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Original Article

CAT104 silence behaves as a tumor suppressor in human leukemia cells by down regulating miR-182 expression

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Abstract: Background: LncRNAs and miRNAs are found to play crucial roles in the tumorigenesis of acute myeloid leukemia (AML). We aimed to investigate the functions and mechanisms of lncRNA-CAT104 and miR-182 in AML. Methods: Expression of CAT104, miR-182, and ZEB1 in K562 and HL60 cell lines was respectively or synchronously altered by transfection. Expressions of CAT104, miR-182 and ZEB1 in cell were then analyzed by qRT-PCR. Cell viability, migration, invasion and apoptosis were evaluated by MTT, transwell assays and flow cytometry, respectively. Protein expressions of ZEB1 and factors related with apoptosis and two signal pathways (Wnt/β-catenin and JNK) were detected by western blot. Results: CAT104 expressed highly in K562 and HL60 cells compared to embryonic kidney cell line HEK293 ($P < 0.001$). Knockdown of CAT104 inhibited cell viability, migration and invasion, but increased cell apoptosis of K562 and HL60 cells through inhibitionof miR-182 ($P < 0.05$). miR-182 promoted cell survival, migration and invasion through upregulatingthe expression of ZEB1 ($P < 0.05$). miR-182 silence deactivated Wnt/β-catenin and JNK signal pathways by downregulating the expression of ZEB1 in K562 and HL60 cells. Conclusion: LncRNA-CAT104 expressed highly in leukemia cells and its silence inhibited cell survival, migration and invasion by downregulating miR-182 expression. miR-182 functioned as an oncogene by upregulating ZEB1 via which miR-182 silence deactivated Wnt/β-catenin and JNK signal pathways in leukemia cells.

Keywords: Acute myeloid leukemia, CAT104, miR-182, ZEB1, Wnt/β-catenin, JNK

Introduction

Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells, characterized by that abnormal white blood cells in the bone marrow grow rapidly and interfere with the production of normal blood cells [1]. AML is the most common acute leukemia affecting adults, but is rare in children [2]. Chromosomal abnormalities, mutations in specific gene and noncoding RNAs such as microRNAs (miRNAs) have been considered to be possible causes for AML development [3-5]. Cure rates of AML in clinical trials ranged from 20-45% [6]. Thus, a comprehensive understanding of the pathogenesis of AML is critical for developing novel therapeutic strategies.

Noncoding RNAs are found to show abnormal expression patterns in cancerous tissues which include short noncoding RNAs called miRNAs [7] and long noncoding RNAs (lncRNAs) [8, 9]. miRNAs, a class of single-stranded RNA with 20-24 nucleotides in length, have been widely reported to post-transcriptionally regulate gene expression by binding the 3' untranslated region (3'UTR) of target mRNAs [10]. Their abnormal expression plays a key role in the hallmarks of cancer, such as cell proliferation, apoptosis, migration and invasion [11-13]. These miRNAs are considered to be oncogenes or tumor suppressor genes. LncRNAs, typically more than 200 nucleotides in length, have been recently found to regulate expression of gene not only atthe post-transcriptional level, but also at transcriptional and epigenetic levels [14]. Especially, lncRNAs have been discovered to function as miRNA sponges, altering miRNA expression levels by binding to and sequester-

ing miRNAs [15, 16]. Therefore, lncRNA contributed to a large range of functions such as modulation of apoptosis and invasion [17], reprogramming of induced pluripotent stem cells [18] and marker of cell fate [19]. A recent study found that a new lncRNA, CAT104, expressed highly in breast cancer cells and was related with the survival of breast cancer cells [20]. However, the effect of CAT104 on other cancers especially AML has not been revealed so far.

In the present study, we aimed to investigate the role of CAT104 in leukemia cells. In addition, we also explored the regulatory effect of miR-182 on leukemia cells. Our study indicates that CAT104 silence functions as a tumor suppressor in leukemia cells by downregulating miR-182 expression. miR-182 plays an oncogenic role in AML by upregulating ZEB1 expressionvia which miR-182 silence deactivates JNK and Wnt/β-catenin signal pathways. Our study may open new avenues in the research of AML pathogenesis for developing novel therapeutic strategies.

Materials and methods

Cell culture

Human leukemia cell lines K562 and HL60, human osteosarcoma cell line MG63, human osteoblast cell line hFOB1.19 and human embryonic kidney cell line HEK293 were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). MG63, hFOB1.19 and HEK293 cells were cultured in the Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Grand Island, NY, USA), and K562 and HL60 cells were maintained in RPMI 1640 medium (Gibco). Both DMEM and RPMI 1640 media contained 10% of fetal bovineserum (FBS, Gibco), 100 U/ml of penicillin (Gibco) and 100 μg/ml of streptomycin (Gibco). All cells were cultured at 37°C in a humidified 5% CO_2 incubator.

Cell transfection

The short-hairpin RNA (shRNA) directly against human lncRNA-CAT104 was ligated into the U6/GFP/Neo plasmid (GenePharma, Shanghai, China) and was referred as sh-CAT104. The plasmid carrying a non-targeting sequence was used as a negative control (NC) of sh-CAT104 and was referred as sh-NC. The sequences of full-length ZEB1 were constructed in the pEX plasmid (GenePharma) and were referred as pEX-ZEB1. The empty vector was used as a NC of pEX-ZEB1 and was referred as pEX. The short interfering RNAs (siRNA) targeting ZEB1 was referred as si-ZEB1. Non-targeting siRNA for negative control was referred as si-NC. si-ZEB1, si-NC, miR-182 mimic, mimic control, miR-182 inhibitor and inhibitor control were all synthesized by GenePharma Co. K562 and HL60 cells were transfected using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer protocol. After 72 h of transfection, the stably transfected cells were selected by the culture medium containing 0.5 mg/ml G418 (Sigma-Aldrich, St Louis, MO, USA).

Real-time quantitative reverse-transcriptase PCR (*qRT-PCR)*

Total RNA was extracted from cells using Trizol reagent (Invitrogen) according to the manufacturer's instructions. For the test of miR-182 level, the Taqman MicroRNA Reverse Transcription Kit and Taqman Universal Master Mix II (Applied Biosystems, Foster City, CA, USA) were used in turns for reverse transcription and qPCR with manufacturer's protocols. U6 (Applied Biosystems) was used as an internal standard. To test the expression levels of CAT104 and mRNA of ZEB1, we used The ExiLERATE universal cDNA synthesis and SYBR® Green Master Mix Kits (Exiqon, Vedbaek, Denmark) in turns according to manufacturer's protocols. The GAPDH (Exiqon) was used for normalizing. The changes in expression were calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell viability assay

For cell viability assay, a 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used. Cells (5×10^3) were seeded in a 96-wells plate and 30 μl of MTT (5 mg/ml; Klamar, Shanghai, China) solution was added in the cell culture for 4 h incubation at 37°C and 5% CO_2 conditions. The media were discarded, and cells were lysed in 200 μl dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) to allow the formed formazan crystals to dissolve. The optical density was measured at 490 nm with a microplate reader (Bioteck, Winooski, VT, USA).

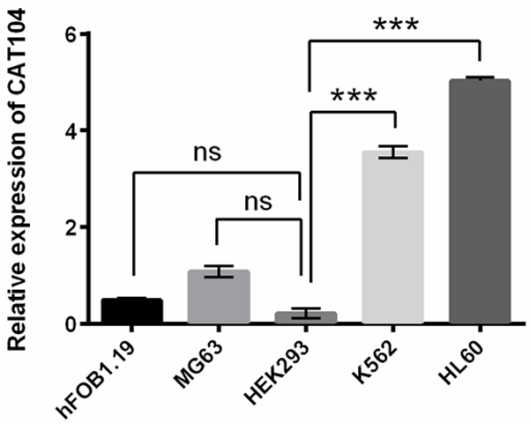

Figure 1. Different expression levels of CAT104 in several cell lines. Expression levels of CAT104 were detected by qRT-PCR in human osteoblast cell line hFOB1.19, human osteosarcoma cell line MG63, human embryonic kidney cell line HEK293 and human leukemia cell line K562 and HL60. ns, no significant; ***P < 0.01.

Cell apoptosis assay

Cell apoptosis analysis was performed using Annexin V-FITC/PI apoptosis kit (Dojindo Molecular Technologies, Kyushu, Japan). Briefly, cells were washed in cold phosphatebuffered saline (PBS) and resuspended in 100 µl of binding buffer containing 5 µl of Annexin V-FITC and 5 µl of PI. Cells were then incubated for 10 min at room temperature in the dark. Flow cytometry analysis was done by using a FACS can (Beckman Coulter, Fullerton, CA, USA). The number of early apoptotic cells (Annexin-V positive and PI negative) were analyzed by using the FlowJo software (Flowjo LLC, Ashland, OR, USA).

Cell migration and invasion assays

Cell migration was determined by using a 24-wells transwell culture chamber with 8 µm PET membranes (Millipore, Bedford, MA, USA). Cells (1×10^5) were suspended in 200 µl of serum-free DMEM medium on the upper compartment, and 600 ml of complete medium containing 10% FBS (Gibco) was added to the lower compartment. After incubation at 37°C for 24 h according to the manufacturer's protocol, the chamber was carefully removed. Non-traversed cells on the upper surface of the membrane were wiped off carefully with a cotton swab. Traversed cells on the lower side were fixed with methanol and then stained with crystal violet. We counted the number of traversed cells microscopically in five randomly chosen fields and got the average.

Cell invasion was determined also using a 24-wells transwell culture chamber with 8 µm PET membranes (Millipore). Diluted Matrigel (BD, San Jose, CA, USA) was added vertically at the center of the membrane in the upper chamber and incubated at 37°C for 4 h to facilitate gel formation. Cells (5×10^4) in 200 µl of serum-free DMEM medium were plated onto the upper compartment, while complete medium containing 10% FBS was added to the lower chamber. After processing the invasion chambers for 24 hours (37°C, 5% CO_2) in accordance with the manufacturer's protocol, the non-invading cells were removed with a cotton swab; the invading cells were fixed in methanol, then stained with crystal violet solution and counted microscopically from five randomly chosen fields to get the average. The data are presented as relative migration or invasion rates.

Western blot

Proteins used for western blot was extracted using RIPA lysis buffer (Beyotime, Shanghai, China) and quantified using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Protein bands were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride membrane (PVDF, Millipore). After 2 h of blocking with 5% skim milk (Nestlé, Shuangcheng, China) in Tris Buffered Saline with Tween (TBST), the membrane was incubated with primary antibodies (all from Abcam, Cambridge, UK) at 4°C overnight. Primary antibodies included anti-Bcl-2 (ab59348, 1:1000), anti-Bax (ab182733, 1:2000), anti-pro-caspase-3 (ab44976, 1:500), anti-cleaved-caspase-3 (ab13847, 1:500), anti-caspase-9 (ab202068, 1:2000), anti-ZEB1 (ab203829, 1:1000), anti-JNK (ab179461, 1:1000), anti-p-JNK (ab124956, 1:5000), anti-c-Jun (ab32137, 1:5000), anti-p-c-Jun (ab32385, 1:5000), anti-Wnt3a (ab28472, 1:1000), anti-Wnt5a (ab72583, 1:500), anti-β-catenin (ab6302, 1:4000) and anti-GAPDH (ab8245, 1:1000). GAPDH antibody was used as the endogenous protein for reference. After washing, the membrane was incubated with secondary antibody marked by horseradish peroxidase for 1 h at room temperature. Secondary antibodies included goat anti-mouse IgG (ab6789, 1:5000; Abcam) and goat anti-rabbit IgG (ab6721, 1:5000; Abcam). The sig-

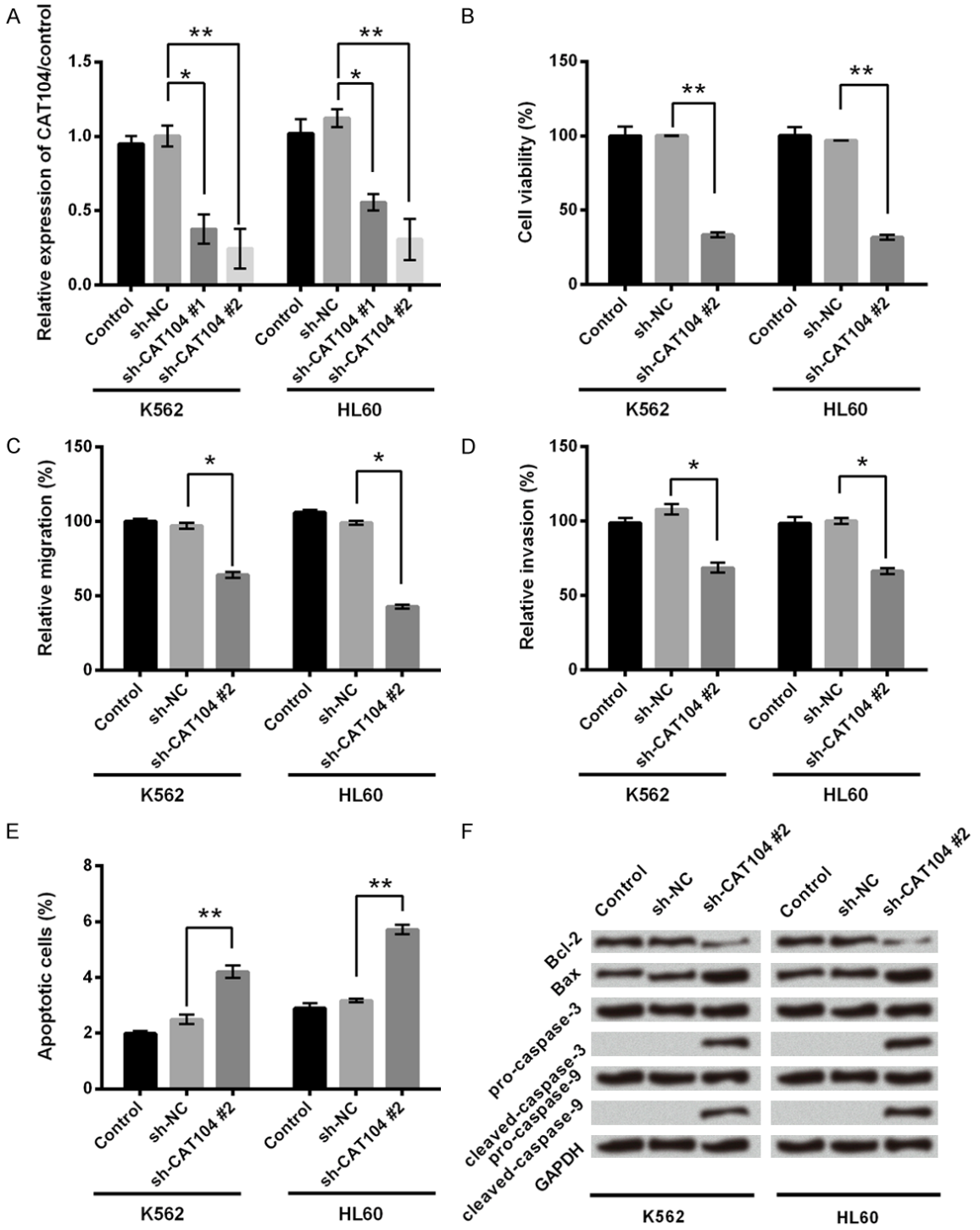

Figure 2. Knockdown of CAT104 inhibited cell viability, migration and invasion and promoted cell apoptosis in K562 and HL60 cells. A. Expression level of CAT104 was measured by qRT-PCR in K562 and HL60 cells with or without transfection with sh-CAT104/sh-NC. B. Cell viability was determined by a MTT assay in K562 and HL60 cells with or without transfection with sh-CAT104/sh-NC. C, D. Migration and invasion assays were used to determine the effect of CAT104 on cell migration and invasion. E. Apoptosis rate was determined by flow cytometry in K562 and HL60 cells with or without transfection. F. Western blot was used to determine the expression level of apoptosis related proteins in K562 and HL60 cells with or without trasfection. *$P < 0.05$, **$P < 0.01$.

nals were developed by using enhanced chemiluminescence (ECL) reagents (GE Healthcare, Little Chalfont, UK) according to the manufacturer's instructions.

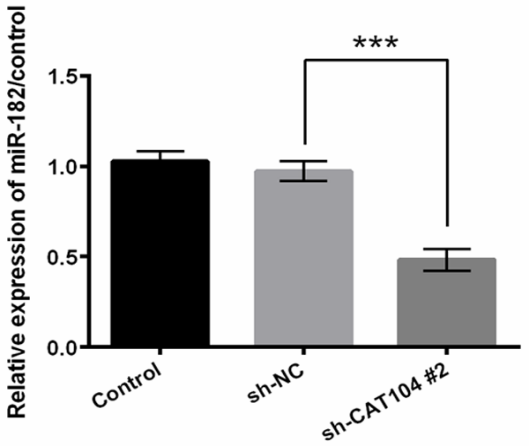

Figure 3. CAT104 affected the expression of miR-182. Expression level of miR-182 was measured by qRT-PCR in K562 and HL60 cells with or without transfection with sh-CAT104/sh-NC. ***P < 0.001.

Statistical analysis

All experiments were repeated three times. Data are presented as the mean ± SD. Statistical analyses were performed by using SPSS 19.0 statistical software (SPSS, Chicago, IL, USA) and a one-way analysis of variance (ANOVA) to calculate the *P*-values. A *P*-value of < 0.05 was considered to be a statistically significant result.

Results

Expression levels of CAT104 in several cell lines

We detected the expression levels of CAT104 in several cell lines which included human leukemia cell lines K562 and HL60, human osteosarcoma cell line MG63, human osteoblast cell line hFOB1.19 and human embryonic kidney cell line HEK293 by qRT-PCR. As shown in **Figure 1**, the expression of CAT104 in the cell line HEK293 was not statistically different from that expressed in cell lines hFOB1.19 and MG63 (P > 0.05). However, CAT104 expression was much higher in K562 and HL60 cell lines than HEK293 cell line (P < 0.001), indicating CAT104 was highly expressed in human leukemia cell lines K562 and HL60.

Knockdown of CAT104 inhibited cell viability, migration and invasion, but promoted cell apoptosis of leukemia cells

The sh-CAT104 was transfected into K562 and HL60 cell lines to knock down CAT104. After transfection, expression levels of CAT104 were detected by qRT-PCR. As shown in **Figure 2A**, transfection of sh-CAT104 significantly set off a decrease in expression levels of CAT104 in both K562 and HL60 cells compared to transfectionof sh-NC (P < 0.05 or P < 0.01). The higher transfection efficiency was observed in the second group of sh-CAT104, which was selected for further experiments.

The effect of CAT104 on cell viability of K562 and HL60 cells was investigated by a MTT assay. As shown in **Figure 2B**, transfection of sh-CAT104 significantly reduced cell viability in both K562 and HL60 cells (P < 0.01). Cell migration and invasion of K562 and HL60 cells transfected with sh-CAT104 or sh-NC were detected by transwell assay with or without Matrigel, respectively. Inhibition of CAT104 caused significant decreases in therates of cell migration and invasion of K562 and HL60 cells (P < 0.05, **Figure 2C** and **2D**).

To reveal the effect of CAT104 on cell apoptosis of K562 and HL60 cells, we performed an Annexin V FITC/PI apoptosis assay and detected apoptosis related proteins by western blot. **Figure 2E** demonstrated that transfection of sh-CAT104 significantly caused increases in the ratesof apoptosisin both K562 and HL60 cells (P < 0.01). Western blot showed that knockdown of CAT104 caused a decrease in the expression of the antiapoptotic protein Bcl-2 and increases in the expressions of proapoptotic protein Bax, executioner cleaved-caspase-3 and initiator cleaved-caspase-9 (**Figure 2F**). These results indicated that inhibition of CAT104 promoted cell apoptosis and reduced cell viability, migration and invasion of K562 and HL60 cells.

CAT104 positively regulated the expression of miR-182

miR-182 plays an important role in the process of many cancers [21, 22]. In the next step, we set out to identify the effect of CAT104 on miR-182. As shown in **Figure 3**, transfection of sh-CAT104 significantly caused a decrease in the expression of miR-182 (P < 0.001). This result supported that CAT104 positively regulated the expression of miR-182.

CAT104 silence behaved as a tumor suppressor in leukemia cells through downregulating the expression of miR-182

We further investigated the association between CAT104 and miR-182 in cell viability,

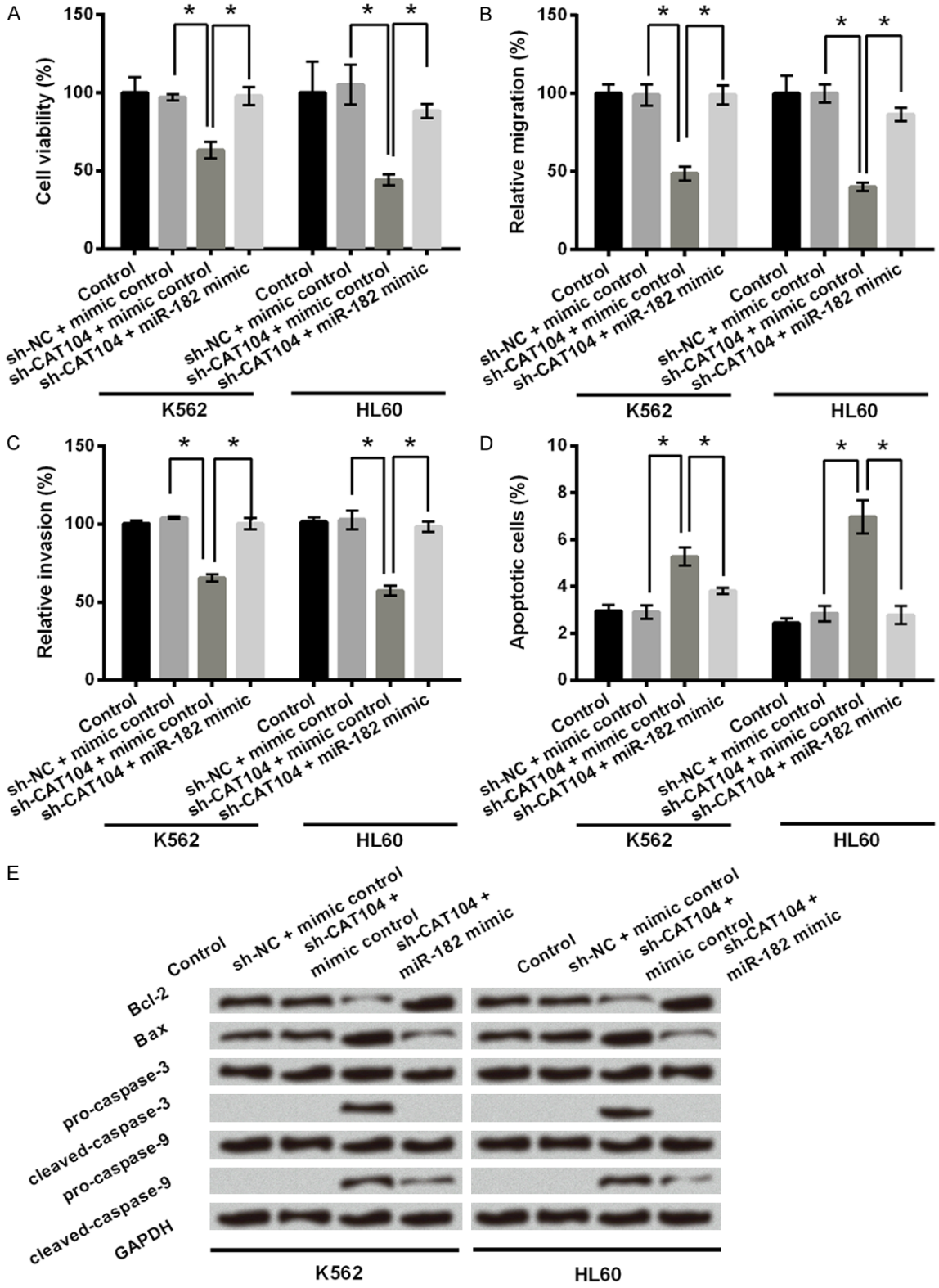

Figure 4. CAT104 silence inhibited cell viability, migration and invasion and promoted cell apoptosis in K562 and HL60 cells through regulating miR-182 expression. A. K562 and HL60 cells were co-transfected with sh-NC/sh-CAT104 and miR-182 mimic/mimic control. Cell viability was determined by a MTT assay. B, C. Migration and invasion assays were used to determine the effect of CAT104 and miR-182 on cell migration and invasion. D. Apoptosis rate was determined by flow cytometry in co-transfected K562 and HL60 cells. E. Western blot was used to determine the expression level of apoptosis related proteins in co-transfected K562 and HL60 cells. *P < 0.05.

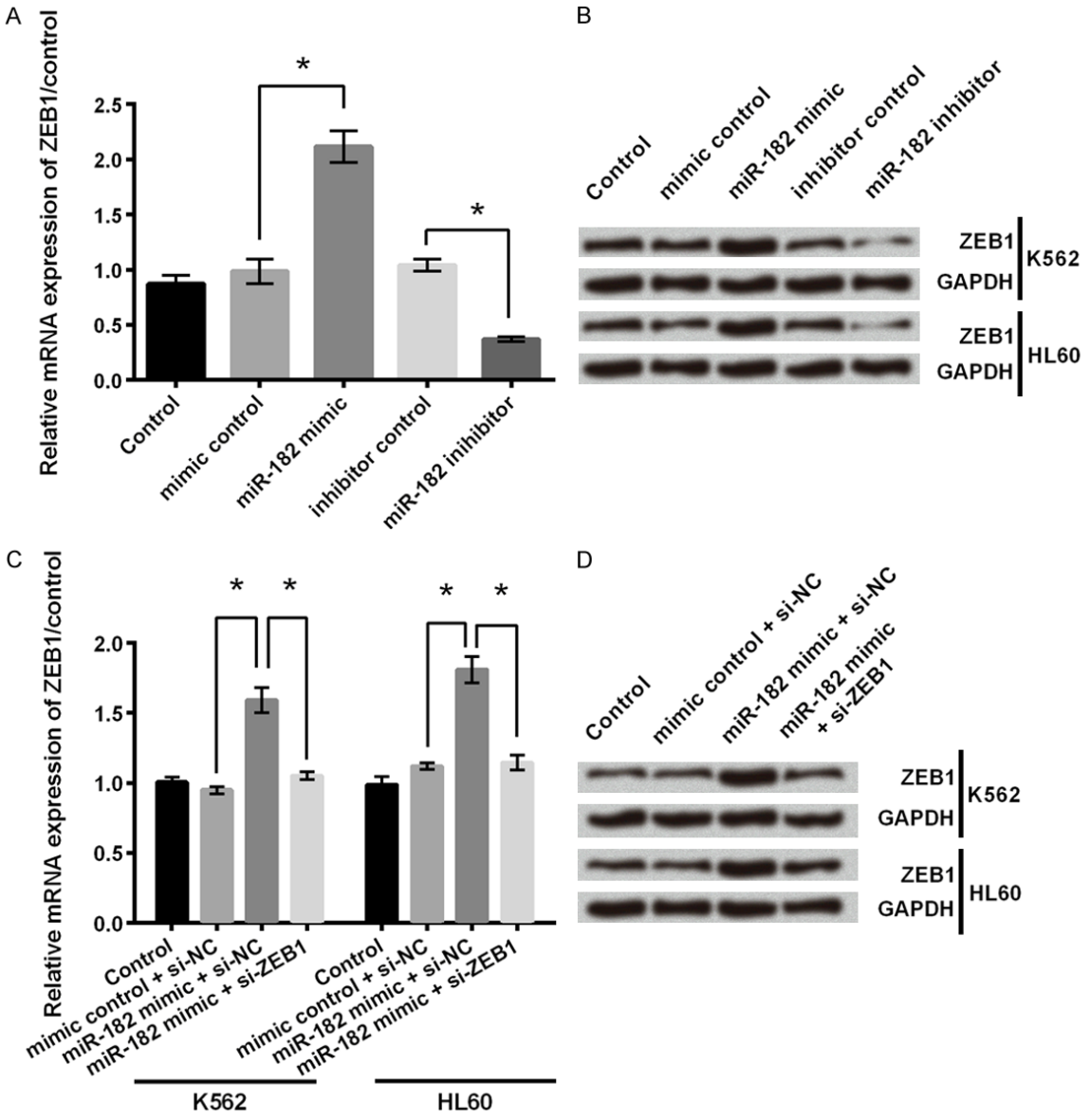

Figure 5. miR-182 regulated the ZEB1 expression in both mRNA and protein levels. ZEB1 expression level was determined by both (A) qRT-PCR and (B) western blot in K562 and HL60 cells transfected with mimic control, miR-182 mimic, inhibitor control or miR-182 inhibitor. ZEB1 expression level was determined by both (C) qRT-PCR and (D) western blot in K562 and HL60 cells with or without co-transfection with si-NC/si-ZEB1 and mimic control/miR-182 mimic. *P < 0.05.

migration, invasion and apoptosis of K562 and HL60 cells. Knockdown of CAT104 alone reduced cell viability of K562 and HL60 cells as described above (P < 0.05, **Figure 4A**). However, co-transfection of sh-CAT104 and miR-182 mimic reversed the effect of CAT104 inhibition on cell viability (P < 0.05). This reversal also occurred in cell migration, invasion and apoptosis of K562 and HL60 cells (**Figure 4B-E**). Knockdown of CAT104 alone reduced cell migrationand invasion of K562 and HL60 cells (P < 0.05), while co-transfection of sh-CAT104 and miR-182 mimic caused increases in cell migration and invasion (P < 0.05, **Figure 4B** and **4C**). By analyzing the rate of cell apoptosis and expression levels of apoptosis related proteins, we observed that inhibition of CAT104 alone elevated cell apoptosis rates (P < 0.05, **Figure 4D**), decreased the expression of the Bcl-2 and increased expressions of Bax, cleaved-caspase-3 and cleaved-caspase-9 (**Figure 4E**). These effects were reversed by co-transfection of sh-CAT104

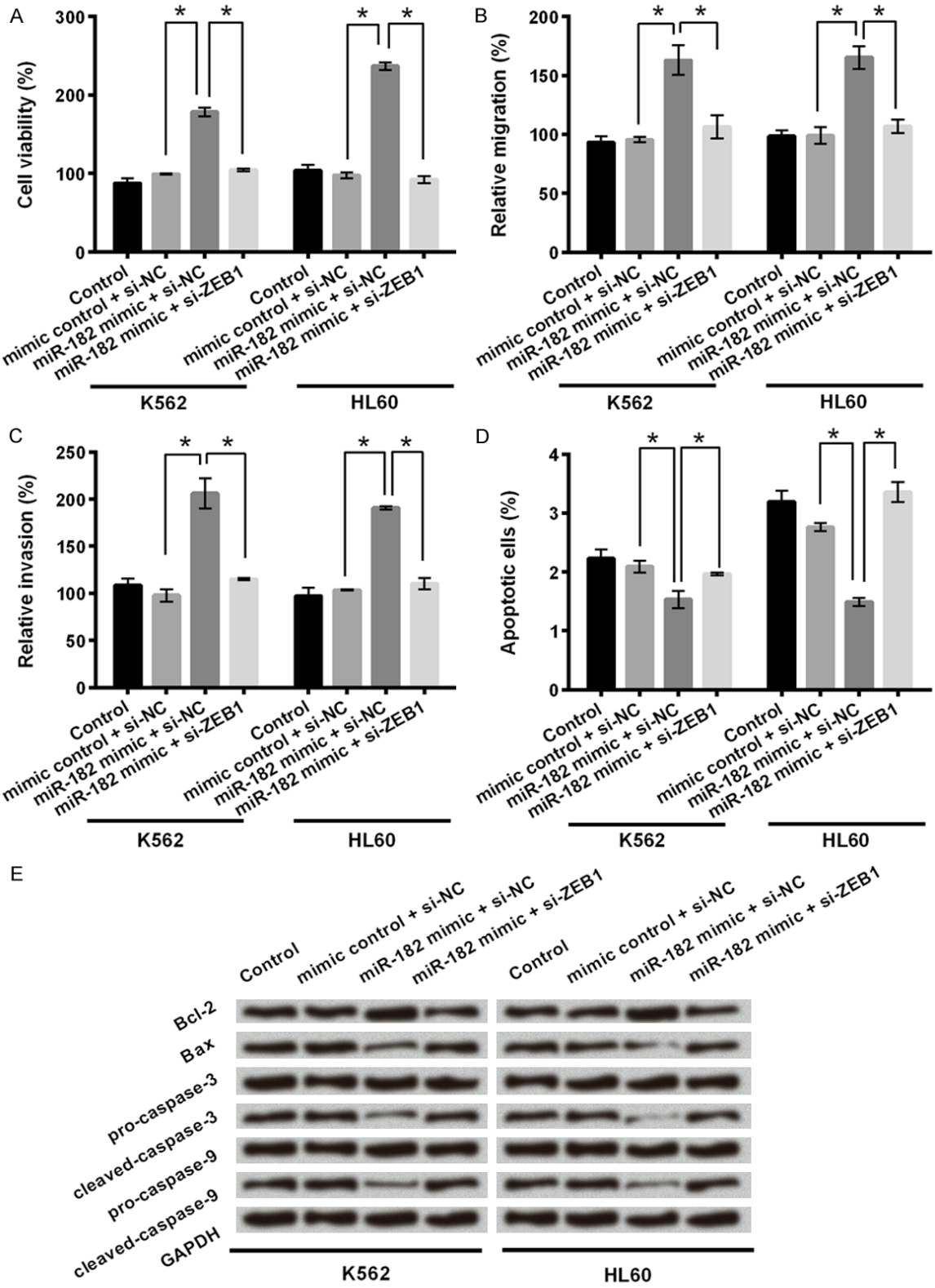

Figure 6. miR-182 affected cell viability, migration, invasion and apoptosis in K562 and HL60 cells through regulating ZEB1 expression. A-C. K562 and HL60 cells were co-transfected with si-NC/si-ZEB1 and miR-182 mimic/mimic control. MTT, and transwell assays were used to determine cell viability, migration and invasion respectively in co-transfected cells. D. Apoptosis rate was determined by flow cytometry in co-transfected cells. E. Western blot was used to determine the expression levels of apoptosis related proteins in co-transfected K562 and HL60 cells. *P < 0.05.

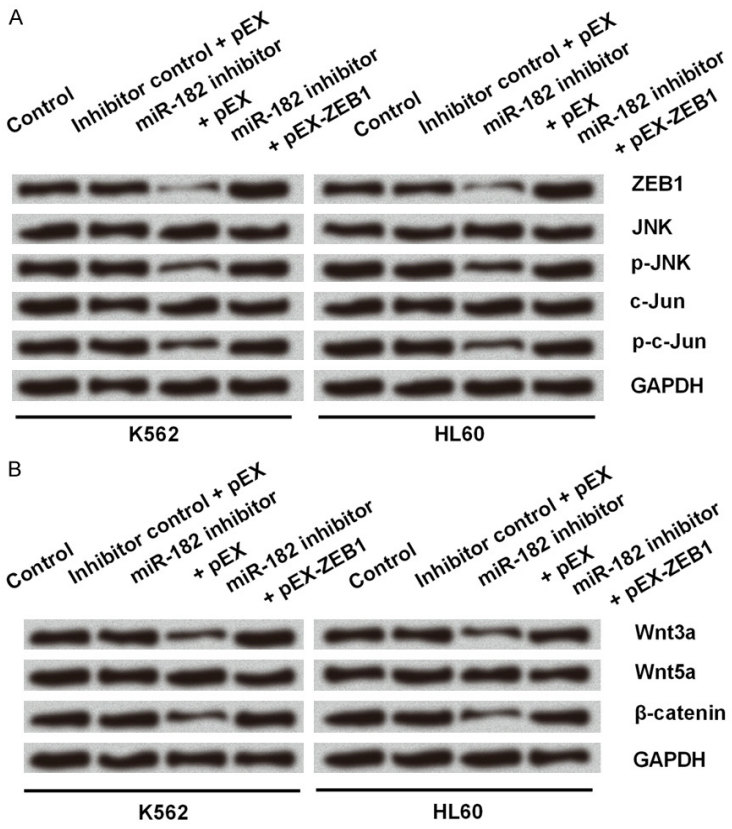

Figure 7. MiR-182 silence deactivated JNK and Wnt/β-catenin signaling pathways through downgulating ZEB1 expression. A. K562 and HL60 cells were co-transfected with pEX/pEX-ZEB1 and miR-182 inhibitor/inhibitor control. Expressions of ZEB1 and proteins related to JNK signaling pathway were measured by western blot in transfected cells. B. Protein expression levels of Wnt3a, Wnt5a and β-catenin in transfected cells were determined by western blot.

and miR-182 mimic ($P < 0.05$). Considering CAT104 positively regulated the expression of miR-182, these findings suggested that knockdown of CAT104 inhibited cell viability, migration and invasion, and promoted cell apoptosis in leukemia cells maybe through downregulating the expression of miR-182.

miR-182 upregulated the expression of ZEB1

ZEB1 is reported for its contributing role in cancer invasion and metastasis [23]. We next identified the regulatory effect of miR-182 on ZEB1 in both mRNA and protein levels. As shown in **Figure 5A**, overexpression of miR-182 caused an increase in the expression of ZEB1 mRNA, while inhibition of miR-182 had an inverse effect ($P < 0.05$). Western blot showed that the protein expression level of ZEB1 was increased by overexpression of miR-182, while was decreased by miR-182 suppression (**Figure 5B**). These results indicated that miR-182 upregulated the expression of ZEB1 in both mRNA and protein levels in K562 and HL60 cells.

miR-182 functioned as an oncogene in leukemia cells through upregulating the expression of ZEB1

We further investigated the association between miR-182 and ZEB1 in cell viability, migration, invasion and apoptosis of K562 and HL60 cells. As shown in **Figure 5C** and **5D**, overexpression of miR-182 alone caused an increase in the expression of ZEB1 in both mRNA and protein levels ($P < 0.05$). However, accompanied by si-ZEB1, miR-182 mimic could not increase the expression of ZEB1 in both mRNA and protein levels any more ($P < 0.05$). Then we evaluated cell viability, migration, invasion and apoptosis in the case of overexpression of miR-182 with or without inhibition of ZEB1 expression. As shown in **Figure 6A**, overexpression of miR-182 increased cell viability in both K562 and HL60 cells, while miR-182 overexpression and inhibition of ZEB1 expression simultaneously decreased cell viability ($P < 0.05$). The same trend was found in migration and invasion assays (**Figure 6B** and **6C**). Overexpression of miR-182 alone increased cell migration and invasion rates in both K562 and HL60 cells ($P < 0.05$). In contrast, the migration and invasion rates of these two cell lines co-transfected with miR-182 mimic and si-ZEB1 were decreased ($P < 0.05$).

In apoptosis assay (**Figure 6D**), the apoptosis rates of K562 and HL60 cells transfected with miR-182 mimic were significantly decreased compared to that of cells transfected with mimic control ($P < 0.05$). On the contrary, co-transfection ofmiR-182 mimic and si-ZEB1 dramatically resulted in increases of the apoptosis rates of these two cell lines ($P < 0.05$). The same trend was observed in analyzing expres-

sion of apoptosis related proteins by western blot (**Figure 6E**). Overexpression of miR-182 alone increased the expression of the Bcl-2 and decreased expressions of Bax, cleaved-caspase-3 and cleaved-caspase-9. These effects were reversed by co-transfection of miR-182 mimic and si-ZEB1. These results suggested that overexpression of miR-182 promoted cell viability, migration and invasion and inhibited cell apoptosis in leukemia cells through upregulating ZEB1 expression.

MiR-182 silence deactivated JNK and Wnt/β-catenin signal pathways through downregulating ZEB1 expression

Finally, we sought to investigate the underling mechanisms in which miR-182 and ZEB1 involved. Firstly we measured the expression levels of JNK and c-Jun proteins in the case of miR-182 inhibition with or without ZEB1 overexpression. As shown in **Figure 7A**, inhibition of miR-182 alone decreased expressions of ZEB1, p-JNK and p-c-Jun. However, co-transfection with miR-182 inhibitor and pEX-ZEB1 causedincreases in expressions of ZEB1, p-JNK and p-c-Jun compared to that with miR-182 inhibitor and pEX. These results supported that knockdown of miR-182 deactivated JNK signal pathway possibly through downregulating ZEB1.

Next, we measured the expression levels of Wnt3a, Wnt5a and β-catenin proteins in the case of miR-182 inhibition with or without overexpression of ZEB1. As shown in **Figure 7B**, inhibition of miR-182 alone decreased expressions of Wnt3a and β-catenin proteins, while it had no effect on Wnt5a. However, co-transfection with miR-182 inhibitor and pEX-ZEB1 caused increases in expressions of Wnt3a and β-catenin proteins compared to that with miR-182 inhibitor and pEX. These results supported that knockdown of miR-182 also could inhibit Wnt/β-catenin signal pathway through downregulating ZEB1.

Discussion

More and more lncRNAs were found to play important rolesin AML. For example, Wang et al identified RUNXOR as a novel lncRNA involved in a long range DNA interaction of RUNX1 in AML [24]. LncRNA-CCD26 has been shown to control the growth of myeloid leukemia cells through the regulation of KIT expression [25]. Another lncRNA (ZNF571-AS1) may be involved in AML via JAK/STAT signal pathways [26]. Compared to the huge number of lncRNA (about 27,919) [27], amount of lncRNAs which we have understood isonly the tip of the iceberg. CAT104 was identified recently as a novel lncRNA involved in predicting the survival of breast cancer [20]. However, the effect of CAT104 on AML is unknown. In the present study, we for the first time found that CAT104 was upregulated in human leukemia cell lines (K562 and HL60). In addition, knockdown of CAT104 significantly increased cell apoptosis and inhibited cell viability, migration and invasion of K562 and HL60 cells. These findings supported that lncRNA-CAT104 might intervene in pathogenesis and function as an oncogene in AML.

Recent reports supported that lncRNAs could potentially interact with other classes of noncoding RNAs especially miRNAs and modulate their regulatory role [28]. For instance, lncRNA-CCAT1 negatively regulated miR-218-5p to promote the development of gallbladder cancer [29]. However, how lncRNA-CAT104 exerts oncogenic functions in AML is not clear. In the present study, we found CAT104 positively regulated the expression of miR-182, which in turn affected cell survival, migration and invasion of leukemia cells.

miR-182 has been recognized as anonco-miRNAin various cancers such as breast cancer [22], urinary bladder cancer [30], and cervical cancer [21]. However, there is little research regarding the role of miR-182 in AML. Our data indicated that miR-182 promoted cell growth and inhibited cell apoptosis in leukemia cells. Therefore we concluded that miR-182 also behaved as an onco-miRNA in AML. We further found miR-182 exerted its oncogenic functions through upregulating the expression of ZEB1. When ZEB1 was knocked down, miR-182 would lose its oncogenic functions. ZEB1 has been reported to be a master inducer of epithelial-mesenchymal transition (EMT) and its expression is related to growth, survival, and drug resistance of tumor cells [23]. It has emerged as one of the master regulators for metastasis [31] and played critical roles in tumour initiation at distant sites [32] and invasion [23]. Consistent with these reports, our study indicated that ZEB1 played an essential role in the viability, migration, invasion and apoptosis of leukemia cells.

In order to reveal the underling mechanism of miR-182 in AML, we tested the association between miR-182, ZEB1 and Wnt/β-catenin and JNK signal pathways. Activated JNK regulates several important cellular functions including cell growth, differentiation, survival and apoptosis by activating some molecules such as c-Jun [33]. However there is little research focused on the association between miR-182 and JNK signal pathway. We found miR-182 silence deactivated JNK signal pathways inleukemia cells. On the other hand, the canonical Wnt-pathway can elevate the level of β-catenin to activate transcription of specific target genes and lead to tumor development [34]. Wnt/β-catenin signal pathway was reported to be activated by miR-182-5p in human bladder cancer [35]. Similarly, we found miR-182 silence deactivated Wnt/β-catenin signal pathway in leukemia cells. In addition, miR-182 silence deactivated JNK and Wnt/β-catenin signal pathways by downregulating ZEB1 in AML.

In conclusion, we demonstrated that lncRNA-CAT104 silence exerted anti-cancer functions in human leukemia cells by downregulating miR-182 expression. miR-182 may play an oncogenic role in human leukemia cells through upregulating ZEB1 expression. Furthermore, miR-182 silence deactivates Wnt/β-catenin and JNK signal pathways via downregulating ZEB1 expression. The illustration of relationships among these molecules may promote understanding the pathogenesis of AML. In addition to trying to downregulate miR-182 levels, designing strategies to manipulate the expression of lncRNA-CAT104 may represent an alternative therapeutic approach for the treatment of AML. However, further research is still needed.

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Disclosure of conflict of interest

None.

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