Original Article

Eukaryotic initiation factor 3B downregulation suppresses cell proliferation, migration and invasion while it induces cell apoptosis by blocking the β-catenin pathway in endometrial cancer

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Abstract: This study aimed to evaluate the effect of eukaryotic initiation factor 3B (EIF3B) downregulation on cell proliferation, apoptosis, migration and the β-catenin pathway in endometrial cancer. EIF3B mRNA and protein expressions were determined in human endometrial cancer cell lines (Ishikawa, HEC-1A, RL95-2 and EFE-184) and in the normal human endometrial epithelial cell line (HEEC). EIF3B siRNA and a control siRNA were transfected into HEC-1A cells, then cell proliferation, apoptosis, apoptotic marker (C-caspase 3 and Bcl-2) expressions, cell migration, and invasion were determined. β-catenin and cyclin E1 (CCNE1) expressions were also measured. EIF3B mRNA and protein expressions were increased in the Ishikawa, HEC-1A and RL95-2 cell lines, but they were similar in the EFE-184 cell line compared to the HEEC cell line. In HEC-1A cells, EIF3B siRNA suppressed cell proliferation, but it elevated the cell apoptosis rate compared to the control siRNA, and EIF3B siRNA also enhanced C-caspase 3 expression, but it inhibited Bcl-2 expression. Also, EIF3B siRNA reduced cell migration and cell invasion compared to the control siRNA in HEC-1A cells. More interestingly, EIF3B siRNA reduced β-catenin and CCNE1 mRNA as well as protein expressions compared with the control siRNA in HEC-1A cells. In conclusion, EIF3B downregulation suppresses cell proliferation, migration, and invasion, but it induces cell apoptosis by blocking the β-catenin pathway in endometrial cancer.

Keywords: Endometrial cancer, eukaryotic initiation factor 3B, proliferation, apoptosis, β-catenin

Introduction

Endometrial cancer, a common gynecologic malignancy characterized by abnormal uterine bleeding, increased vaginal discharge, and a thickened endometrium, is the fourth most prevalent gynecologic cancer in America and the second most common gynecologic cancer in China [1, 2]. Due to extended lifespans and the increased mental stress of modern women, the incidence of endometrial cancer rises annually, which creates substantial economic burdens to society [2, 3]. Generally, treatments for endometrial cancer mainly include surgery, radiotherapy, chemotherapy, hormonal therapy, and a couple of novel treatments (such as targeted therapy and immunotherapy) [4, 5]. Despite the favorable efficacy of these treatments, the prognosis of advanced endometrial cancer patients remains undesirable [1, 6]. Therefore, it is important to investigate the underlying pathology of endometrial cancer and explore novel therapeutic targets.

Eukaryotic initiation factor 3 (EIF3), the largest eukaryotic initiation factor that consists of 12 different polypeptide subunits with a molecular weight of 550-700 kDa, modulates various cellular activities (including proliferation, differentiation and migration) by regulating the interaction between ribosomes and microRNAs during the protein translation, and subsequently participates in the pathological processes of various diseases [7-10]. As one of the important subunits of EIF3, EIF3B has been found to promote tumorigenesis by promoting cancer cell
proliferation while inhibiting apoptosis in several cancers (such as non-small-cell lung cancer, ovarian cancer, glioblastoma, and osteosarcoma) [11-14]. Considering the carcinogenic effect of EIF3B in a number of cancers, we hypothesized that it might also play an important role in promoting endometrial cancer progression. However, there is limited information about the role of EIF3B in the pathology of endometrial cancer.

Therefore, the objective of the current study was to evaluate the effect of EIF3B downregulation on cell proliferation, apoptosis, migration, invasion, and the β-catenin pathway in endometrial cancer.

Materials and methods

Cell source

Human endometrial cancer cell line Ishikawa was purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). Human endometrial cancer cell lines HEC-1A and RL95-2 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Human endometrial cancer cell line EFE-184 was purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). In addition, normal human endometrial (uterine) epithelial cell line (HEEC) was purchased from Lifeline® Cell Technology (CA, USA).

Cell culture

The Ishikawa cell line was cultured in 90% Minimum Essential Medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA). The HEC-1A cell line was cultured in 90% McCoy’s 5A (modified) Medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA). The RL95-2 cell line was cultured in 90% Dulbecco’s Modified Eagle Medium/Nutrient Mixture F-12 Medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA). The EFE-184 cell line was cultured in 90% Roswell Park Memorial Institute (RPMI) 1640 Medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA). The HEEC cell line was cultured in ReproLife Cell Culture Medium (Lifeline® Cell Technology, USA).

Measurement of EIF3B expression in human endometrial cancer cell lines

The mRNA expression of EIF3B in human endometrial cancer cell lines, including Ishikawa, HEC-1A, RL95-2, and EFE-184, was measured by quantitative polymerase chain reaction (qPCR), while the protein expression of EIF3B in these cell lines was measured by Western Blot. Also, the mRNA and protein expressions of EIF3B in the normal human endometrial epithelial cell line HEEC were detected by qPCR and Western Blot as well, respectively.

Transfection

EIF3B siRNA and control siRNA were constructed by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) and were transfected into HEC-1A cells with HilyMax (Dojindo, Japan). Then, the mRNA and protein expressions of EIF3B were measured at 48 hours after the transfection by qPCR and Western Blot, respectively.

Cell proliferation measurement

The HEC-1A cells were digested and seeded in a 96-well plate with three replicate wells after the transfection, then the CCK-8 solution was added to the cells at 0 hr, 24 hr, 48 hr, and 72 hr, respectively. After that, the cells were incubated at 37°C for another 2 hr, and the absorbance was subsequently measured.

Cell apoptosis measurement

The cell apoptosis rate was measured at 48 hr after the transfection using an Annexin V-FITC Apoptosis Detection Kit (BD, USA). The HEC-1A cells were rinsed twice at 48 hr after the transfection and then stained at room temperature for 30 min with Annexin V-FITC and propidium iodide. Subsequently, the cells were quantified using a Flow Cytometer (BD, USA) and analyzed by FlowJo Software 7.6 (FlowJo, LLC). In addition, the apoptotic markers Cleaved caspase 3 (C-caspase 3) and Bcl-2 protein expressions were measured at 48 hr after the transfection by Western Blot.

Cell migration measurement

The cells were incubated in 24-well plates at 24 hr after the transfection. When the cells re-
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β-catenin pathway measurement

EIF3B was previously reported to exhibit oncogenic functions by regulating the β-catenin pathway in several cancers [14, 15], so we hypothesized that EIF3B inhibition might suppress human endometrial cancer by blocking the β-catenin pathway. Then the β-catenin and cyclin E1 (CCNE1) mRNA and protein expressions were measured at 48 hr after the transfection using qPCR and Western Blot, respectively.

qPCR

Total RNA in the HEC-1A cells was extracted using an RNaseasy Protect Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The cDNA was then synthesized using a QuantiTect Rev. Transcription Kit (Qiagen, Germany) and subjected to qPCR using a QuantiNova SYBR Green PCR Kit (Qiagen, Germany). The reaction conditions were as follows: 94°C for 5 min, then 94°C for 5 s and 61°C for 30 s in 40 cycles. After that, the mRNA expression of EIF3B, β-catenin, and CCNE1 were calculated using the 2^−ΔΔCt method with glyceraldehyde-phosphate dehydrogenase (GAPDH) as an internal reference. The primers utilized in this study are listed in Table 1.

Western blot

Protein samples in HEC-1A cells were extracted using RIPA Lysis Buffer (Beyotime, China), and then quantified with a Bicinchoninic Acid Kit for Protein Determination (Sigma, USA). An equal amount of protein from each sample was separated by NuPAGE™ 4-12% Bis-Tris Protein Gels (Invitrogen, USA) and transferred to a polyvinylidene fluoride membrane (PVDF) (Millipore, Germany). After blocking, the membrane was incubated with primary antibodies at 4°C overnight and subsequently incubated with a secondary antibody for 1 hr at room temperature. Finally, the bands were visualized using an EasyBlot ECL kit (Sangon, China) followed by exposure to x-ray film (Kodak, USA). GAPDH was utilized as an internal control. The primary antibodies and the secondary antibody are depicted in Table 2.

Table 1. Primers applied in qPCR

<table>
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<th>Gene</th>
<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
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<td>EIF3B</td>
<td>CGTATGCGCTGCTGCTGCTCATTAA</td>
<td>CCTTGGTGCTGCTGCTGCTGAA</td>
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<td>β-catenin</td>
<td>TGACGAGCCACCAGAACAGA</td>
<td>GCACAGAAAGACCACTGAAG</td>
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<tr>
<td>CCNE1</td>
<td>CCTGAGCTGCTGCTGCTGCTGAA</td>
<td>GTTCCTGAGCTGCTGCTGACAGA</td>
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<tr>
<td>GAPDH</td>
<td>GACACAGCTCATGCCATCACCAC</td>
<td>ACGGCTGCTCCACCACCT</td>
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<tr>
<th>Antibody</th>
<th>Company</th>
<th>Dilution</th>
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<tr>
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<td>Abcam (UK)</td>
<td>1:10000</td>
</tr>
<tr>
<td>Rabbit monoclonal to beta Catenin</td>
<td>Abcam (UK)</td>
<td>1:6000</td>
</tr>
<tr>
<td>Rabbit monoclonal to Cyclin E1 (CCNE1)</td>
<td>Abcam (UK)</td>
<td>1:2000</td>
</tr>
<tr>
<td>Rabbit polyclonal to Caspase-3</td>
<td>Abcam (UK)</td>
<td>1:500</td>
</tr>
<tr>
<td>Rabbit monoclonal to Cleaved Caspase-3</td>
<td>Abcam (UK)</td>
<td>1:500</td>
</tr>
<tr>
<td>Rabbit polyclonal to Bcl-2</td>
<td>Abcam (UK)</td>
<td>1:100</td>
</tr>
<tr>
<td>Rabbit monoclonal to GAPDH</td>
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Table 2. Antibodies applied in Western Blot

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<tr>
<td>Rabbit monoclonal to GAPDH</td>
<td>Abcam (UK)</td>
<td>1:5000</td>
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Cell invasion measurement

The cell invasive ability was measured at 24 hr after the transfection using a Transwell assay. In brief, HEC-1A cells were suspended at 24 hr in a serum-free medium after the transfection. Then the cell suspension was transferred to the upper chamber (Costar, USA) and coated with Matrigel Matrix (BD, USA), and the lower chamber was filled with a medium containing 10% FBS. After 24 hr, the remaining cells were removed from the upper membrane, and the cells that travelled to the lower face of the membrane were fixed with 10% formaldehyde (Sigma, USA) then stained at room temperature with 5% crystal violet (Sigma, USA) for 15 min. The invasive cells were counted with an inverted microscope (Olympus, Japan, × 100).

Goat Anti-Rabbit IgG H&L (HRP) (Sangon, China) followed by exposure to x-ray film (Kodak, USA). GAPDH was utilized as an internal control. The primary antibodies and the secondary antibody are depicted in Table 2.
Statistics

The statistical analysis and graphs were performed and drawn using GraphPad Prism 6 software (GraphPad Int., USA). The data were mainly presented as the mean ± standard deviation (SD). Comparisons among groups were determined using one-way ANOVA followed by Tukey’s multiple comparison test, and comparisons between two groups were determined using a t test. A P value < 0.05 was considered significant in this study.

Results

The comparison of EIF3B expression between human endometrial cancer cells and normal human endometrial (uterine) epithelial cells

The EIF3B mRNA expressions were increased in human endometrial cancer cell lines Ishikawa (P < 0.05), HEC-1A (P < 0.001) and RL95-2 (P < 0.001) compared with the normal human endometrial (uterine) epithelial cell line HEEC, but there was no difference between the human endometrial cancer cell line EFE-184 and HEEC (P > 0.05) (Figure 1A). The EIF3B protein expression was also elevated in Ishikawa, HEC-1A and RL95-2 cells compared with the HEEC cells (Figure 1B). These data suggested that EIF3B was upregulated in the endometrial cancer cells compared with the normal endometrial (uterine) epithelial cells.

The effect of EIF3B downregulation on cell proliferation, the apoptosis rate, and the apoptotic marker expressions in HEC-1A cells

EIF3B mRNA (P < 0.001) (Figure 2A) and protein (Figure 2B) expressions were decreased in HEC-1A cells transfected with EIF3B siRNA compared with HEC-1A cells transfected with the control siRNA, suggesting the transfections were successful. After the transfections, cell proliferation (Figure 2C) at 48 hr (P < 0.05) and 72 hr (P < 0.05) was suppressed, but the cell apoptosis rate (P < 0.01) (Figure 2D, 2F) was promoted in HEC-1A cells transfected with EIF3B siRNA compared with the HEC-1A cells transfected with control siRNA. Meanwhile, the apoptotic marker C-caspase expression was increased, but the Bcl-2 expression was reduced (Figure 2E) in the HEC-1A cells transfected with EIF3B siRNA compared with the HEC-1A cells transfected with control siRNA. These experiments indicated that EIF3B downregulation suppressed cell proliferation but stimulated cell apoptosis in endometrial cancer cells.
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The effect of EIF3B downregulation on cell migration and invasion in HEC-1A cells

Both cell migration (P < 0.01) (Figure 3A, 3B) and cell invasion (P < 0.01) (Figure 3C, 3D) were reduced in the HEC-1A cells transfected with EIF3B siRNA compared with the HEC-1A cells transfected with the control siRNA, implying that EIF3B downregulation suppressed cell migration and invasion in endometrial cancer cells.

The effect of EIF3B downregulation on the β-catenin pathway in HEC-1A cells

EIF3B was previously reported to exhibit onco-genic functions by regulating the β-catenin pathway in several cancers other than endometrial cancer [1, 2], so we therefore hypothesized that EIF3B downregulation might suppress endometrial cancer development by blocking the β-catenin pathway. To test our hypothesis, we determined β-catenin and CCNE1 (a downstream gene of the β-catenin pathway) mRNA and protein expressions after the downregulation of EIF3B, and we found that both β-catenin (P < 0.01) (Figure 4A, 4C) and CCNE1 (P < 0.05) (Figure 4B, 4C) mRNA as well as their protein expressions were reduced in HEC-1A cells transfected with EIF3B siRNA compared with HEC-1A cells transfected with the control siRNA. Moreover, β-catenin signaling is well known as an oncogenic pathway in many cancers including endometrial cancer, so our data implied that EIF3B downregulation inhibited endometrial cancer progression by blocking the β-catenin pathway.

Discussion

There were some interesting observations in the current study. Firstly, EIF3B was upregulated in endometrial cancer cells compared to normal endometrial (uterine) epithelial cells. Secondly, EIF3B downregulation suppressed cell proliferation, migration, and invasion, but it promoted cell apoptosis. Thirdly, EIF3B downregulation suppressed endometrial cancer progression by blocking the β-catenin pathway.

Figure 2. The impact of EIF3B downregulation on cell proliferation, the cell apoptosis rate, and apoptotic marker expressions in HEC-1A cells. Comparison of EIF3B mRNA expression (A) and protein expression (B) between the EIF3B siRNA group and the control siRNA group. Comparison of cell proliferation (C) and apoptosis (D, F) between the EIF3B siRNA group and the control siRNA group. Apoptotic marker expressions (E) in the EIF3B siRNA and control siRNA groups. Comparisons between two groups were determined using a t test. A P value < 0.05 was considered significant. *P < 0.05, **P < 0.01, ***P < 0.001. EIF3B, eukaryotic initiation factor 3B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Figure 3. The effect of EIF3B downregulation on cell migration and invasion in HEC-1A cells

Figure 4. The effect of EIF3B downregulation on the β-catenin pathway in HEC-1A cells

Figure 5. The effect of EIF3B downregulation on endometrial cancer progression by blocking the β-catenin pathway.
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The 12-subunit mammalian EIF3 is the largest and most complex translation initiation factor, and it has been found to play crucial role in various steps of protein translation (such as pro-
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moting the assembly of the 43S pre-initiation complex, promoting re-initiation after the translation of short upstream open reading frames and controlling translation termination as well as ribosomal recycling) [7, 9]. These functions of EIF3 have been demonstrated to play important roles during the progression of complicated diseases (including liver fibrosis, growth disorders and cancers) [8, 10, 12, 16, 17]. As one of the most important subunits of EIF3, EIF3B is fundamental in the formation of the complete EIF3 complex, and its downregulation has been found to cause a broad-spectrum, patternless downregulation of almost all other EIF3 subunits by approximately 40%-70%, suggesting that the dysregulated EIF3B is able to influence the complete EIF3 complex formation and other EIF3 subunit expressions, thereby mediating key signaling pathways or gene expressions during the pathological processes of many diseases [7, 11, 14, 15, 18]. Therefore, unveiling the function of EIF3B in the pathology of diseases enables a full understanding the etiology of some diseases and facilitates the discovery of novel therapeutic targets.

In recent years, EIF3B has been reported to be implicated in the tumorigenesis of several cancers (such as osteosarcoma, colon cancer, ovarian cancer, bladder cancer, and prostate cancer) [11-13, 19]. For example, an in vitro study reveals that EIF3B is upregulated in glioblastoma cells (U87, U251 and A172 cell lines), and the knockdown of EIF3B suppresses cell proliferation and stimulates cell apoptosis via GO/G1 arrest [20]. In another study, EIF3B expression was also found to be elevated in non-small cell lung cancer cells (A549, NCI-H1299, NCI-H16650 and PC9 cell lines) compared with normal lung epithelial cells (DEAS-2B cell line); also, the upregulation of EIF3B accelerates cell proliferation and inhibits cell apoptosis, but the downregulation of EIF3B suppresses cell proliferation and induces cell apoptosis [12]. These studies suggest that EIF3B promotes the development of several cancers by regulating cancer cell proliferation and apoptosis. Considering the carcinogenic effect of EIF3B in these cancers and the malignancy of endometrial cancer, we hypothesized that EIF3B also exhibited a tumorigenic effect in endometrial cancer. However, no relevant study has been found so far. Therefore, we first measured EIF3B expression in endometrial cancer cells, and then we discovered that EIF3B expression was enhanced in endometrial cancer cells (Ishikawa, HEC-1A and RL95-2 cell lines) compared to normal human endometrial (uterine) epithelial cells (HEEC cell line), which might be explained as follows: Considering that the endometrial cancer cells proliferated much faster than the normal human endometrial (uterine) epithelial cells, they would express more EIF3B to facilitate the faster protein expression (protein expression required EIF3B) and to support the faster cell proliferation [10, 18]. Meanwhile, we also investigated the impact of EIF3B downregulation on cell proliferation, apoptosis, migration, and invasion in endometrial cancer, and we discovered that EIF3B downregulation reduced cell proliferation, migration and invasion while it stimulated cell apoptosis in endometrial cancer cells (HEC-1A cell line), indicating that EIF3B downregulation inhibited the tumorigenesis of endometrial cancer. The possible explanation might be that: (1) EIF3B downregulation might reduce the expressions of various structural and functional proteins (such as tubulin and enzymes), whose existences are paramount for cell proliferation, migration and invasion [2, 4]. (2) EIF3B downregulation inhibiting endometrial cancer gene expression might also be done by inducing cell cycle arrest or by regulating a few signaling pathways such as the β-catenin pathway (as indicated by our later experiment).

EIF3B was previously reported to exhibit oncomgenic functions via regulating the β-catenin pathway in a few cancers [14, 15]. For instance, a recent study discovered that silencing EIF3B expression inhibits cell proliferation and interferes in the cell cycle by blocking the β-catenin pathway in esophageal squamous cell carcinoma cells (EC109 and KYSE510 cell lines) [15]. Another similar study disclosed that EIF3B downregulation also inhibits clear-cell renal-cell carcinoma progression via inactivating the β-catenin pathway [14]. Based on these previous findings, we hypothesized that EIF3B downregulation suppressed endometrial cancer tumorigenesis by mediating the β-catenin pathway as well. However, few studies have reported on this. Therefore, we investigated the β-catenin and CCNE1 (a downstream gene of β-catenin pathway) expressions after EIF3B downregulation, and we discovered that both the β-catenin and CCNE1 expressions were
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decreased after the EIF3B downregulation compared to the control, implying that EIF3B downregulation inhibited endometrial cancer development, and this might be achieved by blocking the β-catenin pathway. Briefly, our study illustrated the impact of EIF3B downregulation on the carcinogenesis of endometrial cancer and unveiled its molecular mechanisms in endometrial cancer pathology, which contributes to the discovery of novel therapeutic targets for this cancer.

In summary, EIF3B downregulation suppresses cell proliferation, migration, and invasion, but it induces cell apoptosis via blocking the β-catenin pathway in endometrial cancer.

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Disclosure of conflict of interest

None.

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