JMJD2B Promotes Epithelial–Mesenchymal Transition by Cooperating with β-Catenin and Enhances Gastric Cancer Metastasis

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Abstract

Purpose: This study investigated the role of histone demethylase Jumonji domain–containing protein 2B (JMJD2B) in promoting epithelial–mesenchymal transition (EMT) and underlying molecular mechanisms in the progression of gastric cancer.

Experimental Design: The induction of EMT by JMJD2B in gastric cancer cells and its underlying mechanisms were examined by a series of assays. In vivo and in vitro assays were performed to clarify invasive potential of JMJD2B in gastric cancer cells. The expression dynamics of JMJD2B were detected using immunohistochemistry in 101 cases of primary gastric cancer tissues.

Results: Inhibition of JMJD2B by specific siRNA suppresses EMT of gastric cancer cells, whereas ectopic expression of JMJD2B induces EMT. Importantly, JMJD2B is physically associated with β-catenin and enhances its nuclear localization and transcriptional activity. JMJD2B, together with β-catenin, binds to the promoter of the β-catenin target gene vimentin to increase its transcription by inducing H3K9 demethylation locally. JMJD2B inhibition attenuates migration and invasion of gastric cancer cells in vitro and metastasis in vivo. The expression of JMJD2B was positively correlated with tumor size (P = 0.017), differentiation status (P = 0.002), tumor invasion (P = 0.045), lymph node metastasis (P = 0.000), distant metastasis (P = 0.024), and tumor–node–metastasis (TNM) stage (P = 0.002) in patients with gastric cancer.


Introduction

Gastric cancer is one of the most common malignancies worldwide and ranks second in terms of global cancer-related mortality (1). Although the overall incidence of gastric cancer has declined in the United States, the incidence remains high in Asian countries (2). Once into the stage of locally advanced or metastatic stage, surgery and combination chemotherapies play a minor role to improve the survival rate (3). Thus, a better understanding of the progression of gastric cancer is urgent. It is critical to identify the new diagnostic and prognostic markers and find novel targeted therapies.

Epithelial–mesenchymal transition (EMT), a developmental process in which epithelial cells show reduced intercellular adhesion and acquire migratory fibroblastoid properties, is considered to be critical for invasive and metastatic progression in cancer (4–6). The process of EMT is associated with the downregulation of epithelial markers, abnormal translocation of β-catenin, and aberrant upregulation of mesenchymal markers (7). Repression of epithelial markers [e.g., E-cadherin (CDH1)], abnormal translocation of β-catenin, and aberrant upregulation of mesenchymal markers [e.g., vimentin and N-cadherin (CDH2)] are typical gene expression changes observed during EMT (5–7). These processes are initiated by zinc-finger transcriptional repressors such as Snail (also known as Snail-1), which suppresses E-cadherin expression (5). Vimentin, a type III intermediate filament normally expressed in cells of mesenchymal origin, has been reported as a mesenchymal marker. Upregulation of vimentin is associated with a poor clinical prognosis of many tumors including gastric cancer. β-Catenin/T-cell factor (TCF) binds to the vimentin promoter and transactivates its expression, thereby increasing tumor cells invasive potential (8–10). Although great research efforts have been made on EMT program, the underlying molecular mechanism is still not fully understood.
Mounting evidence indicates that histone posttranslational modifications play a crucial role in diverse biologic processes, and disruption of these processes has been linked to diseases such as cancer (11, 12). Abnormalities in the methylation of histones by histone demethylases have been implicated in various cancers (13). Jumonji domain–containing protein 2B (JMJD2B), also known as KDM4B, is a jmjC domain–containing histone demethylase, which belongs to JMJD2 family. JMJD2B primarily targets the trimethylated lysine 9 of histone H3 (H3K9) and has been reported to play an important role in many biologic processes such as heterochromatin formation, X-chromosome inactivation, homeotic gene silencing, and transcriptional regulation (14–16). Recently, increasing studies have revealed an epigenetic role of JMJD2B in stem cells differentiation (17), inflammation (18), and tumorigenesis (19–26). It is reported that JMJD2B interacts with estrogen receptor α (ERα) and is recruited to ERα target sites, which demethylates H3K9me3 and facilitates transcription of ER-responsive genes including MYB, MYC, and CCND1 to play an important role in the development and progression of breast cancer (22). It is also reported that JMJD2B/MLL2 complex is copurified with ERα, with H3K9 demethylation, and H3K4 methylation in ERα–activated transcription, which provides a mechanism for the role of JMJD2B in breast carcinogenesis (23). In our previous study, we demonstrated that JMJD2B promoted proliferation and survival of gastric cancer cells (24). However, whether and how JMJD2B is involved in gastric cancer invasion and metastasis arouses our interest.

In the present work, we found that JMJD2B promoted EMT and enhanced metastasis and progression of gastric cancer. We identified that JMJD2B physically interacted with β-catenin and was recruited to the promoter of vimentin and induced H3K9 demethylation on vimentin promoter increasing vimentin transcription. Moreover, we also found that JMJD2B inhibition attenuated nuclear accumulation of β-catenin and decreased its transcriptional activity. Knockdown of JMJD2B in gastric cancer cells inhibits cells migration and invasion both in vitro and in vivo. Finally, we demonstrated that JMJD2B expression positively correlated with the tumor metastasis status in patients with gastric cancer. Collectively, we identified potential invasive activities of JMJD2B in gastric cancer cells, and revealed a novel transcriptional mechanism for regulating vimentin expression, suggesting that JMJD2B might be a promising therapeutic target for blocking progression of gastric cancer.

RNA extraction, reverse transcription, and qRT-PCR
The method of RNA extraction and reverse transcription was used as described (27). The quantitative real-time PCR (qRT-PCR) was performed using the ABI7000 Fast Real-Time PCR System (Applied Biosystems) with the SYBR Green Kit (Applied Biosystems) according to the manufacturer’s instructions. The primers used in this study were listed in the Supplementary Table S1.

Western blot analysis and coimmunoprecipitation
Total cellular proteins were extracted and Western blot analysis was performed as described (24). Cellular proteins (80 μg) were separated for Western blot analysis. The procedure was described in detail in Supplementary Materials and Methods.

HEK293T cells were transfected with different plasmids and then treated cells as well as BGC823 cells were lysed in NP40 lysis buffer (supplemented with protease
inhibitors) for 30 minutes on ice. Extracts were clarified by centrifugation at 13,000 × g for 10 minutes at 4°C for coimmunoprecipitation. After centrifugation, the supernatant was collected and preclarified by incubating with protein G-PLLIS-Agarose Immunoprecipitation beads (Santa Cruz Biotechnology) at 4°C for 1 hour. And then they were incubated with special antibodies against Flag (Zhongshan Golden Bridge Biotechnology), HA (Zhongshan Golden Bridge Biotechnology), JMJD2B (A301-478A; Bethyl), β-catenin (ab32572; Abcam), and IgG (Santa Cruz Biotechnology) at 4°C on a rocker platform. The antibody-coated beads were then incubated with the lysates at 4°C overnight. Immunoprecipitates were collected, washed, lysed, and boiled. The boiled samples were analyzed by Western blot analysis as described above.

Luciferase reporter activity assay

The luciferase reporter activity assay was described in detail in Supplementary Materials and Methods.

ChIP

Chromatin immunoprecipitation (ChIP) assay was performed as described previously (28). Briefly, HEK293T cells transfected with different plasmids and BGC823 cells treated with JMJD2B siRNA and control siRNA were cross-linked and lysed in 1% (v/v) formaldehyde containing medium for 10 minutes at 37°C, followed by sonication to make soluble chromatin with DNA fragments between 200 and 1,000 bps. Immunoprecipitation was performed overnight at 4°C with specific antibodies, or irrelevant control antibody. The protein A/G Sepharose beads (Millipore) were added for 1 hour at 4°C with rotation to collect the antibody/histone complex for immunoprecipitation. The protein-DNA complex was eluted, and reverse cross-linked. Following treatment with Protease K (Sigma-Aldrich), DNA was extracted with phenol–chloroform and precipitated with ethanol. The recovered DNA was resuspended in Tris-EDTA buffer, and used for PCR amplification with the following specific primers listed in the Supplementary Table S1.

Immunofluorescence assay

The immunofluorescence assay was described in detail in Supplementary Materials and Methods.

Scratch wound–healing assay

Scratch wound–healing assay was used to assess cell migration and described in detail in Supplementary Materials and Methods.

Matrigel invasion assay

Matrigel invasion assay was described in detail in Supplementary Materials and Methods.

Immunohistochemistry

Immunohistochemical analysis was performed as previously described (24). The procedure was described in detail in Supplementary Materials and Methods. The pathologic scores were assigned by two independent pathologists, who were blinded to the final pathologic interpretation. According to the intensity and percentage of JMJD2B staining, JMJD2B expression was scored as 0 (no appreciable staining in tumor cells), 1 (mild, positive staining in the nucleus of 0%–29% tumor cells), 2 (moderate, positive staining in the nucleus of 30%–69% tumor cells), and 3 (strong, positive staining appreciable in the nucleus of more than 70% tumor cells).

Patients

The paraffin-embedded pathologic specimens from 101 patients with primary gastric cancer were used in the study, which were approved by the local ethics committee. The patients underwent gastrectomy at Qilu Hospital, Shandong University (Jinan, PR China) between 2005 and 2011; none of the patients had received chemotherapy or radiotherapy before surgery. We confirmed the diagnosis of the specimens by histopathologic examination. These patients included 77 (76.2%) men and 24 (23.8%) women, with a mean age of 60 years. The pathologic tumor–node–metastasis (TNM) status of all gastric cancer was assessed according to the criteria of the seventh edition of American Joint Committee on Cancer/International Union Against Cancer TNM classification system.

Mouse model

BGC823 cells were injected into nude mice through tail vein and metastatic lung tissues were evaluated. The detailed procedure was described in Supplementary Materials and Methods.

Statistical analyses

Differences between experiment groups were analyzed by Mann–Whitney U test or Student t test. The relationships between JMJD2B expression and clinicopathologic features of patients with gastric cancer were analyzed by the χ² test. All the tests were two-tailed and analysis using the SPSS statistical software package (standard version 18.0). P values of less than 0.05 in all cases were considered statistically significant.

Results

JMJD2B inhibition induces the epithelial morphology of gastric cancer cells

In our previous study, we identified the functional role of JMJD2B in gastric cancer cell proliferation, survival, and tumorigenesis (24). Interestingly, we noted that knocking down JMJD2B expression by siRNA in HGC27 and MGC803 gastric cancer cells (spindle-shaped malignant gastric epithelial cell lines with high expression of JMJD2B and loosen cell–cell adhesion) not only inhibited cell proliferation, but also induced tight cell–cell contacts and cobblestone-like appearance that represented epithelial morphology (Fig. 1A). This observation indicated that JMJD2B might play a role in EMT and promoting gastric cancer cell invasion.
JMJD2B repression results in downregulation of mesenchymal markers and upregulation of epithelial markers

To address whether JMJD2B affected the EMT program, we knocked down JMJD2B expression using JMJD2B-specific siRNA in the gastric cell lines AGS, BGC823, HGC27, and MGC803, and determined the expression of the epithelial marker (E-cadherin) and mesenchymal markers (vimentin and Snail) by Western blot analysis and qRT-PCR (Fig. 1B and C1–C3). JMJD2B inhibition led to decreased vimentin and Snail protein expression and evaluated E-cadherin protein expression (Fig. 1B). The mRNA profile is consistent with protein expression (Fig. 1C1–C3). Furthermore, ectopic expression of JMJD2B using HA-JMJD2B vector transient transfection upregulated the vimentin and Snail expression, whereas reduced E-cadherin expression both in mRNA in BGC823 cells (Fig. 1D) and protein level in AGS and BGC823 cells (Fig. 1E). Collectively, JMJD2B plays an important role in regulating EMT marker expression in gastric cancer cells.

JMJD2B is physically associated with β-catenin and recruited to the vimentin promoter

JMJD2B regulated vimentin at mRNA level (Fig. 1C1–C3 and D). To identify whether JMJD2B regulated vimentin expression at the transcriptional level, we performed a luciferase assay. Our results showed that VimProMut-Luc luciferase activity was decreased in JMJD2B-depleted BGC823 cells (Fig. 2A), whereas opposite results were obtained in JMJD2B-overexpressed BGC823 cells (Fig. 2B). Previous study shows that β-catenin targets the vimentin gene for its transcription in gastric cancer (10). Given that JMJD2B regulates vimentin expression at the transcriptional level, we hypothesized a potential involvement of JMJD2B and β-catenin in vimentin transcription. We assessed the luciferase activity driven by the VimProMut-Luc luciferase vector in which putative binding site of β-catenin/TF on the vimentin promoter were inactivated. VimProMut-Luc luciferase activity failed to be repressed in JMJD2B-depleted BGC823 cells (Fig. 2A), whereas its activity also failed to be enhanced in JMJD2B-overexpressed BGC823 cells (Fig. 2B), suggesting that the regulation of JMJD2B on vimentin transcription was dependent on β-catenin. To further characterize the interaction between JMJD2B and β-catenin, we performed a coimmunoprecipitation experiment in flag-β-catenin and HA-JMJD2B expression vectors transfected HEK293T cells, and observed the physical interaction of JMJD2B with β-catenin (Fig. 2C). Consistently, their interaction at endogenous levels was shown in BGC823 cells (Fig. 2D). Furthermore, ChIP assay showed that JMJD2B occupied the vimentin promoter in HEK293T cells transfected with the HA-JMJD2B vector (Fig. 2E). In BGC823 cells, silencing JMJD2B abolished the binding of JMJD2B to the vimentin promoter coupled with the reduced binding of β-catenin. As predicted, a marked
reduction of H3K9me2 and sharp accumulation of H3K9me3 at the vimentin promoter was observed following JMJD2B knockdown (Fig. 2F). Taken together, JMJD2B is physically associated with β-catenin and is recruited to the vimentin promoter to demethylate H3K9me3 there, thereby facilitating vimentin induction.

**JMJD2B inhibition attenuates TGF-β1–mediated β-catenin nuclear accumulation**

TGF-β1–induced β-catenin nuclear translocation and its target genes transactivation is one of the key factors activating the EMT program (29–31). Gene ontology analysis revealed that JMJD2B regulates Wnt and TGF-β pathway in tumorigenesis (19). We found that JMJD2B inhibition attenuated the nuclear accumulation of β-catenin and its target gene vimentin expression in BGC823 cells (Fig. 3A and B). Furthermore, TGF-β1 mediated β-catenin nuclear translocation and vimentin expression was also reduced when JMJD2B expression was inhibited in BGC823 cells (Fig. 3C and D). Thus, JMJD2B is required for β-catenin nuclear retention and its transcription activity during EMT process.

**JMJD2B inhibition results in diminished invasion and metastasis in vitro and in vivo**

To further study the role of JMJD2B in gastric cancer cell migration and invasion, we performed wound-healing
assay and Transwell invasion assay in HGC27 and MGC803 cells. When JMJD2B was inhibited, there was a striking decrease of gastric cancer cell migration and invasion \((P < 0.01; \text{Fig. 4A and B and Supplementary Fig. S1A–S1C}).\)

We next asked whether JMJD2B knockdown inhibits tumor metastasis in vivo. JMJD2B siRNA and control siRNA–treated BGC823 cells (\(1 \times 10^6\) cells/mouse) were injected into immunodeficient nude mice through tail vein. The mice were sacrificed after 30 days and then their lungs for metastasis were examined. The lungs from mice injected with control siRNA-treated BGC823 cells were larger and heavier than those from JMJD2B knockdown mice \((P < 0.05; \text{Fig. 4C}).\) The representative picture of lungs (Supplementary Fig. S1D) shows grossly cystic lung micrometastasis in the control and JMJD2B-depletion groups. More tumor colonies were observed in the lungs from the control group than that from JMJD2B knockdown group (Fig. 4D).

**JMJD2B expression positively correlates with metastasis in human gastric cancer**

Finally, we determined whether the plot originating from the cell lines and animal experiments has any clinical relevance. Nonmetastatic gastric cancer (NMGC) and metastatic gastric cancer (MGC) samples were collected from patients with gastric cancer. JMJD2B and vimentin protein expression was determined in 101 cases of paraffin-embedded primary gastric cancer tissues using immunohistochemical staining. As expected, strong nuclear staining of JMJD2B was observed in MGC tissues, especially in metastatic lymph nodes (MLN), whereas less and weak nuclear staining was found in NMGC (Fig. 4E). Furthermore, cytoplasmic staining of vimentin was consistent with the expression trends of JMJD2B in MGC and NMGC tissues (Fig. 4E). Correlation analysis demonstrated no distinguished relationship between JMJD2B expression and the patients’ age.
and gender ($P > 0.05$). However, JMJD2B expression was positively correlated with tumor size ($P = 0.017$), differentiation status ($P = 0.002$), invasion ($P = 0.045$), lymph node status ($P = 0.000$), distant metastasis ($P = 0.024$), and TNM stage ($P = 0.002$; Table 1) of patients with gastric cancer. Moreover, a significant positive correlation between the expression of JMJD2B and vimentin was analyzed in 101 cases of gastric cancer tissues ($P = 0.004$; Table 2). Taken together, JMJD2B enhanced the metastasis of gastric cancer.

**Discussion**

Emerging evidence has indicated that aberrant alterations in the methyl status of histones play important roles in tumorigenesis (11, 12, 32). JMJD2B, a member of the JMJD2 family of histone demethylase, has been shown to exhibit oncogenic activities and overexpressed in human cancers (19–26, 33, 34). In our previous study, we found that JMJD2B facilitated gastric cancer cell proliferation, survival, and xenografted tumor growth in vivo. However, whether and how JMJD2B is involved in gastric cancer progression and metastasis is still unknown. In the present study, we provide evidence that JMJD2B is a critical positive regulator of EMT and thereby contributes to the progression and metastasis of gastric cancer. JMJD2B inhibition using specific siRNA induces epithelial morphology of invasive gastric cancer cells with upregulation of epithelial markers and downregulation of mesenchymal markers (Fig. 1 and Supplementary Fig. S2A). Moreover, JMJD2B silence results in diminished invasion and metastasis of gastric cancer cells in vitro and in vivo. Furthermore, overexpression of JMJD2B is observed in primary gastric cancer specimens and JMJD2B expression positively correlates with advanced disease.
Collectively, these results indicate that JMJD2B is an important epigenetic factor in the progression of gastric cancer.

EMT represents a critical event in the transition from early to invasive carcinomas (6, 35, 36). The upregulation of the mesenchymal marker vimentin is significantly associated

### Table 1. Correlation between JMJD2B expression and clinicopathologic features in patients with gastric cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>0—1</th>
<th>2–3</th>
<th>P (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>77</td>
<td>19 (24.7%)</td>
<td>58 (75.3%)</td>
<td>0.699</td>
</tr>
<tr>
<td>Women</td>
<td>24</td>
<td>5 (20.8%)</td>
<td>19 (79.2%)</td>
<td></td>
</tr>
<tr>
<td>Age y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>44</td>
<td>8 (18.2%)</td>
<td>36 (81.8%)</td>
<td>0.247</td>
</tr>
<tr>
<td>≥60(^b)</td>
<td>57</td>
<td>16 (28.1%)</td>
<td>41 (71.9%)</td>
<td></td>
</tr>
<tr>
<td>Tumor size (diameter in cm)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3</td>
<td>26</td>
<td>11 (42.3%)</td>
<td>15 (57.7%)</td>
<td>0.017</td>
</tr>
<tr>
<td>≥3, ≤5</td>
<td>39</td>
<td>9 (23.1%)</td>
<td>30 (76.9%)</td>
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</tr>
<tr>
<td>&gt;5</td>
<td>36</td>
<td>4 (11.1%)</td>
<td>32 (88.9%)</td>
<td></td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>23</td>
<td>11 (47.8%)</td>
<td>12 (52.2%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Moderate</td>
<td>25</td>
<td>7 (28.0%)</td>
<td>18 (72.0%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>53</td>
<td>6 (11.3%)</td>
<td>47 (88.7%)</td>
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</tr>
<tr>
<td>pT Status</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>7 (50.0%)</td>
<td>7 (50.0%)</td>
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<tr>
<td>2</td>
<td>9</td>
<td>2 (22.2%)</td>
<td>7 (77.8%)</td>
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<tr>
<td>3–4</td>
<td>78</td>
<td>15 (19.2%)</td>
<td>63 (80.8%)</td>
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<td>pN Status</td>
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<tr>
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<td>39</td>
<td>18 (46.2%)</td>
<td>21 (53.8%)</td>
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</tr>
<tr>
<td>1</td>
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<td>27</td>
<td>2 (7.40%)</td>
<td>25 (92.6%)</td>
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<tr>
<td>pM0</td>
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<td>24 (27.6%)</td>
<td>63 (72.4%)</td>
<td>0.024</td>
</tr>
<tr>
<td>pM1</td>
<td>14</td>
<td>0 (0.00%)</td>
<td>14 (100%)</td>
<td></td>
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<tr>
<td>pTNM Stage</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>I</td>
<td>20</td>
<td>9 (45.0%)</td>
<td>11 (55.0%)</td>
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<td>13</td>
<td>6 (46.2%)</td>
<td>7 (53.8%)</td>
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<tr>
<td>III</td>
<td>54</td>
<td>9 (16.7%)</td>
<td>45 (83.3%)</td>
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</tr>
<tr>
<td>IV</td>
<td>14</td>
<td>0 (0.00%)</td>
<td>14 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** 0—1, low-JMJD2B expression; 2–3, high-JMJD2B expression.

\(^a\)χ\(^2\) test.

\(^b\)Mean age.

### Table 2. Association between expression of JMJD2B and vimentin in gastric cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>0—1</th>
<th>2–3</th>
<th>P (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vimentin staining</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>38</td>
<td>15 (39.5%)</td>
<td>23 (60.5%)</td>
<td>0.004</td>
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<tr>
<td>2–3</td>
<td>63</td>
<td>9 (14.3%)</td>
<td>54 (85.7%)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)χ\(^2\) test.
with aggressive phenotype of gastric cancer (7, 10). We found that JMJD2B regulates vimentin expression at the transcription level and JMJD2B expression positively correlates with vimentin expression in primary gastric cancer tissues. It has been demonstrated that JMJD2B regulates gene expression by decreasing trimethylation of histone H3K9 on the target gene promoter (18, 25, 26). Indeed, we found the occupancy of JMJD2B on the vimentin promoter coupled with the accumulation of repressive H3K9me3 on vimentin promoter following JMJD2B depletion.

Histone modifiers often regulate target gene transcription through interaction with their transcription factors (22, 36–39). Gene ontology analysis reveals that JMJD2B regulates Wnt/β-catenin, TGF-β, Notch, and phosphoinositide 3-kinase (PI3K) pathways, all of which play important roles in tumorigenesis or cancer progression (19). β-Catenin can directly promote EMT phenotype besides its involvement in TGF-β1-mediated EMT (6, 8, 10). Our present findings demonstrate that JMJD2B is involved in β-catenin/TGF-β1-mediated EMT. JMJD2B knockdown reduces the nuclear accumulation of β-catenin and the decrease of the transcription of its target gene vimentin. Moreover, TGF-β1-mediated nuclear accumulation of β-catenin is also diminished by JMJD2B inhibition. JMJD2B physically interacts with β-catenin at the vimentin promoter, enhancing its transcription. To further verify the effect of JMJD2B on TCF/β-catenin–dependent transcription, the TOPflash/FOPflash TOP/FOP reporter system was used. JMJD2B inhibition slightly decreased the TOP/FOP ratio in JMJD2B-depleted BGC823 cells (Supplementary Fig. S2E1 and S2E2). Although a significant decrease was found in JMJD2B-depleted BGC823 cells compared with control cells when treated with TGF-β1 or β-catenin plasmid (Supplementary Fig. S2E1 and S2E2). Ectopic expression of JMJD2B significantly increased the TOP reporter activity when cotransfected with β-catenin plasmid in BGC823 cells (Supplementary Fig. S2E3). The above results indicate that JMJD2B is involved in TGF-β/Wnt-mediated TCF/β-catenin transcription activity and imply its transcriptional activation of vimentin expression through cooperating with β-catenin. Collectively, our result reveals novel mechanisms through which JMJD2B regulates the transcription of β-catenin target genes and EMT program. On one hand, JMJD2B is recruited to the promoter of β-catenin target gene in which it demethylates H3K9me3 and facilitates the transcription of β-catenin target gene. On the other hand, JMJD2B promotes the nuclear translocation and accumulation of β-catenin and thereby enhances its transcription activity. A positive feedback loop is formed to amplify the EMT effect. As is known, β-catenin nuclear accumulation is a hallmark of β-catenin transcriptional activation. Although whether JMJD2B has any effect on β-catenin protein expression is worth being considered. Immunoblotting showed that inhibition of JMJD2B expression had no obvious effect on overall expression of β-catenin, and nuclear β-catenin was reduced in JMJD2B-depleted BGC823 cells (Supplementary Fig. S2B1 and S2B2). However, it is still unclear how JMJD2B stimulates the nuclear translocation of β-catenin and more research work on the detailed mechanism needs to be done.

Cancer-associated EMT process involves multiple factors and pathways (26, 39). In our experiments, we also found the upregulation of E-cadherin and downregulation of Snail in JMJD2B-depleted gastric cancer cells. Therefore, it is likely that other mechanisms are also involved in JMJD2B mediated EMT program in addition to its regulation of vimentin via cooperation with β-catenin. Luciferase activity assay showed no significant effect on the E-cadherin promoter (pEcad–1008/+49) by JMJD2B knockdown or overexpression (Supplementary Fig. S2C). Therefore, JMJD2B might not regulate E-cadherin expression simply dependent on Snail. It was reported that Snail promoted Wnt target gene expression cooperating with β-catenin (40). We assumed that Snail played a role in JMJD2B mediated EMT program by regulating vimentin via cooperation with β-catenin. To address this hypothesis, we inhibited Snail expression by specific siRNA targeting Snail and Western blot analysis showed a subtle reduction of vimentin expression. The upregulation of vimentin by JMJD2B overexpression was also partially rescued when Snail was knocking down (Supplementary Fig. S2D). It indicated that Snail played a role in JMJD2B-mediated vimentin regulation. Moreover, a decrease of JMJD2B expression was observed in Snail-depleted BGC823 cells (Supplementary Fig. S2D). It suggested that a feedback loop between JMJD2B and Snail might exist. Besides, Snail might also participate in the EMT progress by regulating other EMT related markers through other mechanisms.

JMJD2B was shown to regulate Wnt/β-catenin, TGF-β, Notch, and PI3K pathways by gene ontology analysis (19). Our data also showed the involvement of JMJD2B in Wnt/TGF-β1–regulated gene expression. It was reported that Snail expression could be regulated through Wnt/β-catenin, TGF-β, and PI3K pathways (41, 42). Therefore, it is plausible to presume that JMJD2B induces Snail expression through these pathways. However, more work needs to be done to explore the detailed mechanism in future work.

Because the oncogenic potential of JMJD2B has been demonstrated in breast cancer (19, 22, 23), more attention has been drawn on the role of JMJD2B in tumorigenesis. However, little is known about its function and underlying mechanism in tumor metastasis and progression. Our results show that JMJD2B promotes EMT through Wnt/β-catenin pathway and increases the metastasis and progression of gastric cancer. Furthermore, higher JMJD2B expression positively correlates with the aggressive phenotype of gastric cancer. Recently, a novel inhibitor targeting the histone demethylase LSD1 has been developed and a suppressive effect on tumor growth in colon cancer cells was demonstrated (43). Therefore, the identification of small-molecule inhibitors targeting JMJD2B will be of interest and importance. JMJD2B may be a novel target for reversing EMT and prevention and treatment of gastric cancer metastasis and recurrence.
Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of, the article entitled ‘MV2B promotes epithelial–mesenchymal transition by cooperating with β-catenin and enhances gastric cancer metastasis.’

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Zhao, W. Li, W. Zang, Z. Liu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Zhao, W. Li, W. Zang
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References


JMJD2B Promotes Epithelial–Mesenchymal Transition by Cooperating with β-Catenin and Enhances Gastric Cancer Metastasis

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