Advancement of KIF20B in the Process of Invasion and Metastasis of Colon Cancer Induced by EMT Through Gli1

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ABSTRACT

The aim of this study was to determine the role of KIF20B in the metastasis of colon cancer cells. CCK-8 method and Transwell cell test were used to determine the proliferation, invasion, and migration of cells. Western blotting was used to determine the changes in expressions of vimentin ZO-1, E-cadherin, β-catenin, N-cadherin and Gli1. We found that at 48h~96h, compared with the control group, the proliferation ability of the group with overexpression of KIF20B was significantly increased (P<0.05), but decreased in the group with low expression of KIF20B (P<0.05). The invasion and migration ability of the group with overexpression of KIF20B were increased compared with the control group (P<0.05). Compared with the control group, the expression levels of Vimentin, N-cadherin and Gli1 in the group with overexpression of KIF20B were significantly increased, while the expression levels of ZO-1, E-cadherin, and β-Catenin were decreased (P<0.05). It can be concluded that KIF20B can promote the EMT, proliferation, and metastasis of colon cancer cells in vitro.

INTRODUCTION

Colon cancer is a malignancy with a high risk of poor prognosis, as approximately 50% of patients may experience metastasis within five years after diagnosis. (Christou et al., 2022). In-depth understanding of the molecular mechanism of colon cancer metastasis is of great importance for improving patient survival. Epithelial-mesenchymal transition (EMT) is an important event during tumor invasion and metastasis (Song et al., 2017; Sannino et al., 2017), which is associated with anti-apoptosis, immune escape, and stem cell characteristics, etc. (Ribatti, 2017; Brabletz et al., 2018). Members of the Kinesin superfamily proteins (KIF) are involved in mitosis, meiosis, and macromolecular transport, for example, KIFC1 is required for tubulin acetylation and actin-dependent spindle migration in mouse oocyte meiosis. KIF20B is a protein that is specifically phosphorylated during G2ram M conversion. KIF20B is an M-phase microtubule-associated protein, which plays a critical role in cytokinesis. In addition, the high expression of KIF20B is associated with tumor node metastasis (TNM) status, and lymph node metastasis in patients with pancreatic cancer (Chen et al., 2021). KIF20B, which is overexpressed in hepatocellular carcinoma, plays a role in cell proliferation (Cao and Zhu, 2021). However, due to colon cancer is extremely complex, the mechanism of its action in colon cancer remains unclear. In this study, colorectal cancer cell lines were selected to explore the effect of KIF20B on colorectal cancer metastasis and its mechanism of action.

MATERIALS AND METHODS

Experimental materials

Human colon cancer cell line SW480 (Shanghai Beinuo Biotechnology Co., Ltd.) was purchased from the American Type Culture Collection (ATCC, USA). The cells were cultured in DMEM/F12 medium (Lonza, Germany) supplemented with 10% Fetal Bovine Serum (Sigma-Aldrich, F7524), 1% L-glutamine (Lonza, Germany) and 1% Antibiotic-Antimycotic (Gibco, USA) at 37 °C and 5%
CO₂ atmosphere.

**Experimental reagents and instruments**

The reagents and instruments used in this experiment are as follows: fetal bovine serum (Hangzhou jiangbine Biotechnology Co., Ltd.), penicillin streptomycin double anti-body solution (Wuhan Ploysay Life Technology Co., Ltd., China), DMEM medium (Ponosyl Biotech Co., Ltd., China), phosphate buffer (Thermo Fisher Scientific, China), real time PCR assay instrument (Shenzhen Sanli Technology Co., Ltd., China, Model: tl988-iv), biomicroscope (Suzhou Jingtong Instrument Co., Ltd., China, model: xsp-11cd), ultra-low temperature freezer (Zhongke meishong Low Temperature Technology Co., Ltd., China, model: dw-hl398), cell culture incubator (Shanghai Baju Industrial Co., Ltd., China, Model: bpn-240rwp), and low temperature high-speed centrifuge (Shanghai Luziangyi Centrifuge Instrument Co., Ltd., China, Model: tgl-17m).

**Experimental plan**

SW480 cells were divided into the control group, the group with high expression of KIF20B, and the group with low expression of KIF20B, with five replicate wells set for each group. These cells were transfected with the KIF20B overexpression plasmid plvx IRES-kif20b in the group with high expression of KIF20B and the culb4 knockdown plasmid pgipz-shkif20b in the group with low expression of KIF20B. After 48 h of culture, the cells in each group were collected for relevant assays.

**Cell culture**

The SW480 cells were maintained in DMEM cell culture medium and subcultured at approximately 75% confluence. The cells were converted into a cell suspension, centrifuged to remove the supernatant, and resuspended in complete culture medium before being cultured further.

**Cell transfection**

The cells were seeded into 6-well plates at a density of 10,000 cells per well and incubated in a cell incubator until the cell density reached approximately 70%. The cells were transfected with miR-873-5p inhibitors, ZEB1 inhibitors, or blank plasmids and then cultured in complete medium with serum for 4 h before continuing the culture for an additional 48 h.

The proliferation ability of cells was determined by CCK-8 method. The cells were subcultured and plated in 96-well plates at a density of 10,000 cells per well. At 0, 48, and 96h, 20μL of CCK-8 solution was added to each well, and the plates were incubated in the dark for 2 h. The absorbance at 520nm was determined by enzyme labeling instrument.

**Cell invasion test**

The matrigel was reconstituted with pre-cooled fetal bovine serum and diluted to an appropriate concentration. Then, 200 μL of the diluted Matrigel was added to the upper chamber, while 400 μL of complete medium containing 10% FBS was added to the lower chamber. The cells from the transfection and control groups were seeded into the upper chamber at a density of 10,000 cells per well and incubated in the cell incubator for 24 h. After incubation, the cells were stained and observed under a microscope.

**Cell migration test**

The cells were suspended in serum-free medium and seeded into the upper chamber of the culture plate at an appropriate density. Then, 400 μL of complete medium containing 10% FBS was added to the lower chamber. The culture plate was incubated in the incubator for 24 h, after which the cells were stained and observed under a microscope.

The cells were lysed, and the lysate was centrifuged to obtain the supernatant. The supernatant was then mixed with BCA working solution to determine the total protein content. The total protein content was separated by SDS-PAGE electrophoresis, transferred onto a PVDF membrane, and blocked. The membrane was then incubated with primary antibodies against rabbit vimentin, ZO-1, E-cadherin, β-catenin, N-cadherin, and Gli1 at a dilution of 1:300, followed by washing with TBST buffer and incubation with secondary antibodies. The membrane was then washed again and incubated with ECL developer before being placed into an automatic gel imaging system for visualization. GAPDH expression was used as a reference for normalization, and the gray value of each protein band was analyzed using Image J software.

**Observation indices**

The changes of cell proliferation were measured by CCK-8 method and cell clone formation test. CCK-8 analysis was used to determine cell viability. A cell suspension of 100 liters was prepared in 96-well plates, and they were then pre-cultivated. After 24, 48, or 72 h of incubation, 10L of CCK-8 was added to each sample. Finally, the absorbance at 450nm was measured via microplate reader after 24, 48, or 72 h. For the colony formation experiment, cells were cultured in 35-mm dishes for 12 days and stained with crystal violet.

Transwell cell invasion and migration assay was carried out to determine the changes in cell invasion and migration. Cells were resuspended in serum-free medium solution and added to the upper chamber of the Petri dish and in the lower chamber in medium with 15% serum. 24 h to 48 h later, cells in the upper chamber were wiped and
fixed to stain cells in the lower chamber. Observe under the microscope and perform cell counts.

The expressions of Vimentin, ZO-1, E-cadherin, β-catenin, N-cadherin and Gli1 related to EMT were measured by Western blotting. Cells from each group were collected and total cellular protein was extracted via PMSF and RIPA. The BCA kit was applied to measure the protein concentration. Blocked membranes with 10% skimmed milk powder in TBS for 1 h, incubated with primary antibodies against Vimentin, ZO-1, E-cadherin, β-catenin, N-cadherin, Gli1, and GAPDH overnight at 4°C. After washing, incubated for 1 h at 37°C with IR fluorescence-labeled secondary antibody diluted in TBST. The PVDF membranes were immersed in 1mL of color development solution for approximately 10 min away from light and the results were observed. The ImageJ software was utilized to analyze the protein expression levels.

**Statistical analysis**

SPSS22.0 software package was used to analyze the statistical data. The quantitative data of cell proliferation, invasion and migration followed a normal distribution, which were expressed by (x̄±s). Group comparisons were performed using one-way analysis of variance (ANOVA) followed by post-hoc multiple comparison testing for means. Differences between two groups were tested using the LSD-t test, and statistical significance was set at P<0.05. Compared with control group aP<0.05; compared with KIF20B group bP<0.05.

**RESULTS**

**Expression level of KIF20B**

The expression level of KIF20B in the group with high expression of KIF20B was increased compared to the control group, while it was decreased in the group with low expression of KIF20B (P< 0.05) (Fig. 1).

**Table I. Changes of cell proliferation ability in different groups (x̄±s).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample (n)</th>
<th>0h (x̄±s)</th>
<th>48h (x̄±s)</th>
<th>96h (x̄±s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0.41±0.02</td>
<td>0.73±0.05</td>
<td>1.16±0.08</td>
</tr>
<tr>
<td>KIF20B high expression</td>
<td>5</td>
<td>0.40±0.03</td>
<td>1.04±0.09a</td>
<td>2.51±0.24a</td>
</tr>
<tr>
<td>KIF20B low expression</td>
<td>5</td>
<td>0.39±0.03</td>
<td>0.58±0.07ab</td>
<td>0.86±0.10ab</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>0.229</td>
<td>54.527</td>
<td>66.428</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.768</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Changes of invasion and migration ability**

The invasion and migration ability of the group with high expression of KIF20B was significantly enhanced, and those of the group with low expression of KIF20B was lower compared with the control group (Table II).
Table II. Invasion and migration ability (X±s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample (n)</th>
<th>Number of invasive cells</th>
<th>Number of migrating cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>126.95±15.17</td>
<td>106.69±13.58</td>
</tr>
<tr>
<td>KIF20B high expression</td>
<td>5</td>
<td>174.08±20.76(^a)</td>
<td>137.47±25.51(^a)</td>
</tr>
<tr>
<td>KIF20B low expression</td>
<td>5</td>
<td>85.86±10.52(^b)</td>
<td>77.58±12.37(^b)</td>
</tr>
<tr>
<td>F</td>
<td>26.645</td>
<td></td>
<td>14.864</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table III. Expression levels of EMT-related proteins Vimentin, ZO-1, E-cadherin, β-catenin, N-cadherin and Gli1 in different groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Vimentin</th>
<th>ZO-1</th>
<th>E-cadherin</th>
<th>β-catenin</th>
<th>N-cadherin</th>
<th>Gli1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.02±0.05</td>
<td>1.03±0.04</td>
<td>1.04±0.05</td>
<td>1.03±0.04</td>
<td>1.01±0.04</td>
<td>0.97±0.06</td>
</tr>
<tr>
<td>KIF20B high expression</td>
<td>2.31±0.21</td>
<td>0.68±0.04</td>
<td>0.43±0.03</td>
<td>0.57±0.06</td>
<td>1.63±0.17</td>
<td>2.65±0.28</td>
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<tr>
<td>KIF20B low expression</td>
<td>0.69±0.05</td>
<td>1.26±0.07</td>
<td>1.46±0.23</td>
<td>1.26±0.08</td>
<td>0.83±0.09</td>
<td>0.64±0.07</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Expressions of EMT-related proteins Vimentin, ZO-1, E-cadherin, β-catenin, N-cadherin and Gli1

Compared with the control group, the expression levels of Vimentin, N-cadherin and Gli1 in the group with high expression of KIF20B were increased, and while those of ZO-1, E-cadherin and β-catenin were decreased. Meanwhile the expression levels of Vimentin, N-cadherin and Gli1 in the group with low expression of KIF20B were decreased than the control group, and the expressions of ZO-1, E-cadherin and β-catenin were increased (Table III and Fig. 3).

Fig. 3. Expression level of EMT-related protein and Gli1 in cells of each group.

DISCUSSION

The KIF protein has been identified in humans, and it contains a highly conserved motor domain that binds to microtubules (Lawrence et al., 2004); therefore, upregulation of mKIF20B may promote tumorigenesis, whereas knockdown of KIF20B can cause mitotic arrest, reduce cell proliferation, and inhibit tumor growth in xenograft models (Liu et al., 2012). Inhibition of KIF20B expression can restore the sensitivity of microtubule targeted drugs. Knockdown of KIF20B inhibits the hepatocellular carcinoma cells growth via stabilizing p53 and blocking STAT3 phosphorylation. The combination of KIF20B knockdown and paclitaxel treatment showed a greater therapeutic effect compared to treatment with paclitaxel alone (Liu et al., 2018). KIF20B is overexpressed and abnormally activated in pancreatic cancer (Chen et al., 2021), breast cancer (Gao et al., 2018), hepatocellular carcinoma (Cao and Zhu, 2021), prostate cancer (Moamer et al., 2019), and promotes tumor cell invasion and metastasis (Duan et al., 2016). KIF20B promoted the ability of proliferation invasion and metastasis of lung adenocarcinoma (LUAD) cells. At the same time, KIF20B was found to be upregulated and was significantly associated with poor prognosis and survival status in patients with LUAD (Zhang et al., 2019). In this study, it was found that the KIF20B promoted the migration and invasion of colon cancer cells. This suggests that KIF20B may be a prognostic biomarker of colon cancer.

Epithelial-mesenchymal transition (EMT) is a crucial process involved in tumor cell metastasis and is closely linked to tumorigenesis. EMT is involved in the regulation of various tumor cell characteristics, such as drug resistance, anti-apoptosis, immune evasion, stemness, and more (Yeung and Yang, 2017; Bhatia et al., 2017). The members of KIF family have the regulation ability of EMT.
Overexpression of KIF20A can enhance the migration and invasion of cervical cancer cells, which is related to the induction of EMT-related protein Vimentin expression and inhibition of E-cadherin expression (Yang et al., 2021; Tan et al., 2020; Park et al., 2020). In our study, the overexpression of KIF20B significantly promoted the colon cancer EMT in vitro. These data suggest that KIF20B plays a key role in the EMT, but its mechanism of action remains unclear.

Hedgehog (Hh) signaling pathway plays an important role in colon cancer progression. Binding of Hh to its receptors and PTCH leads to the release of SMO proteins. This, in turn, blocks SUFU and activates Gli1 signaling (Wang et al., 2012). Gli1 protein, as a key transcription factor of Hh, can also be activated AKT, ERK and mTOR in a SMO-independent manner (Teperino et al., 2014). It remains unclear whether KIF20B regulates the malignant behavior of rectal cancer through the modulation of Gli1 signaling. In our study, overexpression of KIF20B promoted the expression of Gli1 in colon cancer cells. These data suggest that KIF20B plays a key role in the EMT via Gli1 in colon cancer cells.

To sum up, in vitro studies have shown that KIF20B overexpression promotes EMT, proliferation, and metastasis of colon cancer cells, possibly through the regulation of Gli1 expression. KIF20B provides experimental basis for anti-tumor EMT and metastasis.

Fundings

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IRB approval

This research was carried out with the approval of Research Guidance Workshop Committee (The First Affiliated Hospital of Wenzhou Medical University).

Ethics statement

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

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES


