p38 (SB 203580) and Src (PP2) inhibitors were purchased from Calbiochem (San Diego, CA) and were dissolved in DMSO as stock solutions. Primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals were purchased from Sigma unless otherwise specified.

RNA isolation and reverse transcription-PCR

Total RNA was isolated after transduction with Trizol reagent (Invitrogen, USA). A nanodrop spectrophotometer (Gene, USA) was used to measure the concentration of total RNA. After oligo (dT)-primer reverse transcription of the RNA, the resulting single stranded cDNA was amplified using TaqDNA polymerase and buffer (Promega, Madison, WI) with two-step Reverse Transcription-PCR (RT-PCR) according to the manufacturer's instructions. GAPDH was used as an internal control. Heparanase primers: forward primer 5'-TTCGATCCCAAGAAGGAATCAAC-3', reverse primer 5'-GTAGTGATGC2CATGTAACTGAATC-3', amplified fragment length is 585 bp. GAPDH primers: forward primer 5'-GTCTTCACCACCATGGAGAAGGCT-3', reverse primer 5'-CATGCCAGTGAGCTTCCCGTTCA-3', amplified fragment length is 392 bp. cDNA was amplified on a PCR thermal controller with an initial denaturation at 95°C for 3 min, followed by cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, 30 cycles, and a final extension step of 72°C for 10 min. The amount of starting cDNA was adjusted using GAPDH intensity. The intensity of each band was quantified using Scion Image software (Scion, Frederick, MD). The experiment was repeated for three times.

Western blot

Cell total protein was extracted with CytoBusterTM Protein Extraction Reagent (Novagen). Twenty micrograms of total proteins were separated by SDS-PAGE and transferred onto a PVDF membrane. The membrane was then incubated with antibodies specific for p38, Src, heparanase, p-p38, p-Src and GAPDH were detected at dilution of 1:500, followed by horseradish peroxidase (HRP) conjugated anti-mouse IgG or anti-rabbit IgG at a dilution of 1:1000. Immunocomplexes were visualized by the DAB. Blots were developed using the enhanced chemiluminescence (ECL) reagents (Amersham Pharmacia, Bucking-hamshire, UK) and visualized using the Gene-Genius Imaging System (Syngene, Frederick, MD, USA).

Knockdown of heparanase by shRNA

Heparanase knockdown was performed using plasmid vector from Genechem (Shanghai, China). The plasmid vectors containing the following four different shRNA target sequences: shH1: 5'-CTGTCAGCCCGAATGGGAA-3', shH2: 5'-CTTGCCACCTTTAATGGAA-3', shH3: 5'-GCTTTATGTGGCTGGATAA-3', and shH4: 5'-GACTTCGATCCTTTACCTGA-3'. The scrambled sequence plasmid vector as control. Briefly, 150 000 cells were plated per six-well in 1640 with 10% FBS and were allowed to attach overnight. Equivocal amounts of shRNA vectors were incubated with Transfection Reagent (Lipofect 2000, Invitrogen) as the manufacturer's instructions. Cells

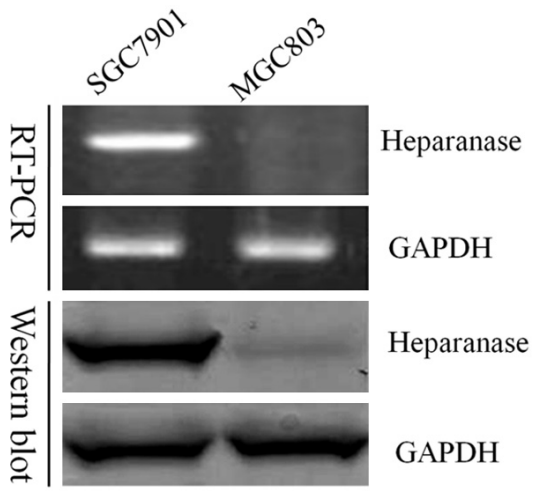


Figure 1. The expression of Heparanase in human gastric cancer cell lines: heparanase mRNA and protein expression is higher in human gastric cancer SGC-7901 cells than in human gastric cancer MGC-803 cells by RT-PCR and western blot, respectively. The results are the representative of three independent experiments.

were maintained for 48 h before experiments, the heparanase mRNA and protein expression of cells were assessed by RT-PCR and western blotting, respectively.

Scratch migration assay

SGC-7901 and MGC-803 cells cells were transfected in 6-well plate with shRNAs and post-transfection, small linear wounds were created by gently striking a pipette tip across the monolayers. The healing of the wounds through cell migration was assessed by measuring the wound distance. Human recombinant heparanase protein group (5 and 10 μg/ml), heparanase protein + pp2 group, heparanase protein + SB 203580 group and control group were set up; they were added for 3 hrs ahead of schedule. Cells migration into the wounded empty space was followed after 48 hrs and was detected. These experiments were repeated three times and mean values of migration distance were calculated.

Matrigel invasion assay

The Boyden chamber technique (transwell analysis of 6-well plate) was performed. Briefly, the 8 μm pore size filters were coated with 100 μl of 1 mg/ml matrigel (dissolved in serum-free RPMI1640 medium). 600 μl of RPMI-1640 medium containing 10% FBS was added to the lower chambers. 2 ml serum-free homogeneous signal cell suspensions (1×10^5 cells/mL) of SGC-7901 cells transfected with shRNAs for 48 h, and MGC-803 cells treated with 5 and 10 μg/ml human recombinant heparanase protein for 24 hrs, and control cells were added to the upper chambers, allowed to invade for 4 hrs at 37°C in a 5% CO_2 incubator. Remaining attached to the upper surface of the filters was carefully removed with cotton swabs. The invading cells on the lower surface were fixed with methyl alcohol and stained with H&E. The total number of cells from four microscopic fields selected at random (×200, magnification) was counted for each membrane and the mean number of cells was calculated in each field. The experiment was repeated three times.

Statistical analysis

The results are expressed as mean ± standard deviation. Statistical analysis was performed using student's *t* test by SPSS13.0 software. $P<0.05$ was considered statistically significant.

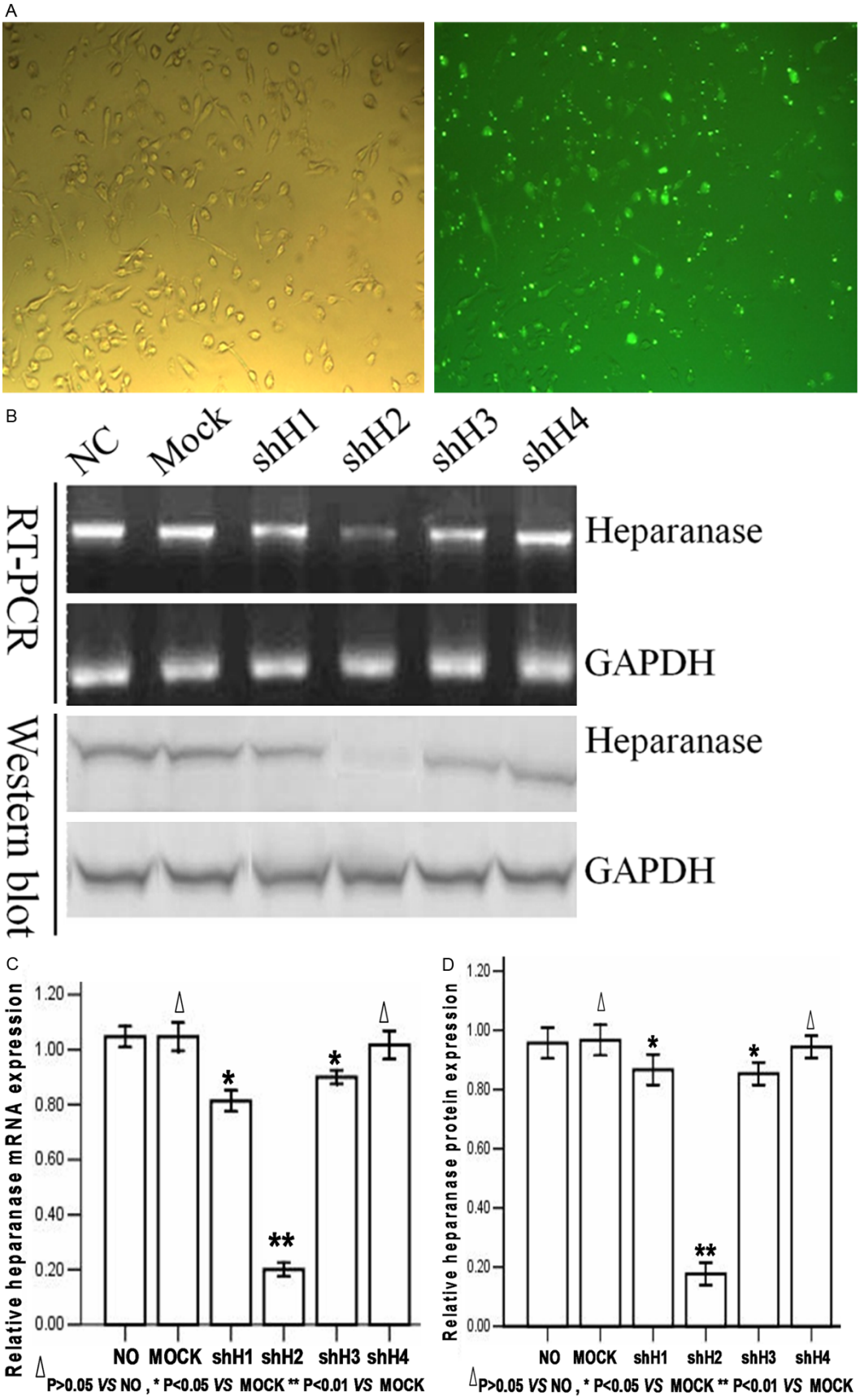
Results

Expression of heparanase in human gastric carcinoma cells

We first evaluated the endogenous expression of Heparanase in human gastric cancer cell lines, we found that human gastric cancer SGC-7901 cells contained high level of Heparanase mRNA and protein, and human gastric cancer MGC-803 cells contained low level of heparanase mRNA and protein by RT-PCR and western blot (**Figure 1**).

Down-regulation of heparanase abolished migration and invasion and decreases the expression of p-Src and p-p38

In order to clarify heparanase influencing invasion and migration of human gastric cancer cells correlated with promoting Src and p38 phosphorylation. First, we transfected shRNA vector (**Figure 2A**) and knocked down the heparanase expression in human gastric cancer SGC-7901 cells to observe the migration and matrigel invasion of SGC-7901 cells and the expression of p-Src and p-p38. We founded that heparanase expression was knocked down


A
B
NC
Mock
shH1
shH2
shH3
shH4
RT-PCR
Heparanase
GAPDH
Western blot
Heparanase
GAPDH
C
Relative heparanase mRNA expression
1.20
1.00
0.80
0.60
0.40
0.20
0.00
NO MOCK shH1 shH2 shH3 shH4
P>0.05 VS NO , * P<0.05 VS MOCK ** P<0.01 VS MOCK
D
Relative heparanase protein expression
1.20
1.00
0.80
0.60
0.40
0.20
0.00
NO MOCK shH1 shH2 shH3 shH4
P>0.05 VS NO , * P<0.05 VS MOCK ** P<0.01 VS MOCK

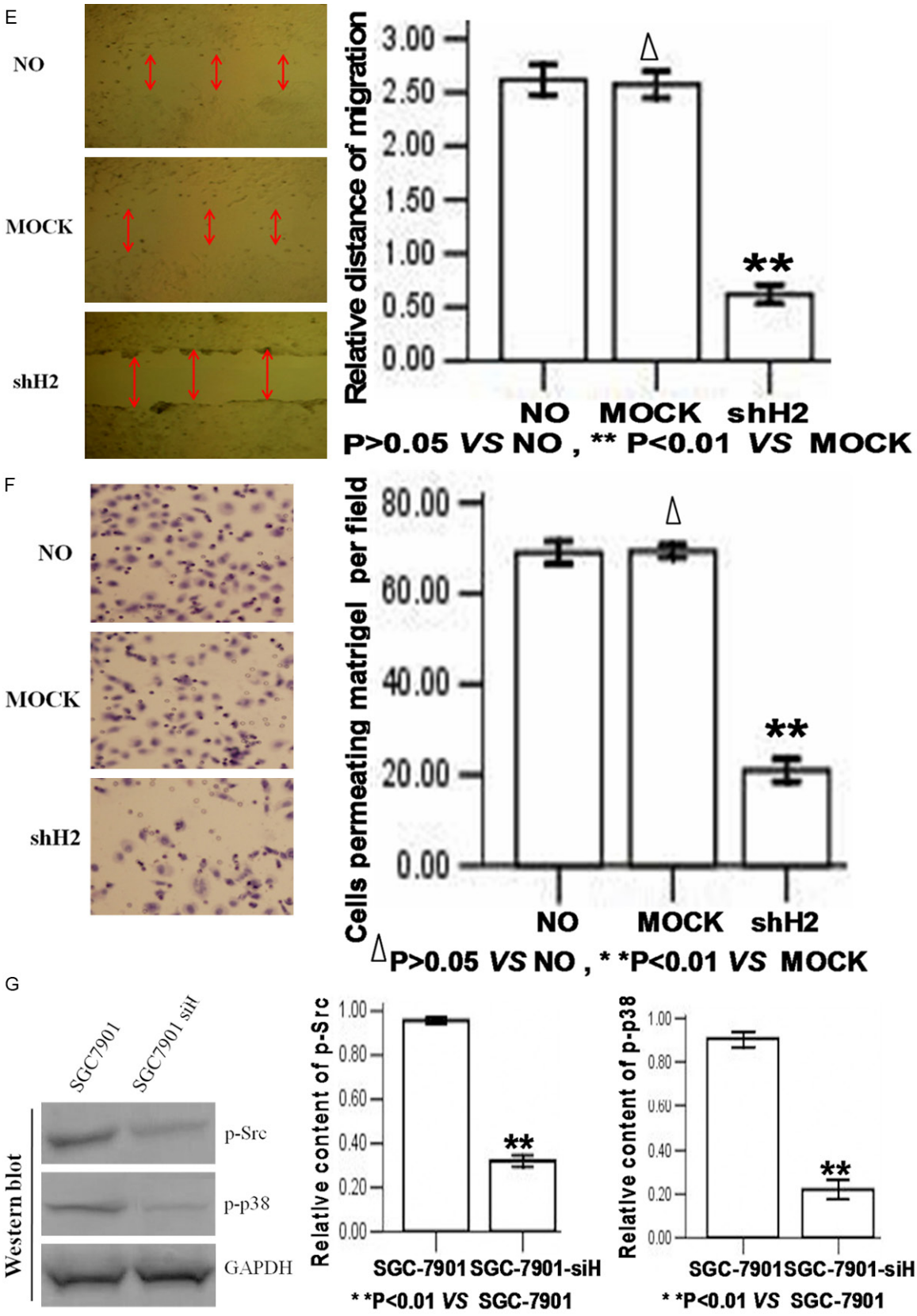


Figure 2. Validation of transduction efficiencies and knockdown efficacies of Heparanase shRNA-encoding vector in SGC-7901 cells. Knockdown of heparanase abolished migration and matrigel invasion of human gastric cancer cells and decreases the expression of phospho-Src (p-Src) and phospho-p38 (p-p38). A: 48 hrs post-transfection,

transduction efficiencies of shRNA vector were confirmed to be over 85% by fluorescence microscopic detection of vector expression of GFP. B-D: All four shRNA-encoding constructs and the knockdown efficacies were further confirmed by RT-PCR and Western blots for Heparanase. E: The scratch wound assays was performed. The experiment was repeated three times. Cell migration into the wounded empty space was followed after 24 hrs. The bar graph indicates the quantitative relative migration distance of human gastric cancer SGC-7901 cells. F: *In vitro* cell invasion through Matrigel was assessed in gastric cancer SGC-7901 cells; homogeneous single cell suspensions (1×10^5 cells/well) were added to the upper chambers and allowed to invade for 4 hrs. Migrated cells were stained with HE and examined by light microscopy. The results indicated that transfection of siH inhibited the invasion of SGC-7901 cells. The bar graph indicates the mean number of cells permeating matrigel per field. G: The protein was extract and western blot was performed to assay Src and p38 phosphorylation levels. The results indicate knockdown of heparanase decreases Src and p38 phosphorylation levels. The bar graph indicates the quantitative relative content of phospho-Src (p-Src) and phospho-p38 (p-p38). All the results are the representative of three independent experiments. A two sided *P*-value <0.05 was considered as statistically significant. ***P*<0.01 as significantly different from control group (Mock). Transfected with heparanase-specific shRNAs (shH) or non-target shRNA (mock). The untransfected cells served as a control (No).

significantly (approaching 80%) by shH2 of the selected shRNA sequences by RT-PCR and western blot (**Figure 2B-D**) and knockdown of heparanase abolished migration and matrigel invasion of human gastric cancer SGC-701 cells (**Figure 2E**, **2F**). To determine whether knockdown of heparanase altered Src and p38 activation, we quantified p-Src and p-p38 levels by western blot. p-Src and p-p38 was significantly decreased in heparanase knockdown human gastric cancer SGC-7901 cells compared to control (**Figure 2G**).

Heparanase protein enhanced the ability of migration and matrigel invasion and activation of Src andp38 phosphorylation

Scratch migration assay indicated that the migration distance of human gastric carcinoma MGC-803 cells was significantly longer in 5 μg/mL and 10 μg/mL human recombinant heparanase protein group than in control group (*P*<0.05; *P*<0.01), the migration distance was significantly longer in 10 μg/mL group than in 5 μg/mL group (*P*<0.05) (**Figure 3A**). These results suggested that human recombinant heparanase protein enhanced the migration capability of MGC-803 cells and the migration was enhanced with increasing heparanase protein concentration. In matrigel invasion assay, The number of human gastric carcinoma MGC-803 cells to invade through Matrigel-coated filters were statistically significantly increased in 5 μg/mL (*P*<0.05) and 10 μg/mL (*P*<0.01) heparanase protein group compared with control group, and 10 μg/mL group compared with 5 μg/mL group was significantly increased (*P*<0.05). These results demonstrated heparanase protein enhanced the matrigel invasion ability of gastric carcinoma MGC-803 cells in dose-dependent manner (**Figure 3B**). To determine whether human recombinant heparanase protein altered Src and p38 activation, we quantified p-Src and p-p38 levels by western blot. P-Src and p-p38 were significantly increased in human gastric carcinoma cells treated with 10 μg/mL human recombinant heparanase protein for 24 h by western blot assay (**Figure 3C**).

Src and p38 kinases inhibitors attenuated heparanase protein enhancing the migration and invasion of MGC-803 cells

The expression of p-Src and p-p38 protein were inhibited in human gastric carcinoma SGC-7901 and MGC-803 cells treated with 5 μmol/L pp2 and 1 μmol/L SB 20358 for 24 h by western blot assay, respectively. These results demonstrated that Src kinases inhibitor pp2 and p38 kinases inhibitor SB 203580 were able to inhibit phosphorylation of Src and p38 (**Figure 4A**, **4B**).

In the scratch test, MGC-803 cells were treated with 5 μmol/L pp2 or 1 μmol/L SB 203580 for 3 h, and then were added with 10 μg/mL recombinant human heparanase protein for 24 h. The results of cell migration distance were significantly shorter in 5 μmol/L pp2 or 1 μmol/L SB 203580 group, despite with heparanase together group compared with the 10 μg/mL heparanase protein alone (all *P*<0.01) (**Figure 4C**). In the matrigel invasion assay for 24 h with the same experimental group, the number of MGC-803 cells to invade through Matrigel-coated filters were statistically significantly decreased in 5 μmol/L pp2 or 1 μmol/L SB 203580, with 10 μg/mL recombinant human heparanase together group, compared with cells treated control group (All *P*<0.01) (**Figure 4D**). These results suggest that both src kinases inhibitor

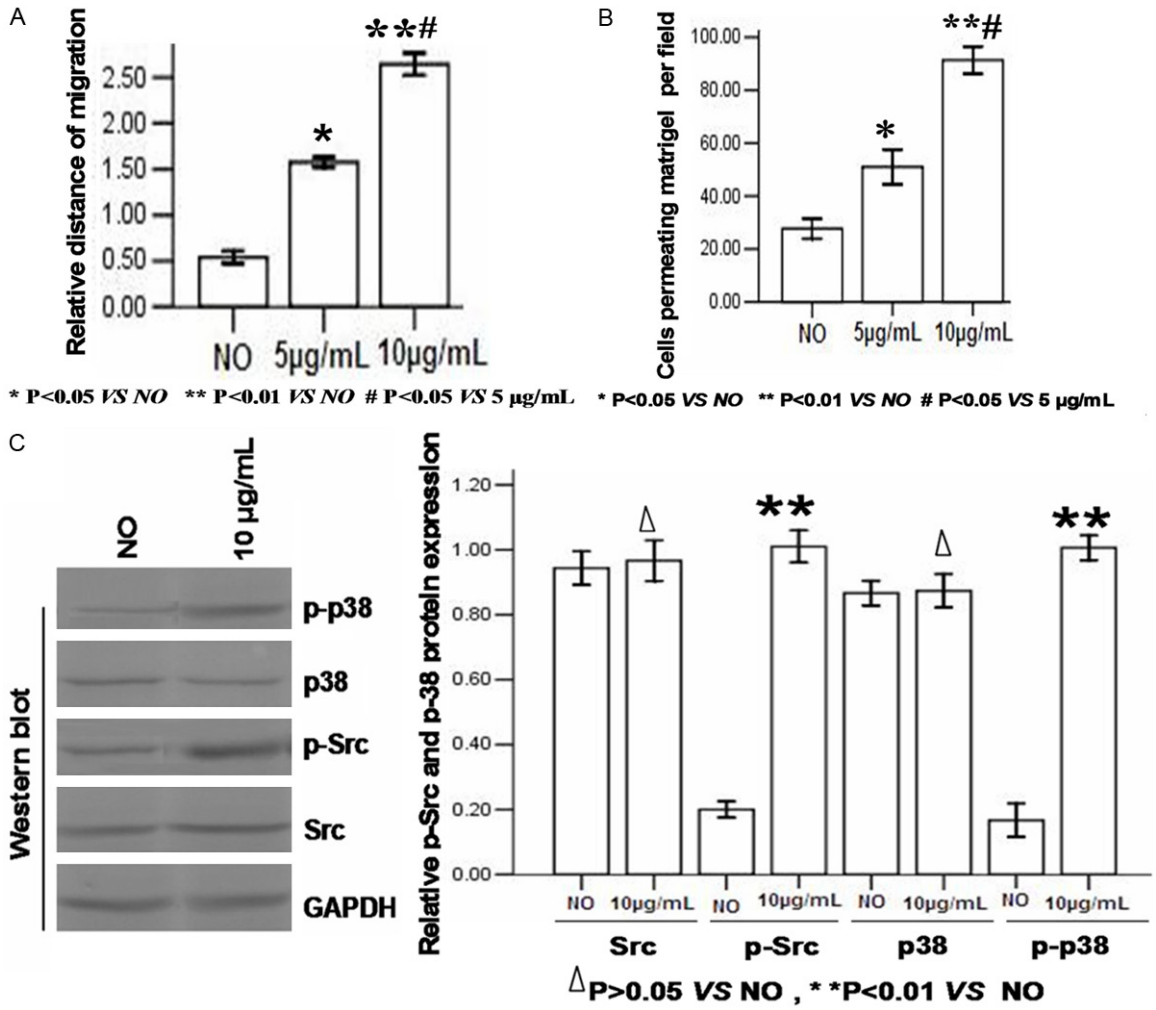


Figure 3. Human recombinant heparanase protein enhances the ability of migration and matrigel invasion and expression of p-Src and p-p38 protein of human gastric carcinoma cells. A: The migration distance was significantly longer in human gastric carcinoma MGC-803 cells treated with 5 μg/mL and 10 μg/mL human recombinant heparanase protein. The bar graph indicates the relative migration distance. B: In vitro cell matrigel invasion assay, the number of cells permeating matrigel per field was significantly higher in human gastric carcinoma MGC-803 cells treated with 5 μg/mL and 10 μg/mL human recombinant heparanase protein. The bar graph indicates the mean number of cells permeating matrigel per field. C: The expression of p-Src and p-p38 was higher in human gastric carcinoma MGC-803 cells treated with 5 μg/mL and 10 μg/mL human recombinant heparanase protein. The bar graph indicates the quantitative relative content of p-Src and p-p38. All the experiment was repeated three times. A two sided *P*-value <0.05 was considered as statistically significant. ***P*<0.01 as significantly different from control group (Mock). Transfected with heparanase-specific shRNAs (shH) or non-target shRNA (mock). The untransfected cells served as a control (No).

pp2 or p38 kinases inhibitor SB 203580 could attenuate human recombinant heparanase protein enhancing the migration and invasion of human gastric carcinoma MGC-803 cells.

Src and p38 kinases inhibitors effect on the expression of p38/Src protein phosphorylation and heparanase protein

Human gastric cancer SGC-7901 cells were treated with 5 μmol/L pp2 and 1 μmol/L SB 203580 for 24 h, respectively. The results showed the expression of heparanase protein did not significantly change by Western blot assay (**Figure 5A**). The expression of p-p38 was downregulation in human gastric cancer SGC-7901 and MGC-803 cells treated with the specific inhibitor pp2 of Src kinase (5 μmol/L) for 24 h (**Figure 5B**), but the expression of p-Src didn't significantly change in human gastric cancer cells treated with the specific inhibitor

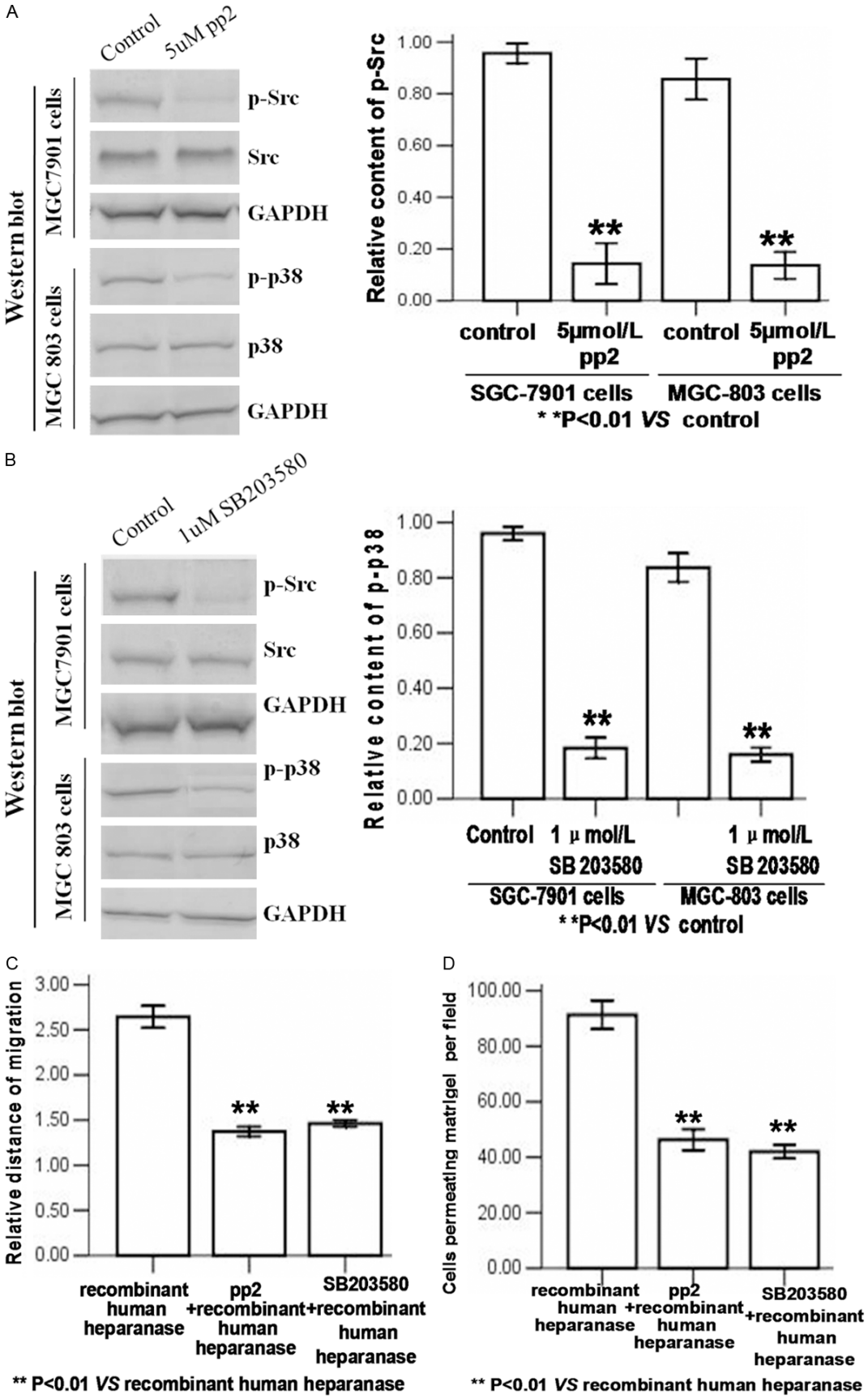

A
Control
5uM pp2
Western blot
MGC7901 cells
MGC 803 cells
p-Src
Src
GAPDH
p-p38
p38
GAPDH
Relative content of p-Src
1.00
0.80
0.60
0.40
0.20
0.00
**
control
5μmol/L pp2
SGC-7901 cells
MGC-803 cells
* *P<0.01 VS control
B
Control
1uM SB203580
Relative content of p-p38
Control
1 μ mol/L SB 203580
SGC-7901 cells
MGC-803 cells
* *P<0.01 VS control
C
Relative distance of migration
3.00
2.50
2.00
1.50
1.00
0.50
0.00
recombinant human heparanase
pp2 +recombinant human heparanase
SB203580 +recombinant human heparanase
** P<0.01 VS recombinant human heparanase
D
Cells permeating matrigel per field
100.00
80.00
60.00
40.00
20.00
0.00
** P<0.01 VS recombinant human heparanase

Figure 4. Src and p38 kinases inhibitors induced expression of p-Src and p-p38 protein and attenuated heparanase protein enhancing the migration and invasion of MGC-803 cells. A: The expression of p-Src was significantly inhibited in human gastric carcinoma SGC-7901 and MGC-803 cells treated with 5 μmol/L pp2 for 24 h compared with human gastric carcinoma SGC-7901 and MGC-803 cells treated without pp2 (NO) by western blot assay. The bar graph indicates the quantitative relative content of phospho-Src (p-Src). B: The expression of p-p38 was significantly inhibited in human gastric carcinoma SGC-7901 and MGC-803 cells treated with 5 μmol/L pp2 for 24 h compared with human gastric carcinoma SGC-7901 and MGC-803 cells treated without pp2 (NO) by western blot assay, but the expression of p38 was not influenced. The bar graph indicates the quantitative relative content of phospho-Src (p-Src). C: The distance of migration was significantly shorter in gastric cancer MGC-803 cell treated with 5 μmol/L pp2 and 10 μg/mL recombinant human heparanase together or 1 μmol/L SB 203580 and 10 μg/mL recombinant human heparanase protein together than in gastric cancer MGC-803 cell treated with the simple 10 μg/mL recombinant human heparanase protein. The bar graph indicates the relative distance of migration. D: In the matrigel invasion assay, the mean number of cells permeating matrigel per field was significantly decreased in gastric cancer MGC-803 cells treated with 5 μmol/L pp2 and 10 μg/mL recombinant human heparanase together or 1 μmol/L SB 203580 and 10 μg/mL recombinant human heparanase together compared with gastric cancer MGC-803 cells treated with simple 10 μg/mL recombinant human heparanase protein. The bar graph indicates the mean number of cells permeating matrigel per field. All the experiment was repeated three times. A two sided *P*-value <0.05 was considered as statistically significant.

SB 203580 of p38 kinase (1 μmol/L) (**Figure 5C**).

Discussions

A large number of studies have shown that heparanase promote tumor invasion, angiogenesis and metastasis. There are two mechanisms on this aspect. One is dependent on the activity of heparanase, and the other is that heparanase can cause cascade of the signal transduction, cause the expression of tumor invasion, angiogenesis and metastasis related factors, thereby promoting tumor invasion, angiogenesis and metastasis. But the signal transduction pathways regulated by heparanase remain be studied and this research is also currently a hot point.

Src is the first proto-oncogene discovered, but its oncogenic activity is weak, and large amount of data have been generated supporting the multiple function intracellular tyrosine kinases role of Src as a key messenger in many important cellular pathways. Src plays a central hub role in cellular signal transduction pathways, and it is a downstream and is activated from some upstream receptors and molecules and then transduce signals to downstream molecules, involved in tumor formation, growth, survival, cellular migration, metastasis, osseous metastasis and so on [14-18]. For example, Src receive signals from upstream platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), epidermal growth factor receptor2 (HER2), Met and interleukin-6 receptor (IL-6R) and is activated, and then activated Src activates signal transduction pathway including Ras, MAPK and signal transducer and activator of transcription3 (STAT3), and then they activates other factors to promote cellular proliferation and differentiation and are related with growth, invasion, metastasis, and angiogenesis [19-21]. Activated Src can up-regulate some factors expression including focal adhesion kinases (FAK), p130CAS and paxillin concerned with cellular migration and invasion to promote cells migration and conduce tumor metastasis [22, 23]. Activated Src can up-regulate HIF-1α and then HIF-1α can up-regulate VEGF, or Activated Src can directly up-regulate VEGF to promote angiogenesis [24].

MAPKs are a class of ubiquitous intracellular serine/threonine protein kinase. Some studies have confirmed that, MAPKs signal transduction pathway exists in most cells, which plays a crucial role in the process of transducing signal from the cell membrane to the nucleus and causing cell biological reactions. At present, multiple parallel MAPKs signaling pathway are found, different extracellular stimuli use different MAPKs signaling pathways. It has been found that the existence of the following three parallel MAPKs pathways in mammalian cells, extracellular signal-regulated kinase (ERK) signaling pathway, c-Jun N-terminal kinase (JNK) also known as stress-activated protein kinase (SAPK) signaling pathway and p38MAPKs pathway. Recent studies have shown that MAPKs signal transduction pathways are involved in cell migration and the induction of other proteases relative with invasion and metastasis of tumor and induce the expression of angiogenic factors relative with angiogenesis [25, 26]. For example, breast cancer cells with inhibition of

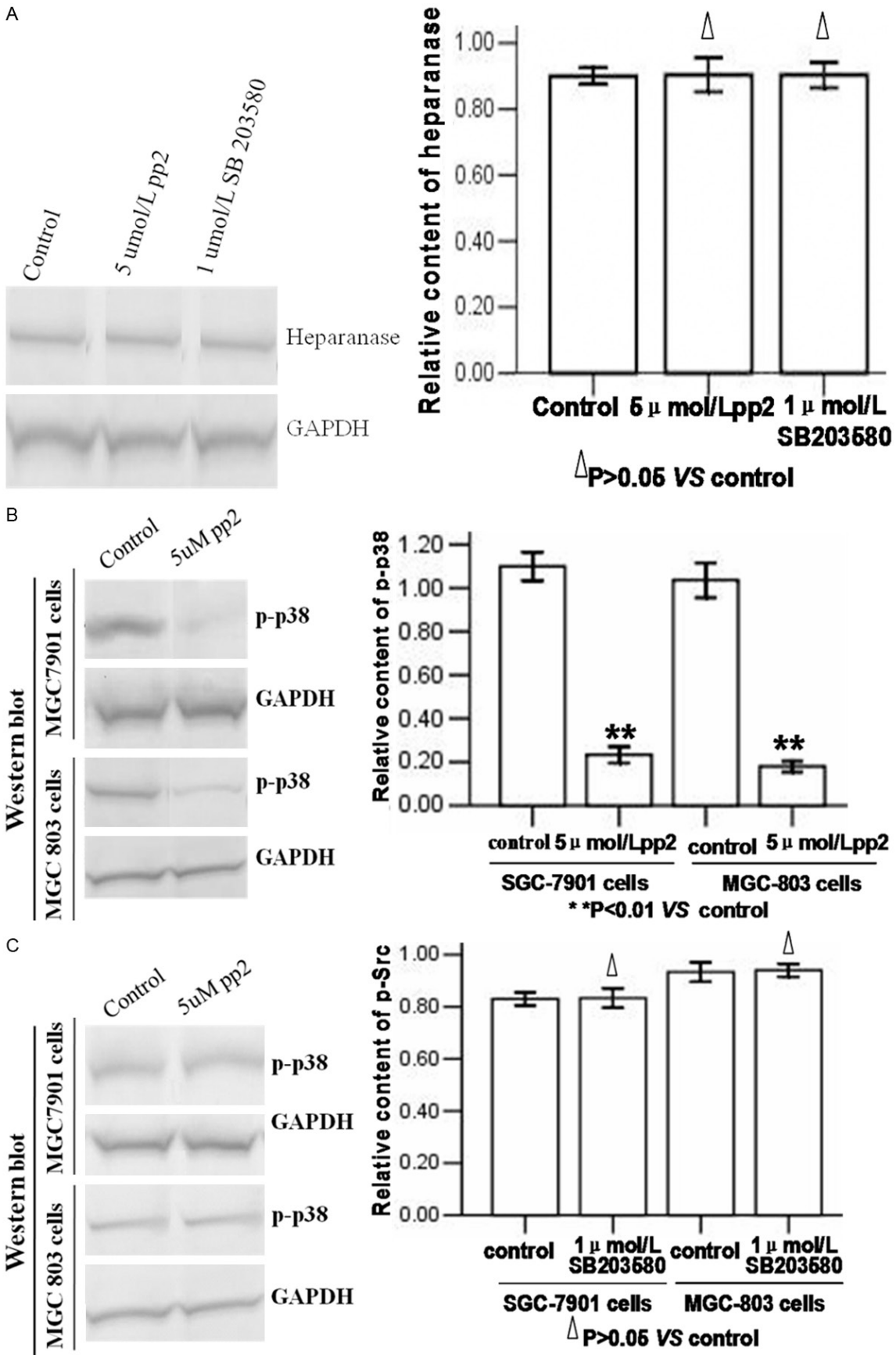

A
Control
5 umol/L pp2
1 umol/L SB 203580
Heparanase
GAPDH
Relative content of heparanase
1.00
0.80
0.60
0.40
0.20
0.00
Control
5 μ mol/Lpp2
1 μ mol/L SB203580
P>0.05 VS control
B
Control
5uM pp2
Western blot
MGC7901 cells
MGC 803 cells
p-p38
GAPDH
p-p38
GAPDH
Relative content of p-p38
1.20
1.00
0.80
0.60
0.40
0.20
0.00
**
**
control
5 μ mol/Lpp2
control
5 μ mol/Lpp2
SGC-7901 cells
MGC-803 cells
**P<0.01 VS control
C
Control
5uM pp2
Western blot
MGC7901 cells
MGC 803 cells
p-p38
GAPDH
p-p38
GAPDH
Relative content of p-Src
1.00
0.80
0.60
0.40
0.20
0.00
control
1 μ mol/L SB203580
control
1 μ mol/L SB203580
SGC-7901 cells
MGC-803 cells
P>0.05 VS control

Figure 5. Src and p38 kinases inhibitors effect on the expression of p38/Src protein phosphorylation and heparanase protein. A: The expression of heparanase protein did not change in human gastric cancer SGC-7901 cells treated with 5 μmol/L pp2 or 1 μmol/L SB 203580 for 24 h compared with human gastric cancer SGC-7901 cells treated without 5 μmol/L pp2 or 1 μmol/L SB 203580 (NO) by Western blot assay. The bar graph indicates the relative content of heparanase protein. B: The expression of p-p38 significantly decreased in human gastric cancer SGC-7901 and MGC-803 cells treated with the specific inhibitor pp2 of Src kinase (5 μmol/L) for 24 h compared with treated without the specific inhibitor pp2 of Src kinase by western blot assay. The bar graph indicates the relative content of p-p38. C: The expression of p-Src did not significantly change in human gastric cancer SGC-7901 and MGC-803 cells treated with the specific inhibitor SB 203580 of p38 kinase (1 μmol/L) for 24 h compared with human gastric cancer SGC-7901 and MGC-803 cells treated without the specific inhibitor of p38 kinase by western blot assay. The bar graph indicates the relative content of p-p38. All the experiment was repeated three times. A two sided *P*-value <0.05 was considered as statistically significant.

p38alpha have decreased MMP-9 activity and exhibit decreased bone metastasis in mice [27]. In the human THP-1 monocytes, inhibition of ERK1/2 reduced the expression of MMP-9, inhibition of p38 and JNK enhance the expression of MMP-9 through increased ERK1/2 phosphorylation [28].

In this study, we knocked down the heparanase expression by RNA interference in human gastric cancer cells with high heparanase mRNA and protein expression and observed the migration and matrigel invasion of human gastric cancer cells were inhibited and the expression of p-Src and p-p38 were decreased. We also found that human recombinant heparanase protein significantly enhanced the ability of migration and matrigel invasion and the expression of p-Src and p-p38 protein in human gastric cancer cells; Src kinase inhibitor pp2 and p38 kinase inhibitor SB 203580, significantly inhibited Src and p38 kinase phosphorylation of human gastric cancer cells, respectively, while they did not change the expression of heparanase protein in human gastric cancer cells. The inhibitors of Src kinase and p38 kinase can significantly reduce human heparanase recombinant proteins enhancing the migration and invasion of human gastric cancer cells. These results indicates that, heparanase may regulate the phosphorylation of Src kinase and p38 kinase, rather than Src kinase and p38 kinase regulate the expression of heparanase protein in human gastric cancer cells; heparanase may promote the migration and invasion of human gastric cancer cells through upregulating the phosphorylation of Src kinase and p38 kinase. Furthermore, we founded that the specific inhibitor pp2 of Src kinase inhibited the expression of p-p38, while the specific inhibitor of p38 kinase had no effect on the expression of p-Src in human gastric cancer cells, these results indicates that Src kinases may regulate p38 kinase phosphorylation in human gastric cancer cells. All these results indicate that heparanase may be involved in regulating human gastric cancer cells invasion and migration through regulating Src kinase or p38 kinase, or Src kinases firstly and then p38 kinase in human gastric cancer cells. Heparanase-Src kinase or heparanase-p38 kinase or heparanase-Src kinase-p38 kinase or all these may be the invasion and migration-related signal transduction pathway which exists in gastric cancer. The down stream factors correlated with these signal transduction pathway are needed to study. Moreover, recent studies have shown that adding exogenous heparanase can enhance endothelial cell invasion and mobility through Akt signaling-dependent pathway independent on the enzyme activity of heparanase [12, 29]; heparanase activity does not rely on the activity of enzyme and increase adhesion of glioma, lymphoma and T-cell through the β1-integrin-mediated, which is relative with Akt, Pyk2, and ERK activation [30]; in human MDA-MB-435 breast cancer cells, B16 melanoma cells, rat C6 glioma cells and human embryonic kidney 293 cells, the high expression of heparanase increased VEGF expression through activation of Src kinase, thus promoting the formation of new blood vessels in these tumors [11]; heparanase induce GEF-H1 signaling regulates the cytoskeleton dynamics of brain metastatic breast cancer cells [31]. All these results substantiate roles for heparanase, eliciting signal transduction cascades to induced some factors to promote the invasion, angiogenesis and metastasis of tumor.

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Disclosure of conflict of interest

None.

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