Interference of STAT 5b expression enhances the chemo-sensitivity of gastric cancer cells to gefitinib by promoting mitochondrial pathway-mediated cell apoptosis

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Abstract. Signal transducer and activator of transcription (STAT) 5, including STAT 5a and STAT 5b, was reported to play important roles in the malignant biological behaviors of tumors. However, their roles in gastric cancer, especially for STAT 5b remain unknown. This study aimed to detect the expression of STAT 5b in gastric cancer cells and analyze the role and possible mechanism of STAT 5b in the chemo-sensitivity of gastric cancer cells to gefitinib. A total of 69 patients with gastric carcinomas were analyzed for the expression of STAT 5b in carcinomas and para-carcinomas by immunohistochemistry. Cultured MGC-803 and MKN-45 cells were exposed to gefitinib and/or STAT 5b siRNA. Mitochondrial proteins including Bcl-2, Bax, caspase-3 and caspase-9 were extracted using special kits for detecting mitochondrial pathway-related apoptosis proteins. The results showed that STAT 5b expression was significantly increased in gastric carcinomas compared with para-carcinomas, with a positive rate of 49/69 in carcinomas and 27/69 in para-carcinomas (P=0.001). Gefitinib exposure reduced the relative viabilities of MGC-803 and MKN-45 cells in a concentration- and time-dependent manner, and cell apoptosis increased significantly (P<0.05) with gefitinib treatment (4 mM, 24 h). STAT 5b expression was significantly downregulated by treatment with gefitinib (4 mM, 24 h). Interference of STAT 5b expression by siRNA targeting enhanced the chemo-sensitivity of gastric cancer cells to gefitinib by promoting mitochondrial pathway-mediated apoptosis. Bax, caspase-3 and caspase-9 expression were upregulated, and Bcl-2 expression was downregulated in the combined treatment group (gefitinib+siRNA) compared with the gefitinib (4 mM, 24 h) only group in the MGC-803 and MKN-45 cells (P<0.05). Overall, STAT 5b was upregulated in gastric carcinomas compared with para-carcinomas. Interference of STAT 5b expression by siRNA targeting enhanced the chemo-sensitivity of gastric cancer cells to gefitinib by promoting mitochondrial pathway-mediated cell apoptosis. These findings may be useful for developing new approaches for the treatment of gastric cancer.

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Introduction

Signal transducer and activator of transcription (STAT) proteins have garnered more attention recently as latent cytoplasmic transcription factors. Six STAT family members have been identified thus far: STAT 1, STAT 2, STAT 3, STAT 4, STAT 5 (including STAT 5a and STAT 5b), and STAT 6. They are believed to regulate many cellular process including cell proliferation, differentiation, apoptosis and survival (1). STAT 1, STAT 3 and STAT 5 (including STAT 5a and STAT 5b) were reported to play important roles in the malignant biological behavior of tumors (2-4). Regarding STAT 5a and STAT 5b, evidence has identified some of their similarities and characteristics, including their gene encoding, distinct regulatory features and functions. Based on recent studies, despite some overlapping functions, STAT 5a and STAT 5b generally play different physiological functions (5). However, the roles of STAT 5a and STAT 5b in malignant tumors, especially in gastric cancer, which is one of the major causes of cancer-associated mortalities worldwide and has become one of the great social harms to human health due to its poor prognosis (6,7), remain largely unknown.

Gefitinib, also known as Iressa, is commonly used to treat locally advanced or metastatic non-small-cell lung cancer (NSCLC). Use of gefitinib has been widely promoted for its anticancer effects (8). Gefitinib is a selective inhibitor of the EGFR tyrosine kinase, which is usually expressed in the solid tumors of epithelial cells. Inhibition of the EGFR tyrosine kinase can disrupt tumor growth, metastasis, and angiogenesis and can promote apoptosis of cancer cells (9). Thus, gefitinib is effective in treating certain types of epithelial-derived solid tumors rich in tyrosine kinase, including gastric cancers.

However, the clinical significance and molecular mechanism of gefitinib in gastric cancers remain largely unknown.

In this study, we aimed to investigate the expression of STAT 5b in gastric cancer tissues and to analyze the role and possible mechanism of STAT 5b in the chemo-sensitivity of gastric cancers to gefitinib. The results showed the increased expression of STAT 5b in gastric carcinoma clinical samples but not in para-carcinoma samples. The expression of STAT 5b was downregulated significantly by treatment with gefitinib (4 mM, 24 h). Interference of STAT 5b expression by siRNA targeting enhanced the chemo-sensitivity of gastric cancer cells to gefitinib, the mechanism of which involved promoting mitochondrial pathway-mediated apoptosis. The results provide important theoretical guidance for the clinical prevention and treatment of gastric cancers.

Materials and methods

Patients and specimens. A total of 69 patients with gastric carcinomas (48 male and 21 female cases) obtained from Shandong Provincial Qianfoshan Hospital Affiliated with Shandong University during the period March, 2013 to December, 2013 were studied. All the cases in this study were treated with primary carcinoma and para-carcinoma resection. Written informed consent was obtained from all the patients prior to surgery. The study was approved by the Ethics Committees of Shandong University. Resected tissues were fixed by immediate immersion in formalin for immunohistochemistry (IHC) or were rapidly frozen by immediate immersion in liquid nitrogen, and stored at -80˚C for protein extraction. The mean age of the patients was 65.8 years (range, 46-83 years). Tissue specimens were embedded in paraffin for the experiments by the same investigator. Clinicopathological characteristics, including gender, age, T stage, N stage, clinical stage and pathological grading were studied. T stage, N stage, clinical stage and pathological grading were defined based on the TNM Staging Classification for Carcinoma of the Stomach (7th edition, 2010) from the American Joint Committee on Cancer (AJCC).

Immunohistochemistry. Paraffin-embedded gastric carcinoma sections (4 μm) were dewaxed and subjected to antigen retrieval by water bathing in 0.01 M citric buffer (pH 6.0) at 95-98˚C for 15 min. To quench endogenous peroxidase, the sections were incubated in 3% hydrogen peroxide solution (H_2O_2) for 30 min. Non-specific binding was prevented by incubating the samples in 10% normal goat serum for 30 min in a humid chamber. The slides were incubated overnight at 4˚C in antibodies for STAT 5b (Epitomics, Burlingame, CA, USA; rabbit polyclonal antibody, 1:300). For the negative control, an equal volume of PBS was used instead of the primary antibody solution. After washing, the primary antibodies were detected with appropriate secondary antibodies for 30 min at 37˚C. A solution of 3,3'-diamino-benzidine tetrahydrochloride (DAB) was used to visualize positive staining, whereas hematoxylin was used to counterstain the nucleoli.

Interpretation of immunohistochemical staining. The expression of STAT 5b as visualized by immunohistochemical staining was evaluated in five random areas of the slide sections at a magnification of x100. Validation was performed blindly and without knowledge of the eventual clinical parameters. When differences between inter-observers occurred, the slides in question were jointly re-examined by two investigators.

Cell culture. MGC-803 and MKN-45 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 100 U/ml penicillin, and 100 mg streptomycin at 37˚C in a humidified atmosphere composed of 95% air and 5% CO_2. Passage digestion was conducted using a 0.25% trypsin-0.02% EDTA digestive solution.

Cell treatment and assessment of cell viability. To determine the optimal gefitinib co-culture conditions, cells ($5x10^4$/ml) were subcultured in a 96-well cell culture cluster (Corning, NY, USA) and were treated with different concentrations of gefitinib (0, 1, 2, 4 and 8 mM) for 24, 48 and 72 h. MTT (5 mg/ml, 20 μl) was added to each well 4 h before each of the indicated time-points. After 4 h of incubation at 37˚C, the cells were resuspended in dimethylsulfoxide (DMSO). The effects of gefitinib on the two cell lines were measured using the Cell Counting Kit-8 (CCK-8). Optical density (OD) values were measured at 570 nm using an ELISA reader (Multiskan MK3, Shanghai Bio-excellent, Shanghai, China). The relative cell viability was calculated according to the formula: Relative cell viability (%) = OD experiment/OD control x 100% (OD blank was tared to zero).

Interference of STAT 5b expression. STAT 5b siRNA (10 μM) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and interference of STAT 5b expression was conducted according to the manufacturer's instructions. Briefly, 1, 2, or 3 μl of 10 μM STAT 5b siRNA was added to each well of confluent cells. After a 6-h incubation, the transfection complexes were discarded, and the cells were cultured in growth medium for 48 h. Protein extracts were used to detect STAT 5b expression levels in the different STAT 5b siRNA (10 μM) dosages.

Flow cytometry. Cells incubated with or without gefitinib (4 mM, 24 h) or with the gefitinib+siRNA combination were trypsinized. After washing, the cells were resuspended in 200 μl binding buffer containing Annexin V FITC (5 μl) and PI (10 μl) for 15 min at room temperature. Then, 300 μl binding buffer was added before analysis with a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA, USA).

Protein extraction and western blot analysis. Total protein was extracted from primary tissues or cultured cells using radioimmune precipitation (RIPA) protein lysis buffer according to appropriate protocols. The Bradford method was used to determine the protein concentration of the supernatant. The samples (40 μg of total protein each) were used for western blot analysis with primary antibodies (STAT 5b, 1:1,000; β-actin, 1:2,500). The STAT 5b and β-actin bands were visualized at apparent molecular weights of 90 and 43 kDa, respectively.

Statistical analysis. Statistical analyses were performed using SPSS statistical software (version 13.0; SPSS Inc., Chicago, IL, USA). To test the associations between STAT 5b expres-

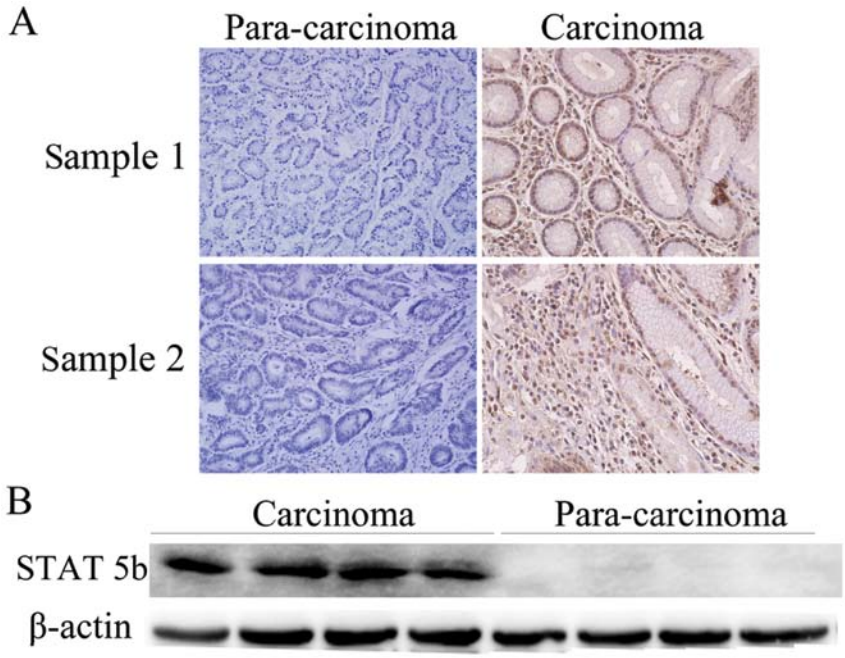

Figure 1. Increased expression of STAT 5b was detected in clinical gastric carcinoma samples but not in para-carcinoma samples. (A) Two randomly selected samples showed an increased expression of STAT 5b in gastric carcinomas compared with that in para-carcinomas based on IHC. (B) Upegulation of STAT 5b was confirmed by western blot analysis in the protein extracts of gastric carcinomas and para-carcinomas.

Table I. Protein expression of STAT 5b in gastric carcinomas as assessed by immunohistochemical staining.

Variables	Negative	Positive	Total	P-value
Carcinoma	20	49	69	0.001
Para-carcinoma	42	27	69	

sion and various clinicopathological characteristics, including gender, age, T stage, N stage, clinical stage and pathological grading stages, we used a non-parametric test for the comparisons of differences between groups. A χ^2 test was applied for trend variables. P<0.05 was considered to indicate statistical significance.

Results

Increased expression of STAT 5b is detected in clinical gastric carcinomas but not in para-carcinomas. Sixty-nine gastric carcinoma and corresponding para-carcinoma clinical samples were collected in this study. IHC was conducted to evaluate STAT 5b expression between carcinomas and para-carcinomas. Fig. 1A shows images from two randomly selected samples, for which STAT 5b expression was significantly increased in gastric carcinomas compared with para-carcinomas. As shown in Table I, the statistical analysis revealed that this difference in STAT 5b expression was significant, with 49/69 carcinomas and 27/69 para-carcinomas positively staining for STAT 5b (P=0.001).

Western blot analysis was also conducted to evaluate STAT 5b expression in the protein extracts of gastric carcinomas or para-carcinomas. As shown in Fig. 1B, STAT 5b was also found to be significantly upregulated in carcinomas compared with para-carcinomas when β-actin was used as a loading control.

Associations between STAT 5b expression and clinicopathological parameters, including gender, age, T stage, N stage, clinical stage and pathological grading, in gastric carcinomas are shown in Table II. The results showed no significant association between STAT 5b expression and gender (P=0.774) or age (P=1.001). Significant associations were found between STAT 5b expression and T stage (P=0.037), N stage (P=0.014), clinical stage (P=0.014) and pathological grading (P=0.005).

Gefitinib reduces the relative viability of gastric cancer cells in a concentration- and time-dependent manner. The effects of gefitinib on cultured MGC-803 and MKN-45 gastric cancer cells, were measured using the CCK-8 kit. Gefitinib, a common anticancer chemotherapeutic, was shown to reduce the relative viability of gastric cancer cells. As shown in Fig. 2A and B, cultured MGC-803 and MKN-45 cells were exposed to different concentrations of gefitinib (0, 1, 2, 4 and 8 mM) for 24, 48 and 72 h to assay their relative cell viabilities using the CCK-8 kit. Taking the cell viability of the control sample (without gefitinib) as 100%, the cell viabilities of MGC-803 and MKN-45 cells were reduced in a concentration- and time-dependent manner. The cell viabilities of MGC-803 and MKN-45 cells treated with gefitinib (4 mM, 24 h) were (54.01±6.72)% and (56.13±5.02)%, respectively.

The effects of 4 mM gefitinib on MGC-803 and MKN-45 cells were also studied in cells incubated with gefitinib for 0, 24, 48 and 72 h. The cell viabilities markedly decreased after 24 h (P<0.05) (Fig. 2C).

Cell apoptosis of MGC-803 and MKN-45 cells treated with gefitinib (4 mM, 24 h) increases significantly. Cell apoptosis

Table II. Associations between STAT 5b expression and clinicopathological parameters in gastric carcinomas.

Characteristics	No.	STAT 5b		P-value
		Negative	Positive (%)	
Gender				
Male	48	13	35 (72.9)	0.774
Female	21	7	14 (66.7)	
Age				
<60	21	6	15 (71.4)	1.001
≥60	48	14	34 (70.8)	
T stage				
T1	18	8	10 (55.6)	0.037
T2	14	6	8 (57.1)	
T3	25	6	19 (76.0)	
T4	12	0	12 (100)	
N stage				
N0	24	11	13 (54.2)	0.014
N1	10	5	5 (50.0)	
N2	18	2	16 (88.9)	
N3	17	2	15 (88.2)	
Clinical stage				
1	12	6	6 (50.0)	0.014
2	22	10	12 (54.5)	
3	34	4	30 (88.2)	
4	1	0	1 (100)	
Pathological grading				
1	11	8	3 (27.3)	0.005
2	22	3	19 (86.4)	
3	29	7	22 (75.9)	
4	7	2	5 (71.4)	

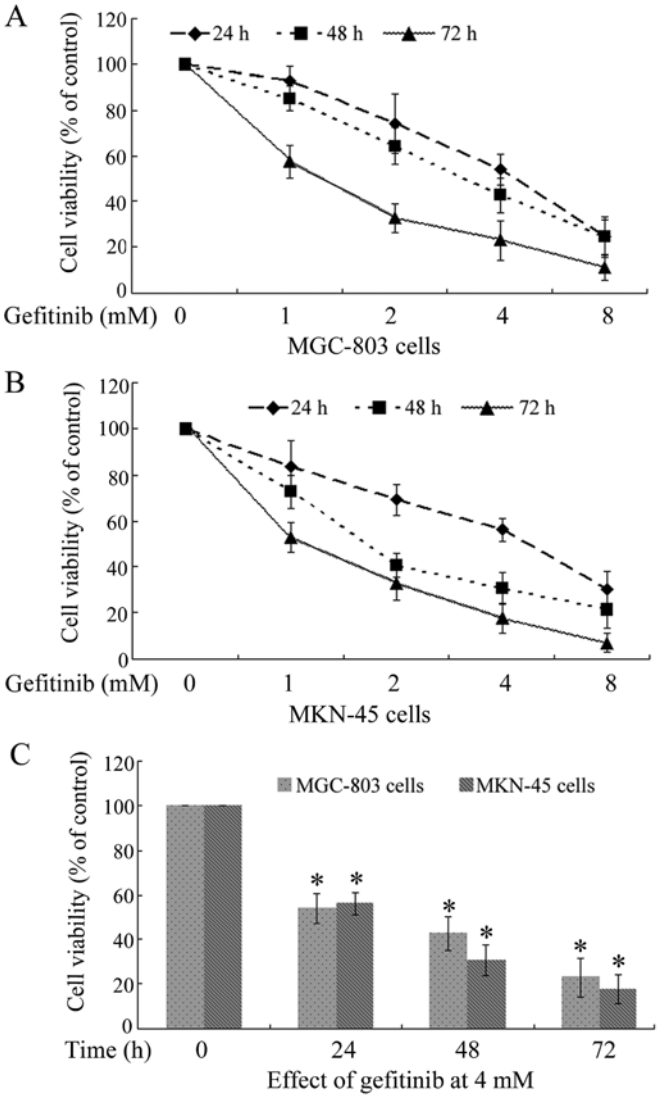

Figure 2. Effects of gefitinib to cultured MGC-803 and MKN-45 cells were measured using a CCK-8 kit. (A and B) Cultured MGC-803 and MKN-45 cells were exposed to different concentrations of gefitinib (0, 1, 2, 4 and 8 mM) for 24, 48 and 72 h. The cell viabilities of MGC-803 and MKN-45 cells decreased in concentration- and time-dependent manners. (C) Effect of 4 mM gefitinib in both MGC-803 and MKN-45 cells were also studied in cells incubated with gefitinib for 0, 24, 48 and 72 h. The cell viabilities markedly decreased after 24 h (*P<0.05).

of MGC-803 and MKN-45 cells treated with gefitinib (4 mM, 24 h) and assayed using flow cytometry (FCM). The apoptotic rates of normal MGC-803 cells and normal MKN-45 cells were (1.53±0.31)% and (2.2±0.53)%, respectively. Cell apoptotic rates increased significantly in MGC-803 and MKN-45 cells treated with gefitinib, with rates of (9.33±1.45)% in MGC-803 cells and (10.17±1.66)% in MKN-45 cells (Fig. 3).

STAT 5b expression is downregulated significantly in MGC-803 and MKN-45 cells treated with gefitinib (4 mM, 24 h). MGC-803 and MKN-45 cells were exposed to gefitinib (4 mM) for 24 h. Protein extracts were collected, and the expression of STAT 5b was assayed by western blot analysis (Fig. 4A). Taking the expression in the control cells as 100%, the relative expression levels of STAT 5b in MGC-803 and MKN-45 cells treated with gefitinib (4 mM, 24 h) were (48.2±9.68)% and (35.78±10.85)%, respectively, as assessed by a quantitative analysis using ImageJ software (Fig. 4B). The differences in expression were statistically significant (P<0.05).

Interference of STAT 5b expression by siRNA targeting enhanced the chemo-sensitivity of gastric cancer cells to gefitinib. To assess the role of STAT 5b in the chemo-sensitivity of gastric cancer cells to gefitinib, we interfered with the expression of STAT 5b by siRNA (10 μM) targeting. Optimal conditions for interference were assessed by incubating MGC-803 and MKN-45 cells with 1, 2 and 3 μl STAT 5b siRNA. The results indicated that incubation with 3 μl of 10 μM STAT 5b siRNA significantly interfered with STAT 5b expression (Fig. 5A).

The chemo-sensitivity of gastric cancer cells to gefitinib (4 mM, 24 h) with or without STAT 5b siRNA (10 μM, 3 μl) was detected using the CCK-8 kit. Fig. 5B showed that the cell viabilities significantly decreased in the combined treatment group (gefitinib+siRNA) compared with the gefitinib (4 mM, 24 h) only group in MGC-803 and MKN-45 cells (P<0.05).

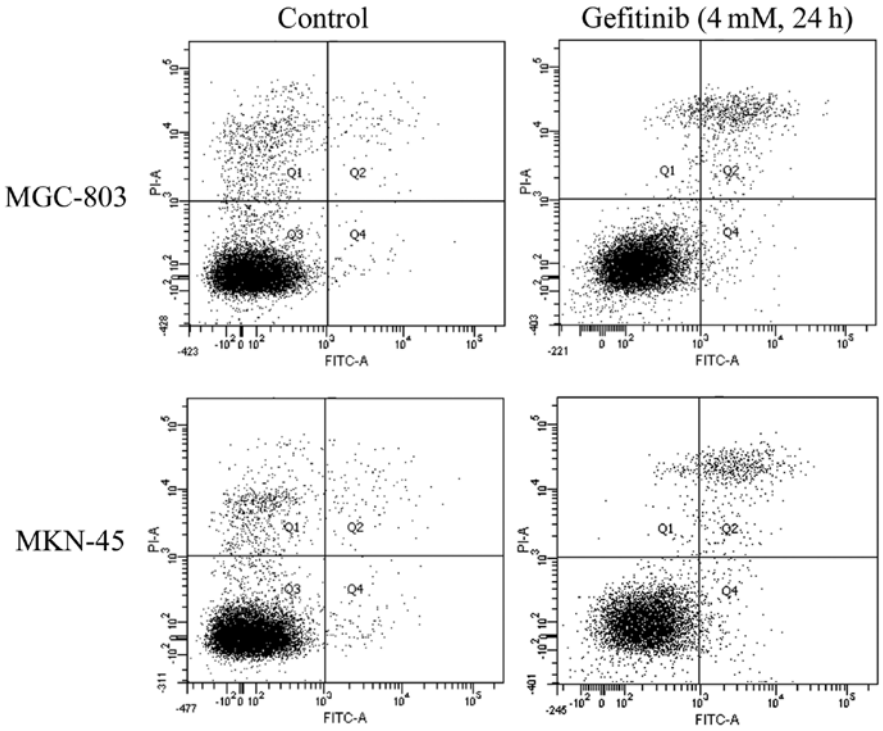

Figure 3. Cell apoptosis of MGC-803 and MKN-45 cells treated with gefitinib (4 mM, 24 h) and assayed by flow cytometry. Cell apoptosis increased significantly in the MGC-803 and MKN-45 cells treated with gefitinib (4 mM, 24 h).

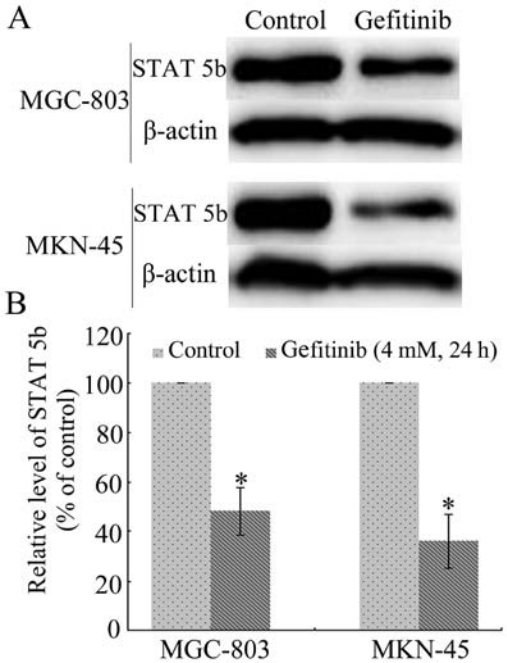

Figure 4. Expression of STAT 5b is significantly downregulated in MGC-803 and MKN-45 cells treated with gefitinib (4 mM, 24 h). The differences in expression were statistically significant (*P<0.05).

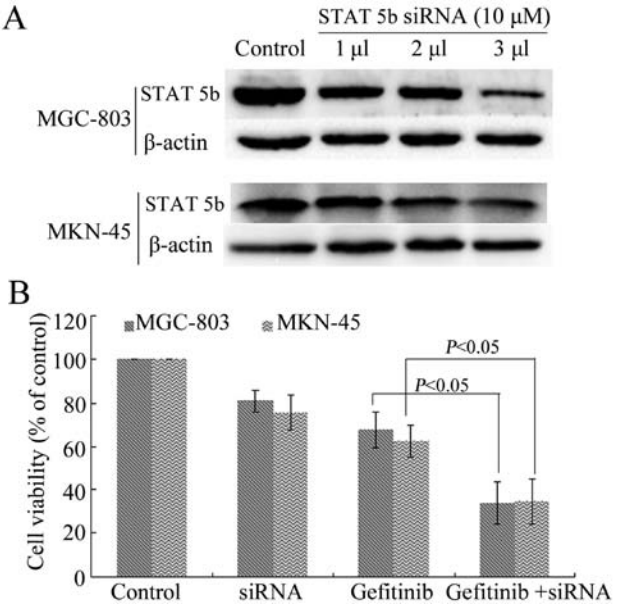

Figure 5. Interference of STAT 5b expression enhances the sensitivity of gastric cancer cells to gefitinib. (A) STAT 5b siRNA (10 μM) was used to interfere with the expression of STAT 5b in MGC-803 and MKN-45 cells. STAT 5b siRNA (10 μM, 3 μl) interfered significantly with STAT 5b expression. (B) Interference of STAT 5b expression by siRNA (10 μM, 3 μl) targeting enhances the sensitivity of gastric cancer cells to gefitinib (4 mM, 24 h), with cell viabilities decreasing significantly in the combined treatment group (gefitinib+siRNA) compared with the gefitinib (4 mM, 24 h) only group in the MGC-803 and MKN-45 cells (P<0.05).

Interference of STAT 5b expression by siRNA targeting enhances gefitinib-induced apoptosis in gastric cancer cells. As shown in Fig. 6, the cell apoptosis of MGC-803 and MKN-45 cells in the combined treatment group (gefitinib+siRNA) increased significantly compared with the gefitinib (4 mM, 24 h) only group, as determined by flow cytometry.

Interference of STAT 5b expression influences the expression of mitochondrial pathway-related apoptosis proteins in MGC-803 and MKN-45 cells. The mitochondrial pathway is

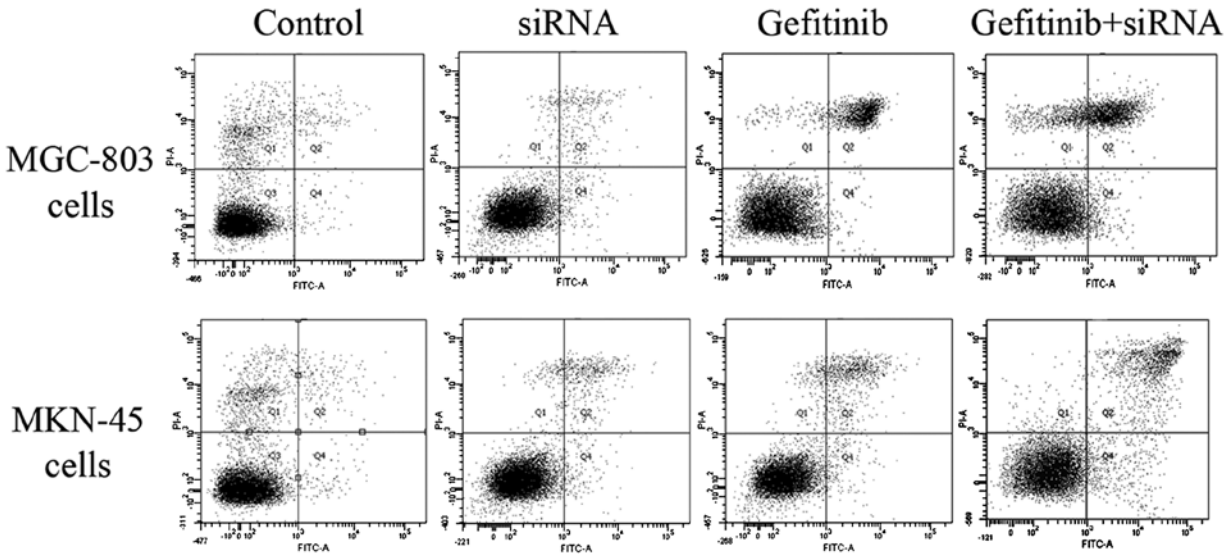

Figure 6. Cell apoptosis of MGC-803 and MKN-45 cells in the combined treatment group (gefitinib+siRNA) increased significantly compared with the gefitinib (4 mM, 24 h) only group, as assessed by flow cytometry.

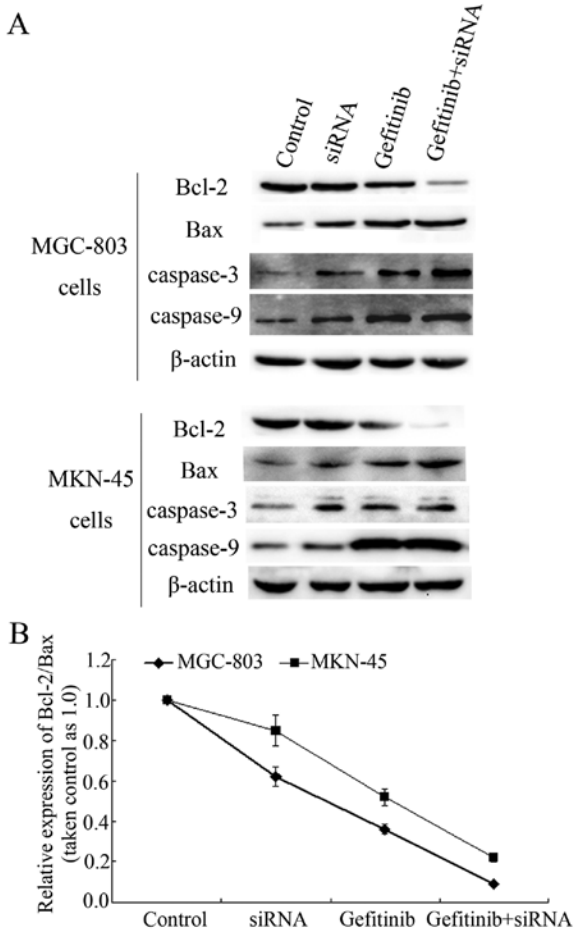

Figure 7. Interference of STAT 5b expression influences the expression of mitochondrial pathway-related apoptosis proteins in MGC-803 and MKN-45 cells. (A) Interference of STAT 5b expression by siRNA (10 μM, 3 μl) targeting upregulated the mitochondrial expression of Bax, caspase-3 and caspase-9 and downregulated the expression of Bcl-2. The expression of these proteins was significantly enhanced in the combined treatment group (gefitinib+siRNA) compared with the gefitinib (4 mM, 24 h) only group in the MGC-803 and MKN-45 cells. (B) Interference of STAT 5b expression by siRNA (10 μM, 3 μl) targeting significantly decreased the Bcl-2/Bax expression ratio (P<0.05).

a major apoptosis mechanism. Mitochondrial proteins were extracted using a special kit for extracting mitochondrial proteins. Mitochondrial pathway-related apoptosis proteins, including Bcl-2, Bax, caspase-3 and caspase-9, were assayed. The results showed that the interference of STAT 5b expression by siRNA (10 μM, 3 μl) targeting upregulated the expression of Bax, caspase-3 and caspase-9 and downregulated the expression of Bcl-2. The expression of these proteins was enhanced significantly in the combined treatment group (gefitinib+siRNA) compared with the gefitinib (4 mM, 24 h) only group in the MGC-803 and MKN-45 cells (Fig. 7A).

The relative expression of Bcl-2 and Bax is usually used to assess sensitivity to apoptosis. In the present study, we analyzed Bcl-2/Bax levels under gefitinib with or without STAT 5b siRNA. The results showed that interference of STAT 5b expression significantly decreased the Bcl-2/Bax expression ratio (P<0.05) (Fig. 7B).

Discussion

In this study, we found evidence that a molecular mechanism links STAT 5b expression to gastric cancers. We found an increased expression of STAT 5b in both gastric carcinomas and MGC-803 and MKN-45 cells treated with gefitinib. Additionally, we found that interference of STAT 5b expression by siRNA targeting enhances the chemo-sensitivity of gastric cancer cells to gefitinib by promoting mitochondrial pathway-mediated cell apoptosis, indicating a potential role and mechanism for STAT 5b in the chemo-sensitivity of gastric cancers.

As a main member of the STAT family, STAT 5 has been reported to play an important role in many biological processes. For example, STAT 5 plays essential roles in body growth, lipid metabolism, and the cell cycle pertaining to hepatosteatosis, fibrosis, and hepatocellular carcinoma through its action in the GH-STAT 5 axis. STAT 5 has been reported to mediate GH signals, and GH-STAT 5 signaling has been found to be important in hepatic physiology and pathophysiology (10). Additionally, STAT 5, including STAT 5a and STAT 5b, has

been shown to be important in some types of solid tumors (11-13). In preclinical prostate cancer (PCa) models, STAT 5a/b promotes PCa growth and progression. STAT 5a/b is critical for PCa cell viability *in vitro* and for tumor growth *in vivo* and promotes the metastatic dissemination of cancer in nude mice (14). We reported the upegulation of STAT 5b in gastric cancers compared with the para-carcinomas and found a significant association between STAT 5b expression and the clinicopathological parameters, including T stage, N stage, clinical stage and pathological grading. These results indicate the critical importance of STAT 5b in gastric cancers. To the best of our knowledge, this study is the first to describe a specific role of STAT 5b in gastric cancers. However, findings of other studies on the function of STAT 5 are inconsistent with our results. According to studies based on 100 patients who underwent gastrectomy due to gastric adenocarcinoma, there was no statistically significant association between STAT 5 expression and TNM staging and survival, as assessed by IHC (15). Those authors did not refer specifically to STAT 5a or STAT 5b but to a general STAT 5, which may account for this inconsistency. Mounting evidence indicates that the role of STAT 5a or STAT 5b is different in various tumors. Studies in breast cancer reported that loss of STAT 5a, but not STAT 5b, represents a new independent marker of poor prognosis in node-negative breast cancer and may be a predictor of response to antiestrogen therapy, if validated in randomized clinical trials (16). Of note is that STAT 5a was also detected in our gastric carcinomas and para-carcinomas, and an increased expression was found in gastric carcinomas. However, no significant association was observed between STAT 5a expression and the above clinicopathological parameters (data not shown). This result indicates the different effects of STAT 5a and STAT 5b in gastric cancers. Results of the present study have shown that, STAT 5b, but not STAT 5a, plays an important role in the progression of gastric cancer.

Apoptosis, known as programmed cell death, is a programmed mechanism of cell death that ensures normal development. STAT 5 is believed to be critical in the process of cell apoptosis. Repression of STAT 5a and STAT 5b expression in the chronic myelogenous leukemia (CML) cell lines, K-562, with unmodified or chemically modified siRNAs has been shown to induce apoptosis and is believed to constitute a potential new and alternative curative method for supporting therapy of the CML-diagnosed patients (17). Avoidance of apoptosis is an important contributor to the survival of tumor cells, and an important aim in cancer treatment is targeted towards tumor cell apoptosis. In this study, the gastric cancer cell lines MGC-803 and MKN-45 were exposed to gefinitib, a selective inhibitor of the EGFR tyrosine kinase. Cell apoptosis was induced and expression of STAT 5b was upregulated in gefinitib-treated cells, indicating the possible importance of STAT 5b in the chemo-sensitivity of gastric cancers by promoting apoptosis. Previous studies have reported the effect of STAT 5b in the apoptotic-sensitivity of many solid tumors, such as colorectal cancer (18), breast cancer (19), and lymphoid and non-lymphoid malignancies (20). These studies on other cancer types are consistent with our novel finding of an effect of STAT 5b on the apoptotic-sensitivity of gastric cancers.

Two apoptotic pathways have been described in mammalian cells, the extrinsic pathway, also known as the death receptor pathway, and the intrinsic pathway, known as the mitochondrial pathway (21,22). In the extrinsic pathway, an extracellular signal such as Fas ligand, a member of the tumor necrosis factor (TNF) family, is targeted to cell surface receptors. In the intrinsic pathway, intracellular signals such as DNA damage and mitochondrial injury are involved. At the same time, many proteins, mostly in the mitochondrial cytoplasm, are involved in the intrinsic pathway. The B-cell lymphoma-2 (Bcl-2) and caspase family members are important in the intrinsic pathway, and the Bcl-2 family is the major target of STAT 5 in anti-apoptotic pathways (23,24). Most Bcl-2 family members, including Bcl-2, Bcl-XL, Bcl-w, Mcl-l, Bfl 1/A-1 and Bcl-B, play anti-apoptotic roles, with a subset classified as pro-apoptotic (BAX, BAK and BID). The pro-apoptotic BAX was first identified as an inhibitory binding partner of Bcl-2, and the ratio of Bcl-2 to BAX expression is usually used as an index for predicting apoptosis (25). In this study, we detected the expression of Bcl-2 and BAX in mitochondria, and the Bcl-2/BAX ratio was also calculated. The results show that Bax expression was upregulated and Bcl-2 expression was downregulated in gefitinib-treated cells and in the gefitinib+STAT 5b siRNA-combined treatment group, indicating the pro-apoptotic role of STAT 5b in the gefitinib treatment of gastric cancer. Caspase-3 and caspase-9 are also two common pro-apoptotic molecules (24,26). Our study, based on the detection of caspase-3 and caspase-9, also confirmed the pro-apoptotic role of STAT 5b in the gefitinib-mediated treatment of gastric cancer. To the best of our knowledge, this is the first study to describe the pro-apoptotic role of STAT 5b in the gefitinib-mediated treatment of gastric cancer, especially in the mitochondrial apoptotic pathway.

This study had some limitations. For example, gefitinib is as a type of molecular-targeted drugs, however, its targeted molecular EGFR was not studied. Additionally, the change of phosphorylated STAT 5b (p-STAT 5b) was not completed in the present study, because phosphorylation is the main approach to play its role for STAT 5b. It is not clear whether p-STAT 5b was changed accordingly. These limitations indicate potential avenues for future studies.

We showed the increased expression of STAT 5b in both gastric carcinomas and in MGC-803 and MKN-45 cells treated with gefitinib. We found that interference of STAT 5b expression by siRNA targeting enhances the chemo-sensitivity of gastric cancer cells to gefitinib by promoting mitochondrial pathway-mediated cell apoptosis, indicating a potential role and mechanism for STAT 5b in the chemo-sensitivity of gastric cancers. This study has the potential to increase our understanding concerning the molecular pathophysiology of gastric cancer and introduces gefitinib as a potential medication to be included in the treatment regimen of gastric cancer chemotherapy.

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