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Molecular mechanism of KIF21B in pediatric acute T lymphoblastic leukemia

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Abstract

Our study aims to explore the molecular mechanism of KIF21B (Kinesin family member 21B, KIF21B) in pediatric acute T-lymphocytic leukemia. Jurkat cell lines were selected into over-expression group and knockdown knockdown group. western blotting, cell flow cytometry, CCK-8 and immunofluorescence were used to detect our results. The protein level of KIF21b in over-expression group was significantly higher than knockdown group (P<0.05); The G1 phase of KIF21B cells in over-expression group was obviously shortened, while the G1 phase of KIF21B cells in knockdown group was distinctly prolonged (P<0.05); The proliferation rate of cells in over-expression group was distinctly higher than knockdown group (P<0.05); The TORC1 signaling pathway related proteins in over-expression group were obviously higher than knockdown group (P<0.05); Over-expression of KIF21b can activate the MTORC1 signaling pathway, while knocking down KIF21b significantly inhibits the activity of the MTORC1 signaling pathway; KIF21B makes regulations on activating the MTORC1 pathway by affecting the localization of the MTORC1 complex in cells. Our results indicated that Over-expression of KIF21b affects the localization of mTOR protein cells and activates the MTORC1 signaling pathway to promote the occurrence and development of T-ALL. KIF21B has important potential value in disease diagnosis and treatment.

Keywords: pediatric acute T-lymphocytic leukemia; KIF21B; molecular mechanism

Introduction

Acute lymphoblastic leukemia (ALL) is an invasive tumor, including type B and type T acute lymphoblastic leukemia [1]. T-cell acute lymphoblastic leukemia (T-ALL) is a kind of serious blood disease with high malignant degree and great harm, accounting for 15% of ALL cases in children [2, 3]. After the disease, a large amount of leukemia cells accumulate in the bone marrow, inhibiting the normal hematopoietic function, leading to decreased immunity and infection, and then causing fever, fatigue, dizziness and other symptoms [4, 5]. At present, the clinical treatment is mainly through chemical drugs, and the commonly used drugs include daunorubicin hydrochloride and cytarabine hydrochloride. Although daunorubicin hydrochloride and cytarabine hydrochloride can inhibit the progression of the disease to some extent, it is still hard to cure the disease and has large toxic and side effects [6, 7]. Continuous exploration of new treatment schemes has always been an important clinical work, and clarifying the pathogenesis is of great significance for the treatment of diseases [8]. KIF21B (Kinesin family member 21B) protein belongs to the Kinesin-4 motor protein superfamily, which is a motor protein that binds to microtubules and may affect the growth, development and functional maintenance of neurons by adjusting the transport of substances inside neurons [9]. KIF21B has relation to the development and occurrence of a variety of tumors, which can be employed as a potential biomarker and has the potential to predict the stage of tumor development [10]. Related studies have confirmed that KIF21B is strongly related to the occurrence of T-ALL, but the specific mechanism is still unclear. In view of this, the molecular mechanism of KIF21B in T-ALL was studied and reported as follows.

1. Material and methods

1.1 Materials

Jurkat cells were bought from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). Streptomycin (Multicellular, USA), DMEM medium (Gibco, UK), 10% fetal bovine serum (FBS, Gibco), trypsin (Beyotime, China), TRIzol reagent (Life Technologies, USA), Trizol LS (Life Technologies), Primescript RT Reagent (Takara, Japan), Agilent 2100

Bioanalyzer (Thermo Fisher Scientific), PCR primers (RiboBio, China), qRT-PCR status kit (RiboBio), lentivirus (Genechem, China), PVDF membrane (Merck Millipore, USA), Lipofectamine® 3000 transfectant (Invitrogen), Cell counting kit-8 (MCE, USA), dNTP MIX (Shanghai Haoran Biotechnology,

China), SYBR Green PCR (Applied Biosystems, USA), Magic XP Western Protein (Shanghai Solarbio Biotech, China), Tanon 4600 series chemiluminescence/fluorescence image analysis system (Tanon, China), 4-12% prefabricated polyacrylamide gel (Shanghai Solarbio), tween20 (Shanghai Solarbio), Reverse transcription kit (Beijing Biolaibo Technology, China), TAE protein loading buffer (4×) (Beijing Biolaibo Technology), Trisgly SDS transfer membrane buffer (Shanghai Yase Biotechnology, China).

Low-temperature high-speed centrifuge (Shandong Biobase, China), low-temperature low-speed centrifuge (Shandong Biobase), -80℃ refrigerator (Jinan laibao Medical Equipment, China), ordinary refrigerator (Midea, China), inverted optical microscope (Shanghai Batuo Instrument, China), digital display water bath (Shanghai Analytical Instrument, China), high-precision analytical balance (Shanghai Yoke, China), ice machine (Bingyue, Shanghai, China), and enzyme-linked immunosorbent assay (Promega, USA).

1.2 Cell culture

Cells were incubated in an incubator with 5% CO_2 at 37°C, and changed the medium every 48h. Cell passage was performed when the cell seeding density reached 80%. 1ml of trypsin was added to a 10-cm cell culture dish prewashed with PBS and incubated in an incubator for 5 minutes. The cell morphology was observed under a microscope. When the cell morphology became spherical and did not detage from the culture dish, the trypsin was discarded and the digestion was terminated by adding medium containing 10% FBS. The dish was gently blown to detach the cells from the dish. Exfoliated cells were collected, equilibrated and centrifuged at 1000 RPM for 5 minutes at 25 ℃. Cell precipitation was seen after discarding the supernatant. Resuspend the cells by adding 1ml of medium and add to a new culture dish. After shaking thoroughly, the cells were put into an incubator for continued culture. It is stored in aliquots frozen and can be used for 12 months. This study obtains approval of the Institute of hospital Ethics.

1.3 RNA extraction and sequencing

Isolate total RNA from patients' serum employing Trizol LS (Life Technologies). The concentration and quality of isolated RNA were estimated by an Agilent 2100 Bioanalyzer (Thermo Fisher Scientific). The OD 260/280 absorbance ratios of the samples ranged from 1.8 to 2.0. The final RNA preparation was resuspended in ribonuclease-free water and then reserved at -80°C. The pretreated RNA was sequenced through an Illumina chastity filter and sequenced and counted through an Illumina NextSeq 500, and the resulting matrix was used for sequence analysis. Raw Illumina NextSeq 500 sequencing reads through the Illumina Chastity filter were applied to conduct sequence analysis.

Then extract total RNA by TRIzol reagent (Takara, Japan), about 1000 ng of RNA was reversely transcribed into cDNA applying Primescript RT reagent (Takara). qRT-PCR was accomplished in accordance with manufacturer's instructions. The PCR primers used were as follows:

β-actin forward, 5'-TCACCCACACTGTGCCCATCTACGA -3';

β-actin reverse, 5'-CAGCGGAACCGCTCATTGCCAATGG -3';

KIF21B forward, 5'- CGGGCGGGAATTTGTGAGA -3';

KIF21B reverse, 5'- TCTGTCCGTAGACTGCATCTG -3';

GAPDH forward, 5'- GTAGGGCGGGAGTTTGTGAG -3';

GAPDH reverse, 5'- CTGGGGCTTTCCACTAGCATC -3'.

1.4 Lentiviral and siRNA transfection

The lentivirus used for knockdown or over-expression of KIF21B was constructed on the basis of vector GV280. In addition, the sequence of carrier elements was HU6-MCS-ubiquitin-EGFP-IRES-puromycin. And the siRNA for RAPTOR was designed specifically and bought from Ribobio. Employ Lipofectamine® 3000 transfectant (Invitrogen) to transfect siRNA into cells. KIF21B gene high expression lines (over-expression group) and knockdown cell lines (knockdown group) were constructed, and all operations shall be performed strictly in accordance with the instructions.

1.5 Cell proliferation

Cell proliferation assay were conducted through CCK-8 to detect cell survival and growth. 2×10^3 cells were incubated in each well of a 96-well plate containing 100μL of medium. Afterwards, add 10μL CCK-8 into each well, and after 2 hours of incubation in the dark, the absorbance was determined at 450nm by a microplate reader.

1.6 Immunoblotting

Cells were obtained and then lysed in cold lysis buffer solution (Beyotime, China). Proteins in cell lysates were isolated by SDS-PAGE and then transferred to PVDF membranes. Apply a Tanon 4600 series chemiluminescence/fluorescence image analysis system (Tanon) so as to detect and quantify the gray scale ratios of target proteins .

1.7 Immunofluorescence

Cells were inoculated on glass coverslips and incubated for different periods depending on the experiment's purpose. After that, cells accepted double staining and employed DAPI as a nuclear stain agent. Employ a confocal laser scanning microscope to get images.

1.8 Immunoprecipitation

According to the Abcam (https://www.abcam.com/protocols/immunoprecipitation-protocol-1) method. BeyoMag™ protein A+G magnetic beads (Beyotime) were used for co-immunoprecipitation (co-IP) experiments. Cell lysates were cultivated together with antibody-linked magnetic beads, after that, proteins were eluted from these beads.

1.9 Inspection indicators

(1) KIF21B protein expression: the expression level of KIF21B protein in different groups was analyzed by Western Blotting.

(2) The cell cycle: analyze and compare the cell cycle's change of KIF21B in different groups.

(3) Cell proliferation: analyze and compare the proliferation of KIF21B cells in different groups.

(4) MTORC1 signaling pathways: KIF21B effects on MTORC1 signaling pathways.

(5) Mechanism: analyze the specific mechanism of action of KIF21B.

1.10 Statistical methods

In statistical analyses, data are typically indicated as mean value plus or minus standard deviation ($\bar{x} \pm s$) from more than three independent experiments. For pairwise comparisons between groups, the Student's t-test is usually used; For experiments with three or more groups, one-way analysis of variance (ANOVA) is usually applied. In the clinical sample analysis, the chi-square test is usually used. All experiments should be independently performed at least 3 times. Statistical analysis were typically conducted with the use of GraphPad Prism software or SPSS software, and the results are presented graphically. $P < 0.05$ was significant.

2. Results

2.1 Protein expression

The protein level of KIF21b in over-expression group was markedly higher than knockdown group (Fig 1, $P < 0.05$).

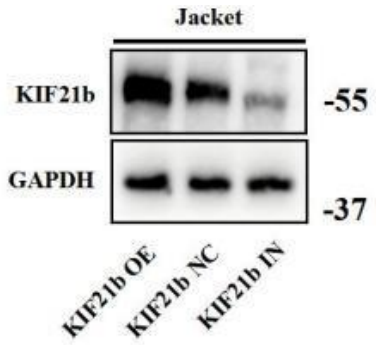

Figure 1: KIF21b protein level in 3 groups of cells

2.2 Cell cycle

The G1 phase was significantly shortened in KIF21B over-expression group and prolonged in KIF21B knockdown group (Fig 2, $P < 0.05$).

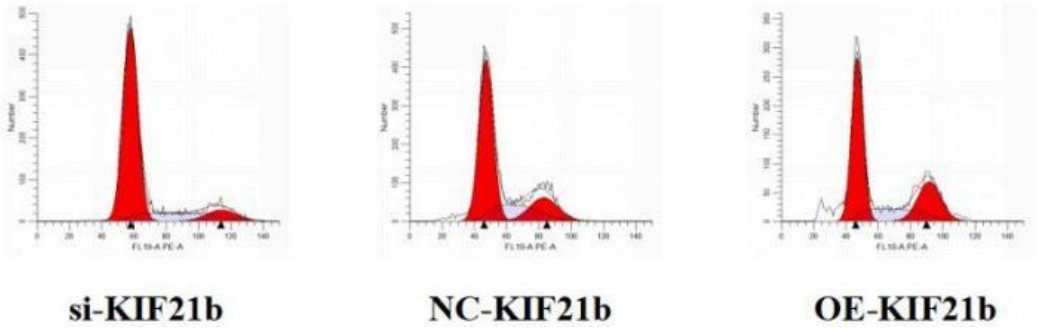

Figure 2: Cell cycle changes in 3 groups

2.3 Cell proliferation

The cell proliferation rate of the over-expression group was observably higher than knockdown group (Fig 3, $P < 0.05$).

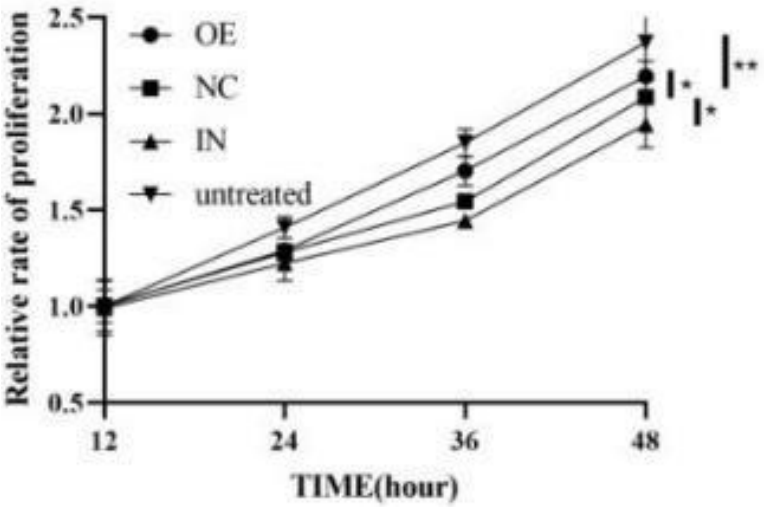

Figure 3: Cell proliferation in 3 groups

MTORC1 signaling pathway

The protein related to TORC1 signaling pathway in over-expression group was signally higher related to knockdown group ($P < 0.05$). Over-expression of KIF21b could activate the MTORC1 signaling pathway, while knockdown of KIF21b significantly inhibited the activity of MTORC1 signaling pathway. (Fig 4)

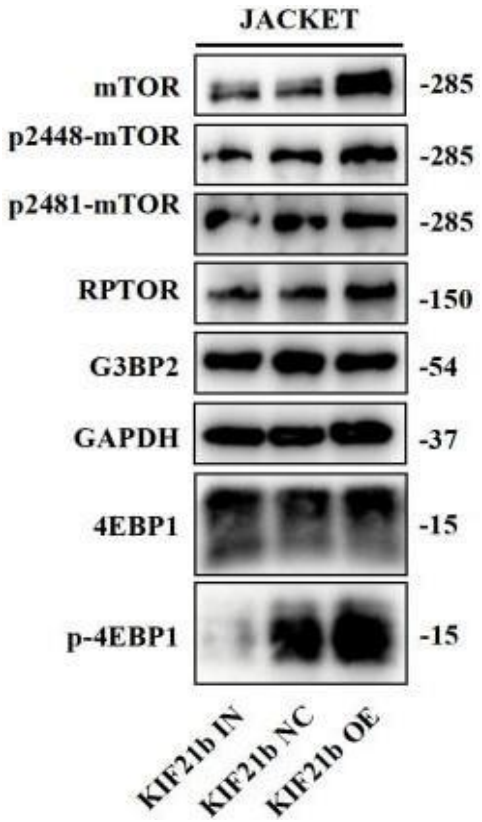

Figure 4: Expression of MTORC1 signaling pathway-related proteins in 3 groups

2.4 Pathway of Action

KIF21B makes regulation on activating of MTORC1 pathway by affecting the localization of MTORC1 complex in cells. (Fig 5)

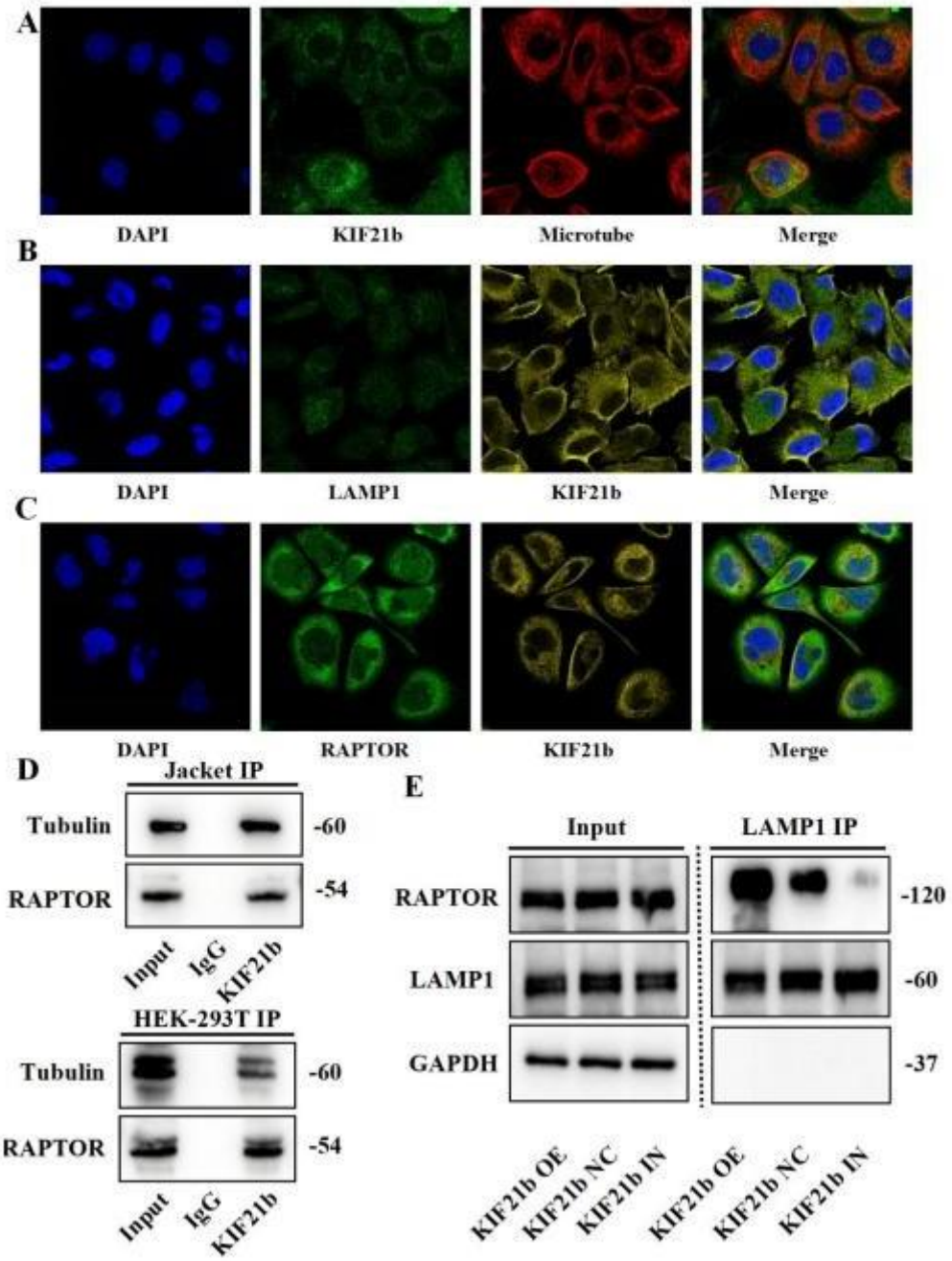

Figure 5: KIF21B promotes RAPTOR lysosomal localization. A. Cellular immunofluorescence assay showed that KIF21B was co-localized with tubulin; B. Cellular immunofluorescence assay showed that KIF21B was co-localized with the lysosomal surface protein LAMP1. C. Cellular immunofluorescence assay showed that KIF21B co-localized with RAPTOR, a key protein of MTORC1 signaling pathway. D. Co-immunoprecipitation assays showed that KIF21B binds TUBULIN to RAPTOR protein. E. Co-immunoprecipitation assay showed that the binding of LAMP1 to RAPTOR was affected by KIF21B.

3. Discussion

T-ALL is a common type of leukemia, which threatens the physical and mental health of children seriously. Affected by many factors, the incidence of pediatric T-ALL in China has been increasing, which has attracted wide attention [11, 12]. With the continuous development and advancement of medical technology, the prognosis of children with T-ALL has been significantly

improved. The overall survival and 5-year event-free survival rate of T-ALL have increased to more than 80% and 90%, respectively, and the cumulative risk of recurrence has been reduced to less than 10% [13, 14]. However, the long-term efficacy is still not obvious, and the treatment effect still needs to be further improved in order to further improve the treatment outcome and quality of life of children with ALL [15]. A clear pathogenesis is the prerequisite for formulating scientific and reasonable treatment plans [16, 17]. KIF21B belongs to kinesins superfamily and is an ATP-dependent microtubule motor protein involved in the transport of intracellular membrane organelles [18]. Related researches have shown that KIF21B gets involved in genesis and development process of T-ALL, but the exact mechanism is still unclear [19, 20].

In this study, cell lines with high and knockdown of KIF21B gene were constructed using Jurkat cells, and the expression level of KIF21B protein was verified by Western Blotting. Flow cytometry revealed that the proportion of cells with high expression of KIF21B in G1 phase was significantly reduced, and the early apoptosis of cells was reduced. KIF21B knockdown cells showed increased G1 phase and early apoptosis. This suggests that KIF21B may affect cell proliferation rate by affecting the cell cycle. The rate of cell proliferation usually reflects the rate of tumor development and malignancy. CCK8 assay showed that high expression of KIF21B significantly increased cell proliferation rate, while knockdown of KIF21B significantly inhibited cell proliferation. This suggests that KIF21B may affect the occurrence and progression of T-ALL by affecting the cell proliferation rate. Over-expression of KIF21B can affect the cell cycle to a certain extent. After knockdown of KIF21B, the cell cycle was arrested in G1 phase, thereby reducing the rate of cell proliferation. The results of immunofluorescence assay showed that KIF21B interacts with tubulin, and the change of KIF21B expression level may affect the function of tubulin and the localization of some proteins in cells. By analyzing KIF21B related cell signaling pathways in the pan-cancer database, we found that KIF21B was closely related to cell proliferation and glucose metabolism regulation, and mTOR signaling pathway was enriched by GSEA analysis. The MTOR pathway is a critical intracellular signaling system participated in regulating a variety of cell growth, metabolic and proliferation processes. MTOR, the target of mammalian rapamycin, belongs to the phosphatidylinositol 3-kinase-related kinase (PIKK) family and is a serine/threonine protein kinase. The MTOR signaling pathway mainly consists of two complexes: MTORC1 and MTORC2, each with different functions and regulatory mechanisms. Our study shows that KIF21B binds RAPTOR, a key protein of MTORC1 complex, through tubulin and affects RAPTOR localization in cells. Western Blotting showed that over-expression of KIF21B promoted the activation of MTORC1 pathway, while knockdown of KIF21B inhibited the MTORC1 signaling pathway.

4. Conclusion

Over-expression of KIF21b affected the cellular localization of mTOR to activate MTORC1 signaling pathway to promote acute T-cell lymphoblastic leukemia and predict poor clinical outcome. However, this study was limited to the cellular level, and the results may have a certain bias. Animal experiments should be further supplemented in future studies to explore the pathogenesis of T-ALL from multiple perspectives, so as to provide reliable evidence for clinical practice.

5. Acknowledgements

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