Research Article

KIF23 Promotes Gastric Cancer by Stimulating Cell Proliferation

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1. Introduction

Gastric cancer (GC) is one of the most aggressive malignant tumors with low early diagnosis and high metastasis. Despite progress in treatment, to combat this disease, a better understanding of the underlying mechanisms and novel therapeutic targets is needed. KIF23, which belongs to the KIF family, plays a vital role in various cell processes, such as cytoplasm separation and axon elongation. Nowadays, KIF23 has been found to be highly expressed in multiple tumor tissues and cells, suggesting a potential link between KIF23 and tumorigenesis. Herein, we reported that KIF23 expression was correlated with poor prognosis of gastric cancer and found an association between KIF23 and pTNM stage. An in vitro assay proved that the proliferation of gastric cancer cells was significantly inhibited, which is caused by KIF23 depletion. Additionally, knockdown of KIF23 resulted in a marked inhibition of cell proliferation of gastric cancer in mice, with significant downregulation of Ki67 and PCNA expression. In conclusion, these data indicate that KIF23 is a potential therapeutic target for gastric cancer treatment.
2. Materials and Methods

2.1. Antibodies and Primers. Rabbit anti-MKLP1 (1:200 dilution for immunohistochemistry, ab235955, Abcam plc, Cambridge, UK), mouse anti-β-actin (1:5000 dilution, ab2672, Abcam plc, Cambridge, UK), rabbit anti-Ki67 (1:1000 dilution for western blot and 1:100 dilution for immunohistochemistry, ab16667, Abcam plc, Cambridge, UK), and rabbit anti-proliferating cell nuclear antigen (PCNA) (1:1000 dilution for western blot and 1:100 dilution for immunohistochemistry, ab18197, Abcam plc, Cambridge, UK) were used.

The qRT-PCR primer sequences of KIF23 are as follows: forward, 5′-AGACAGAAAGGCGAGGATG-3′, and reverse, 5′-GGAGACGAATTGGTGGTG-3′. The qRT-PCR primer sequences of GAPDH are as follows: forward, 5′-GGTTGAGCACAGGGTCTTAT-3′, and reverse, 5′-GGITGACAGCACAGGGTACCTTAT-3′.

Ready-to-package AAV shRNA clones of KIF23 were bought from Addgene plc. The shRNA sequences targeting KIF23 were as follows: sense, 5′-AAACGACCTTTAAGACCCAGGCG-3′, and control shRNA did not match any known human coding cDNA.

2.2. Immunohistochemistry. Gastric cancer tissues were obtained from patients in Baoding First Central Hospital, and immunostaining was performed to analyze protein expression in clinical samples. Tissues were formalin-fixed and paraffin-embedded, and then sections were made in 5 μm. Those sections were deparaffinized in xylene and rehydrated in alcohol (100%, 100%, 95%, 85%, and 75%). The antigen was retrieved by citric acid buffer in a microwave for 15 min and then cooled at room temperature. Endogenous peroxidase was blocked using a blocking reagent for 5 minutes at room temperature and washed three times using PBS buffer. Then, sections were incubated with an anti-KIF23 antibody at 4°C overnight. On the second day, sections were washed three times in PBS and incubated with a secondary antibody-HRP (ProteinTech, Sanying, Wuhan, China, 1:1000) for 1 hour at room temperature, washed, and stained with diaminobenzidine (DAB, Cell Signaling Technology, Pudong, Shanghai, China) for 5 min. The images were collected with a microscope (Nikon, Tokyo, Japan).

The KIF23 protein is mainly located in the cytoplasm and the nucleus of tumor cells. The tissues were scored according to the staining intensity of tumor cells. Positive cells were scored as follows: the proportion of positive tumor cells < 10%—score 0, the percentage of positive tumor cells being 10% to 30%—score 1, the positive percentage of tumor cells being 30%-70%—score 2, and the positive percentage of tumor cells > 70%—score 3. The positive staining was evaluated zero for negative staining, 1 for weak positive staining, 2 for moderate positive staining, and 3 for strong positive staining. The final scores were calculated by multiplying the proportion and intensity. According to the distributions of the final scores, we divided the expression of KIF23 into the high- (>3) and low- (0-3) expression groups. The sections of each patient were observed within 10 visual fields, and two experienced pathologists read the sections without getting the pathological grade and clinical data. The results were judged by a double-blind method.

2.3. Cell Culture and Transfection. The two types of human gastric cancer cell lines MGC-803 and SGC-7901 were bought from ATCC (Chicago, USA). Both of them were maintained in RPMI 1640 culture medium, supplemented with 10% of fetal bovine serum at 37°C in a 5% CO₂ incubator.

The KIF23 shRNA plasmids were transfected into cancer cell lines with InvitroGen Lipofectamine® 2000 (Thermo Fisher Scientific Inc.). The specific shRNA with the sequence of AAGCTTGCCTATTGACATAGAC (Cat# SH817843, Vigen Biosciences, Rockville, USA) to target KIF23 and scrambled sequence was used as a negative control. 100,000 cells per well were plated in six-well plates according to the manufacturer’s protocol, and 3 groups were set, including the shKIF23 group which was transfected with shRNA targeting KIF23, the negative control group which was transfected with a scrambled sequence, and the mock group which was treated without transfection (data not shown). Silencing efficiency was measured by RT-PCR and western blot after 48-hour transfection. These reduced cells were used to explore the association between KIF23 and cell proliferation and cell invasion. Then, the cell lines with stable depletion of KIF23 were screened and used for the in vitro and in vivo assays.

2.4. qRT-PCR Assay. Total RNA was extracted from MGC-803 and SGC-7901 cells using TRIzol Reagent (Invitrogen). Then, total RNA was reverse-transcribed by M-MLV reverse transcriptase (Promega). Quantitative real-time PCR was performed using SYBR mixture (Takara), and the relative expression of KIF23 was normalized to GAPDH.

2.5. Western Blot Assay. Protein samples extracted from cells or tumor tissues were separated by SDS-PAGE and sequentially transferred onto NC membranes, followed by blocking with 5% fat-free dry milk. The membranes were then incubated with primary antibodies for detection of KIF23, Ki67, PCNA, and β-actin for two hours. Subsequently, the membranes were incubated with HRP-conjugated secondary antibodies for 1 hour. Signals were visualized with an ECL kit.

2.6. Colony Formation. Both MGC-803 and SGC-7901 cells were plated in 6-well plates with a density of 4000 cells each well and grown for 14 days. The colonies were then fixed with methanol and stained with 0.1% crystal violet for 25 minutes. Images were then photographed, and colony numbers were sequentially calculated.

2.7. MTT Assay. MGC-803 and SGC-7901 cells were plated in 96-well plates with a density of 2000 cells per well and cultured for 48 hours. Subsequently, cells were incubated with MTT for 4 hours and removed from the medium. Then, cells were washed with PBS twice. MTT was then extracted by 150 μL DMSO, and the absorbance value at a wavelength of 570 nm was quantified.
2.8. In Vivo Tumor Growth Assay. The surgery of mice was approved by the Institutional Animal Care Committee of Baoding First Central Hospital. Nude BALB/c mice (6-8 weeks, 18-22 g) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). To measure tumor growth in vivo, MGC-803 cells were stably transfected with the control or KIF23 shRNAs and injected subcutaneously into the right flank of female nude mice. After tumors (50-60 mm$^3$) had established (about 2 weeks), the tumor volume was measured every 2, 3, or 4 days with a caliper and calculated as (length $\times$ width$^2$)/2.

2.9. Statistics. Data was analyzed using SPSS 22.0 software (SPSS Inc., IBM Corp., Armonk, NY). For immunohistochemistry experiments, the association between KIF23 expression and clinicopathological features was assessed using the $\chi^2$ test. The survival was correlated with tumor progression and KIF23 expression by the Kaplan-Meier method and log-rank test. Data are shown as mean $\pm$ standard deviation (SD) in in vitro and in vivo experiments. Student’s $t$-test is used for statistical comparison. $P < 0.05$ was considered statistically significant.

3. Results

3.1. KIF23 Expression Was Associated with the Prognosis of Gastric Cancer. To explore the potential role of KIF23 in gastric cancer, we detected the expression of KIF23 in tumor tissues of gastric cancer patients who underwent surgical resection by immunohistochemistry. The surgical samples ($n = 82$) were classified into low- and high-KIF23 expression groups, according to the staining intensity of KIF23, while the expression of KIF23 was low in the normal paracancer
According to the results, 30 tumor tissues showed low expression levels of KIF23, whereas 52 of them showed a high expression level. The difference in clinicopathological characteristics was analyzed between the low-KIF23 expression and high-KIF23 expression groups. Data such as patient age, patient gender, tumor size, tumor grade, and lymph node metastasis were recorded, whereas no significant difference was found in these aspects between the KIF23 low-expression and high-expression groups, suggesting no marked association between KIF23 expression and these characteristics (Table 1). However, the KIF23 expression level in tumor tissues was remarkably correlated with the pTNM stage and recurrence (Table 1).

We further explored the association between KIF23 and poor prognosis in gastric cancer patients. We found that the expression of KIF23 was associated with the overall survival rate and disease-free survival rate (Figure 1(c)). The patients with high expression of KIF23 had shorter OS and DFS time ($P < 0.05$, respectively). In conclusion, immunohistochemistry assays, clinicopathological characteristics, and survival analyses indicated that KIF23 was positively correlated with poor prognosis of gastric cancer.

### Table 1: Relationships of KIF23 and clinicopathological characteristics in 82 patients with gastric cancer.

| Feature                  | All (n = 82) | KIF23 expression | $\chi^2$ | $P$     |
|--------------------------|-------------|------------------|---------|--------|
| Age (year)               |             |                  |         |        |
| <50                      | 48          | 14               | 3.577   | 0.059  |
| ≥50                      | 34          | 16               |         |        |
| Gender                   |             |                  |         |        |
| Male                     | 52          | 20               | 0.216   | 0.642  |
| Female                   | 30          | 10               |         |        |
| Tumor grade              |             |                  |         |        |
| Low                      | 50          | 15               | 2.395   | 0.122  |
| High                     | 32          | 15               |         |        |
| Tumor size               |             |                  |         |        |
| <5 cm                    | 36          | 16               | 1.708   | 0.191  |
| ≥5 cm                    | 46          | 14               |         |        |
| pTNM stage               |             |                  |         |        |
| I-II                     | 60          | 26               | 4.389   | 0.036* |
| III-IV                   | 22          | 4                |         | 0.278  |
| Lymph node metastasis    |             |                  |         |        |
| Yes                      | 28          | 8                | 1.177   | 0.278  |
| No                       | 54          | 22               |         |        |
| Recurrence               |             |                  |         |        |
| Yes                      | 40          | 10               | 4.518   | 0.034* |
| No                       | 42          | 20               |         |        |

3.2. KIF23 Suppression Could Inhibit Cell Proliferation of Gastric Cancer In Vitro. To explore the functional role of KIF23 in gastric cancer, we used shRNA targeting KIF23 to decrease the expression level of KIF23 in two types of gastric cancer cell lines, MGC-803 and SGC-7901. The qRT-PCR (Figure 2(a)) and western blot (Figure 2(b)) assay confirmed that the transfection of KIF23 shRNA effectively inhibited the expression of KIF23 in both MGC-803 and SGC-7901 cells, respectively.

Cell proliferation is a vital process in cancer development. To explore the potential of KIF23 in the proliferation of gastric cancer cells, we performed colony formation assays. We found that the transfection of KIF23 shRNA plasmid significantly inhibited the proliferation of MGC-803 and SGC-7901 cells, respectively, consistent with significantly decreased cell colony numbers (Figure 3(a)). Furthermore, a significantly decreased absorbance value of 570 nm in MGC-803 and SGC-7901 cells was detected after performing MTT assays (Figure 3(b)). To further confirm the results about KIF23 ablation which inhibited the proliferation of gastric cancer, we then examined the expression levels of Ki67 and PCNA, respectively. Both Ki67 and PCNA could reflect the proliferation degree. Data indicated that the expression of Ki67 and PCNA resulted in a significant decrease in KIF23-depleted gastric cancer cells, compared with the control (Figures 3(c) and 3(d)). Thus, these results demonstrated that KIF23 depletion could inhibit cancer cell proliferation in vitro.

3.3. KIF23 Knockdown Could Inhibit Gastric Cancer Proliferation in Mice. In order to explore the protumor effect of KIF23 in vivo, MGC-803 cells were infected with shKIF23 lentivirus to stably knock down the expression of KIF23. shControl or shKIF23 cells were injected subcutaneously into...
nude mice, and then the tumor volume was measured per week and after 2 weeks following injection. The tumor volumes in the KIF23-depleted group were remarkably smaller than those in the control (Figure 4(a), left), and representative photographs of tumors from each group were shown (Figure 4(a), right). Additionally, we examined the KIF23 expression in tumor tissues of mice, performing western blot and immunohistochemistry assays. All these data confirmed that the expression of KIF23 in the shKIF23 group was markedly decreased compared with the control (Figures 4(b) and 4(c)).

We further explored the potential mechanism about KIF23 and gastric cancer proliferation. Immunohistochemistry assays were performed using tumor tissues, and interestingly, the results showed that the expression level of Ki67 and PCNA in the KIF23-ablated group was significantly reduced, suggesting a significant inhibition of cell proliferation (Figure 4(d)). These data demonstrated that KIF23 was involved in the proliferation of gastric cancer in vivo.

4. Discussion

Gastric cancer is a malignant tumor that originated from the gastric mucosal epithelium, and its incidence ranks first among all kinds of malignant tumors in China [18, 19]. Most of the early gastric cancer can receive radical treatment under surgical therapy, and the 5-year survival rate is over 90% [20]. However, the diagnosis and treatment rate of early gastric cancer in China is less than 10%, far lower than that in other Southeast Asian countries, such as Japan (70%) and South Korea (50%) [21]. Early diagnosis and early treatment of cancer are the main strategies to reduce mortality and improve survival [22]. In recent years, many new therapeutic targets for gastric cancer, such as STK33 and HNF4, have been found, and their effectiveness needs to be further confirmed [23, 24]. In this study, we demonstrated the association between KIF23 and gastric cancer and considered it to be a new target for gastric cancer treatment. Of course, more elaborate mechanisms need to be confirmed by further experiments.

As a member of the KIF family, KIF23 plays as a molecular motor, which is plus-end-directed and relied on the microtubule [14, 25]. Several studies suggest that KIF23 may play a potential role in cell proliferation [14]. During mitosis, KIF23 is involved in the formation of the midbody and further affects cytokinesis by recruiting regulatory factors involved in cell division [12, 26, 27]. KIF23 has the special localization in the nucleus. When the nuclear localization
Figure 3: Knockdown of KIF23 significantly inhibited the proliferation in gastric cancer cells. (a) Photographs of the colony formation assay showed MGC-803 (a) and SGC-7901 cells (b) that were transfected with the control or KIF23 shRNA. Cultivation lasted 2 weeks. (b) The MTT assay revealed the difference between the control and KIF23-depleted gastric cancer cells. (c) The western blot assay showed that the expression level of Ki67 was markedly decreased in MGC-803 and SGC-7901 cells. (d) The western blot assay revealed that the PCNA expression was significantly downregulated in MGC-803 and SGC-7901 cells. Results are presented as mean ± SD (*P < 0.05).
signal of KIF23 is mutated, the cell cycle is markedly blocked, suggesting that KIF23 may also influence the process of cell proliferation through transcription [26]. Although KIF23 may be involved in the regulation of cell proliferation, it is still unclear whether KIF23 regulates tumor cell proliferation and further affects the occurrence and development of tumor. Here, we found that KIF23 suppression could inhibit gastric cancer development by affecting cell proliferation, which confirmed the previous conclusion and provided a novel therapeutic target for the treatment of gastric cancer.

KIF23 affects the progression of various tumors. Consistent with our data on the role of KIF23 in gastric cancer, KIF23 has been reported to affect the proliferation of glioma both in vivo and in vitro, which was regulated by TCF-4, suggesting a similar mechanism [28], whereas KIF23 could inhibit the development of lung cancer and promote
apoptosis of lung cancer cells in vivo [13, 29]. We only examined its function on the proliferation of gastric cancer cells, and the effect on apoptosis needs further study. Previous data have shown that KIF23 is associated with the expression of a variety of oncopgenes that influence tumor cell division, chromosome segregation, proliferation, and metastasis [30]. In hepatocellular carcinoma, KIF23 is found to be upregulated and correlated with poor prognosis [30]. KIF23 was also reported to be highly expressed in non-small-cell lung cancer, and patients with low expression of KIF23 tended to have better prognosis [17]. In conclusion, KIF23 is involved in the occurrence and development of various tumors, mainly through affecting the proliferation, invasion, and apoptosis of cancer cells [13, 28]. We demonstrated that KIF23 suppression could inhibit cancer development by affecting the proliferation of gastric cancer cells, but more detailed molecular mechanisms still needed to be further studied.

Collectively, our data argued that the expression of KIF23 was increased in tumors of gastric cancer patients. KIF23 was correlated with the pTNM stage of gastric cancer, suggesting its role in the pathogenesis of gastric cancer. In fact, patients with gastric cancer with high KIF23 expression had worse prognosis. It has been confirmed by in vitro and in vivo assays that KIF23 was involved in the development of gastric cancer by affecting the proliferation of the cancer cells. Our data indicated that KIF23 might serve as a potential molecular target for personalized medicine in gastric cancer.

Data Availability
The dataset supporting the conclusions of this article is included within the article.

Ethical Approval
All applicable international, national, and/or institutional guidelines for the care and use of human specimens and animals were followed. The animal study was carried out in accordance with the guidelines approved by the animal experimentation ethics committee of the hospital. The protocol was approved by the committee, all surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Conflicts of Interest
The authors declare that they have no competing interests.

Authors’ Contributions
Xiao-Long Li and Ya-Ming Ji carried out the experiment of molecular biology and drafted the manuscript. Rui Song and Xiao-Ning Li carried out the animal experiment and participated in the sequence alignment. Lan-Shuan Guo participated in the design of the study and performed the statistical analysis. All authors conceived the study and participated in its design and coordination and helped in drafting the manuscript. All authors read and approved the final manuscript. Xiao-Long Li and Ya-Ming contributed equally to this work.

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