E2F1-mediated RAB34 upregulation accelerates the proliferation and inhibits the cell cycle arrest and apoptosis of acute myeloid leukemia cells

XIAOJIE XIONG[1] and GANG JIAN[2]

[1]Clinical Laboratory and [2]Department of Pharmacy, The First Affiliated Hospital of HaiNan Medical University, Haikou, Hainan 570102, P.R. China

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Abstract. Acute myeloid leukemia (AML) is a malignant disease that is mainly arisen from myeloid stem/progenitor cells. The pathogenesis of AML is complex. Ras-related protein member RAS oncogene GTPases (RAB) 34 protein has been reported to serve an important role in the development of cancer. However, to the best of our knowledge, the role of RAB34 in AML has not been previously reported. The GEPIA database was used to predict the expression levels of RAB34 in patients with AML. Reverse transcription-quantitative PCR and western blotting were used to detect the expression of RAB34 in AML cell lines. Cell transfection with short hairpin (sh)RNAs targeting RAB34 was used to interfere with RAB34 expression. Cell Counting Kit-8 and 5-ethynyl-2'-deoxyuridine staining were used to measure cell proliferation. Flow cytometry was used to investigate cell cycle distribution and apoptosis. Western blotting was used to assess the protein expression levels of RAB34 and E2F transcription factor 1 (E2F1), and cell cycle- and apoptosis-associated proteins, including Bcl-2, Bax, CDK4, CDK8 and cyclin D1. The potential binding between E2F1 and RAB34 was then verified by luciferase reporter and chromatin immunoprecipitation assays. Subsequently, cells were co-transfected with RAB34 shRNA and the E2F1 overexpression plasmid before cell proliferation, cell cycle and apoptosis were analyzed further. The expression of RAB34 was found to be significantly increased in AML cell lines. Knocking down RAB34 expression in AML cells was found to significantly inhibit cell proliferation, induce cell cycle arrest and promote apoptosis. E2F1 activated the transcription of RAB34 and E2F1 elevation reversed the impacts of RAB34 silencing on cell proliferation, cell cycle and apoptosis in AML. Therefore, these findings suggest that E2F1-mediated RAB34 upregulation may accelerate the malignant progression of AML.

Correspondence to: Dr Gang Jian, Department of Pharmacy, The First Affiliated Hospital of HaiNan Medical University, 31 Longhua Road, Haikou, Hainan 570102, P.R. China
E-mail: jiangang890@126.com

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Introduction

Acute myeloid leukemia (AML) is a malignant disease that is mainly derived from myeloid stem/progenitor cells (1). It is characterized by the aberrant proliferation of primitive and naive myeloid cells in the bone marrow and peripheral blood, the clinical manifestations of which can include anemia, hemorrhage, infection and fever, organ infiltration and/or lipid metabolic abnormalities (2). The majority of AML cases are severe and the prognosis is unfavorable, which can become fatal if not diagnosed and treated in the early stages (3). AML accounts for ~25% of all types of childhood leukemias (4). To the best of our knowledge, the pathogenesis of AML remains unclear.

Ras-related protein member RAS oncogene GTPases (RAB) 34 is a gene that encodes a protein belonging to the RAB family, which are small GTPases involved in intracellular protein transport (5). Previous studies have found that RAB34 is associated with the occurrence and development of hepatocellular carcinoma, non-small cell lung cancer and breast cancer through the driving of tumor cell proliferation and migration (6-8). Compared with that in paracancerous tissues, RAB34 expression in human hepatocellular carcinoma tissues was found to be upregulated, where it was associated with poorer prognosis (7). In addition, knocking down RAB34 expression was observed to inhibit hepatocellular carcinoma cell proliferation and migration (7). Plasmacytoma variant translocation oncogene 1 was also demonstrated to promote the proliferation and migration of non-small cell lung cancer by targeting the microRNA (miR)-148/RAB34 signaling axis (6). RAB34 was found to be overexpressed in invasive breast cancer MDA-MB-231 and BT549 cells, where RAB34 silencing could inhibit cell migration, invasion and adhesion (8). However, the role of RAB34 in AML remains unclear.

E2F1 is a member of the cell cycle-related transcription factor family (9). A recent study has shown that long non-coding RNA (lncRNA) lncSIK1 can block the expression

of E2F1 and suppress the E2F1-mediated transcription of LC3 and DNA damage-regulated autophagy modulator to alleviate aggressive autophagy in the myeloid leukemia Molm13 cell line, which delayed the progression of AML *in vitro* (10). In another study, lncRNA NR-104098 was previously found to effectively inhibit enhancer of zeste homolog 2 (EZH2) transcription by directly binding to E2F1 and recruiting E2F1 to the EZH2 promoter, which ultimately inhibited proliferation whilst inducing the differentiation of AML cells (11). The proliferating cell nuclear antigen clamp-associated factor was previously demonstrated to accelerate G_1/S transition in neuroblastoma cells by activating the E2F1/pituitary tumor-transforming gene 1 protein signaling pathway (12).

Therefore, it was hypothesized in the present study that RAB34 is highly expressed in AML, where it can be regulated by E2F1 and is associated with poor prognosis, such that RAB34 regulation may affect the malignant behavior of AML cells.

Materials and methods

Database. The 'Boxplots' module of the GEPIA database (gepia.cancer-pku.cn) was used to analyze the expression of RAB34 in the tissues of patients with AML and the 'Survival Analysis' module of the GEPIA database was to analyze the correlation between RAB34 and the overall survival rate of patients with AML (13). HumanTFDB (http://bioinfo.life.hust.edu.cn/) was used to predict the binding sites of E2F1 on the promoter of RAB34 (14).

Cell culture. Human bone marrow stromal HS-5 cells (cat. no. BFN60808921) and AML MOLM-14 cells (cat. no. BFN60810333) were purchased from Qingqi (Shanghai) Biotechnology Development Co., Ltd. AML MV4-11 (cat. no. CRL-9591) and HL-60 cells (cat. no. CCL-240) were purchased from the American Type Culture Collection. The AML Kasumi-1 cell line (cat. no. SCSP-5015) was purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. The cells were cultured in RPMI-1640 medium supplemented with 10% FBS (both from Thermo Fisher Scientific, Inc.) and 100 IU/ml penicillin + 100 μg/ml streptomycin (Sigma-Aldrich; Merck KGaA) at 37˚C with 5% CO_2.

Reverse transcription-quantitative PCR (RT-qPCR). RNA samples were extracted from cells with chloroform and a TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed to cDNA using the HiScript II 1st Strand cDNA Synthesis Kit (cat. no. R211-01; Vazyme Biotech Co., Ltd.). The conditions for reverse transcription were as follows: 37˚C for 10 min, followed by 85˚C for 5 sec and then storage at 4˚C. qPCR was subsequently performed using the HiScript II One Step qRT-PCR SYBR Green kit (Vazyme Biotech Co., Ltd.), 1 μl cDNA and amplification primers. The thermocycling conditions used were as follows: Denaturation at 95˚C for 30 sec, followed by 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec. The following primer pairs sequences were designed by Guangzhou RiboBio Co., Ltd.: RAB34 forward, 5'-GTCTCCGATTCCCCATCACC-3' and reverse, 5'-ATGCGGACAACATCCCCAAT-3'; E2F1 forward, 5'-GGGGGAGAAGTCACGCTATG-3' and reverse, 5'-AAACATCGATCGGGCCTTGT-3' and GAPDH forward, 5'-CATGAGAAGTATGACAACAGCCT-3' and reverse, 5'-AGTCCTTCCACGATACCAAAGT-3'. The mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (15) and normalized to that of the internal reference gene GAPDH.

Western blotting. Total protein was extracted from the cells using RIPA lysis buffer (Cell Signaling Technology, Inc.) and quantified using a BCA kit (Beyotime Institute of Biotechnology). Total protein (30 μg/lane) was separated by 10% SDS-PAGE and transferred onto a PVDF membrane. Membranes were blocked with 5% milk for 1 h at 37˚C and then incubated in the primary antibodies, against RAB34 (1:1,000; cat. no. PA5-99697; Thermo Fisher Scientific, Inc.), Bcl-2 (1:1,000; cat. no. ab32124; Abcam), Bax (1:1,000; cat. no. 182733; Abcam), CDK4 (1:1,000; cat. no. ab108357; Abcam), CDK8 (1:1,000; cat. no. ab229192; Abcam), cyclin D1 (1:1,000; cat. no. ab16663; Abcam), E2F1 (1:1,000; cat. no. ab288369; Abcam) or GAPDH (1:1,000; cat. no. ab9485; Abcam), overnight at 4˚C. On the next day, membranes were incubated with the HRP-conjugated secondary antibody (goat anti-rabbit; 1:5,000; cat. no. ab6721; Abcam) for 2 h at 37˚C. Finally, all the membranes were visualized using a BeyoECL Plus kit (Beyotime Institute of Biotechnology) on an Odyssey Infrared imaging system (Bio-Rad Laboratories, Inc.) and semi-quantified using ImageJ software (version 1.42; National Institutes of Health).

Cell transfection. Lentivirus short hairpin (sh)RNA RAB34 sequences (shRNA-RAB34#1 and shRNA-RAB34#2) which were ligated into the plasmid of U6/GFP/Neo and scrambled control shRNA (shRNA-NC) were established and synthesized by Jimon Biotechnology (Shanghai) Co., Ltd. After 48 h of transfection, 2 μg/ml puromycin (Beyotime Institute of Biotechnology) was added to create stably transfected HL-60 cell lines at a multiplicity of infection of 10, followed by maintenance with 0.5 μg/ml puromycin. The E2F1 overexpressing lentivirus (Oe-E2F1) tagged with Flag (with the E2F1 gene inserted into the pcDNA3.1 vector), was purchased from Jimon Biotechnology (Shanghai) Co., Ltd. An empty vector served as the negative control (Oe-NC), and the interim cell line used was the 293T cell line which was purchased from the American Type Culture Collection. The 2nd generation system was used. ShRNAs (500 ng/μl) and pcDNA3.1 vectors (4 μg) were transfected into HL-60 cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 37˚C. The sequences of two human shRNA-RAB34 and the shRNA-NC were as follows: Sh-RAB34#1, 5'-CCGCGTAATCGTAGGAACTAT-3'; sh-RAB34#2, 5'-CGCGTAATCGTAGGAACTATC-3'; and shRNA-NC, 5'-CAACAAGATGAAGAGCACCAA-3'. After 48 h incubation at 37˚C, transfected cells were harvested and utilized for further experiments.

Cell counting kit-8 (CCK-8) assay. Cell viability was assessed using a CCK-8 assay kit (Beyotime Institute of Biotechnology) following the manufacturer's protocol. After 48 h of transfection, HL-60 cells were seeded into 96-well plates at the density of 1,000 cells/well. RPMI-1640 medium containing 10 μl CCK-8 was then added for 4 h. The absorbance in each

well at 450 nm was then measured using a microplate reader (Bio-Rad Laboratories, Inc.).

5-ethynyl-2'-deoxyuridine (EdU) staining. EdU staining was used to analyze the cancer cell proliferation according to the following protocol. Briefly, HL-60 cells were transfected and then incubated with EdU (20 mmol/l; cat. no. KGA331-1000; Nanjing KeyGen Biotech, Co., Ltd.) at 37˚C for 2 h to infiltrate thymine into the DNA molecule being synthesized during DNA replication. The cells were fixed with 4% paraformaldehyde for 20 min at room temperature. DAPI (10 μmol) was used to stain the nucleus for 5 min at room temperature. Finally, the images were captured with a fluorescence microscope (Olympus Corporation; magnification, x200).

Cell cycle analysis. Flow cytometry was used to observe the cell cycle distribution. Briefly, HL-60 cells ($4x10^5$) were collected after 48 h of transfection and were stained with 50 μg/ml PI (Dojindo Molecular Technologies, Inc.) for 15 min at room temperature. Cell cycle distribution and sub-G_1 DNA content were analyzed using a BD Accuri™ C6 flow cytometer (BD Biosciences) and the data were analyzed with ModFit 2.0 software (Verity Software House, Inc.).

Cell apoptosis analysis. Cell apoptosis was evaluated through flow cytometry using a cellular Annexin V-FITC/PI Kit [cat. no. 70-AP101-100; Hangzhou Multi Sciences (Lianke) Biotech Co., Ltd.] according to the manufacturer's protocol. Cells were plated into six-well plates at a density of $4x10^5$ cells/well. After 48 h of transfection, cells ($4x10^5$ cells/well) were incubated with 200 μl binding buffer and double stained with Annexin V-FITC and PI at 4˚C in the dark for 20 min. The cells were then assessed using BD FACSCalibur™ (BD Biosciences) and the data were analyzed with ModFit 2.0 software (Verity Software House, Inc.).

Luciferase reporter assay. The pGL3 plasmid (Promega Corporation) containing the RAB34 promoter region element was generated by site-directed mutagenesis and subcloned into the pGL3-basic luciferase reporter vector. A mutant type (MUT) and wild-type (WT) RAB34 promoter vector were produced by GeneCopoeia, Inc.. The Lipofectamine® 2000 transfection reagent was used to co-transfect the HL-60 cells with 400 ng aforementioned plasmids and 100 nM Oe-E2F1 or the Oe-NC plasmids. After 48 h incubation at 37˚C, the luciferase activity was assayed using a Dual-Luciferase Reporter Assay system (Promega Corporation) according to the manufacturer's protocol. The relative luciferase activity was calculated by normalizing the luminescence intensity of the firefly luciferase activity to that of the *Renilla* luciferase activity.

Chromatin immunoprecipitation (ChIP) assay. The cells were treated with 4% formaldehyde for 10 min at room temperature to generate DNA-protein cross-links. A Bioruptor® (Diagenode, Inc.) was applied to sonicate cell lysates (20 kHz; 4 pulses of 12 sec each, followed by 30 sec rest on ice between each pulse) to generate chromatin fragments with an average size of 500 bp. A total of 40 μl protein A/G agarose beads (cat. no. sc-2003; Santa Cruz Biotechnology, Inc.) was added to the lysates and the lysates (500 μg) were then immunoprecipitated for 6 h with 5 μg specific antibody against E2F1 (ab245308; Abcam) at 4˚C. Normal IgG antibody (ab172730; Abcam) was used as a control. The next day, 30 μl protein G agarose beads were added and the precipitate was collected after incubating at 4˚C for 6 h and centrifuged at 1,000 x g at 4˚C for 3 min. The precipitate was washed with 5X lysis buffer and resuspended in 150 μl 1X ChIP Elution Buffer. Chromatin from the beads were eluted with gentle vortexing (1,200 rpm) at 65˚C for 30 min. DNA was purified using the DNA Purification kit (cat. no. D0033; Beyotime Institute of Biotechnology). Relative enrichment was evaluated using RT-qPCR. The primers used were as follows: RAB34 forward, 5'-GTCTCCGATTCCCCA TCACC-3' and reverse, 5'-ATGCGGACAACATCCCCAAT-3'.

Statistical analysis. Data were presented as mean ± standard deviation and analyzed using the one-way ANOVA with Tukey's post hoc test with GraphPad Prism 5.0 (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference. Each test was repeated ≥ three times.

Results

RAB34 is highly expressed in patients with AML and is associated with the prognosis of AML. GEPIA database analysis revealed that RAB34 was highly expressed in the tissues of patients with AML compared with normal tissues (Fig. 1A), where higher RAB34 expression levels were significantly associated with poorer overall survival in patients with AML (Fig. 1B). RT-qPCR and western blotting showed that RAB34 expression was markedly increased in AML cell lines (Fig. 1C and D). Since the expression level of RAB34 was the highest in HL-60 cells, HL-60 cells were selected for subsequent experiments.

RAB34 knockdown inhibits the proliferation of AML cells, induces cell cycle arrest and apoptosis. The RAB34 shRNA plasmid was constructed before the knockdown efficiency was detected by RT-qPCR and western blotting (Fig. 2A and B). Since the degree of knockdown efficiency in the shRNA-RAB34#2 group was higher, shRNA-RAB34#2 was chosen for subsequent experiments, which were divided into the control, shRNA-NC and shRNA-RAB34 groups. Cell proliferation was detected by CCK-8 assay and EdU staining, which showed that compared with that in the shRNA-NC group, cell proliferation in the shRNA-RAB34 group was markedly decreased (Fig. 2C and D).

Flow cytometry analysis of the cell cycle distribution showed that compared with that in the shRNA-NC group, the proportion of cells in the G_0/G_1 phase in the shRNA-RAB34 group was significantly increased, whilst that in the S phase was significantly decreased (Fig. 3A). The expression of cell cycle marker proteins CDK4, CDK6 and cyclin D1 was next detected by western blotting, which showed that knocking down RAB34 expression significantly inhibited the expression of CDK4, CDK6 and cyclin D1 (Fig. 3B). Flow cytometry and western blotting were subsequently used to analyze cell apoptosis and the results showed that RAB34 knockdown significantly promoted cell apoptosis (Fig. 3C). In addition, significant decreases in the expression of the apoptosis marker

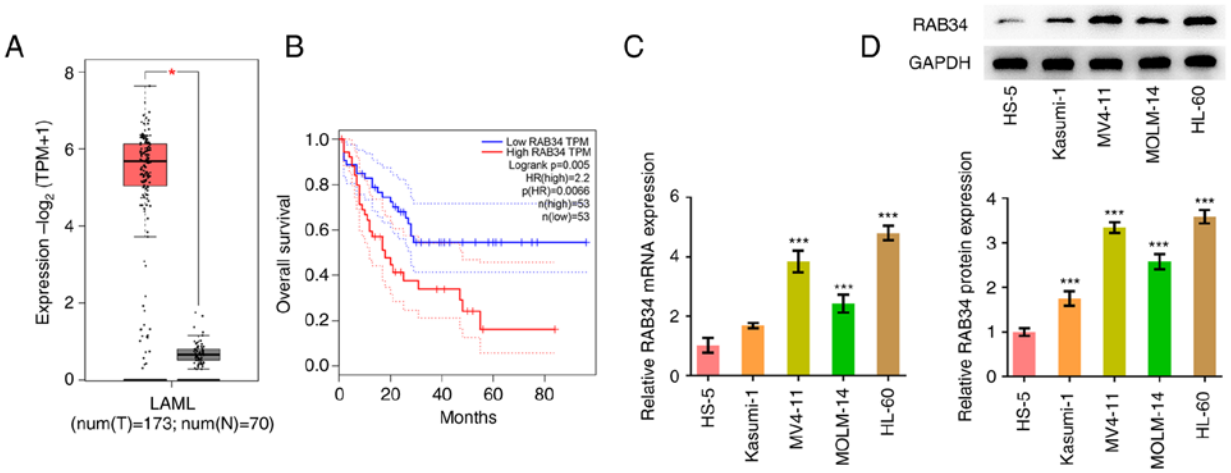

Figure 1. RAB34 is highly expressed in AML patients and is associated with the prognosis of AML. (A) GEPIA database showed that RAB34 was highly expressed in the tissues from AML patients. *P<0.05. (B) GEPIA database showed that higher RAB34 expression levels were significantly associated with poorer overall survival in patients with AML. (C) Reverse transcription-quantitative PCR and (D) western blotting showing that RAB34 expression is markedly increased in AML cell lines. ***P<0.001 vs. HS-5. RAB34, Ras-related protein member RAS oncogene GTPases 34; AML or LAML, acute myeloid leukemia; HR, hazard ratio; TPM, transcript per million.

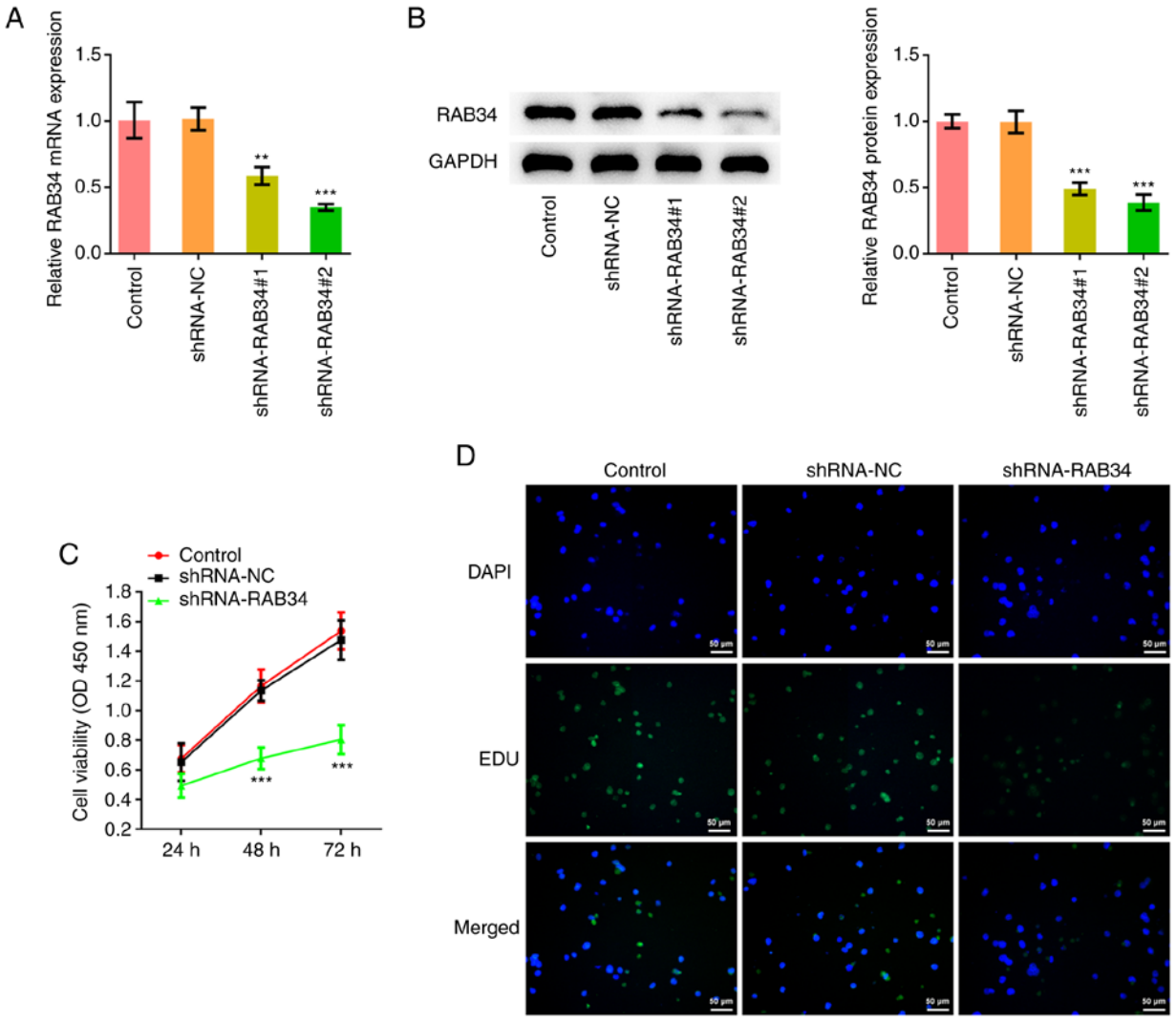

Figure 2. Knocking down RAB34 expression inhibits the proliferation of AML cells, induces cell cycle arrest and apoptosis. (A) Reverse transcription-quantitative PCR and (B) western blotting show that RAB34 expression is significantly decreased in AML cell lines after RAB34 knockdown. Cell proliferation was detected via (C) CCK-8 assay and (D) 5-ethynyl-2'-deoxyuridine staining. Scale bars, 50 μm. **P<0.01 and ***P<0.001 vs. shRNA-NC. shRNA, short hairpin RNA; NC, negative control; EdU, 5-ethynyl-2'-deoxyuridine; RAB34, Ras-related protein member RAS oncogene GTPases 34; AML, acute myeloid leukemia; OD, optical density.

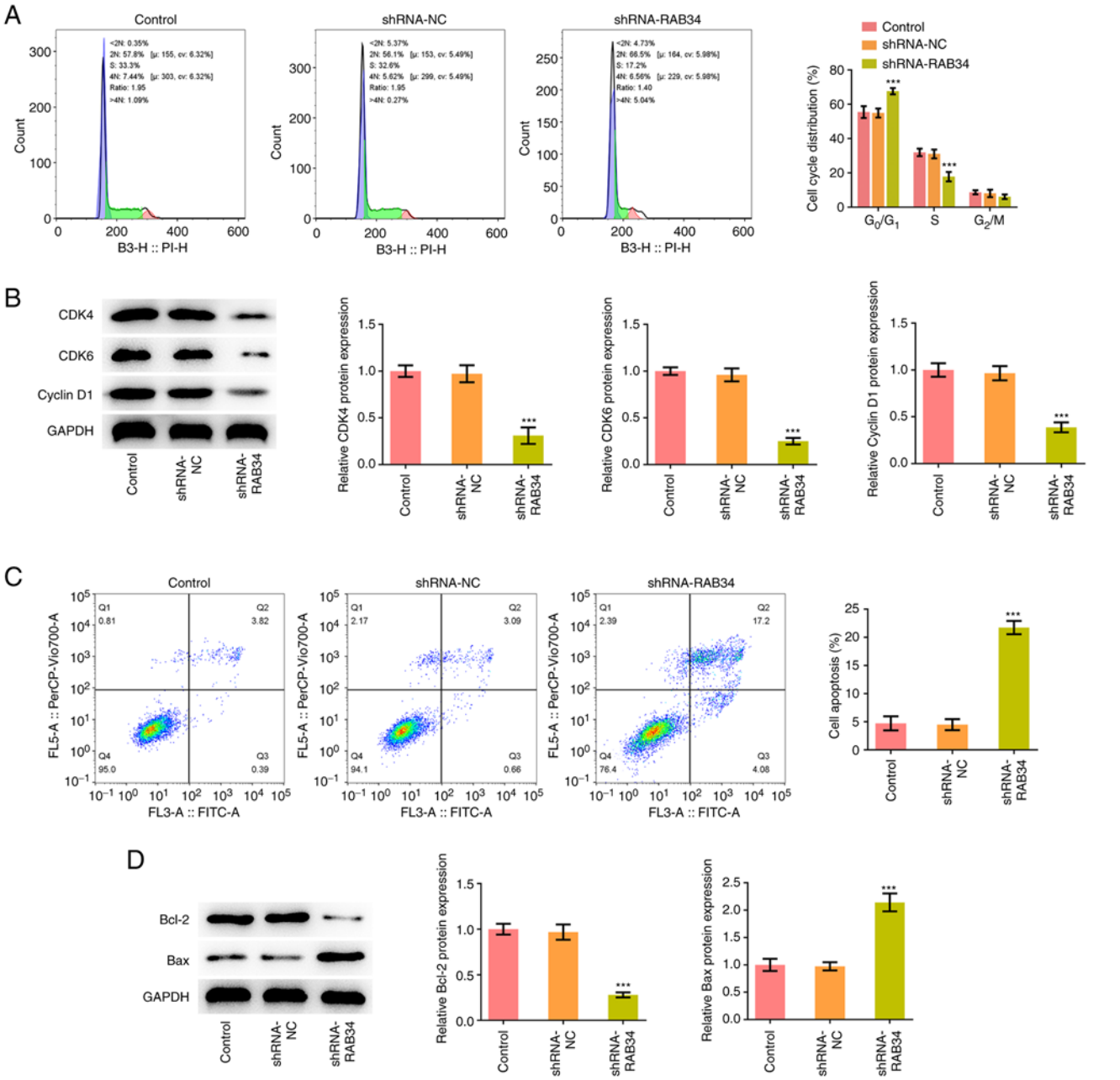

Figure 3. RAB34 knockdown induces HL-60 cell cycle arrest and apoptosis. (A) Flow cytometry analysis of the cell cycle distribution. (B) Expression of cell cycle marker proteins CDK4, CDK6 and cyclin D1 as measured by western blotting. (C) Flow cytometry analysis of cell apoptosis. (D) Expression of cell apoptosis marker proteins Bcl-2 and Bax as detected by western blotting. ***P<0.001 vs. shRNA-NC. shRNA, short hairpin RNA; NC, negative control; RAB34, Ras-related protein member RAS oncogene GTPases 34.

protein Bcl-2 and an significant increases in the expression of Bax were observed (Fig. 3D).

E2F1 activates RAB34 transcription. Based on the binding motif of E2F1, the HumanTFDB website predicted the potential binding for the transcription factor E2F1 on the RAB34 promoter (Fig. 4A). The expression of E2F1 in HL-60 cells was significantly increased compared with HS-5 cells as shown through RT-qPCR and western blotting results (Fig. 4B and C). Subsequently, the E2F1 overexpression plasmid was constructed and it was discovered than E2F1 expression was significantly elevated by transduction of Oe-E2F1 plasmids, which indicated successful transfection (Fig. 4D and E). Through the luciferase reporter assay, it was demonstrated that the luciferase activity of RAB34-WT was markedly enhanced after E2F1 was overexpressed (Fig. 4F). The ChIP assay also demonstrated that the RAB34 promoter was enriched in the E2F1 antibody (Fig. 4G). In addition, RT-qPCR and western blotting showed that RAB34 expression in HL-60 cells was significantly increased after overexpressing E2F1 (Fig. 4H and I).

Overexpression of E2F1 reverses the effect of RAB34 knockdown on the proliferation and apoptosis of AML cells. The regulatory mechanism of RAB34 on the proliferation and apoptosis of AML cells was next investigated. Cells were

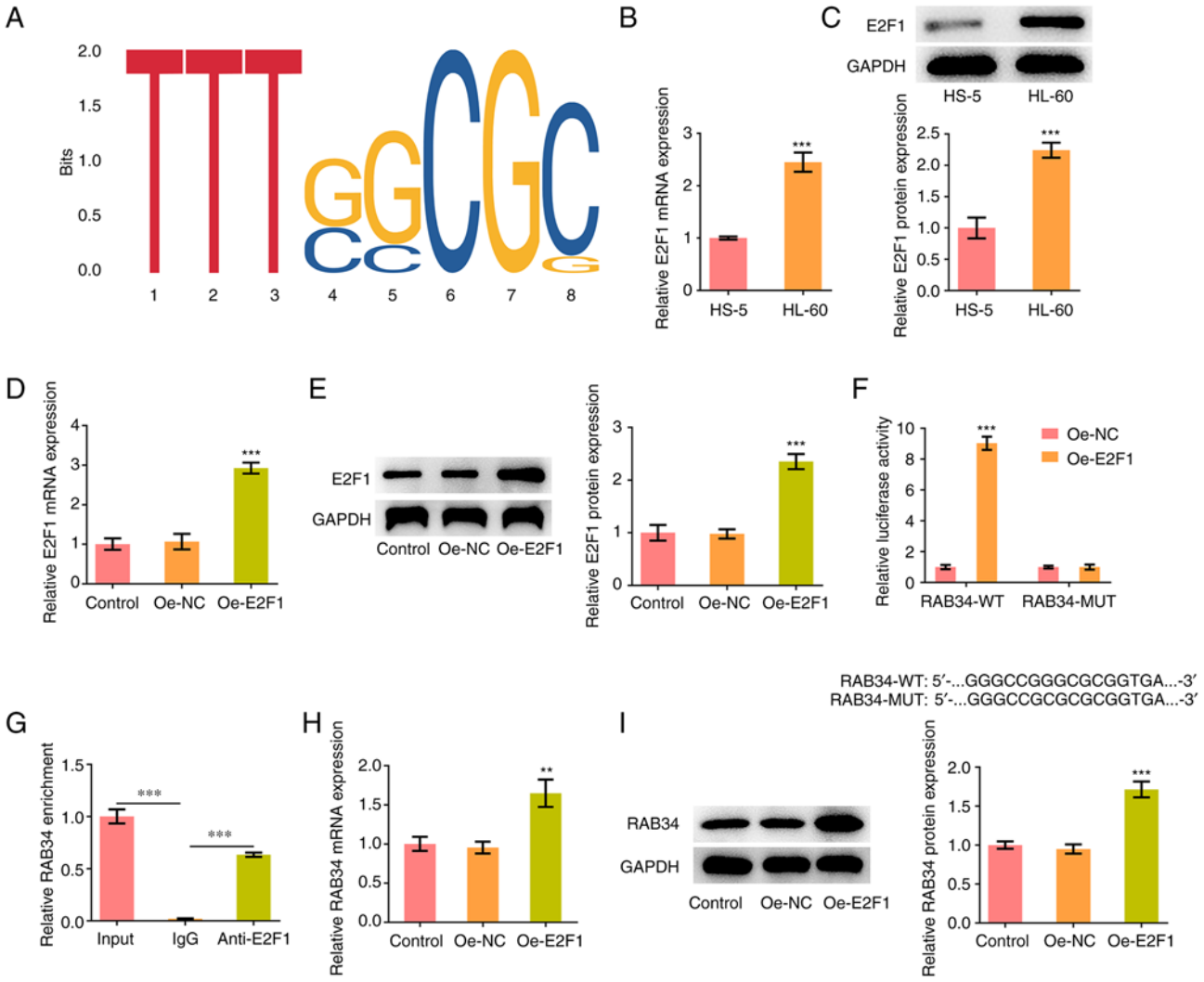

Figure 4. E2F1 activates RAB34 transcription. (A) The binding motif of E2F1 with RAB34 promoter predicted using the HumanTFDB website. The x-axis denotes the order of the bases, and y-axis represents the frequency of occurrence of different bases. Increased expression of E2F1 in acute myeloid leukemia cells as measured by (B) RT-qPCR and (C) western blotting. ***P<0.001 vs. HS-5. Transfection efficiency of E2F1 overexpression as measured by (D) RT-qPCR and (E) western blotting. The binding of E2F1 to the RAB34 promoter was demonstrated by (F) luciferase reporter and (G) chromatin immunoprecipitation assays with whole cell lysates as input. The expression of RAB34 was detected by (H) RT-qPCR and (I) western blotting. **P<0.01 and ***P<0.001 vs. Oe-NC. E2F1, E2F transcription factor 1; Oe-E2F1, E2F1 overexpression; Oe-NC, E2F1 overexpression negative control; RAB34, Ras-related protein member RAS oncogene GTPases 34; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; WT, wild type; MUT, mutant.

divided into the control (shRNA-NC), shRNA-RAB34, shRNA-RAB34 + Oe-NC and shRNA-RAB34 + Oe-E2F1 groups. CCK-8 and EdU staining showed that compared with that in the shRNA-RAB34 + Oe-NC group, cell proliferation of the shRNA-RAB34 + OE-E2F1 group was markedly increased (Fig. 5A and B). Flow cytometry showed that compared with that in the shRNA-RAB34 + Oe-NC group, the proportion of cells in the G_0/G_1 phase in the shRNA-RAB34 + Oe-E2F1 group was significantly decreased, whilst that in the S-phase in the shRNA-RAB34 + Oe-E2F1 group was significantly increased (Fig. 5C). Western blotting showed that E2F1 overexpression significantly reversed the inhibitory effects of RAB34 knockdown on CDK4, CDK6 and cyclin D1 protein expression (Fig. 5D).

Subsequently, analysis of apoptosis showed that compared with that in the shRNA-RAB34 + Oe-NC group, the shRNA-RAB34 + Oe-E2F1 group showed a significant decrease in the levels of apoptosis (Fig. 6A). Furthermore, shRNA-RAB34 + Oe-E2F1 co-transfection significantly increased Bcl-2 expression whilst decreasing Bax expression, compared with those in the shRNA-RAB34 + Oe-NC group (Fig. 6B).

Discussion

The molecular mechanism of cancer occurrence and development has been widely concerned and discussed. Identifying novel biomarkers and potential drug targets in cancer will greatly promote and enrich the early diagnosis and treatment of cancer (16). At present, delaying cancer onset and prevention of further metastasis by cancer cells may be the more urgent aim to be achieved instead of the complete eradication of cancer.

RAB34 is an important regulator of vesicle transport, which exerts specific physiological functions by binding to GTP or GDP since guanine nucleoside releasing protein on the donor membrane recognizes the specific Rab protein in the cytosol,

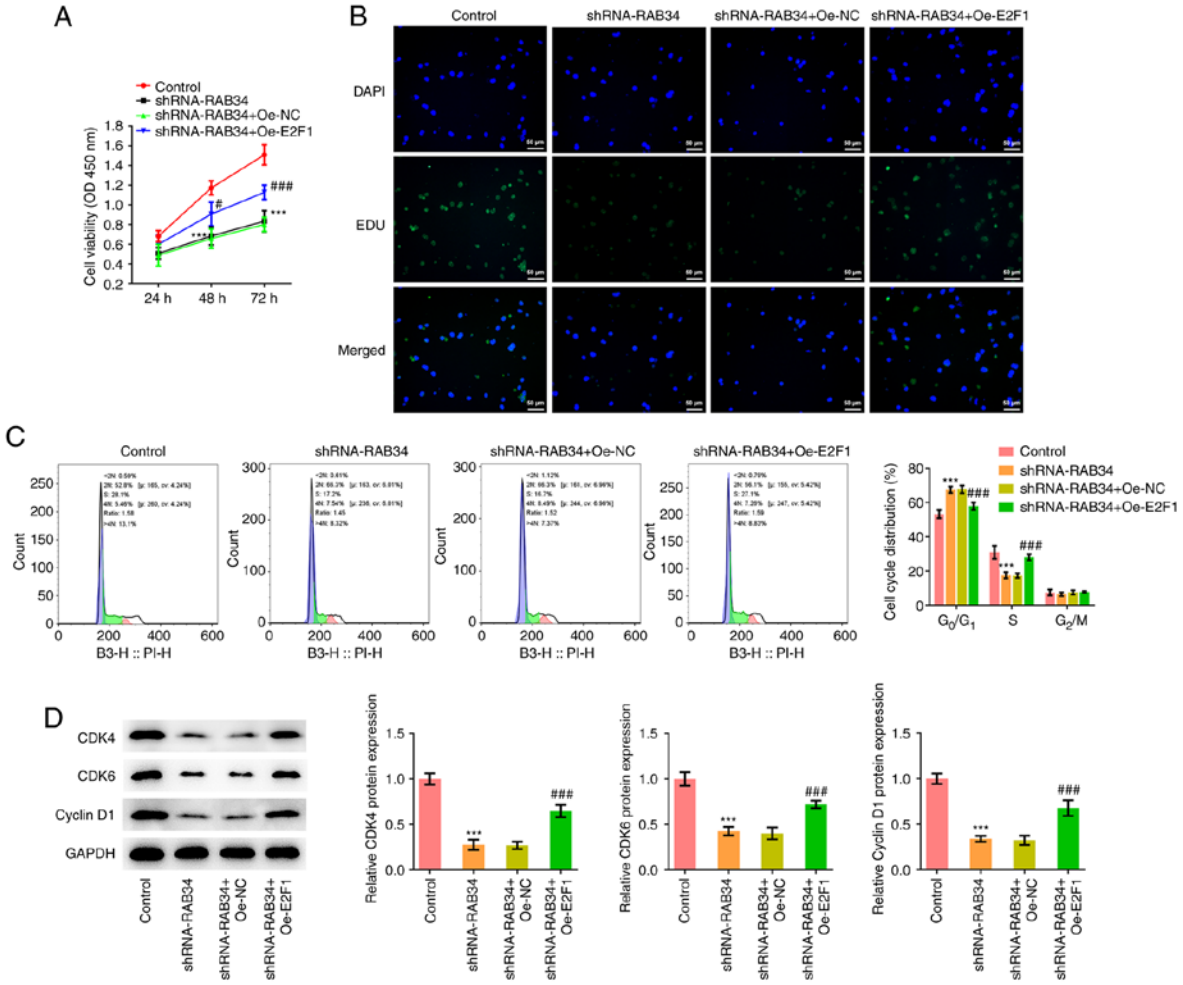

Figure 5. Overexpression of E2F1 reverses the effect of RAB34 knockdown on the proliferation of acute myeloid leukemia cells. Cell proliferation was detected by (A) Cell Counting Kit-8 assay and (B) 5-ethynyl-2'-deoxyuridine staining. (C) Flow cytometry analysis of cell cycle distribution. (D) Expression of cell cycle marker proteins CDK4, CDK6 and cyclin D1 as measured by western blotting. ***P<0.001 vs. control (shRNA-NC). #P<0.05 and ###P<0.001 vs. shRNA-RAB34 + Oe-NC. E2F1, E2F transcription factor 1; Oe-E2F1, E2F1 overexpression; Oe-NC, E2F1 overexpression negative control; RAB34, Ras-related protein member RAS oncogene GTPases 34; EdU, 5-ethynyl-2'-deoxyuridine; sh, short hairpin; NC, negative control.

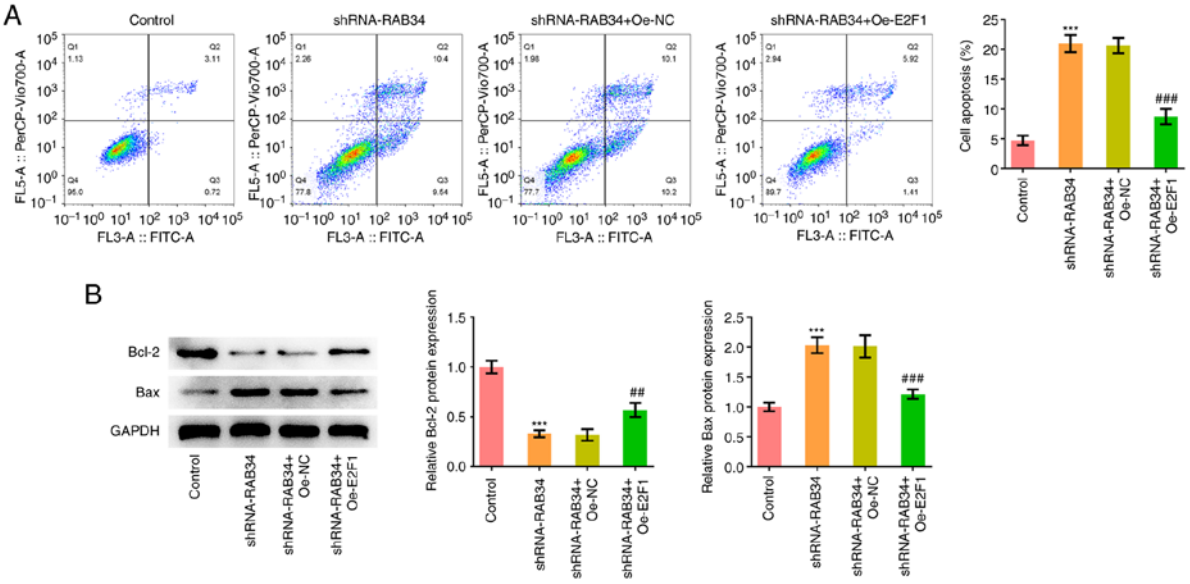

Figure 6. Overexpression of E2F1 reverses the effect of RAB34 knockdown on the proliferation of acute myeloid leukemia cells. (A) Flow cytometry analysis of cell apoptosis. (B) Expression of cell apoptosis marker proteins Bcl-2 and Bax as measured by western blotting. ***P<0.001 vs. Control (shRNA-NC). ##P<0.01 and ###P<0.001 vs. shRNA-RAB34 + Oe-NC. E2F1, E2F transcription factor 1; Oe-E2F1, E2F1 overexpression; Oe-NC, E2F1 overexpression negative control; RAB34, Ras-related protein member RAS oncogene GTPases 34; sh, short hairpin; NC, negative control.

which induces the release of GDP and binding to GTP, thus changing the configuration of Rab protein (17). Because vesicle transport is an important part of the normal physiological activities of cells, abnormal expression of RAB proteins frequently leads to the occurrence of cancer (18). A previous study has shown that the expression of RAB34 is associated with the gradual progression of glioma, where the prognosis of patients with high-grade glioma is poor (19). In addition, the expression of miR-9 was found to be downregulated in gastric cancer, where its target was the tumor-associated protein RAB34 (20). These results suggest that RAB34 serves a role as an oncogene in tumor development. Through GEPIA database analysis, the present study found that RAB34 expression was significantly increased in patients with AML, where high RAB34 expression was significantly associated with poorer overall survival. Subsequently, AML cell lines were selected and the expression of RAB34 was measured. It was found that RAB34 expression was also markedly increased in the AML cell lines. To further determine the regulatory effect of RAB34 on the proliferation, cell cycle progression and apoptosis of AML cell lines, the expression of RAB34 was knocked down in AML cells. It was found that the viability and proliferation of AML cells were decreased, cell cycle arrest occurred and the levels of cell apoptosis were significantly increased. These results suggest that RAB34 can also serve as an oncogene in AML.

By using the HumanTFDB database, the present study identified the potential binding motif of E2F1 with the RAB34 promoter. It was also demonstrated that E2F1 could transcriptionally regulate RAB34 expression in AML cells. E2F is an important family of transcription factors that can regulate gene expression (21). E2F1 is a member of the E2F family, which is a central factor involved in the cell cycle progression and apoptosis (22). Its regulatory role in cell progression has been widely studied and reported. Previous studies have found that E2F1 is highly expressed in a variety of tumor cells, such as lung (23), prostate (24) and breast cancer (25). E2F1 is a pro-oncogene and serves an important role in the occurrence and development of tumors (23-25). In addition, E2F1 and miR-223 have been reported to form an autoregulatory negative feedback loop in AML (26). The present study found that the expression of E2F1 in the AML cell line HL-60 was significantly increased. In addition, overexpression of transcription factor E2F1 reversed the effects of RAB34 knockdown on the proliferation and apoptosis of AML cells.

The present study has certain limitations. Experiments were performed only in cell lines according to the database conclusions. In the future, these findings should be further explored in animal models and clinical human tissue samples. In addition, the effect of RAB34 on the sensitivity of AML cells to chemotherapeutic drugs was not considered in the present study. This aspect should also be explored further in future experiments.

In conclusion, the present results demonstrated that E2F1-mediated RAB34 upregulation may regulate the proliferation, cell cycle progression and apoptosis of AML. This provides a theoretical basis for understanding the pathogenesis of AML and designing the targeted therapy of AML.

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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

XX conceived and designed the study. XX and GJ performed the experiments and wrote the manuscript. All authors read and approved the final manuscript. XX and GJ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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