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Original Paper

p38MAPK Signaling Enhances Glycolysis Through the Up-Regulation of the Glucose Transporter GLUT-4 in Gastric Cancer Cells

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Key Words
Gastric cancer • p38MAPK signaling • Glycolysis • Glucose transporter (GLUT)-4

Abstract
Background/Aims: Previous studies have shown that p38MAPK is involved in gastric cancer, yet the underlying mechanism remains unclear. ***Methods:*** q-PCR, Western blot and immunohistochemistry were used to explore the expression of PP2A and the phosphorylation of p38MAPK in gastric cancer tissues and normal gastric tissues. Activated p38MAPK in the gastric cancer cell line MKN45 using activator, then q-PCR, glucose uptake assay and colony formation assay were performed to determine whether p38MAPK promotes gastric cancer through the enhancement of glycolysis. After transfection of p38MAPK dominant negative mutation (p38DN) into MKN45 cells or MKN45 cells treated with an inhibitor of p38MAPK, Western blot was performed to detect the expression of GLUT-4. The knock down of MEF2α in MKN45 cells by siRNA was followed by Western blot and luciferase reporter assay to investigate the underlying mechanism of the role of p38MAPK in the promotion of gastric cancer. Finally, q-PCR, Western blot and immunohistochemistry were performed to examine GLUT-4 expression in gastric cancer tissues and normal gastric tissues. ***Results:*** We found that p38MAPK activation significantly increases GLUT-4 expression and promotes glucose uptake and cell growth in gastric cancer cells. Inhibition of p38MAPK abrogates the up-regulation of GLUT-4. MEF2α knockdown abolishes p38MAPK-mediated GLUT-4 up-regulation. PP2A, an inhibitor of p38MAPK, is down-regulated in gastric cancer tissues, which might contribute to the activation of p38MAPK. ***Conclusions:*** Our data indicate that the abnormal activation of p38MAPK promotes glycolysis within gastric cancer cells through the upregulation of GLUT-4 in a MEF2a-dependent manner.

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Introduction

Gastric cancer is one of the most common malignant tumors, with approximately 989,000 cases diagnosed in 2008. The incidence rates of gastric cancer are high in Asia, Europe and America [1, 2]. In China, gastric cancer is the third most common cancer and is the first cause of cancer-related death. Gastric cancer accounts for approximately 300,000 cases of cancer-related deaths annually in China [3, 4]. Currently, the most effective treatment for gastric cancer is surgical resection combined with chemotherapy and/or radiotherapy. Although great progress has been made with respect to surgical techniques, chemotherapy and radiotherapy, the prognosis of gastric cancer remains far from satisfactory because most patients are diagnosed at an advanced stage when the tumors are largely unresectable; additionally, distant metastasis and local recurrence frequently occur in patients who undergo radical surgical resection [4, 5]. Although extensive research has been conducted into the possible mechanisms that underlie the development of gastric cancer, the exact mechanism remains ambiguous. An understanding of the related mechanisms is essential for us to seek new efficient targets and to develop novel treatments to manage gastric cancer.

The members of the mitogen-activated protein kinase (MAPK) family are important protein kinases with conserved function in all eukaryotes. p38MAPK is an important member of MAPK family as this pathway is known to exert key roles in the development of different types of cancers through the promotion of the migration and invasion of cancer cells. For example, in breast cancer cells the phosphorylation of p38MAPK is highly elevated, and treatment with p38MAPK inhibitor significantly inhibits invasiveness of breast cancer cells [6]. The activation of p38MAPK is also involved in bladder cancer and hepatocellular carcinoma [7, 8]. In gastric cancer, many studies have demonstrated that p38MAPK is significantly activated compared with matched normal gastric tissues, and it is thought to be related with the enhanced metastasis of gastric tumors [9]. However, the mechanism that underlies the promotion of gastric cancer development by p38MAPK remains unclear.

Dysregulated metabolic pathways are common in various cancer cells and have been accepted as one of the important hallmarks of cancer [10]. The famous Warburg effect, which was proposed by Otto Warburg in the last century, states that glucose consumption becomes a priority for cancer cells even in the presence of oxygen [11, 12]. Now, high glucose uptake is used to diagnose or monitor the treatment responses of cancers by imaging the uptake of 2-18 F-deoxyglucose with PET-CT in the clinic [10]. Because previous studies have revealed that p38MAPK activation is involved in the development of gastric cancer [13], we wondered whether p38MAPK exerts its effects through the regulation of metabolic pathways.

In this study, we analyzed the activation of p38MAPK in human gastric cancer tissues and found that p-p38MAPK is highly expressed in gastric cancer specimens. After the application of MKK6EE (a p38 MAPK activator), activated p38MAPK in the gastric cancer cell line MKN45 promoted glucose uptake and cell growth in MNK45 cells through the up-regulation of GLUT-4 in a MEF2a-dependent manner. Furthermore, we found that in gastric cancer, PP2A (an inhibitor of p38MAPK) is down-regulated. These data indicate that p38MAPK signaling enhances glycolysis through the up-regulation of GLUT-4 in gastric cancer cells, which suggests that p38MAPK could be a potential target for the clinical treatment of gastric cancer.

Materials and Methods

Tumor samples

76 pairs tumor tissues and matched normal tissues were collected between January 2011 and December 2012 from the Department of general surgery, the Second Hospital of Jilin University, Changchun, China. All samples were obtained during the operation and immediately frozen in liquid nitrogen and stored at -80°C until use or fixed in 4% paraformaldehyde. This study was approved by the Ethics Committee of the Second Hospital of Jilin University. Written informed consent was obtained from all the patients in

accordance with standards established by the ethical committee. All the 76 pairs samples were subjected to analysis and representative results were shown.

Immunohistochemistry

Tumor or matched normal tissues were fixed with 4% paraformaldehyde, then the samples were embedded in paraffin and sectioned. Then the slides were incubated in 1 mM EDTA buffer (PH 8.0), heated by microwave for 15 min to retrieve antigen, and incubated in 3% methanolic hydrogen peroxide solution for 30 min to eliminate endogenous peroxidase activity. Followed incubated with the indicated primary antibody (1:200-500) overnight at 4°C, the slides were incubated with biotin-conjugated secondary antibodies for 1 h at room temperature. Then the slides were incubated with peroxidase-conjugated biotin–streptavidin complex (Vector Labs, Burlingame, USA) for 20 min and visualized with diaminobenzidine (Sigma-Aldrich, Munich, Germany). In our study, we randomly chose 20 pairs of tumor tissues and the related normal tissues for immunohistochemistry analysis. Representative results were shown in our manuscript.

Cell culture

The human gastric cancer cell line MKN45 was obtained from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China. MKN45 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin. Cultures were maintained in a humidified 5% CO2 atmosphere at 37 °C.

RNA isolation and q-PCR detection

Total RNA was extracted from the MKN45 cells by using Trizol (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. RNA quality and quantity were determined by NanoDrop ND-1000. DNAse treatment before the cDNA synthesis was done to prevent gemonic DNA contamination. M-MLV Reverse Transcriptase (Promega, Madison, USA) was used to reverse transcribed RNA into cDNA. q-PCR was performed using the SYBR Green qPCR Master Mix (Tiangen, Beijing, China) on the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster, USA). β-actin was used as an internal control. The q-PCR values were based on three independent experiments. Relative gene expression data was calculated by using comparative Ct method. The primers sequences used were as follow:

GLUT-4: Forward 5′-CTTCATCATTGGCATGGGTTT-3′
Reverse 5′-AGGACCGCAAATAGAAGGAAGA-3′;
ENO1: Forward 5′- AAAGCTGGTGCCGTTGAGAA-3′
Reverse 5′-GGTTGTGGTAAACCTCTGCTC-3′;
ALDOA: Forward 5′- ATGCCCTACCAATATCCAGCA-3′
Reverse 5′- GCTCCCAGTGGACTCATCTG-3′;
GPI1: Forward 5′-CCGCGTCTGGTATGTCTCC-3′
Reverse 5′-CCTGGGTAGTAAAGGTCTTGGA-3′;
PGK1: Forward 5′-TGGACGTTAAAGGGAAGCGG-3′
Reverse 5′-GCTCATAAGGACTACCGACTTGG-3′;
β-actin: Forward 5′-CATGTACGTTGCTATCCAGGC-3′
Reverse 5′-CTCCTTAATGTCACGCACGAT-3′.

Transient transfection

For transfection, MKN45 cells were seeded onto 24-well cell culture plates (2×105/well), 24 h later cells were starved for 2-4 h. Then pcDNA3-MKK6EE or vector control at a final concentration of 800 ng per well was transfected into cells with lipofectamine 2000 (Invitrogen) according to the manufacture's protocol. The human MEF2 siRNA and control siRNA were purchased from OriGene. The siRNA transfection was performed as followed. First, MEF2 siRNA and control siRNA were diluted to 2 µM with 200 µl DNase/Rnase free ddH2O, 2 µM siRNA and 200 µl DMEM (serum and antibiotic free) in one tube and 10 µl lipofectamin 2000 and 390 µl DMEM (serum and antibiotic free) in the other tube were prepared as transfection mixture for each transfection, and the tubes were left at room temperature for 5 min. Then, the two mixtures were mixed and left at room temperature for 20 min. Finally, the transfection mixture was mixed with 3.2 ml DMEM (serum and antibiotic free) and applied to the cells which were left for incubation in a humidified incubator (5% CO2, 37°C). The cells were analyzed 24 h after the transfection.

Western blot analysis

For cells, after transfection as indicated, cells were harvested and lysed in RIPA lysis buffer (150 mM NaCl, 10 mM Tris, pH 7.5, 1% NP40, 1% deoxycholate, 0.1% SDS, protease inhibitor cocktail). Then the lysates were centrifuged at 12000 rpm for 10 min and the supernatant was collected. For tissues, 100-200 mg tissues were homogenized in RIPA lysis buffer. The tissue lysates were centrifuged for 10 min at 10,000 rpm and the supernatant was collected.

The protein concentration was measured using the Bio-Rad protein assay kit (Bio-Rad, USA). 30-50 μg protein was subjected to 10% SDS-PAGE and then transferred to PVDF membrane (Millipore, MA, USA). After blocking with 5% skimmed milk, the member was incubated in the primary antibodies as indicated overnight at 4 °C, followed by incubating in HRP-conjugated corresponding secondary antibodies for one hour and visualized by ECL Advanced Solution (GE Healthcare Life Sciences). Tubulin was used as a loading control. Protein band intensity was analyzed by imageJ. Values from 3 independent experiments. GLUT4 levels were normalized to total protein amount. p38MAPK phosphorylation levels were normalized to total p38MAPK amouts. The primary antibodies used in this study as followed, GLUT-4 (1:1000) and MEF2 (1:1000) were from Sigma, p-p38MAPK (1:1000), p38MAPK (1:1000), PP2A (1:500) and Tubulin (1:10000) were from Cell signaling technology (Danvers, USA).

Glucose uptake assay

Glucose uptake assay was performed using 2-[3H] deoxy-D-glucose (2-DG; Abcam, Cambridge, UK). In brief, MKN45 cells were seeded onto 24-well plates, 24 h later cells were transfected with MKK6EE or control plasmid as indicated. 24 h post-transfection cells were incubated in serum free DMEM for another 12 h. Then cells were washed three times with PBS and incubated with 2-DG (10 mM) for 20 min at 37°C. After washing three times with ice-cold PBS, the cells were lysed with 0.1 N NaOH/0.1% sodium dodecyl sulfate (SDS) for 1 h. Radioactivity was measured by liquid scintillation spectroscopy (Aloka, Tokyo, Japan). All experiments were performed in triplicate and in three independent experiments.

MTT assay

The viability of cells was assessed using a MTT assay. Specifically, the cells (1×104 cells per well) were seeded in triplicate in 96-well cell culture plates, 24 h later cells were transfected with MKK6EE or control plasmid as indicated. Subsequently, the cells were subjected to a MTT assay by the addition of 10 μL 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT; 5 mg/mL, Sigma, St. Louis, MO, USA) for 4 h. Then the culture medium was removed, and 100 μL dimethyl sulfoxide (DMSO; St. Louis, MO, Sigma) was added to each well. After been thoroughly mixed for 10 min, the absorbance values at 570 nm were measured using a Spectra Microplate Reader 1420 multilabel counter (Perkin Elmer, Foster City, CA, USA).

Colony formation assay

For colony formation assay, pre-warmed 1 ml 2×RPMI 1640 medium containing 20% FBS and 1 ml 1.2% Bacto-agar (Difco) solution, then two solutions were mixed and transferred onto a 3 cm cell culture dish, incubated the dish at 37°C for 30 min to allow the agar layer to solidify. Melted 0.5 ml 0.6% agar at 50°C and stored the mixture in 40°C water bath. Then 1×10^3 MKN4 cells treated as indicated were added and mixed quickly, the mixture was poured onto the bottom agar and cultured under normal growth conditions for about 15 days to form colonies. Then cell colonies were observed under microscope colonies larger than 50 cells were counted.

Luciferase reporter assay

MKN45 cells were collected and reseeded onto 24 well-plate at 2.5×10^4 cells per well. 24 h later cells were co-transfected with human GLUT-4 3' UTR firefly luciferase reporter plasmid, together with RL plasmids, MKK6EE, MEF2 siRNA or empty vector using lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. 200 ng firefly luciferase reporter gene construct (per well) and 1 ng pRL-SV40 Renilla luciferase constructs (per well) for normalization were cotransfected. 4 h post-transfection fresh culture medium was changed. 24 h after transfection cells were collected and lysed, and luciferase activity was measured with the Dual-Luciferase Reporter Assay system (Promega, Madison, USA).

Statistical analyses

The results represent the mean ± SE where applicable. Student's t test was used to compare two independent groups. For all tests, values of $p < 0.05$ were considered statistically significant.

Results

p38MAPK is highly activated in gastric tissues whereas PP2A, an inhibitor of p38MAPK, is down-regulated

In this study, we first evaluated p-p38MAPK expression in gastric tumor tissues and then compared its expression to that in normal tissues by immunohistochemistry. The results showed that the phosphorylation of p38MAPK was significantly increased in gastric tumor tissues ($p<0.001$; Fig. 1A), which suggests that the activation of p38MAPK might play a role in the development of gastric cancer.

(PP2A) is a member of the phosphoprotein phosphatase (PPP) family and is involved in the regulation of cell homeostasis through the negative regulation of signaling pathways initiated by protein kinases such as Ras/Raf/MAP kinase, JNK and AKT. Because it has been reported that PP2A is a negative regulator of p38MAPK, we wondered whether PP2A is involved in the activation of p38MAPK in gastric cancer. Therefore, we next examined the expression of PP2A in gastric cancer tissues. The q-PCR results showed that the mRNA levels of PP2A in gastric cancer were significantly decreased ($p<0.01$; Fig. 1B). Then, we examined the expression of PP2A protein by Western blot. As shown in Fig. 1C, compared with normal gastric tissues, PP2A protein expression was significantly down-regulated in gastric cancer ($p<0.001$). The data suggest that in gastric cancer, the activation of p38MAPK may occur at least partially through a decrease in PP2A.

In our study, immunohistochemistry was also performed to detect the phosphorylation of p38MAPK in gastric cancer. As shown in Fig. 1D, p-p38MAPK was slightly expressed in normal gastric tissues, while it was highly expressed in gastric tumor tissues, which is in

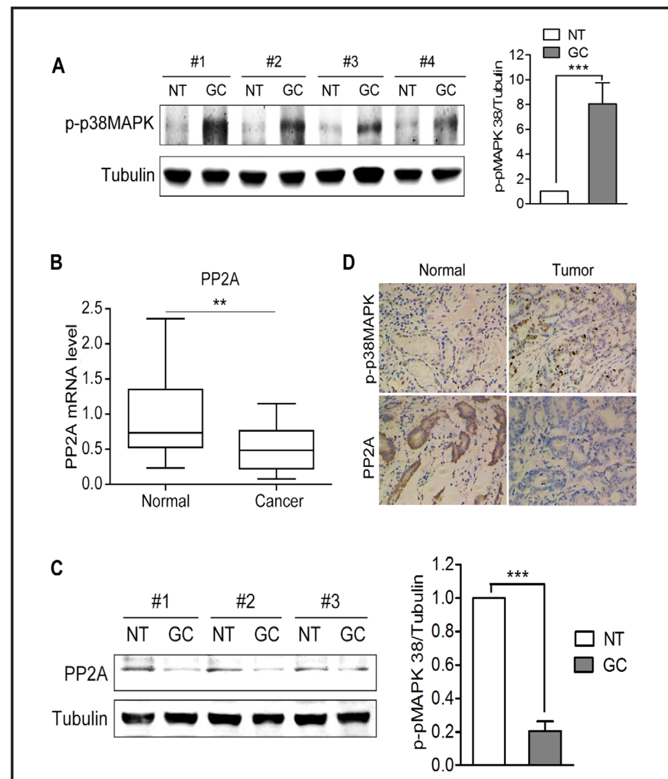

Fig. 1. p38MAPK is highly activated in human gastric cancer specimens. A. Phosphorylation of p38MAPK in human gastric cancer. NT = normal gastric tissues. GC = gastric cancer tissues. n=76, the representative results were shown. B. PP2A mRNA expression in human gastric cancer, n=76. C. PP2A protein expression in human gastric cancer. n=76, the representative results were shown. D. Immunostain of p-p38MAPK in human gastric cancer specimens. n=20, results shown are representative images. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ compared with the control group.

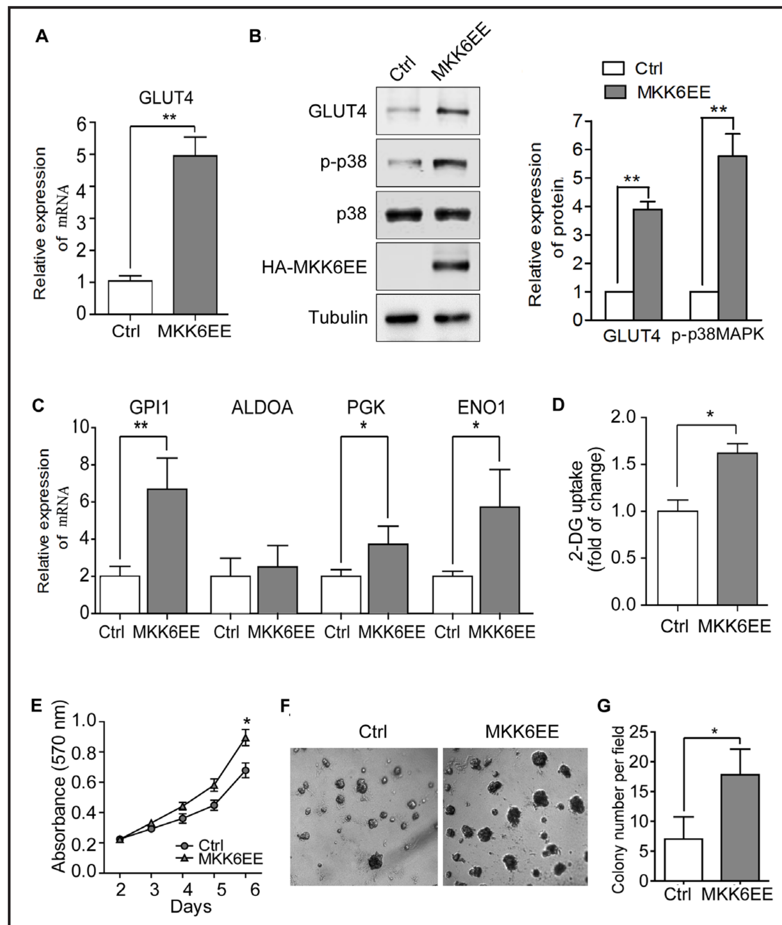

Fig. 2. p38MAPK activation promotes glycolysis and cell growth in gastric cancer cells. A. Overexpression of the constitutively active form of MKK6 (MKK6EE) up-regulates GLUT-4 mRNA transcription. B. Overexpression of MKK6EE in the gastric cancer cell line MKN45 enhances p38MAPK activation and GLUT-4 expression. C. The mRNA expression levels of the glycolysis-related genes GPI1, PGK and ENO1 were upregulated in MKN45 cells that overexpressed MKK6EE. D. p38MAPK activation promotes glucose uptake in MKN45 cells. E. p38MAPK activation promotes growth of MKN45 cells. F, G. p38MAPK activation promoted colony formation and the up-regulation of MKK6EE promoted the clonal growth of MKN45 cells. Ctrl = the control group. Values represent the means ± SE of three independent experiments performed in triplicate. *p<0.05 and **p<0.01 compared with the control group.

accordance with the results of the Western blot. This further demonstrated that the activation of p38MAPK is important in gastric cancer.

Activation of p38MAPK promotes glycolysis and proliferation of gastric tumor cells

To further explore the possible mechanism that underlies the role of p38MAPK in gastric cancer, we transfected the p38MAPK activator MKK6EE into the gastric cancer cell line MKN45; 24 h later, cells were collected and subjected to further analysis. As shown in Fig. 2A, after transfection of MKK6EE, the expression level of GLUT-4 mRNA was increased by approximately 4 times (p<0.01) and the expression of GLUT-4 protein was also significantly up-regulated (p<0.001; Fig. 2B). Meanwhile, the increased expression of MKK6EE's enhanced the phosphorylation of p38MAPK (p<0.01).

GPI1, PGK, ALDOA and ENO1 are important glycolysis-related genes. Here, we also detected the expression of these genes by real time PCR. Among the 4 genes, the levels of 3 genes (GPI1, PGK and ENO1) were significantly higher in cells that overexpressed MKK6EE (p<0.01, p<0.05, p<0.05; Fig. 2C). A glucose uptake assay showed that the overexpression of MKK6EE induced a significant increase in glucose uptake in gastric tumor cells (p<0.05; Fig. 2D and p<0.05; 2E). A soft agarose colony formation assay revealed that the overexpression of MKK6EE promoted gastric cancer cell clonal growth (p<0.05; Fig. 2F, 2G). These data confirmed that the activation of p38MAPK plays a critical role during the development of gastric cancer, which may occur through the up-regulation of GLUT-4.

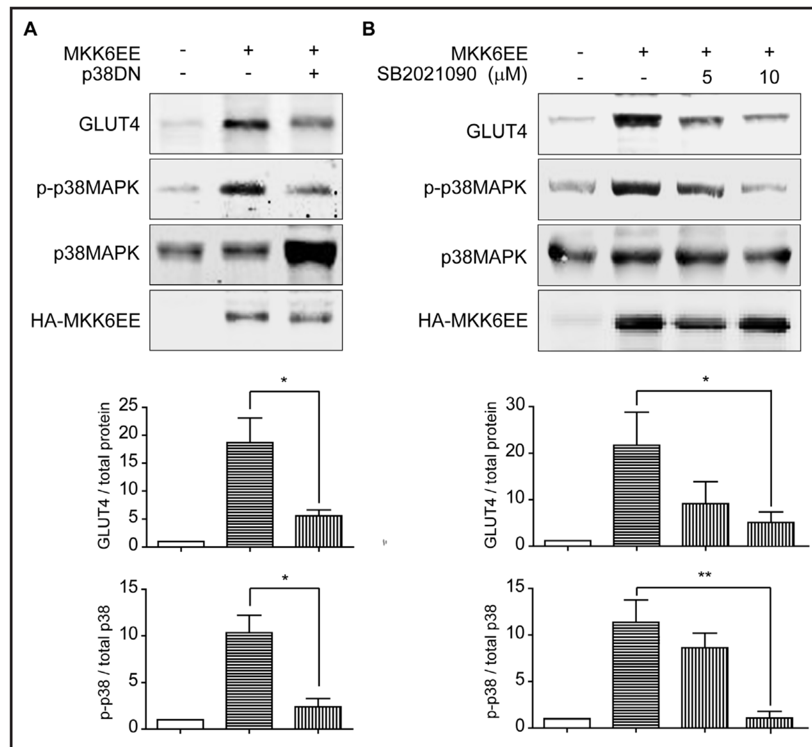

Fig. 3. Inhibition of p38MAPK activity abrogates the up-regulation of GLUT-4. A. A p38MAPK dominant negative mutation (p38DN) decreases GLUT-4 expression. B. The p38MAPK specific inhibitor SB202190 blocks the enhancement of GLUT-4 expression. Values represent the means ± SE of three independent experiments performed in triplicate. *p<0.05 and **p<0.01 compared with the control group.

Inhibition of p38MAPK activity abrogates the up-regulation of GLUT-4

To further elucidate whether the up-regulation of GLUT-4 is related to the activation of p38MAPK, we constructed a p38MAPK dominant negative mutation (p38DN), which may competitively inhibit the function of wild-type p38MAPK. As shown in Fig. 3A, after the co-transfection of MKK6EE with or without p38DN into MKN45 cells, the phosphorylation of p38MAPK induced by MKK6EE was significantly decreased ($p<0.05$), and the up-regulation of GLUT-4 induced by MKK6EE was significantly inhibited as well ($p<0.05$). To further identify that the up-regulation of GLUT-4 was attributed to the activation of p38MAPK, we pretreated MKN45 cells with 5 μM or 10 μM SB2021090 (a specific inhibitor of p38MAPK), and then transfected MKK6EE into the cells. The results showed that SB2021090 effectively inhibited the activation of p38MAPK induced by MKK6EE ($p<0.01$); the up-regulation of GLUT-4 was also abrogated ($p<0.05$; Fig. 3B).

p38MAPKinduced GLUT-4 up-regulation is dependent on MEF2a activity

MEF2a is an important target protein of p38MAPK, and therefore, to further explore the possible mechanism that underlies p38MAPK-induced expression of GLUT-4, we inhibited the expression of MEF2a in MKN45 cells by RNA interference. As shown in Fig. 4A, MEF2a siRNA co-transfected with MKK6EE effectively depressed the expression of MEF2a induced by MKK6EE. The results of the Western blot demonstrated that knockdown of MEF2a showed no effect on the MKK6EE-induced phosphorylation of p38MAPK, while the up-regulation of GLUT-4 induced by MKK6EE was almost completely abolished ($p<0.05$; Fig. 4B). The data suggest that MEF2a plays a critical role in the mediation of p38MAPK-induced GLUT-4. Some studies discovered that MEF2 binding to the GLUT-4 promoter is required for GLUT-4 gene expression [14, 15]. To further determine whether MEF2a mediates p38MAPK-induced GLUT-4 expression via the promotion of GLUT-4 promoter activity, we knocked down MEF2a by siRNA with or without MKK6EE overexpression. As shown in Fig. 4C, in MKN45 cells that overexpressed MKK6EE, GLUT-4 promoter activity was greatly increased. However, the knockdown of MEF2a significantly reduced the activity of the GLUT-4 promoter that was induced by MKK6EE. Our data indicate that MEF2a mediates p38MAPK-induced GLUT-4 expression through the promotion of GLUT-4 promoter activity.

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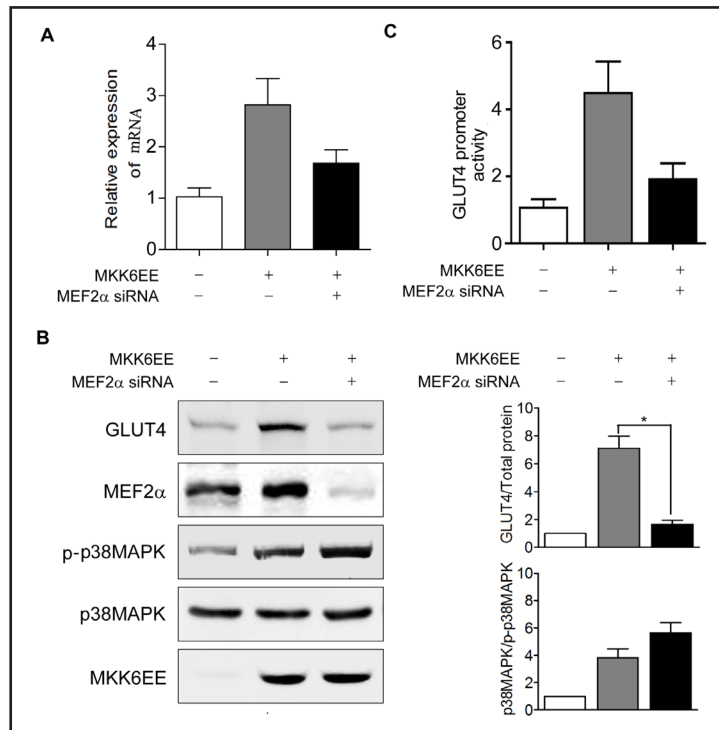


Fig. 4. p38MAPK-induced up-regulation of GLUT-4 is dependent on MEF2a activity. A, B. MEF2a knockdown abolishes p38MAPK-mediated GLUT-4 up-regulation at both the mRNA level (A) and at the protein level (B). C. GLUT-4 transcriptional activity was determined by a luciferase assay. Values represent the means ± SE of three independent experiments. *p<0.05 compared with the control group.

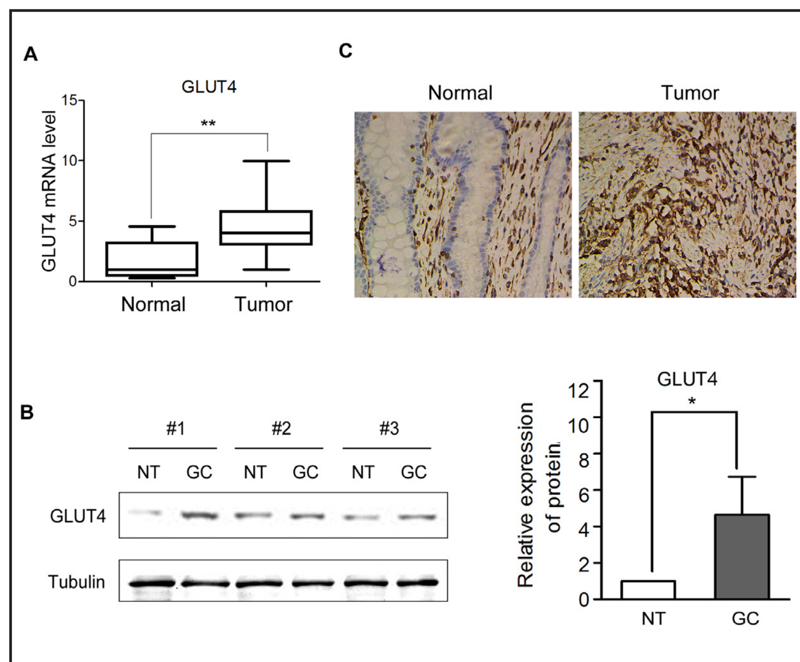


Fig. 5. GLUT-4 is highly expressed in human gastric cancer specimens. A. mRNA levels of GLUT-4 in human gastric cancer. n=76. B. Protein levels of GLUT-4 in human gastric cancer. NT, normal gastric tissues. GC, gastric cancer tissues. n=76, the representative results were shown. C. Immunostain of GLUT-4 in human gastric cancer specimens, n=20, the representative results were shown.the results shown are representative images. Values represent the means ± SE of three independent experiments. *P<0.05, **P<0.01.

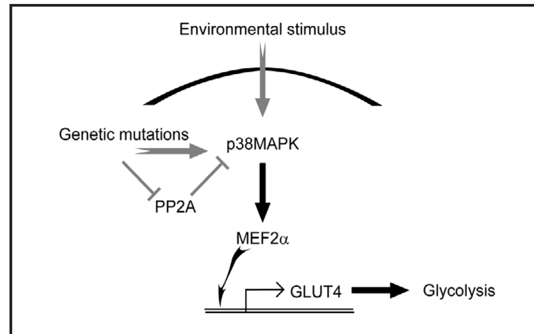
GLUT-4 is highly expressed in human gastric cancer specimens

To verify that the up-regulation of GLUT-4 is associated with gastric cancer, we next detected the expression of GLUT-4 mRNA in gastric cancer tissues. The results of the q-PCR experiment showed that, compared with normal gastric tissues, the expression of GLUT-4 mRNA was clearly up-regulated (p<0.01; Fig. 5A). We also examined the expression of GLUT-4 protein in gastric cancer tissues by Western blot and immunochemistry. The results showed that the expression level of GLUT-4 protein in gastric cancer tissues was also increased (p<0.05; Fig. 5B, 5C).

Discussion

The MAPK family includes three serine/threonine-related protein kinases: c-Jun NH2-terminal kinases (JNKs), extracellular signal-related kinases (ERKs) and p38MAPKs. Numerous studies have indicated that p38MAPKs are critical for the promotion of cell growth and apoptosis [16]. The activation of p38MAPK was reported to be involved in gastric cancer and is correlated with metastasis of gastric tumors [17]. In agreement with these previous studies, we also found that p38MAPK is highly activated in human gastric cancer specimens.

Fig. 6. Scheme of the regulation of GLUT-4 expression in gastric cancer by the p38MAPK/MEF2 pathway.



PP2A is a member of the protein serine/threonine phosphatase super-family, which features proteins that reverse the actions of protein kinases by cleavage of the phosphate from serine and threonine residues of proteins [18]. PP2A is highly conserved in eukaryotic organisms and plays key roles in many biological processes including apoptosis, the cell cycle, and metabolism, among others [19-21]. It has been determined that PP2A is a tumor suppressor gene that is down-regulated in various tumors, such as breast cancer, lung cancer and gastric cancer [22]. Therefore, the PP2A protein is suggested to act as a potential biomarker for the prognostic assessment of many cancers. In addition, reports have shown that PP2A is an inhibitor of p38MAPK that suppresses the activation of p38MAPK and the upstream kinases of the MAPK signaling pathway [23]. In the present study, we found that compared with normal gastric tissues, the expression of PP2A mRNA and PP2A protein was significantly depressed, which was consistent with previous research [22]. Therefore, we proposed that the activation of p38MAPK activated might be related to PP2A inhibition.

Since Otto Warburg first proposed the famous "Warburg effect", which stated that tumor cells prefer to metabolize glucose even in the presence of oxygen, numerous research findings have demonstrated that altered energy metabolism is widespread in various cancer cells. In addition, the increased rate of glucose metabolism has been considered one of the most important hallmarks of cancer [10]. Thus, we wondered whether p38MAPK exerts an affect in gastric cancer cells via the promotion of glycolysis. Our research revealed that the activation of p38MAPK promotes the expression of GLUT-4 and induces a significant increase in glucose uptake in gastric tumor cells. The expression levels of three important glycolysis-related genes, GPI1, PGK and ENO1, were also higher in cells that overexpressed MKK6EE, which suggested the metabolic activation of the glycolytic pathway.

GLUT-4 is an important glucose transporter that is involved in many types of malignant tumors. For example, GLUT-4 is overexpressed in lung carcinoma, and the expression of GLUT-4 is positively correlated with the histologic type, differentiation grade and tumor stage [24]. A tendency for increased GLUT-4 expression is also observed with the advancement of endometrial carcinoma grade [25]. Moreover, a few studies have assessed the expression of GLUT-4 in gastric cancer. By RT-PCR, Noguchi et al. [26] found that, in paired samples of normal gastric mucosa and gastric tumors from 20 individuals, only 40% of normal gastric mucosa samples expressed GLUT-4 mRNA, whereas all gastric cancer samples expressed GLUT-4 mRNA. The examination results from 70 resected gastric cancer specimens showed that GLUT-4 was expressed in the majority of tumors. Here, we also detected an upregulation of GLUT-4 mRNA and protein expression in gastric cancer tissues. This implied that GLUT-4 might be implicated in gastric cancer. Using human gastric cancer cell lines, Noguchi et al. demonstrated that the upregulation of GLUT-4 might be responsible for the ability of the cell to increase its glucose uptake in human gastric cancer [24]. Therefore, we next explored whether GLUT-4 was involved in the effects of p38MAPK in gastric cancer. After the transfection of p38DN into MNK45 cells or through the treatment of MNK45 cells

with SB2021090, we found that the inhibition of p38MAPK abrogated the up-regulation of GLUT-4.

Members of the MEF2 family comprise a group of transcription factors that play an important role in the maintenance and induction of the differentiated phenotype of muscle [27, 28]. It has been well established that MEF2a can bind to the promoter of GLUT-4 and thus regulate GLUT-4 expression. In skeletal muscle, AMPK was demonstrated to be an upstream regulator of MEF2 and can upregulate MEF2 expression. In this study we found that the activation of p38MAPK is involved in upregulation of GLUT-4 in MNK45 cells. Then, we investigated whether p38MAPK regulates GLUT-4 via MEF2a. Our results showed that the knock down of MEF2a by RNA interference in MNK45 cells clearly blocked the expression of GLUT-4 mRNA and protein induced by MKK6EE. The results of the luciferase assay demonstrated that the activation of p38MAPK significantly promoted GLUT-4 promoter activity, while the knock down of MEF2a markedly inhibited GLUT-4 promoter activity induced by p38MAPK. The data suggest that the induction effect of p38MAPK on GLUT-4 is MEF2a-dependent.

Based on the above evidence, we propose that p38MAPK is activated by environmental stimuli, genetic mutations or PP2A inhibition. This leads to increased MEF-2a-dependent expression of GLUT-4, which in turn enhances glycolysis in gastric cancer cells (Fig. 7). In summary, the present study demonstrated that the activation of p38MAPK is involved in the progression of gastric cancer, and determined that p38MAPK signaling enhances glycolysis through the up-regulation of GLUT-4 via MEF-2a, which in turn promotes the development of gastric cancer.

Disclosure Statement

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