Knockdown of eukaryotic translation initiation factor 3 subunit B inhibits cell proliferation and migration and promotes apoptosis by downregulating WNT signaling pathway in acute myeloid leukemia

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Abstract: The study aimed to investigate the effect of eukaryotic translation initiation factor 3 subunit B (EIF3B) on cell proliferation, migration, and apoptosis as well as the underlying mechanism in acute myeloid leukemia (AML). EIF3B expression was detected in AML-193, HL-60, OCI-AML2, and KG-1 cell lines and human primary bone marrow mononuclear cells (BMMC). EIF3B knockdown was realized by transfecting EIF3B ShRNA plasmids, and EIF3B knockdown and WNT2 overexpression were established by transfecting EIF3B ShRNA plasmids and WNT2 overexpression plasmids into KG-1 cells. The effect of EIF3B knockdown, and EIF3B knockdown plus WNT2 overexpression on cell proliferation, apoptosis, migration, glycogen synthase kinase 3B (GSK3B) and catenin beta 1 (CTNNB1) was assessed. EIF3B mRNA and protein expression were higher in AML-193, OCI-AML2 and KG-1 cell lines, but unchanged in the HL-60 cell line compared with human primary BMMC. The expression of WNT2 was decreased by EIF3B downregulation, while it had no effect on EIF3B expression. As for cell activities, EIF3B knockdown inhibited the cell proliferation and migration but promoted apoptosis by inhibiting WNT2 expression. In addition, EIF3B knockdown downregulated the expression of CTNNB1 but upregulated the expression of GSK3B by blocking WNT2 expression in AML, implying an inhibitory effect of EIF3B downregulation on WNT signaling pathway. EIF3B is upregulated and its knockdown inhibits cell proliferation, and migration, while promoting apoptosis by downregulating the WNT signaling pathway in AML.

Keywords: EIF3B, AML, proliferation, migration, apoptosis, WNT signaling pathway

Introduction

According to a 2018 global statistics report, the incidence of leukemia accounts for 2.4% of all cancers globally, and acute myeloid leukemia (AML) is a major pathologic type of leukemia, that is identified as the malignant transformation of clonal hematopoietic stem cells in the bone marrow and peripheral blood [1-3]. Although treatments such as intensive induction chemotherapy and stem cell transplantation contribute to the improvement of the survival in AML patients, long-term mortality and recurrence remain a challenge, which lead to a poor prognosis in AML patients and a heavy burden to social and medical resources [1, 4]. Therefore, it is essential to explore novel therapeutic targets for alternative AML therapies to improve treatment outcomes and prognosis in AML patients.

Eukaryotic translation initiation factor 3 subunit B (EIF3B), as one subunit of the eukaryotic translation initiation factor 3 (EIF3) family participating in the initiation of protein synthesis, has been reported to be dysregulated in several cancers, such as: colon cancer, bladder cancer, prostate cancer, and glioblastoma [5-7]. Knockdown of EIF3B inhibits proliferation but promotes apoptosis in glioblastoma cells [7]. In addition, it is reported that EIF3B promotes malignant progression by regulating the WNT signaling pathway in esophageal squamous cell carcinoma, and existing evidence suggests that WNT signaling is related to the transcription factors of AML which promote the
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aberrant self-renewal ability of hematopoietic cells [8-12]. The regulatory proteins within the WNT signaling pathway, including catenin beta 1 (CTNNB1), glycogen synthase kinase 3B (GSK3B), cyclin D1 (CCND1) and C-Myc, are dysregulated under the induction of AML-related translocation, accelerating the progression of AML [11-13]. Based on the aforementioned evidence and considering that there is still limited information about the function of EIF3B in AML, we speculated that EIF3B might be dysregulated in AML and it might affect AML cell function by regulating the WNT signaling pathway. In the present study we performed cellular experiments to explore the underlying mechanism of EIF3B in AML.

Materials and methods

Cell culture

Human AML cell lines AML-193, HL-60, OCI-AML2 and KG-1 were purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany), AML-193 cells were cultured in 90% Iscove's Modified Dulbecco's Medium (Gibco, USA) and 10% fetal bovine serum (FBS) (Gibco, USA), and HL-60, OCI-AML2 and KG-1 cells were cultured in 90% Roswell Park Memorial Institute 1640 Medium (Gibco, USA) and 10% FBS (Gibco, USA) under 95% air and 5% CO\textsubscript{2} at 37°C.

EIF3B expression in AML cell lines

EIF3B mRNA and protein expressions in AML-193, HL-60, OCI-AML2 and KG-1 cell lines were detected using real-time quantitative polymerase chain reaction (RT-qPCR) and western blot (WB). EIF3B mRNA and protein expressions in human primary bone marrow mononuclear cells (BMMC) were also detected as controls. Human primary BMMC were bought from American Type Culture Collection (Manassas, USA).

Transfections

Control short hairpin RNA (ShRNA), control overexpression EIF3B ShRNA, and WNT2 overexpression plasmids were transfected into KG-1 cells as a Control group; EIF3B ShRNA plasmids and control overexpression plasmids were transfected into KG-1 cells as Sh-EIF3B group; lastly, EIF3B ShRNA plasmids and WNT2 overexpression plasmids were transfected into KG-1 cells as Sh-EIF3B & WNT2 group.

EIF3B and WNT2 expressions after transfection

Protein and mRNA expressions of EIF3B and WNT2 were detected by RT-qPCR and WB respectively at 48 h post transfection.

Counting Kit-8 (CCK-8) assay after transfection

Proliferation of KG-1 cells was detected by CCK-8 assay at 0 h, 24 h, 48 h, and 72 h post transfection using CCK-8 Kit (Dojindo, Japan). In brief, 10 μl CCK-8 and 90 μl serum-free medium were added to each group of KG-1 cells, then the cells were incubated in mixed gas with 95% air and 5% CO\textsubscript{2} at 37°C. Optical density (OD) value was measured by microplate reader (BioTek, USA).

Annexin V/Propidium Iodide (AV/PI) assay after transfection

Apoptosis rate of KG-1 cells was detected by AV/PI assay at 48 h post transfection using FITC Annexin V Apoptosis Detection Kit II (BD, USA). In brief, KG-1 cells in each group were digested by trypsin (Thermo, USA) and washed with phosphate buffer solution (PBS), then suspended in 100 μl binding buffer. Then 5 μl Annexin V (AV) and 5 μl propidium iodide (PI) were added to the cells and then cells were left in dark for 15 min. Subsequently, 400 μl binding buffer was added and apoptosis rate was analyzed using flow cytometry (FCM) (Beckman, USA).

Apoptotic markers expression after transfection

Cleaved Caspase 3 (C-Caspase 3) and B-cell lymphoma-2 (Bcl-2) protein expressions in KG-1 cells were detected at 48 h post transfection by WB assay.

Transwell assay after transfection

Cell migration ability was detected by Transwell assay after transfection. In brief, after fully
hydrating the Chamber (Costar, USA) with 8 μm Polycarbonate film, 200 μl of serum-free medium containing 5×10^5 cells were seeded in the upper chamber. Then the lower chamber was filled with 500 μl 10% FBS medium. The whole chamber was incubated at 37°C for 12 h. The cells were collected from the medium in the lower chamber and the number of cells was counted by flow cytometry (Beckman, USA).

**GSK3B and CTNNB1 expressions after transfection**

Genes downstream of WNT signaling, including GSK3B and CTNNB1 expressions were detected at 48 h post transfection by RT-qPCR and WB respectively.

**RT-qPCR**

Total RNA was extracted from cells using TRIzol™ Reagent (Invitrogen, USA) and then reversely transcribed to cDNA using PrimeScript™ RT Master Mix (Takara, Japan). Following that, RT-qPCR was performed using TB Green™ Fast qPCR Mix (Takara, Japan) to quantify EIF3B, WNT2, GSK3B and CTNNB1 expressions. The result was calculated using 2^-ΔΔCt method with GAPDH as an internal reference. The primers used in RT-qPCR are listed in Table 1.

**Western blot analysis**

Total protein was extracted with RIPA Lysis and Extraction Buffer (Thermo, USA). The protein concentration in each sample was measured using the Pierce™ Rapid Gold BCA Protein Assay Kit (Thermo, USA). 20 μg protein samples were added to NuPAGE™ 4-20% Tris-Acetate Midi Protein Gels (Thermo, USA) and transferred onto Polyvinylidene Fluoride membrane (Millipore, USA). After blocking with BSA (Thermo, USA) for 2 h, the membranes were incubated with the primary antibodies overnight at 4°C. Then, the membranes were incubated with the secondary antibody for 90 min at 37°C. Pierce™ ECL Plus Western Blotting Substrate (Thermo, USA) was used to illuminate the bands and X-ray film (Kodak, USA) was used to visualize the result. The antibodies used in this study are summarized in Table 2.

**Statistics**

Data are shown as mean value ± standard deviation, and comparison among groups was detected by One-Way ANOVA followed by Dunnett’s multiple comparisons test, while comparison between two groups was detected by t test. The statistical software used in this study was SPSS 21.0 Software (IBM, USA), and the graph-making software used in this study was GraphPad Prism 6.01 (GraphPad Int, USA). P < 0.05 was considered significant.

**Results**

EIF3B expression in AML

EIF3B mRNA relative expression was higher in AML-193 (P < 0.01), OCL-AML2 (P < 0.05), and KG-1 (P < 0.001) cell lines but unchanged in the HL-60 cell line (P > 0.05) compared with human primary BMMC (Figure 1A). WB assay revealed that EIF3B protein expression was increased in AML-193, OCL-AML2, and KG-1 cell lines compared with human primary BMMC, but unchanged in the HL-60 cell line (Figure 1B).
Interaction between EIF3B and WNT2 in KG-1 cells

In order to examine the effect of EIF3B on regulating WNT2, the following experiments were carried out in KG-1 cells. EIF3B mRNA was downregulated in Sh-EIF3B group compared with the Control group \( (P < 0.001) \), and EIF3B mRNA expression was similar in the Sh-EIF3B group and Sh-EIF3B & WNT2 group \( (P > 0.05) \) \( (\text{Figure 2A}) \). WNT2 mRNA was downregulated in the Sh-EIF3B group compared with Control group \( (P < 0.01) \). In addition, WNT2 mRNA was upregulated in Sh-EIF3B & WNT2 group compared with Sh-EIF3B group \( (P < 0.01) \) \( (\text{Figure 2B}) \). WB assay indicated that EIF3B protein expression was reduced in Sh-EIF3B group compared with Control group but similar between the Sh-EIF3B & WNT2 group and Sh-EIF3B group. WNT2 protein expression was decreased in the Sh-EIF3B group compared with Control group but elevated in the Sh-EIF3B & WNT2 group compared with Sh-EIF3B \( (\text{Figure 2C}) \). The above findings indicated that knockdown of EIF3B reduced cell proliferation but promoted cell apoptosis by downregulating WNT2 in AML.

EIF3B knockdown reduced cell proliferation while promoting apoptosis by downregulating WNT2 in KG-1 cells

In order to investigate the effect of EIF3B on regulating AML cell function and its interaction with WNT2, cell proliferation and apoptosis were detected after transfection. The cell proliferation ability was unchanged at 24 h \( (P > 0.05) \) but reduced at 48 h \( (P < 0.01) \) and 72 h \( (P < 0.01) \) after transfection in Sh-EIF3B group compared with the control group \( (\text{Figure 3A}) \). Cell proliferation ability was similar at 24 h and 48 h after transfection between the Sh-EIF3B & WNT2 group and Sh-EIF3B group \( (P > 0.05) \) but increased at 72 h after transfection in the Sh-EIF3B & WNT2 group compared with the ShEIF3B group \( (P < 0.05) \). Cell apoptosis rate was increased after transfection in the Sh-EIF3B group compared with Control group \( (P < 0.001) \), while reduced in Sh-EIF3B & WNT2 group compared with Sh-EIF3B group \( (P < 0.01) \) \( (\text{Figure 3B, 3D}) \). WB assay visualized that C-Caspase 3 was upregulated in the Sh-EIF3B group compared with controls but downregulated in the Sh-EIF3B & WNT2 group compared with the ShEIF3B group \( (\text{Figure 3C}) \). Conversely, Bcl-2 was downregulated in the Sh-EIF3B group compared with controls, while upregulated in the Sh-EIF3B & WNT2 group compared with the ShEIF3B group. The above data suggested that knockdown of EIF3B reduced cell proliferation but promoted cell apoptosis by downregulating WNT2 in AML.

EIF3B knockdown reduced cell migration by downregulating WNT2 in KG-1 cells

The number of migrated cells was decreased in Sh-EIF3B group compared with the control group \( (P < 0.01) \) but increased in the Sh-EIF3B & WNT2 group compared with the Sh-EIF3B group \( (P < 0.05) \) \( (\text{Figure 4}) \). The above data show that knockdown of EIF3B decreased cell migration by inhibiting WNT2 in AML.

EIF3B knockdown suppressed WNT signaling pathway in KG-1 cells

In addition, the effect of EIF3B on regulating genes downstream of WNT signaling, including GSK3B and CTNNB1 were evaluated. GSK3B mRNA relative expression was increased in the Sh-EIF3B group compared with the control group \( (P < 0.001) \) but reduced in the Sh-
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Figure 2. EIF3B and WNT relative expression in KG-1 after transfection. Sh-EIF3B decreased EIF3B mRNA expression and protein expression, while Sh-EIF3B & WNT2 had no influence on EIF3B mRNA expression or protein expression (A, C). Sh-EIF3B reduced WNT2 mRNA expression and protein expression, while Sh-EIF3B & WNT2 rescued the effect of Sh-EIF3B on WNT2 expression (B, C). Comparisons of EIF3B mRNA relative expression between the Sh-EIF3B group and control, Sh-EIF3B & WNT2 group, and Sh-EIF3B group were made by t test. P < 0.05 was considered significant. NS, non-significant, **P < 0.01, ***P < 0.001. EIF3B, eukaryotic translation initiation factor 3 subunit B; AML, acute myeloid leukemia; Sh-EIF3B, eukaryotic translation initiation factor 3 subunit B short hairpin RNA plasmids and control overexpression plasmids were transfected into KG-1; Sh-EIF3B & WNT2, eukaryotic translation initiation factor 3 subunit B short hairpin RNA plasmids and WNT2 overexpression plasmids were transfected into KG-1 cells.

Figure 3. Sh-EIF3B increased cell proliferation but inhibited apoptosis by blocking WNT signaling pathway in AML. Sh-EIF3B had no effect on cell proliferation at 24 h but reduced cell proliferation at 48 h and 72 h; Sh-EIF3B & WNT2 rescued the effect of Sh-EIF3B on proliferation at 72 h, but not at 24 h or 48 h (A). Sh-EIF3B increased apoptosis rate, while Sh-EIF3B & WNT2 rescued the influence of Sh-EIF3B on cell apoptosis (B, D). Sh-EIF3B increased the expression of C-Caspase 3, while Sh-EIF3B & WNT2 compensated for the effect of Sh-EIF3B on C-Caspase 3 expression (C). Sh-EIF3B decreased the expression of Bcl-2, while Sh-EIF3B & WNT2 rescued the influence of Sh-EIF3B on Bcl-2 expression. P < 0.05 was considered significant. NS, non-significant, **P < 0.01, ***P < 0.001. EIF3B, eukaryotic translation initiation factor 3 subunit B; AML, acute myeloid leukemia; C-Caspase 3, Cleaved-Caspase 3; Bcl-2, B-cell lymphoma-2; Sh-EIF3B, eukaryotic translation initiation factor 3 subunit B short hairpin RNA plasmids and control overexpression plasmids were transfected into KG-1; Sh-EIF3B & WNT2, eukaryotic translation initiation factor 3 subunit B short hairpin RNA plasmids and WNT2 overexpression plasmids were transfected into KG-1 cells.
EIF3B, as a subunit of the EIF3 family, plays a crucial role in stimulation of cell growth-regulating protein synthesis, which contributes to the oncogenic progression of several cancers [14]. Existing studies show that EIF3B is overexpressed in some malignancies and is a potential oncogene [5-7, 15]. For example, overexpression of EIF3B is observed in SW116 cells of human colon cancer as well as SKOV3 and HO-8910 cells of human ovarian cancer [6, 15]. Another study indicates that EIF3B is upregulated in bladder and prostate cancer compared with normal tissues [5]. According to the previous studies, EIF3B is upregulated in several solid tumors and plays an important role in the process of tumorigenesis; however, the regulatory role of EIF3B in hematologic malignancies including AML remained unknown. In the present study, we observed that EIF3B was overexpressed in AML-193, OCL-AML2, and KG-1 AML cell lines compared with human primary BMMC. The possible explanations were (1) consistent with previous studies, EIF3B might act as an oncogene in AML and accelerate the progression of AML by initiating oncogenic cell activities, which was validated in our cellular experiments. (2) ELB3B might serve as a promoter of some oncogenes in AML, such as CTNNB1, CCND1 and C-Myc, which are related to the self-renewal of leukemic cells and accelerated AML metabolism [13].

Existing evidence suggests that ELB3B is involved in tumorigenesis and affects cancer cell activities in some carcinomas [5-7, 14]. For example, knockdown of EIF3B inhibits cell proliferation and clonability but elevates the cell apoptosis rate in colon cancer SW1116 cells [6]. Another study disclosed that the knockdown of EIF3B reduces cell proliferation and invasion but stimulates cell apoptosis by downregulating the WNT/CTNNB1 signaling pathway in breast cancer [16]. Overexpression of EIF3B is found in immortal fibroblast cells and EIF3B participates in the complex mechanism of tumor formation, which leads to stimulation of tumor growth in human breast carcinoma [14]. In addition, emerging evidence exhibits that the WNT signaling pathway is implicated in the
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AML pathology by enhancing proliferation of hematopoietic stem and progenitor cells [9-11, 17]. Based on previous studies, we speculated that EIF3B might affect the AML cell activities by activating the WNT signaling pathway. We performed experiments that suggested that knockdown of EIF3B inhibited proliferation and migration but promoted apoptosis in AML by blocking the WNT signaling pathway. Possible explanations included: (1) Knockdown of EIF3B decreased WNT2 and other WNT signaling related genes and proteins by regulating protein synthesis, thus its knockdown could inhibit AML cell proliferation and migration while promoting apoptosis by blocking the WNT signaling pathway; (2) EIF3B might affect other carcinogenic pathways by regulating related protein synthesis; thus its knockdown inhibited AML progression, but further studies are needed for validation.

To assess the regulatory effect of EIF3B on the WNT signaling pathway in AML, we selected three genes in the WNT pathway (WNT2, GSK3B and CTNNB1). In detail, WNT2 is overexpressed in AML and it promotes AML development and progression by inducing aberrant gene promoter methylation and subsequently promoting cell growth and reducing cell apoptosis in AML [18]. GSK3B is identified as an anti-cancer gene and the knockdown of GSK3B promotes the progression of AML [17]. Regarding CTNNB1, it contributes to cancer pathology and enhances metastasis in AML [11-13]. In addition, the high expression of CTNNB1 impairs bone marrow hematopoiesis and is related to the elevated self-renewing ability of leukemic cells in AML [13]. Regarding these findings, we propose that EIF3B might regulate the expression of GSK3B and CTNNB1 in AML cells as well, and discovered that knockdown EIF3B upregulated GSK3B but downregulated CTNNB1 by blocking WNT2. The possible reasons were that knockdown of EIF3B might activate the leukemia inhibitory factor through acting synergistically with the WNT antagonists GSK3B and WNT producer CTNNB1. CTNNB1 enhanced the expression of GSK3B and inhibited renewal of hematopoietic stem cells depending on CTNBB1, attenuating the progression of AML [17]. According to our study, knockdown of EIF3B inhibited cell proliferation and migration but elevated apoptosis through blocking the WNT signaling pathway in AML, which suggested that EIF3B might be an innovative therapeutic target for AML treatment.

In conclusion, EIF3B is upregulated, and its knockdown inhibits cell proliferation and migration, while promoting apoptosis by downregulating the WNT signaling pathway in AML.

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Disclosure of conflict of interest

None.
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