KIF5A Promotes Bladder Cancer Proliferation In Vitro and In Vivo

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1. Background

Bladder cancer continues to be a severe health problem, which is ranked as the ninth most diagnosed cancer in the world, and nearly 500,000 people are diagnosed with bladder cancer each year in the world [1]. The mortality from bladder cancer is primarily due to metastasis and recurrence after surgery. Although current clinical treatments such as surgery and chemotherapy have greatly improved, 50-70% of individuals relapse within 5 years after surgery [2]. The high prevalence and postsurgical recurrence of bladder cancer demand a better understanding of the molecular mechanism of cancer progression. Therefore, it is urgent to find novel diagnostic markers and to identify effective therapeutic target to increase the survival rate of patients with bladder cancer.

KIF5A, known as kinesin family member 5A, and the kinesin superfamily is characterized by a set of proteins that share highly conserved motor domain [3]. Several key cellular processes such as mitosis and meiosis involve kinesins, that is to say, they can modulate the cell cycle, proliferation, and differentiation [4]. Kinesin proteins play an important role in the occurrence and development of many human cancers, and these have been confirmed by more and more evidence [5]. In addition, a study has pointed out that drug resistance in human malignancy is also associated with kinesin proteins [6]. More and more kinesins have been discovered with potential anticancer characteristics [7]. Thus, KIF5A generally is accepted as a tumorigenic protein, and targeting KIF5A may be a promising anticancer strategy.

Many researchers have reported that the high expression of KIF5A was associated with poor clinicopathological characteristics and prognosis of solid tumors such as breast cancer [8], prostate cancer [9], and lung cancer [10]. Despite the existing research, no study has evaluated the expression...
of KIF5A in bladder cancer, and little is currently known regarding the clinical significance of KIF5A expression in bladder cancer. Therefore, this study is aimed at evaluating the relationship between KIF5A and bladder cancer. In this study, we demonstrate that KIF5A promotes bladder cancer proliferation and find that the overexpression of KIF5A is associated with poor clinic prognosis suggesting a novel therapeutic strategy for bladder cancer.

2. Materials and Methods

2.1. Clinical Specimen Collection. A total of 115 bladder cancer tissues were acquired from patients undergoing radical cystectomy at The Second Hospital of Tianjin Medical University (Tianjin, China) between 1998 and 2008 (Table 1). Before the radical cystectomy, no patients had received radiotherapy or chemotherapy. All specimens were confirmed by two special urology pathologists, and the histopathological features of the specimens were classified according to the 2002 TNM classification.

2.2. Ethical Statement. This cohort was approved by the Ethics Committee at The Second Hospital of Tianjin Medical University and was carried out in accordance with the principles of the Declaration of Helsinki. A written informed consent was obtained from each patient before operation. All in vivo animal experiments were approved by the Committee on the Ethics of Animal Experimentation of The Second Hospital of Tianjin Medical University. All treatments were followed by the US Public Health Service Policy on the Humane Care and Use of Laboratory Animals. All operations on the animals were conducted under sodium pentobarbital anesthesia and were made to minimize animal suffering system.

2.3. Immunohistochemistry. Tissues from patents and xenografts were fixed in 4% paraformaldehyde, embedded in paraffin, and then cut into 5 μm thickness. The primary antibodies against KIF5A (catalog # ab154414, 1:200 dilution, Abcam PLC, Cambridge, UK) and KIF20B (catalog # PA5-56696, MPHOSPH1 antibody, 1:100 dilution, Thermo Fisher Scientific, New York, USA) were used. For quantitation, average integrated optical density (IOD) was obtained by analyzing five fields, and each slide was evaluated by Image-Pro Plus software (version 6.0) for immunohistochemical staining. All sections were photographed at a magnification of ×100 and ×200. For the results, KIF5A cytoplasmic staining and KIF20B nuclear staining were scored by using 4-point scales (0, no staining; 1+, light staining; 2+, intermediate staining; and 3+, dark staining of linear membrane at low magnification). Furthermore, we divided KIF5A cytoplasmic staining and KIF20B nuclear staining into low (0-1+) and high (2+-3+) expressions, respectively.

2.4. Cell Lines. T24 and 5637 cell lines were purchased from the American Type Culture Collection (Manassas, USA) and were authenticated by isozyme detection, cell vitality detection, and DNA fingerprinting. Both T24 and 5637 cells were cultured in a RPMI 1640 medium (Corning, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL). Both cell lines were regularly authenticated by morphological observation and tested for mycoplasma contamination (MycopAlert, Lonza, Rockland, ME, USA). The cells were incubated at 37°C in a humidified incubator with 5% CO2.

2.5. RNA Interference and Transient Transfection. ShRNAs were cloned into lentiviral pAV-U6-GFP vector. The following shRNA sequences were used: control shRNA, 5′-ACT CAA AAG GAA GTG ACA AGA-3′ and KIF5A shRNA, 5′-AAACACGACTCAAGAGCAAGTTT-3′. Transient transfection was done according to the manufacturer’s procedure.

2.6. RNA Isolation and Quantitative Real-Time PCR (qRT-PCR). Total RNA was extracted using a TRIzol reagent (Invitrogen) as per manufacturer’s instructions. The first-strand cDNA was obtained using the Reverse EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgene). qRT-PCR was implemented by using the SYBR Green Master Mixture reagent (Roche) in ABI7500 real-time

### Table 1: Relationships of KIF5A and clinicopathological characteristics in 115 patients with BC.

| Feature                              | All (n = 115) | KIF5A expression | χ²   | P     |
|--------------------------------------|---------------|-------------------|------|-------|
|                                      | Low (n = 36)  | High (n = 79)     |      |       |
| Age (year)                           |               |                   |      |       |
| <65                                  | 46            | 18                | 2.184| 0.139 |
| ≥65                                  | 69            | 18                | 2.184| 0.139 |
| Gender                               |               |                   |      |       |
| Male                                 | 99            | 30                | 0.332| 0.565 |
| Female                               | 16            | 6                 |      |       |
| Tumor stage                          |               |                   |      |       |
| T2                                   | 54            | 26                | 13.431| 0.000*|
| T3/T4                                | 61            | 10                | 5.830| 0.016*|
| Tumor grade                          |               |                   |      |       |
| Low                                  | 64            | 26                | 1.948| 0.163 |
| High                                 | 51            | 10                | 1.408| 0.235 |
| Lymph node metastasis                |               |                   |      |       |
| Yes                                  | 35            | 8                 | 1.669| 0.196 |
| No                                   | 80            | 28                |      |       |
| Recurrence                           |               |                   |      |       |
| Yes                                  | 59            | 15                | 1.948| 0.163 |
| No                                   | 56            | 21                | 1.408| 0.235 |
| Distant metastasis                   |               |                   |      |       |
| Yes                                  | 33            | 13                | 1.408| 0.235 |
| No                                   | 82            | 23                |      |       |
| Vascular invasion                    |               |                   |      |       |
| Yes                                  | 35            | 12                | 0.208| 0.648 |
| No                                   | 80            | 24                |      |       |
PCR instrument. The expression levels of KIF5A were calculated using the comparative $2^{-\Delta\Delta Ct}$ method by StepOne Software, and GAPDH was used as an internal control. qRT-PCR primers are as follows: KIF5A, forward: 5'-AGGTGTAAGGTGC-3', reverse: 5'-CUCTCTGTT AGGTGTAAGGTGC-3'; and GAPDH, forward: 5'-AACG GATTGGTCGTATGGG-3', reverse: 5'-TCGCTCTCTG GAAGATGGTGAT-3'. All experiments were implemented in triplicate.

### 2.7. Western Blot Analysis

Cells were washed twice with cold PBS and then lysed in RIPA buffer with protease and phosphatase inhibitors (Roche, Complete Mini). Cell debris and insoluble material were removed by centrifugation at 12,000 × g at 4°C for 25 minutes. Then, protein quantitation on 30 μg of the protein was loaded per lane using the Bradford Protein Assay (Bio-Rad, USA), electrophoresed by SDS-PAGE, and then transferred into nitrocellulose membranes. The following membranes were blocked with 5% skim milk at room temperature for 1 h and then incubated with primary antibodies (anti-kinesin 5A antibody with 1:1,000 dilution, ab154414; mouse anti-β-actin with 1:1,000 dilution, ab8226; rabbit anti-Ki67 with 1:1,000 dilution, ab16667; and mouse antiproliferating cell nuclear antigen (PCNA) with 1:500 dilution, ab29; ab72; all of the above antibodies were obtained from Abcam PLC, Cambridge, UK) at 4°C overnight, followed by TBST washed 3 times for 10 minutes each and 1 h incubation with horseradish peroxidase conjugate secondary antibodies at room temperature. The bound antibodies were detected using an enhanced chemiluminescence reagent (32109, Thermo Fisher Scientific).

### 2.8. Colony Formation Assays

Cells were cultured in 6-well plates with a density of 5,000 cells every well. After 2 weeks, colonies were fixed with methanol and then stained with 0.1% crystal violet. Photographs were then obtained, and the number of colonies was counted.

### 2.9. Cell Proliferation Assays

Cells were planted into 96-well tissue culture plates at a density of 5,000 cells/well and incubated at 37°C for 2 h. Cell proliferation was assayed by using a Vybrant MTT cell proliferation assay kit (Thermo Fisher Scientific) according to the operating instruction.

### 2.10. Xenograft Mouse Model

T24 cells ($1 \times 10^5$) with stable expression of shKIF5A were subcutaneously injected into the right flank region of the male euthymic nude mice at 4 weeks old. T24 cells ($1 \times 10^5$) were subcutaneously injected into the left flank of the nude mice as the negative control. The tumor size was measured ($0.5 \times \text{length} \times \text{width}^2$) in mice every 7 days within 21 days and then measured every 3 days. 4 weeks after inoculation, all mice were sacrificed, tumors excised and photographed, and then with 4% paraformaldehyde solution, fixed for immunohistochemistry. All animal studies were carried out under the supervision and guidelines of the Tianjin Medical University Institutional Animal Care and Use Committee.

### 3. Results

#### 3.1. Upregulation of KIF5A in Bladder Cancer Was Associated with Poor Prognosis

Immunohistochemical staining of the tissues showed that KIF5A was mainly localized to the cytoplasm of the human bladder cells (Figure 1(a)), and no KIF5A stain was found in the normal bladder tissue (Figure 1(b)). And the results showed that KIF5A was significantly associated with tumor stage ($P = 0.015$) and tumor grade ($P = 0.016$) of bladder cancer patients. However, no associations were found between KIF5A and other clinical features such as patients’ ages, genders, lymph node metastasis, recurrence, distant metastasis, and vascular invasion (Table 1). Furthermore, the Kaplan-Meier analysis showed that higher KIF5A expression was associated with poorer prognosis. It was found that bladder cancer with higher expression of KIF5A had lower overall patient survival rate and progression-free survival rate (Figure 2(c); $P < 0.05$). The findings indicated that KIF5A is involved in the aggressiveness and progression of bladder cancer.

#### 3.2. Knockdown of KIF5A Inhibited Cell Proliferation In Vitro

To explore the role of KIF5A in the progression of bladder cancer, we stably suppressed KIF5A in two bladder cancer cell lines (T24 and 5637), lentiviruses carrying shRNA-bearing KIF5A (shKIF5A) and control nonspecific shRNA (control). qRT-PCR showed the inhibition of KIF5A expression (Figure 2(a); $P < 0.05$), and western blot analysis showed similar results (Figure 2(b); $P < 0.05$). Colony formation assay showed that bladder cancer cells transfected with shRNA significantly reduced the colony numbers of T24 cells and 5637 cells (Figure 3(a); $P < 0.05$). Consistently, MTT assay results showed that the knockdown of KIF5A obviously inhibited the proliferation of T24 cells and 5637 cells (Figure 3(b); $P < 0.05$). Similarly, the western blot analysis showed proteins that reflect cell proliferation such as Ki67 (Figure 3(c); $P < 0.05$) and PCNA (Figure 3(d); $P < 0.05$) also supported these results. That is to say, KIF5A may act as an oncogene involved in the promotion of bladder cancer cell proliferation.

#### 3.3. Knockdown of KIF5A Suppressed Cell Proliferation In Vivo

Then, we investigated the effect of KIF5A in vivo using a xenograft mouse model. After a 4-week inoculation, the tumor volume produced by the shKIF5A-transfected cells was significantly smaller than the control group.
We implemented a western blot assay using these cancer tissues and found that KIF5A expression was higher in the control group than in the shKIF5A group (Figure 4(b); \( P < 0.05 \)). Immunohistochemistry also gives similar results (Figure 4(c); \( P < 0.05 \)), as well as the western blot assays of Ki67 and PCNA (Figure 4(d); \( P < 0.05 \)). These results illustrated that KIF5A both in vitro and in vivo could promote the proliferation and metastasis of the bladder cells.

3.4. Coexpression Existed between CD164 and CXCR4 in Bladder Cancer Tissues. As the relationship between KIF5A and KIF20B was found in the Internet (https://string-db.org/cgi/network.pl?taskId=vwL0g7pJEEPv) and the abnormally high expression of KIF20B was reported in a study [11], we further investigated the coexpression between them. In our study, we used immunohistochemistry to observe the association between the expressions of KIF5A and KIF20B in tumor tissue by uninterrupted slicing. The typical staining was shown in Figure 5(a): the results found that an obvious positive correlation existed (Figure 5(b)). Therefore, we speculated that KIF5A possibly played roles in bladder cancer through affecting the expression of KIF20B and the relevant pathways.

4. Discussion

Bladder cancer is one of the most common cancers in the world with a high relapse rate and is also a leading cause of cancer-associated deaths [12–14]. However, there is no radical cure for bladder cancer at present, that is to say, once presented with invasion and metastasis, which manifests an abominably ominous prognosis [15]. Meanwhile, the diagnosis and the follow-up monitoring of bladder cancer still remain a challenge because of the lack of disease-specific symptoms [16]. Cystoscopy is accepted as the gold standard for detection and surveillance of bladder cancer; however, it is an invasive procedure that causes discomfort in patients, so some novel ancillary noninvasive biomarkers for detection of bladder cancer have been appearing [17]. Several tumor
suppressor genes have been shown to be mutated in bladder cancer, such as P21, TP53, XPO5, BLCAP, and TRAP1 [18–21], and other tumor-associated genes have been demonstrated to contribute to the pathogenesis of bladder cancer, such as SPAG5 and MORC2 [22, 23]. Despite these knowledges above, the role of the kinesins in bladder cancer remains unclear, and no report has been found. Kinesins are a superfamily of proteins that play important roles in eukaryotic intracellular signaling and cell division [24]. According to the current studies, the genomes of the kinesin superfamily of higher vertebrates contain up to 45 genes that encode different kinesins [25], and groups of kinesin superfamily members exert key roles in the development of cancer, especially in the different stages of mitosis and in the cytoplasmic division in cancer [26]. For example, KIF1B promotes migration and invasion of glioma by inducing the cell surface localization of MT1-MMP [27]. KIF2A drives the proliferation and migration of breast cancer cells and is associated with poor prognosis in breast cancer patients [28]. KIF11 is associated with glioblastoma; a study has found that it can promote tumor cell proliferation, invasion, and self-renewal [29]. It has also been reported that KIF15 can drive the spread of pancreatic cancer through the MEK-ERK signaling pathway [30]. Some scholars have also found that the high expression of KIF18A is associated with the metastasis of breast cancer [31]. The expression level of KIF23 is associated with the prognosis of patients undergoing radical resection of pulmonary carcinoma, and a high expression usually means poor prognosis [32]. Therefore, as shown above, members of the kinesin superfamily play important roles in tumor behavior.

In KIF5A, many researchers have found that it was associated with poor clinicopathological characteristics and prognosis in lots of cancers such as breast cancer, prostate cancer, and lung cancer [8–10]. We also observed that high KIF5A expression was associated with shorter overall survival and progression-free survival rates among patients with bladder cancer, and for the first time, we demonstrated this relationship between the expression of KIF5A and bladder cancer prognosis among patients undergoing radical cystectomy. In this study, we counted 115 cases of patients and first found that high expression of KIF5A is often accompanied by tumor stage and tumor grade; however, this has no association with the patients’ gender, age, lymph node metastasis, distant metastasis, and vascular invasion. These findings are consistent with the results previously seen in breast cancer [8], and our results indicated that KIF5A might be an oncogene that could increase the risk of bladder tumor proliferation.

Additionally, to further explore the KIF5A function, we knockdown KIF5A in the cell lines of bladder cancer. The results showed that KIF5A knockdown significantly inhibited cell growth and colony formation in bladder cancer cell lines in vitro. In vivo, remarkable suppression of tumor growth was also observed after KIF5A was knocked down. Our findings suggest that KIF5A plays an oncogenic role in the progression of bladder cancer by promoting cell growth, which is consistent with previous research in other cancers [10].

In this present study, we showed the clinical significance of KIF5A in bladder cancer. However, there are some limitations to this research. Most importantly, our data are only

![Figure 2](image-url)
based on analyses according to differences in the level of KIF5A using clinical tissue samples and then made verifications in vitro and in vivo. The potential molecular mechanism that KIF5A enhances carcinogenesis in bladder cancer remains for further investigation.

KIF20B, also known as MPHOSPH1 or MPP-1, a member of kinesin-6 family, has been shown to be abnormally high expressed in bladder cancer tissues [11]. In recent years, many studies have indicated that KIF20B played a role in tumor (colorectal cancer, hepatocellular carcinoma, pancreatic cancer, and bladder cancer) progression especially proliferation [11, 33–35], which was closely related to KIF5A in our study. Moreover, we found the positive correlation between KIF5A and KIF20B in the clinical data. In addition, KIF20B/PRC1 complex is reported to play a crucial role in bladder carcinogenesis and that

**Figure 3:** The role of IL1A in cell proliferation ability in T24 and 5637 cell lines. (a) Colony formation assay. Results showed that the stable knockdown of KIF5A by AAV in the T24 and 5637 cells significantly reduced the ability to proliferate in vitro compared to the control cells (*P < 0.05). (b) MTT proliferation assay. OD values were observed to be decreased in the KIF5A silencing group as compared to the control (*P < 0.05). (c) and (d) Western blotting showed that Ki67 and PCNA were significantly reduced in the KIF5A silencing group compared to the control (*P < 0.05). All results are performed of at least three independent experiments.
inhibition of them might become a therapeutic target. Therefore, we suspected that KIF5A might also play roles in tumor progression of bladder cancer through KIF20B/PRC1 signaling pathway. More studies were needed to be verified and the exact mechanism of the functions of KIF5A in bladder cancer should be researched and confirmed in the future.

Figure 4: Inhibitory role of KIF5A silencing in vivo. (a) Xenograft assay. After 7 weeks of treatment, the tumor volume of KIF5A downregulation group was significantly smaller than in the control in subcutaneous and metastatic sites \((^* P < 0.05)\). (b, c) Results suggested that KIF5A protein level was dramatically reduced by our recombinant AAV in the injected tumor xenografts in nude mice by western blot and IHC assays \((^* P < 0.05)\). (d) The expression of proliferation markers Ki67 and PCNA in vivo measured by western blot. Knocking down KIF5A inhibited Ki67 and PCNA expression \((^* P < 0.05)\). All results are performed of at least three independent experiments.

Figure 5: Coexpression between KIF5A and KIF20B in the bladder cancer tissues by immunohistochemistry. (a) The immunohistochemistry of KIF5A and KIF20B in 115 samples was performed. The typical low and high expression staining was shown. (b) \(\chi^2\) tests and correlation analysis (Pearson and Spearman) were performed to analyze the associations between KIF5A and KIF20B. The results found that an obvious positive correlation existed \((P < 0.05)\).
In summary, our results indicated that KIF5A expression was associated with prognosis of bladder cancer and concluded that KIF5A could play an oncogenic role in bladder cancer. KIF5A might be identified as a new possible therapy target for bladder cancer.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflicts of Interest
No potential conflict of interest was reported by the authors.

Authors’ Contributions
Da-Wei Tian, Zhou-Liang Wu, Li-Ming Jiang, Jie Gao, Chang-Li Wu, and Hai-Long Hu conceived and designed the experiments. Da-Wei Tian, Zhou-Liang Wu, Li-Ming Jiang, and Jie Gao performed the experiments. Da-Wei Tian, Zhou-Liang Wu, Li-Ming Jiang, Jie Gao, Chang-Li Wu, and Hai-Long Hu contributed reagents/materials/analysis tools. Da-Wei Tian wrote the paper.

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