EVI-1-Mediated Up-Regulation of IncRNA TUG1 Interacts with miR-186-5p to Promote Proliferation and Drug Resistance in Pediatric Acute Myeloid Leukemia

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Research

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Abstract

Background:

Deregulated lncRNAs have been well-documented to be closely associated with resistance to chemotherapeutic agents in malignancies including acute myeloid leukemia (AML). Herein, we intended to explore the roles and underlying mechanism of lncRNA taurine-upregulated gene 1 (TUG1) in pediatric AML cell adriamycin (ADR) resistance.

Methods:

TUG1 and ecotropic viral integration site-1 (EVI-1) expressions in pediatric AML patients and cells were detected by using qRT-PCR and western blot, respectively. Western blot analysis, flow cytometry and CCK-8 assays were conducted for evaluating the drug sensitivity, apoptosis, and cell viability. Luciferase reporter assay and qRT-PCR were implemented to determine the interaction between miR-186-5p and TUG1. The western blot was employed to analyze the changes of proteins.

Results:

TUG1 was upregulated with a high positive correlation with EVI-1 in pediatric AML patients. TUG1 was transcriptionally activated by EVI-1 and functioned as a miR-186-5p ceRNA to inhibit its expression in AML cells. An enhanced cell proliferation as well as a resistance to ADR in HL60 cells was observed after TUG1 overexpression and miR-186-5p knockdown, while an opposite effect was elicited by TUG1 silencing and miR-186-5p restoration. Mechanistically, robust expression of TUG1 increased P-gp level and induced the PI3K/Akt/mTOR cascade activation in HL60 cells while loss of TUG1 expression exerted reverse effects in HL60/ADR cells. Moreover, TUG1 overexpression abolished miR-186-5p-induced inactivation of the PI3K/Akt/mTOR cascade in HL60/ADR cells.

Conclusion:

Taken together, in pediatric AML, EVI-1-mediated upregulation of the oncogenic lncRNA TUG1 promotes proliferation and ADR resistance by sponging miR-186-5p through the PI3K/Akt/mTOR signals.

Background

Acute leukemia, a heterogeneous group of aggressive hematopoietic system malignancies, is believed to be one of the most frequently diagnosed childhood cancers in China, accompanied by variable clinical outcomes [1]. Acute myeloid leukemia (AML), a common pediatric acute leukemia, originates from abnormal differentiation and rapid proliferation of immature myeloid progenitors, accounting for ~ 30% of leukemia-associated pediatric mortalities [2]. Despite considerable improvements in AML remedy including targeted drugs, the overall prognosis for AML is still hard to be estimated, and the five-year survival rate is only 20–40% [3]. During the recent decades, traditional cytotoxic chemotherapy remains one of the major therapies for AML [4]. Currently, adriamycin (ADR) was a widely used clinical

chemotherapeutic agent for AML [5]. However, the therapeutic inefficiency and frequent recurrence in AML attributed to drug resistance significantly limits the efficacy of chemotherapy [6]. Consequently, an in-depth understanding of the potential mechanism for the chemoresistance in pediatric AML is helpful for developing efficient treatment interventions for overcoming chemoresistance.

Researches on human transcriptome discover that merely less than 2% of the human genome is capable of encoding proteins, while non-coding RNAs (ncRNAs), including recently discovered long non-coding RNA (lncRNAs) and extensively investigated microRNAs (miRNAs), accounts for the vast majority of transcripts [7]. lncRNAs is a group of endogenous single-stranded ncRNAs longer than 200 nt and devoid of the potential of protein encoding [8]. Growing literatures indicate that abnormally expressed lncRNAs closely related to the tumorigenesis of human malignancies, such as AML by modulating a multitude of biological process, including cell development, metastasis, and death [9, 10]. Moreover, increasing experimental data have proposed that deregulated lncRNAs are closely associated with resistance to chemotherapeutic agents in miscellaneous cancers [11]. Originally, Taurine-upregulated gene 1 (TUG1) was acknowledged as an upregulated lncRNA in the developing mouse retinal cells under taurine treatment [12]. Widespread evidence has shown that dysregulation of TUG1 is frequent in diverse carcinomas and is closely implicated in cancer carcinogenesis by exerting either cancer-promoting or tumor-suppressing effects in different cancers [13]. Interestingly, recent studies demonstrated that TUG1 is overly expressed in AML cells and closely correlated with worse outcome, which induces cell proliferation as well as ADR resistance, and represses apoptosis in AML cells [14, 15]. Nevertheless, the detailed regulation of TUG1 on AML resistance to ADR still remains to be more deeply investigated.

Ecotropic viral integration site-1 (EVI-1), situated at chromosome 3q26, was firstly recognized in an integration locus of retrovirus in an AML mouse model [16]. EVI-1 is believed as an oncogenic dual domain zinc finger transcription regulator involved in myeloid leukemia [17]. Moreover, it is widely recognized that EVI-1 is implicated in the maintenance and proliferation of hemopoietic stem cells and myeloid progenitor cell differentiation [18].

We identified that TUG1 expression was increased in pediatric AML patients, which is transcriptionally activated by EVI-1. Moreover, it was demonstrated that depletion of TUG1 hindered cell proliferation and ADR resistance in AML cells by suppressing miR-186-5p through inhibiting the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) signals, contributing to the development of effective treatments to overcome chemoresistance in AML cells.

Materials And Methods

Clinical samples

Prior to any interventional therapies at the First Affiliated Hospital of Zhengzhou University from March 2018 to April 2019, bone marrow (BM) specimens were harvested during the biopsy from 21 pediatric patients who were newly diagnosed with AML and 10 healthy children as normal controls. The French-American-British (FAB) classification was used as the standard criteria for the diagnosis of AML

patients [19, 20]. Signed consent for clinical studies was signed by their guardians prior to the study and our study was approved by Institutional Ethics Board of the First Affiliated Hospital of Zhengzhou University.

Cell culture and transfection

The AML cell lines (HL60 and K562), as well as human bone marrow stromal cell line (HS-5) were get from the American Type Culture Collection (ATCC) (Manassas, VA, USA). These cells were cultivated in RPMI-1640 medium (BOSTER, Wuhan, China) complemented with 10% heat-inactivated fetal calf serum (ExCell Bio, Shanghai, China), together with 1% penicillin/streptomycin in a water-saturated culture incubator flushed with 5% CO₂ and 95% air under 37°C. ADR-resistant HL60 (HL60/ADR) and K562 (K562/ADR) cells were provided by Institute of Haematology, Chinese Academy of Medical Sciences (Tianjin, China). To keep the drug resistance phenotype, HL60/ADR and K562/ADR cells were incubated in culture medium containing 0.1 μM ADR (Sigma, St Louis, MO, USA) and further cultivated without ADR for 2 weeks before experiments.

pcDNA-TUG1 (TUG1), control vector (Vector), siRNA specially targeting TUG1 (si-TUG1), control siRNA (si-NC), miR-186-5p mimics (miR-186-5p), NC-mimic (miR-NC), miR-186-5p antigomir (anti-miR-186-5p), and antigomir control (anti-miR-NC) were all bought from GenePharma Co., Ltd. (Shanghai, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was then taken to perform transient transfection.

Cell viability assay

The cell counting kit-8 (CCK-8) assay was used for cell viability evaluation. 96-well plates were used to carry the logarithmically growing HL60/ ADR and HL60 cells with a density of 5×10^3 cells per well and all the cells were exposed to 8 μM ADR or infected with si-TUG1, TUG1, miR-186-5p, anti-miR-186-5p, or matched controls in the absence or presence of 8 μM ADR. After incubated for the indicated time, the cells were fostered for another 3 h after adding 10 μL CCK-8 solutions. A MultiSkan3 ELISA Reader (Thermo Fisher Scientific, Waltham, MA, USA) was adopted to record the cell viability.

Quantitative real-time polymerase chain reaction (qRT-PCR)

RNAiso Plus (Takara, Dalian, China) was taken to extract total RNA. Using SuperScript III reverse transcriptase (Invitrogen), the synthesis of first strand cDNA was carried out. To examine TUG1 and EVI-1 mRNA expressions, qRT-PCR was implemented on a Chromo4 instrument (Bio-Rad) with THUNDERBIRD SYBR qPCR mix (Toyobo, Osaka, Japan), with GAPDH as the normalization. Additionally, miR-186-5p expression was examined using miScript SYBR Green PCR Kit (Qiagen) on a Chromo4 instrument (Bio-Rad) and normalized to *U6* small nuclear RNA (snRNA). The fold changes were detected according to the $2^{-\Delta\Delta C_t}$ method.

Western blot analysis

RIPA lysis buffer (KeyGEN, Nanjing, China) containing phenylmethanesulphonyl fluoride (PMSF) was used to lyse collected samples and the protein content was quantified by using the BCA™ Protein Assay Kit (Beyotime, Shanghai, China). The 10% SDS-PAGE gel was used to separate collected cell lysates, and separated bands were then electro-transferred onto nitrocellulose membranes. Next, the membranes were exposed to 5% skimmed milk for 1 hour. Then, the blocked membranes were probed with primary antibodies for 12 hours at 4°C and secondary antibody marked by horseradish peroxidase (Abcam, Cambridge, MA, USA) at room temperature. An ECL detection reagent (Solarbio, Shanghai, China) was implemented to measure the signals. The primary antibodies in this study include proliferating cell nuclear antigen (PCNA), Bcl-2, EVI-1 (Abcam); P-glycoprotein (P-gp), PI3K, phosphorylated PI3K (p-PI3K), phosphorylated mTOR (p-mTOR), phosphorylated Akt (p-Akt) and β -actin (Abcam).

Luciferase reporter assay

The fragments from TUG1 cDNA encompassing two potential miR-186-5p-targeting sites and its two mutated (MUT) counterparts were synthesized and subcloned into p-MIR-reporter plasmid (Thermo Fisher Scientific) to produce TUG1 wild type, TUG1 mutant type 1 and TUG1 mutant type 2. Subsequently, HL60/ADR and HL60 cells were cotransfected with TUG1 wild type, TUG1 mutant type 1, or TUG1 mutant type 2 and anti-miR-186-5p, miR-186-5p, or respective controls by means of Lipofectamine 2000 (Invitrogen). A luminometer was used to measure the luciferase activity under the Luciferase Reporter System (Promega, Madison, WI, USA).

Statistics

GraphPad software 6.0 (GraphPad Inc., San Diego, CA, USA) was taken for statistical analysis. All results were displayed as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) and two-tailed Student's *t*-test and were conducted to determine the significance of differences. *P* values < 0.05 between differences were regarded statistically significant.

Results

TUG1 was upregulated with a high positive correlation with EVI-1 in BM samples of pediatric AML patients

Firstly, we detected the TUG1 and EVI-1 expression in collected clinical pediatric AML samples by qRT-PCR. Upregulated expressions of TUG1 and EVI-1 in 21 BM samples of pediatric AML patients were demonstrated when compared with that in 10 BM samples from healthy controls (Fig. 1A and 1B). A positive correlation between TUG1 and EVI-1 expressions in the BM samples of pediatric AML patients was shown in Fig. 1C. These results suggested that TUG1 was upregulated with a significant positive correlation with EVI-1 in pediatric AML patients.

EVI-1 enhanced TUG1 expression in AML cells

The TUG1 and EVI-1 expression in ADR-resistant AML cells and their parental cells were further evaluated. As shown in Fig. 2A and 2B, TUG1 and EVI-1 expressions were both significantly upregulated in HL60 and K562 cells relative to HS-5 cells. Particularly, higher expressions of TUG1 and EVI-1 were observed in HL60/ADR and K562/ADR cells versus those in their parental cells (Fig. 2A and 2B). It is widely recognized that transcription factors could contribute to the activation of lncRNAs in human diseases [21]. Bioinformatics analysis using UCSC (<http://genome.ucsc.edu/>) showed that EVI-1 was a potential transcriptional regulator for TUG1. Determination of EVI-1 effect on TUG1 transcription was accomplished via evaluating the changes of TUG1 expression after delivering 1 µg or 2 µg si-EVI-1 to HL60/ADR and HL60 cells. As a result, EVI-1 expression was dramatically inhibited in HL60/ADR and HL60 cells after si-EVI-1 transfection (Fig. 2C). Of note, introduction with 1 µg or 2 µg si-EVI-1 led to a remarkable reduction of TUG1 expression in these cells (Fig. 2D). Meanwhile, the regulatory effect of TUG1 on EVI-1 expression was analyzed by introducing TUG1, si-TUG1, or respective controls into HL60/ADR and HL60 cells. The expression of TUG1 was observed being strikingly increased followed by delivery with TUG1 and distinctly declined in si-TUG1-treated HL60/ADR and HL60 cells (Fig. 2E). However, no significant change of EVI-1 expression was observed in TUG1- or si-TUG1-transfected cells (Fig. 2F). Thus, we concluded that EVI-1 promoted TUG1 expression by serving as a transcriptional activator.

TUG1 promoted cell proliferation and the resistance to ADR in AML cells

For characterizing the TUG1 regulation on the resistance to ADR in AML cells, TUG1 or Vector were delivered to HL60 cells, and si-TUG1 or si-NC was transfected to HL60/ADR cells. CCK-8 assay proved that ectopic expression of TUG1 dramatically enhanced cell proliferation in HL60 cells compared to control group but TUG1 knockdown by si-TUG1 presented a reverse phenomenon in HL60/ADR cells (Fig. 3A). Consistently, the expression of PCNA, a marker of cell proliferation, was pronouncedly augmented in response to TUG1 overexpression in HL60 cells, but was drastically blocked after TUG1 was silenced in HL60/ADR cells (Fig. 3B). In addition, CCK-8 assay demonstrated that exposure of HL60/ADR and HL60 cells to 8 µM ADR resulted in an evident decrease of cell viability. However, increased expression of TUG1 effectively restored ADR-induced reduction of cell viability in HL60 cells while TUG1 downregulation markedly intensified ADR-induced decrease of cell viability in HL60/ADR cells (Fig. 3C). Also flow cytometry analyses proved that TUG1 upregulation reversed the apoptosis induced by ADR in HL60 cells, whereas TUG1 downregulation accelerated the apoptosis induced by ADR in HL60/ADR cells (Fig. 3D). Furthermore, it was demonstrated that ADR treatment prominently restrained the Bcl-2 expression, Bcl-2 protein, in cells, which was partially reversed following promotion of TUG1 in HL60 cells (Fig. 3E) and further promoted by delivery with si-TUG1 in HL60/ADR cells (Fig. 3F). The above findings demonstrated that TUG1 facilitated cell proliferation and ADR resistance in AML cells.

TUG1 acted as a miR-186-5p sponge in AML cells

Widespread evidence has well-documented the close association between lncRNAs and miRNAs in regulating cellular processes in various types of human malignancies [22]. To clarify the regulatory basis

of TUG1 on AML cells, the bioinformatic database starBase v2.0. was applied to predict the potential target miRNAs binding to TUG1. TUG1 was proposed harboring two binding sequences complementary to miR-186-5p (Fig. 4A), a well-characterized tumor suppressor in different types of tumors. qRT-PCR analysis showed a remarkable reduction of miR-186-5p in HL60 and K562 cells relative to that in HS-5 cells, particularly in HL60/ADR and K562/ADR cells (Fig. 4B). Intriguingly, TUG1 was reversely correlated with the expression of miR-186-5p in 21 BM specimens from pediatric AML patients (Fig. 4C). To double-confirm the TUG1 interaction with miR-186-5p, luciferase reporter assay was conducted. miR-186-5p overexpression greatly reduced but miR-186-5p inhibition greatly boosted the luciferase activity of wild-type reporter vector (TUG1 wild type) in HL60/ADR and HL60 cells (Fig. 4D). However, anti-miR-186-5p and miR-186-5p mimics failed to generate any alternation of mutant reporters (TUG1 mutant type 1 and TUG1 mutant type 2) in these cells (Fig. 4D). Next, HL60/ADR and HL60 cells were introduced with TUG1, si-TUG1, or corresponding controls to investigate whether TUG1 could modulate miR-186-5p expression. As a result, miR-186-5p was apparently repressed in TUG1-overexpressing cells and successfully enhanced in TUG1-silencing cells (Fig. 4E). We deduced that TUG1 sponged miR-186-5p in AML cells.

miR-186-5p upregulation constrained cell proliferation and ADR resistance in AML cells

To determine the functional basis of miR-186-5p in regulating AML progression, anti-miR-186-5p or anti-miR-NC was delivered into HL60 cells and miR-186-5p or miR-NC was introduced into HL60/ADR cells. miR-186-5p silencing notably contributed to cell proliferation in HL60 cells relative to anti-miR-NC group (Fig. 5A). On the contrary, cell proliferation was dramatically suppressed in response to overexpression of miR-186-5p (Fig. 5A). Moreover, miR-186-5p downregulation significantly increased PCNA expression in HL60 cells versus that in anti-miR-NC group while miR-186-5p overexpression showed the opposite effect in HL60/ADR cells (Fig. 5B). The CCK-8 assay was performed to assess the miR-186-5p impact on ADR cytotoxicity. Anti-miR-186-5p effectively restored ADR-induced viability inhibition of HL60 cells while increased expression of miR-186-5p distinctly promoted ADR-induced viability inhibition of HL60/ADR cells (Fig. 5C). In line with the results of CCK-8 assay, ADR-induced decrease of Bcl-2 level in HL60 cells was remarkably rescued following the suppression of miR-186-5p. Conversely, we observed an enhancement of ADR-induced reduction of Bcl-2 expression by promotion of miR-186-5p in HL60/ADR cells (Fig. 5D). Moreover, inhibition of miR-186-5p hindered apoptosis induced by ADR in HL60 cells and robust expression of miR-186-5p elevated ADR-induced apoptosis in HL60/ADR cells (Fig 5E). Altogether, the inhibition of miR-186-5p overexpression on cell proliferation as well as ADR resistance in AML cells was confirmed.

TUG1 increased P-gp expression and activated the PI3K/Akt/mTOR signaling by targeting miR-186-5p in AML cells

Next, western blot analysis manifested that promotion of TUG1 significantly enhanced P-gp level in HL60 cells while TUG1 downregulation elicited the opposite effects in HL60/ADR cells (Fig. 6A and 6B). Additionally, we found that overexpression of TUG1 resulted in a significant increase of the phosphorylation of PI3K, Akt and mTOR in HL60 cells (Fig. 6C) while depletion of TUG1 resulted in a

substantial decrease of p-PI3K, p-mTOR, and p-Akt expression in HL60/ADR cells (Fig. 6D). Moreover, enforced expression of miR-186-5p evidently dampened the protein levels of p-PI3K, p-Akt and p-mTOR in HL60/ADR cells, which was strikingly ameliorated after reintroduction with TUG1 (Fig. 6E). Collectively, these results demonstrated that TUG1 increased P-gp expression and activated the PI3K/Akt/mTOR signals via sponging miR-186-5p in AML cells.

Discussion

It has been proposed that lncRNAs inactivate tumor suppressors and activate oncogenes in tumorigenesis. Considerable evidence shows an upregulation of TUG1, as an tumor-promoting lncRNA, in miscellaneous malignancies, such as osteosarcoma [23], prostate cancer [24], and AML [15]. On the contrary, TUG1 is well-documented to retard tumorigenesis in non-small-cell lung carcinoma [25] and glioma [13]. Accordingly, TUG1 presents great protentional in prognosis and therapeutic strategies for various cancers including AML [9]. Lately, numerous studies have implicated the association between aberrantly expressed lncRNAs and the resistance to chemotherapy of diverse carcinomas [26]. For example, in colorectal cancer cells, TUG1 knockdown re-sensitizes methotrexate (MTX) resistance via miR-186/CPEB2 axis [27]. TUG1 is overexpressed in osteosarcoma cells resistant to cisplatin and underexpression of TUG1 suppresses cisplatin resistance and facilitates apoptosis in cisplatin-treated osteosarcoma cells through the MET/Akt signaling [28]. Intriguingly, it has been previously proved that TUG1 is upregulated in AML cells and tissues resistant to ADR, and underexpression of TUG1 facilitates sensitivity to ADR in ADR-resistant AML cells *in vivo* and *in vitro* by upregulating miR-34a [14]. Our study further focused on exploring the impacts of TUG1 on resistance to ADR in AML cells and the functional basis. We observed upregulated expression of TUG1 in AML cells and pediatric AML patients. Moreover, TUG1 level was higher in ADR-resistant AML cells than their parental cells. Functionally, TUG1 overexpression significantly promoted cell proliferation and ADR resistance, and enhanced P-gp level in HL60 cells while TUG1 knockdown exhibited the opposite effects on HL60/ADR cells, suggesting the oncogenic role of TUG1 in AML.

Mechanistically, increasing experimental data have shown that transcriptional regulation contributes to lncRNA upregulation in human cancers by activating lncRNA transcriptions [29]. Herein, we explored whether overexpression of TUG1 was induced by transcription activation. Bioinformatics prediction presented that EVI-1 was a potential transcriptional activator of TUG1. The present thesis demonstrated that EVI-1 expression was boosted in pediatric AML patients and cells, particularly in the ones resistant to ADR, and positively correlated with the expression of TUG1 in pediatric AML patients. Robust expression of EVI-1 frequently occurs in up to one fifth of pediatric AML and leads to unfavorable prognosis and low survival rate with currently used chemotherapy regimens [30]. The subsequent analyses demonstrated that EVI-1 knockdown suppressed TUG1 expression while TUG1 knockdown or silencing did not affect EVI-1 expression in HL60/ADR and HL60 cells. Therefore, EVI-1 activated the transcription of TUG1 to upregulate the expression of TUG1.

miRNAs are defined as a group of short, endogenous single-stranded ncRNAs (typically 19–25 nucleotides) long with limited or no capacity in encoding proteins [31]. The roles of miRNAs in cancer carcinogenesis and drug resistance have been extensively studied [32]. Substantive studies have suggested that lncRNAs may block the expression levels and activities of miRNAs by competitively binding to miRNAs [33]. According to our bioinformatics analysis, we found that TUG1 contained two potential binding sites pairing with the seed region of miR-186-5p. We further manifested that TUG1 repressed the expression of miR-186-5p via targeting miR-186-5p. As a tumor-specific miRNA located at chromosomal 1p31.1, miR-186-5p is documented to be upregulated and exert cancer-promoting effects in several tumors such as colorectal cancer [34] and lung adenocarcinoma [35]. Conversely, miR-186-5p is down-regulated and suppresses tumorigenesis in human malignancies including non-small cell lung cancer [36], ovarian cancer [37] and osteosarcoma [38]. These results suggest the cancer type-dependent role of miR-186-5p in different tumors. Interestingly, miR-186-5p is reported to be downregulated in AML patients and predict poor prognosis [39]. Herein, a low miR-186-5p expression was observed in AML cells, especially in ADR-resistant AML cells. Moreover, functional experiments revealed that anti-miR-186-5p facilitated cell proliferation and ADR resistance in HL60 cells while miR-186-5p overexpression presented a reverse effect in HL60/ADR cells, indicating that miR-186-5p suppressed oncogenesis in AML.

As we all know, the PI3K/Akt/mTOR signaling cascade is regarded as a key prototypic survival signaling network that participates in modulating diverse physiological processes in hematological malignancies, including cellular apoptosis, metabolism, and cell proliferation [40]. Constitutively activated PI3K/AKT/mTOR pathway is observed in various tumors including AML [41]. Notably, extensive researches within the past decades have demonstrated that the PI3K/Akt/mTOR signals is closely related to drug resistance and its overactivation contributes to resistance to diverse chemotherapeutic agents in human malignancies [42]. Thus, inhibiting the PI3K/Akt/mTOR cascade may be a potential treatment candidate to reverse drug resistance. We proved that the PI3K/Akt/mTOR cascade was activated by TUG1 overexpression in HL60 cells and inhibited after TUG1 downregulation in HL60/ADR cells. Moreover, we further proved that robust expression of miR-186-5p blocked the PI3K/Akt/mTOR signals in HL60/ADR cells while ectopically expressing TUG1 rescued miR-186-5p-induced repression of the PI3K/Akt/mTOR signals in HL60/ADR cells. Altogether, these findings suggested that TUG1 activated the PI3K/Akt/mTOR cascade by suppressing the expression of miR-186-5p in AML cells.

Collectively, we confirmed the upregulation of TUG1 in pediatric AML cells and patients, particularly in AML cells resistant to ADR. Moreover, we firstly claimed that upregulation of TUG1, which was induced by EVI-1, promoted cell proliferation and ADR resistance in pediatric AML via interacting with miR-186-5p through activating the PI3K/Akt/mTOR cascade. Our study provided a novel regulatory mechanism by which TUG1 exerted the oncogenic role in AML progression and TUG1 has the potential to be a potential treatment candidate for conquering drug resistance in AML.

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

E. L. and Y. L. contributed equally to this study. Y. L. designed the study and revised and edited the manuscript. Y. L. and Y. W. analyzed the data, interpreted the results, and wrote the manuscript. E. L., Y. L. and H. Z. designed and guided the whole experiment. L. Z. and Y. T. helped with the sample preparation and performed the experiments. The author(s) read and approved of the final manuscript.

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Ethics approval and consent to participate

Signed consent for clinical studies was signed by their guardians prior to the study and our study was approved by Institutional Ethics Board of the First Affiliated Hospital of Zhengzhou University.

Competing interest

All the authors state that they have no conflicts of interest.

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Figures

Figure 1

The TUG1 and EVI-1 expression profiles in BM samples of pediatric AML patients. qRT-PCR for TUG1 (A) and EVI-1 (B) in BM specimens of 21 pediatric AML recruits and 10 normal controls. (C) Pearson's correlation analysis of the correlation between TUG1 and EVI-1 expressions in BM samples of pediatric AML patients. *P < 0.05.

Figure 2

TUG1 was transcriptionally activated by EVI-1 in AML cells. Western blot and qRT-PCR were applied to determine EVI-1 (A) and TUG1 (B) expressions in AML cells (K562/ADR, K562, HL60/ADR, and HL60) and human bone marrow stromal cell line HS-5, respectively. Western blot and qRT-PCR were implemented to analyze EVI-1 (C) and TUG1 (D) expressions in HL60 and HL60/ADR cells received with si-NC, 1 μ g or 2 μ g si-EVI-1 delivery. qRT-PCR and western blot were taken to evaluate TUG1 (E) and EVI-1 (F) expressions in HL60 and HL60/ADR cells after transfection with TUG1, si-TUG1 or matched controls. * $P < 0.05$.

Figure 3

Impacts of TUG1 on cell proliferation and resistance to ADR in AML cells. (A) HL60 cells were received TUG1 or Vector transfection. HL60/ADR cells were introduced with si-TUG1 or si-NC. Evaluation of cell proliferation at 1, 2, and 3 days by CCK-8 assay. (B) Western blot analysis of PCNA protein level in TUG1 or Vector transfected HL60 cells and si-TUG1 or si-NC introduced HL60/ADR cells. (C) Cells were transfected prior to treatment with 8 μ M ADR for 48 h and CCK-8 assay was utilized for the estimation of cell viability. (D) Flow cytometry assay for apoptosis analysis. (E and F) Western blot for estimation of Bcl-2 protein levels. *P < 0.05.

Figure 4

The association between TUG1 and miR-186-5p in AML cells. (A) Bioinformatics analysis for the putative targeting region prediction and corresponding mutants. (B) qRT-PCR analysis for miR-186-5p expression in AML cells (K562/ADR, K562, HL60/ADR, and HL60) and HS-5 cells. (C) Pearson's correlation analysis for the TUG1 correlation with miR-186-5p expressions in 21 BM samples of pediatric AML patients. (D)

Measurement of luciferase activity by luciferase reporter assay in HL60 and HL60/ADR cells. (E) qRT-PCR analysis of miR-186-5p level in transfected cells. *P < 0.05.

Figure 5

Influences of miR-186-5p on cell proliferation and ADR resistance in AML cells. (A and B) Anti-miR-186-5p or anti-miR-NC was delivered to HL60 cells and miR-186-5p or miR-NC was introduced into HL60/ADR cells, followed by assessment of cell proliferation and PCNA expression by CCK-8 assay and western blot analysis. (C-E) HL60 cells were received with anti-miR-186-5p or anti-miR-NC transfection and HL60/ADR cells were introduced with miR-186-5p or miR-NC in the presence of 8 μ M ADR. At 48 h post-transfection, cell viability, Bcl-2 expression and apoptosis were detected by CCK-8, western blot, and flow cytometry analyses, respectively. *P < 0.05.

Figure 6

TUG1 increased P-gp expression and activated the PI3K/Akt/mTOR pathway by sponging miR-186-5p in AML cells. (A and B) Western blot analysis of P-gp expression in TUG1 or Vector-introduced HL60 cells and si-TUG1 or si-NC-introduced HL60/ADR cells. (C and D) The PI3K, p-PI3K, p-Akt and p-mTOR protein levels were measured by western blot. (E) The protein expressions of PI3K, p-PI3K, p-Akt and p-mTOR were examined by western blot. *P < 0.05.