Knockdown of p54^{nrb} inhibits migration, invasion and TNF-α release of human acute monocytic leukemia THP1 cells

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Abstract. 54 kDa nuclear RNA- and DNA-binding protein (p54^{nrb}) which is also called non-POU domain-containing octamer-binding protein (NONO) is known to be multifunctional involved in many nuclear processes. It was shown that p54^{nrb}/NONO was closely related to the occurrence of erythroleukemia. Whether p54^{nrb}/NONO plays a role in progress of human acute monocytic leukemia remains unknown. In the present study, we examined the effects of p54^{nrb}/NONO silencing on the biological characteristics of human acute monocytic leukemia THP1 cells. The results showed that p54^{nrb} was strongly expressed in THP1 cells, and knockdown of p54^{nrb} slightly promoted proliferation and strongly inhibited motility and invasion of THP1 cells. Moreover, knockdown of p54^{nrb} strongly decreased the release of TNF-α from THP1 cells by inhibiting certain process of TNF-α secretion, specially for the release of TNF-α induced by lipopolysaccharide (LPS). Notably, the infection of negative control shRNA-containing lentiviruses promoted the migration and the release of TNF-α induced by LPS in THP1 cells. All the above results demonstrated that p54^{nrb} slightly inhibited THP1 cell proliferation, but significantly promoted migration, invasion and release of TNF-α induced by LPS in THP1 cells. The present study indicates that p54^{nrb} is a powerful molecule involved in the regulation of cell motility and promotes the migration and invasion of THP1 cells, and it is more likely to be involved in the release of inflammatory mediators and the motility of inflammatory cells.

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Introduction

p54^{nrb}/NONO is known to be involved in a variety of biological events including pre-mRNA splicing, transcriptional regulation (1), nuclear retention of defective RNA (2,3), DNA unwinding and pairing (4), DNA damage repair (5,6). p54^{nrb}/NONO was recently shown to be a component of a novel nuclear domain termed paraspeckles (7,8).

Paraspeckles are ribonucleoprotein bodies found in the interchromatin space of mammalian cell nuclei. The core paraspeckle proteins contain polypyrimidine tract-binding protein-associated splicing factor (PSF)/splicing factor proline/glutamine-rich (SFPQ), p54^{nrb}/NONO and paraspeckle protein 1 (PSPC1). These proteins, together with the long non-protein-coding RNA NEAT1 (MEN-ε/β), associate to form paraspeckles (9,10). The core paraspeckle proteins have been shown to bind to both double and single-stranded DNA and RNA, and have been involved in numerous nucleus events including transcription and splicing. The function relevant to paraspeckles is the involvement of PSF/SFPQ and p54^{nrb}/NONO in the nuclear retention of RNA, specifically preventing A to I hyperedited RNA from leaving the nucleus (11,12). Recent research hints at a more generic retention-release mechanism that exists for transcripts containing hyperedited inverted repeats in their 3' UTR (12,13). Adenosine-to-inosine conversion (A-to-I editing) contributes to extensive transcriptome diversity (14). Disturbance in RNA editing has been implicated in various pathologic disorders, including cancer. Abnormal A-to-I editing was involved in cancer development (15).

Recent studies suggest that as a core paraspeckle protein and multifunctional protein, p54^{nrb}/NONO may be implicated in tumor progress and matastasis. Phosphorylated β-cell differentiation transcription factor HLXB9 promoted insulinoma cell proliferation through interaction with NONO protein (16). Expression of p54^{nrb} was increased in breast cancer with estrogen receptor (17). The protooncogene Spi-1/PU.1 is involved in the erythroleukemic process via impeding the binding of p54^{nrb} to RNA and alters the splicing process (18). Acute myeloid leukemia (AML) is a type of heterogeneous disease derived from haematopoietic stem cells. Whether p54^{nrb}/NONO plays a role in the progress of human acute monocytic leukemia remains unknown. In the present study, we examined the effects of p54^{nrb} silencing on the proliferation, migration, invasion and TNF-α release of human acute monocytic leukemia THP1 cells.

Materials and methods

Materials. Lipopolysaccharide (LPS) from *E. coli*, puromycin, Brefeldin A, propidium iodide (PI), p54^{nrb}/NONO and β-actin antibodies, horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA). Proliferating cell nuclear antigen (PCNA) and TNF-α antibodies were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). RPMI-1640 and fetal bovine serum (FBS) were purchased from Gibco-Life Technologies (Carlsbad, CA, USA). Matrigel and 8 μm pore-sized Transwell chambers were purchased from BD Biosciences (San Jose, CA, USA). RNeasy Mini kit was purchased from Invitrogen (San Diego, CA, USA). ReverTra Ace-α-™ Kit was purchased from Toyobo Co., Ltd. (Osaka, Japan). Human TNF-α Immunoassay Valukine™ ELISA kit was purchased from R&D Systems, Inc. (Minneapolis, MN, USA). Cell Counting kit-8 (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). RIPA lyses buffer was purchased from Beijing Leagene Biotech Co., Ltd. (Beijing, China). Other reagents were produced in China and purchased from local suppliers.

Cell cultures and establishment of p54^{nrb} silencing THP1 cell line. THP1 cells were obtained from the Department of Biochemistry (Guangdong Medical University, Guangdong, China). THP1 cells were maintained in RPMI-1640 media supplemented with 15% FBS and penicillin (100 U/ml) and streptomycin (10 μg/ml). p54^{nrb}-silencing THP1 cell line and negative control THP1 cell line were established in our laboratory. The cell lines were established as follow: packaged lentiviruses containing p54^{nrb}-specific short hairpin RNA (shRNA)-expressing GFP lentiviral vector LV3 (H1/GFP&Puro-NONO) (sense sequence 5'-GGCGAAGUCUU CAUUCAUATT-3') or with negative control shRNA lentiviral vector LV3 (H1/GFP&Puro) (sense sequence 5'-UUCUCCG AACGUGUCACGUUUC-3') were provided by Shanghai GenePharma Co., Ltd., (Shanghai, China). Exponentially growing THP1 cells were infected with p54^{nrb}-specific shRNA-containing lentiviruses or negative control shRNA-containing lentiviruses for 24 h, then the cells were cultured in completed RPMI-1640 media containing 1 μg/ml puromycin for 14 days. The efficiency of p54^{nrb} silencing was determined by reverse transcription polymerase chain reaction (RT-PCR) and western blotting. Stable p54^{nrb}-silencing THP1 cell line (THP1-p54^{nrb}-si) and negative control THP1 cell line (THP1-NC) were maintained in similar media to THP1 supplemented with 0.5 μg/ml puromycin. Cells were grown at 37˚C in a humidified atmosphere of 5% CO_2, 95% air.

RT-PCR. Total RNA was isolated using RNeasy Mini kit (Qiagen). The first strand cDNAs were synthesized using the ReverTra Ace-α-™ kit (Toyobo). The levels of p54^{nrb} transcripts in THP1, THP1-p54^{nrb}-si and THP1-NC cells were assessed by semi-quantitative PCR, respectively. The specific primers for PCR (p54^{nrb}-sense, 5'-ATGCAGAGTAATAAA ACTTTTAACTTGG-3' and p54^{nrb}-antisense, 5'-GTATCGG CGACGTTTGTTTGG-3'. β-actin sense, 5'-GAGACCTTCAA CACCCCAGC-3' and β-actin-antisense, 5'-ATGTCACGCAC GATTTCCCT-3') were synthetized by BGI Tech Solutions Co. (Shenzhen, China). Semi-quantitative PCR was performed with MasterCycler® Gradient Thermal cycler (Eppendorf, Hamburg, Germany). PCR amplification was performed as follows: 94˚C for 2 min, then 30 cycles of 94˚C for 30 sec, 56˚C for 30 sec, 68˚C for 1 min, last 68˚C for 5 min. For PCR analysis β-actin served as loading control. GoldView I-stained bands were visualized by UV using InGenius LHR gel documentation and analysis system (InGene, Fredericktown, MO, USA), and the relative grey scale intensity of p54^{nrb}/β-actin was quantified using ImageJ2x software.

Western blot analysis. THP1, THP1-p54^{nrb}-si and THP1-NC cells (5x10^6 cells/well), respectively were seeded in 6-well culture plates. To test the protein expression of PCNA and p54^{nrb} in THP1, THP1-p54^{nrb}-si and THP1-NC cells, the cells were collected for western blot analysis. To test the effect of p54^{nrb} silencing on expression of TNF-α induced by LPS, the experiments were divided into three groups: i) blank group untreated; ii) LPS treatment group treated with 5 μg/ml LPS for 6 h; iii) BFA and LPS treatment group treated with 5 μg/ml LPS for 6 h and simultaneously protein secretion inhibitor Brefeldin A (10 μg/ml) for last 5 h. Western blot analyses were performed as follow: the cells were collected and lysed with RIPA lyses buffer. Total proteins were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were incubated with 5% skimmed milk in TBST for 2 h at room temperature. After washing with TBST, the membranes were respectively incubated overnight at 4˚C with the first antibodies against human p54^{nrb} (1:800), PCNA (1:1,000), tumor necrosis factor α (TNF-α) (1:1,000) or β-actin (1:1,000), followed by incubation with an HRP-conjugated secondary antibodies at room temperature for 1 h. Enhanced chemiluminescence (ECL) was used to detect the results. The expression of β-actin was used as loading control. The relative grey scale intensity was quantified using ImageJ2x software. Data are representative of three independent experiments.

Cell cycle analysis. THP1, THP1-p54^{nrb}-si and THP1-NC cells were seeded in 6-well plates and cultured for 48 h. After washing with PBS, the cells were fixed with 70% ice-cold ethanol for 12 h. After being washed twice with PBS, the cells were stained with PI for 30 min. The cell cycle distributions were then analyzed by flow cytometry and the percentage of cells in G1/G0, S or G2/M phase was calculated using ModFit LT software. Data represent the mean value derived from triplicate experiments.

Cell proliferation assay. Cell proliferation was assessed by the Cell Counting kit-8 (CCK-8) assay, in accordance with the manufacturer's instructions. Briefly, THP1, THP1-p54^{nrb}-si and THP1-NC cells (2x10^4 cells/well) were seeded in 96-well culture plates and cultured for either 48 or 72 h, then 10 μl CCK-8 reagent was added per well and incubated for 3 h at 37˚C. Absorbance was subsequently measured at 450 nm using Synergy 2 Multi-Mode microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The assay was conducted in quadruplicate for each sample and three parallel experiments were performed.

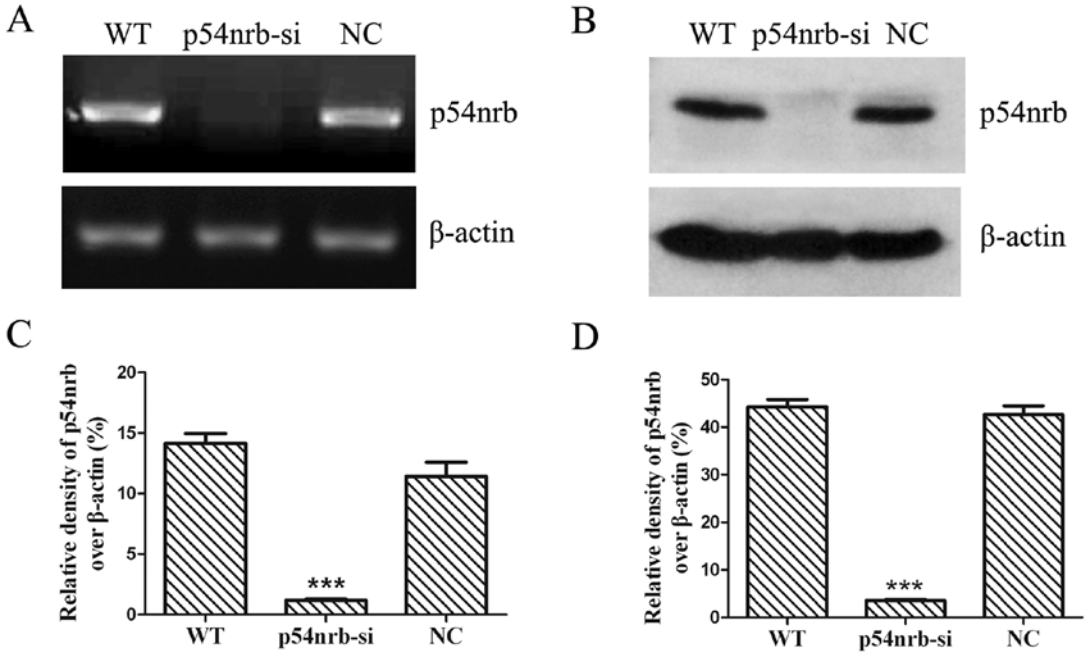

Figure 1. Expression of $p54^{nrb}$ mRNA and protein in THP1, THP1-$p54^{nrb}$-si and THP1-NC cell lines. (A) Wild-type THP1 cells, $p54^{nrb}$ silencing THP1 cells (THP1-$p54^{nrb}$-si) and negative control THP1 cells (THP1-NC) were seeded in 6-well plates and harvested. The expression levels of $p54^{nrb}$ mRNA were assessed by semi-quantitative PCR following reverse transcription of the RNA. β-actin was used as loading control. (B) Western blotting was used to detected $p54^{nrb}$ protein expression in THP1, THP1-$p54^{nrb}$-si and THP1-NC cells. β-actin was used as loading control. (C) The gray values of $p54^{nrb}$ mRNA were determined by InGenius LHR gel analysis system and normalized with the gray value of β-actin. Data are presented from three repeated test. ***P<0.01 vs. wild-type and negative control THP1 cells. (D) The gray values of $p54^{nrb}$ protein were determined by ImageJ2x software and normalized with the gray value of β-actin. Data are presented from three repeated test. ***P<0.01 vs. wild-type and negative control THP1 cells.

Cell invasion and motility assay. In vitro invasion assay was performed by using Matrigel-coated Transwell chambers. Pore-sized polycarbonate membranes (8 μm) were coated with Matrigel (100 $\mu g/cm^2$) and incubated overnight. THP1, THP1-$p54^{nrb}$-si or THP1-NC cells ($1x10^6$ cells) suspended in PRMI-1640 medium supplemented with 1% BSA were respectively seeded in upper chamber which was placed over the lower chamber. The PRMI-1640 medium supplemented with 15% FBS in lower chamber was used as a chemoattractant. Invasion was allowed to proceed for 28 h, then the invaded cells in lower chamber were collected and counted. Transwell motility assay was performed similar to the above invasion assay, with the exception that Transwell insert was not coated with Matrigel. Migration was allowed to proceed for 19 h, then the migrated cells in the lower chamber were collected and counted. All the experiments were repeated three times.

TNF-α release and content assay. THP1, THP1-$p54^{nrb}$-si and THP1-NC cells ($5x10^6$ cells/well) were seeded in 6-well culture plates, then 5 μg/ml LPS was added per well and incubated for 0, 3, 6 and 9 h. The cells were subsequently centrifuged at 2,000 rpm for 15 min, then TNF-α-containing supernatants were collected and measured with Human TNF-α Immunoassay Valukine™ ELISA kit, in accordance with the manufacturer's instruction. The assay was conducted in triplicate for each sample and three parallel experiments were performed.

Statistical analysis. The SPSS version 16.0 software package and GraphPad Prism were used for the statistical analysis and data plotting. The data were expressed as mean ± SD. ANOVA was carried out followed by the Student-Newman-Keuls and LSD tests. P<0.05 was considered to indicate a statistically significant result.

Results

Expression of $p54^{nrb}$ mRNA and protein in THP1, THP1-$p54^{nrb}$-si and THP1-NC cell lines. To test the interference efficiency of $p54^{nrb}$-specific shRNA-containing lentiviruses, THP1 cells, THP1-$p54^{nrb}$-si and THP1-NC cells were harvested. The expression levels of $p54^{nrb}$ mRNA were assessed by semi-quantitative RT-PCR. The result showed that the expression of $p54^{nrb}$ mRNA in THP1-$p54^{nrb}$-si cells was significantly decreased compared with wild-type or negative control THP1 cells (Fig. 1A). The mRNA level of $p54^{nrb}$ in THP1-$p54^{nrb}$-si cells was 89.76% decreased compared with negative control THP1 cells (Fig. 1C). To confirm the efficiency of $p54^{nrb}$ knockdown, the $p54^{nrb}$ proteins were assessed by western blot analysis. The result showed that the expression of $p54^{nrb}$ protein was significantly decreased in THP1-$p54^{nrb}$-si cells compared with wild-type and negative control THP1 cells (Fig. 1B). The expression of $p54^{nrb}$ protein showed 91.59% reduction in THP1-$p54^{nrb}$-si cells compared with negative control THP1 cells (Fig. 1D).

Knockdown of $p54^{nrb}$ promotes THP1 cell proliferation. The effect of $p54^{nrb}$ silencing on THP1 cell cycle distribution was analyzed by flow cytometric assay. As shown in Fig. 2A and B, THP1-$p54^{nrb}$-si cells showed a decrease of cells in G0/G1 phase, and an increase of cells in the S and G2/M phases compared with wild-type or negative control THP1

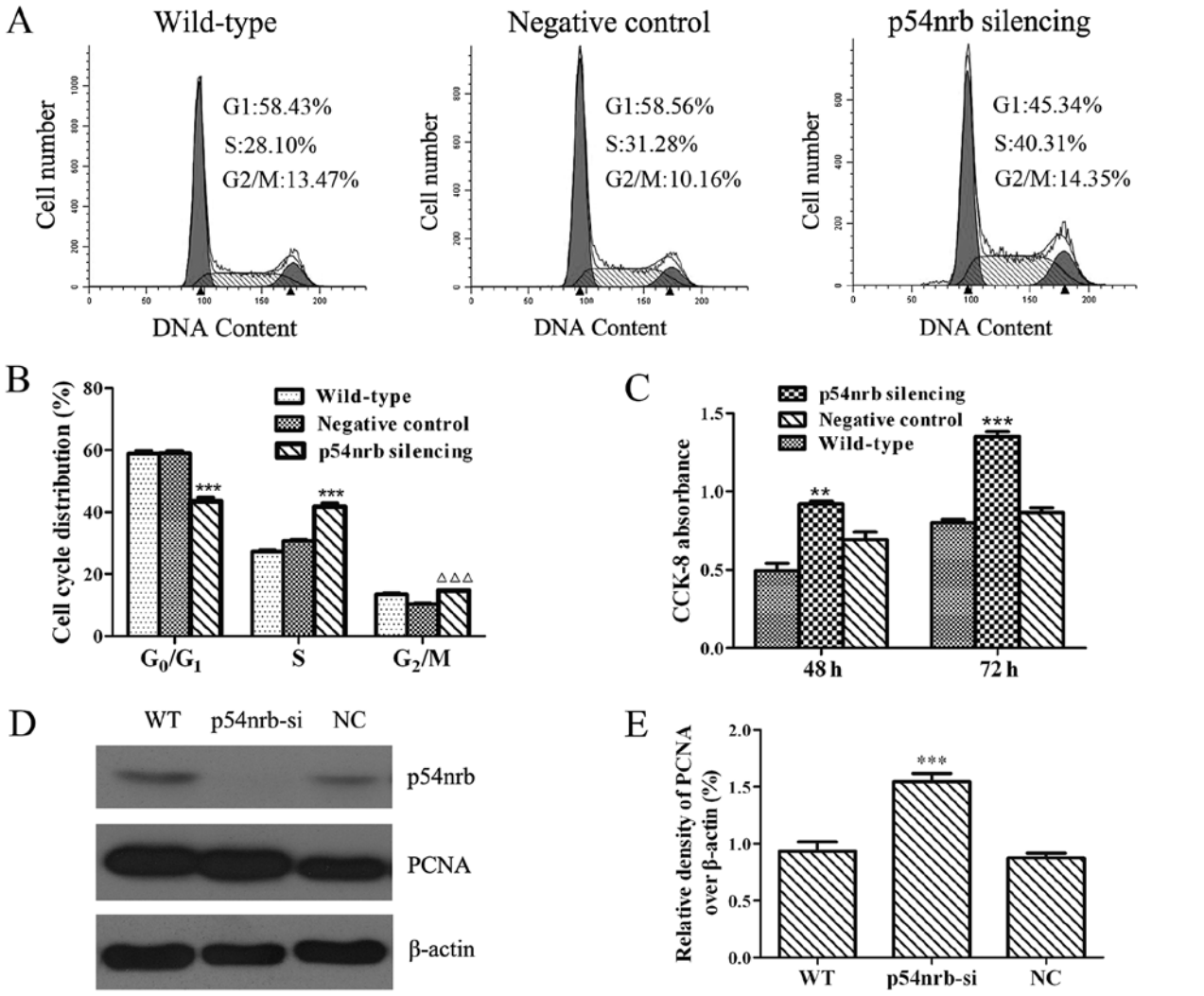

Figure 2. Knockdown of p54^{nrb} promoted THP1 cell proliferation. (A) The effect of p54^{nrb} silencing on THP1 cell cycle distributions was analyzed by flow cytometric assay. (B) The data are expressed as the percentage of cells in G0/G1, S or G2/M phase. ***P<0.01 vs. wild-type and negative control THP1 cells. $^{\triangle\triangle\triangle}$P<0.01 vs. negative control THP1 cells. (C) THP1, THP1-p54^{nrb}-si and THP1-NC cells were seeded in 96-well plates and incubated for 48 or 72 h. Cell proliferation was estimated using CCK-8 assay. Data are presented from three independent experiments. ***P<0.01, **P<0.05 vs. wild-type and negative control THP1 cells. (D) Western blotting was used to detect the expression of PCNA protein, a marker of cell proliferation, in THP1, THP1-p54^{nrb}-si and THP1-NC cells. β-actin was used as loading control. (E) The gray values of PCNA protein were determined by ImageJ2x software and normalized with the gray value of β-actin. Data are presented from three repeated test. ***P<0.01 vs. wild-type and negative control THP1 cells.

cells, which indicated that p54^{nrb} silencing can accelerate cell cycle progression to promote the proliferation of THP1 cells. This alteration was further confirmed by using CCK-8 assay (Fig. 2C). Moreover, expression of PCNA, a marker of cell proliferation, was increased in p54^{nrb}-silencing THP1 cells compared with wild-type or negative control THP1 cells (Fig. 2D and E). These data demonstrated that p54^{nrb} silencing slightly promoted the proliferation of THP1 cells.

Knockdown of p54^{nrb} inhibits the invasion and motility of THP1 cells. To detect the effect of p54^{nrb} silencing on THP1 cell motility, Transwell assay was performed. As shown in Fig. 3A and C, the migration of THP1-p54^{nrb}-si cells was significantly reduced compared with wild-type and negative control THP1 cells. Similar effect was observed for the invasion assay. Matrigel-coated Transwell chambers were used to measure the effect of p54^{nrb} silencing on THP1 cell invasion. The result showed that the invasion of THP1-p54^{nrb}-si cells was significantly reduced compared with wild-type and negative control THP1 cells (Fig. 3B and D). Above results showed that knockdown of p54^{nrb} significantly inhibited the invasion and migration of THP1 cells. The result also displayed that THP1-NC cells infected negative control shRNA lentiviruses displayed stronger migration than wild-type THP1 cells (Fig. 3A and C).

Knockdown of p54^{nrb} inhibits the release of TNF-α induced by LPS from THP1 cells. The effect of p54^{nrb} knockdown on the release of TNF-α induced by LPS from THP1 cells was measured with Human TNF-α Immunoassay Valukine™ ELISA kit. As shown in Fig. 4A, the content of TNF-α in supernatant of THP1-p54^{nrb}-si cells with or without LPS treatment significantly decreased compared with THP1 or THP1-NC cells, which indicated that knockdown of p54^{nrb} significantly inhibited the release of TNF-α from THP1 cells, especially for the release of TNF-α induced by LPS. Moreover, we

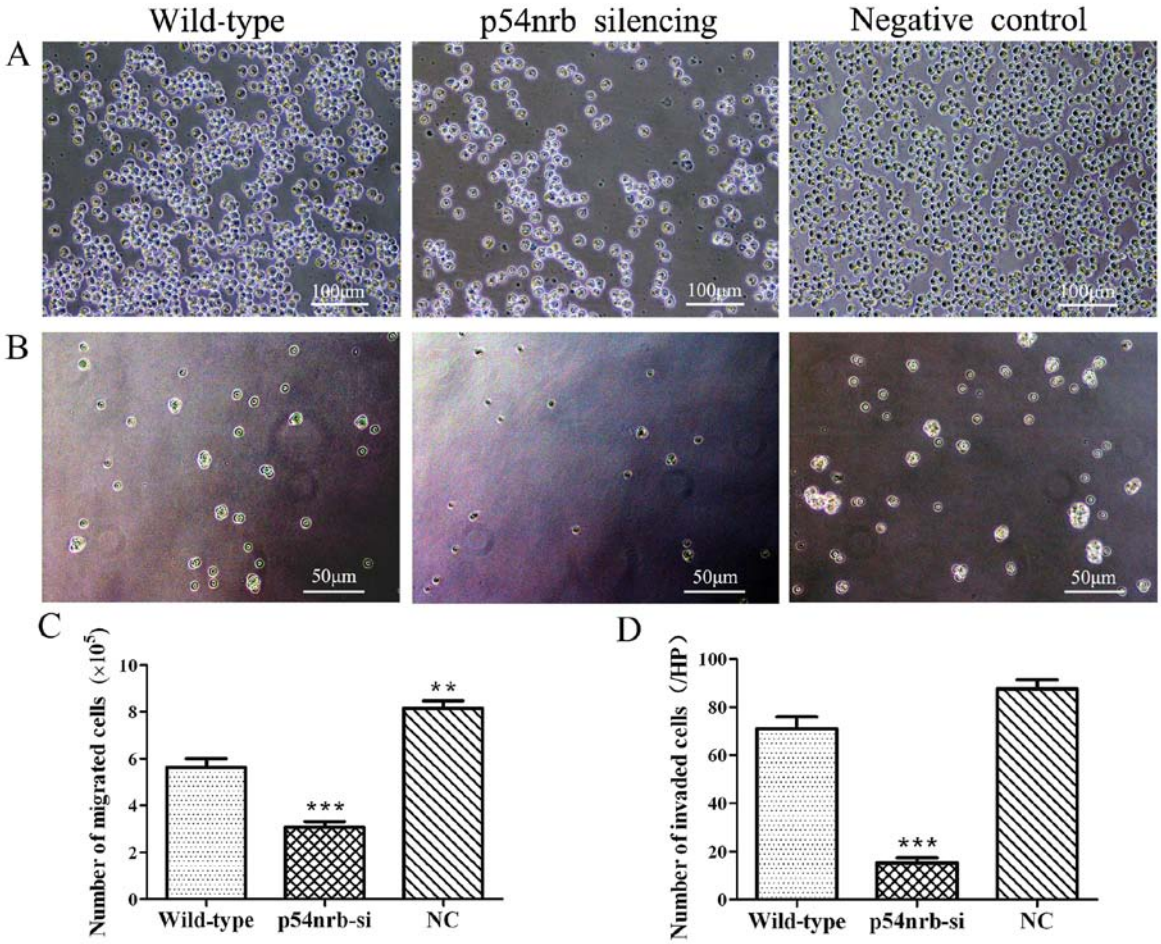

Figure 3. Knockdown of p54^{nrb} inhibited the invasion and motility of THP1 cells. (A) Image of migrated cells. Cell migration was assessed by Transwell motility assay after the THP1, THP1-p54^{nrb}-si and THP1-NC cells were seeded and incubated for 19 h. Original magnification, x200. (B) Image of invaded cells. Cell invasion was assessed by Transwell invasion assay after the THP1, THP1-p54^{nrb}-si and THP1-NC cells were seeded and incubated for 28 h. Original magnification, x100. (C) Number of migrated cells. Migrated THP1, THP1-p54^{nrb}-si and THP1-NC cells in lower chamber were collected and counted in triplicate inserts after the cells were seeded and incubated for 19 h. ***P<0.01 vs. wild-type and negative control THP1 cells; **P<0.05 vs. wild-type THP1 cells. (D) Number of invaded cells. Invaded THP1, THP1-p54^{nrb}-si and THP1-NC cells in lower chamber were collected and counted within five random fields in triplicate inserts after the cells were seeded and incubated for 28 h. ***P<0.01 vs. wild-type or negative control THP1 cells.

found that content of TNF-α in supernatant of THP1-NC cells treated with LPS was remarkably higher than that of wild-type THP1 cells, which indicated that infection of negative control lentiviruses promoted the release of TNF-α induced by LPS from THP1 cells.

In order to explore the mechanism involving in reduction of TNF-α release in p54^{nrb} silencing THP1 cells, we tested the expression of TNF-α protein in THP1, THP1-p54^{nrb}-si and THP1-NC cells. Western blot analysis showed that the protein level of TNF-α was similar in THP1, THP1-p54^{nrb}-si and THP1-NC cells without treatment. After LPS treatment, the protein level of TNF-α in THP1-p54^{nrb}-si cells was significantly higher than that of THP1 and THP1-NC cells. TNF-α can be released from THP1 cells when THP1 cells are stimulated by LPS. Therefore, the protein level of TNF-α was monitored after different THP1 cells were simultaneously treated with LPS and protein secretion inhibitor Brefeldin A. As shown in Fig. 4B, the protein level of TNF-α was similar in THP1-p54^{nrb}-si and THP1-NC cells upon simultaneously treated with LPS and Brefeldin A. The results demonstrated that knockdown of p54^{nrb} decreased the content of TNF-α in culture supernatant by inhibiting certain process of TNF-α secretion from THP1-p54^{nrb}-si cells, instead of effecting the TNF-α protein expression.

Discussion

AML is a heterogeneous disease derived from haematopoietic stem cells. To identify new genes involved in tumor progress is important for the diagnosis and treatment of AML (19). In the present study, we analyzed the effects of p54^{nrb} silencing on the biological characteristics of human acute monocytic leukemia THP1 cells.

Cell cycle and cell proliferation assay showed that knockdown of p54^{nrb} slightly promoted proliferation, and western blotting displayed that knockdown of p54^{nrb} increased the expression of PCNA protein that is a marker of cell proliferation (20). The above results indicated that knockdown of p54^{nrb} slightly promoted the proliferation of THP1 cells. As a core paraspeckle protein and multifunctional protein, p54^{nrb}/NONO was reported to be implicated in tumor progress and metastasis. Previous research demonstrated that p54^{nrb} played diverse roles in cell proliferation. Knockdown of p54^{nrb} in melanoma cell lines led to reduced proliferation rates (21).

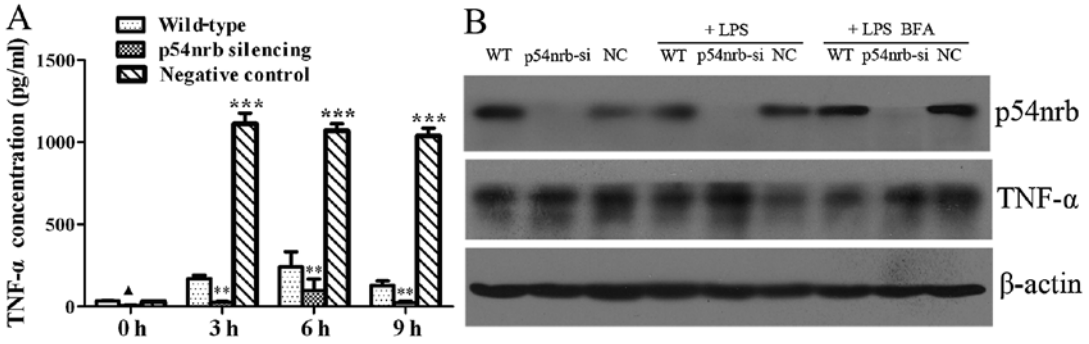

Figure 4. Effect of p54nrb silencing on the release and expression of TNF-α induced by LPS in THP1 cells. (A) Knockdown of p54nrb inhibited the release of TNF-α induced by LPS from THP1 cells. THP1, THP1-p54nrb-si and THP1-NC cells respectively were incubated with 5 μg/ml LPS for 0, 3, 6 and 9 h, then the supernatants were collected and the content of TNF-α in supernatant was measured. The assay was conducted in triplicate for each sample and three parallel experiments were performed. ***P<0.001 vs. wild-type or p54nrb silencing THP1 cells. **P<0.01 vs. wild-type THP1 cells. ▲P<0.05 vs. wild-type and negative control THP1 cells. (B) Effect of p54nrb silencing on expression of TNF-α induced by LPS in THP1 cells. THP1, THP1-p54nrb-si and THP1-NC cells, respectively were seeded in 6-well culture plates. Experiments were divided into three groups: i) blank group untreated; ii) LPS treatment group treated with 5 μg/ml LPS for 6 h; iii) BFA and LPS treatment group treated with 5 μg/ml LPS for 6 h and simultaneously protein secretion inhibitor Brefeldin A (10 μg/ml) for the last 5 h. The protein expression of TNF-α or p54nrb in cells was examined by western blotting. β-actin was used as loading control.

β-cell differentiation transcription factor HLXB9 promoted insulinoma cell proliferation through interaction with NONO protein (16). Spi-1/PU.1 blocks the differentiation of proerythroblast and promotes their malignant transformation in the Friend erythroleukemia, and Spi-1/PU.1 might be involved in leukemogenesis via impeding the binding of p54nrb to RNA and alters the splicing process (18). NONO bound to the p16-Ink4A cell cycle checkpoint gene and potentiated its circadian activation. Loss of NONO abolished this activation and circadian expression of p16-Ink4A and eliminated circadian cell cycle gating. Fibroblasts from NONO gene-trapped mice showed increased proliferation and decreased senescence (22). Our results displayed that knockdown of p54nrb slightly promoted proliferation of THP1 cells, human umbilical vein endothelial cells (HUVEC). Various research demonstrated that p54nrb promotes or inhibits cell proliferation in different tumor cells. These alterations may be responsible for functional diversity of p54nrb protein including mRNA splicing and transcription. As a core paraspeckle protein, p54nrb/NONO involved in the nuclear retention of RNA, specifically for A to I hyper-edited RNA (11,12). The common dysregulation of A-to-I editing in human cancers may contribute to the altered transcriptional program necessary to sustain carcinogenesis (23-25). In hematologic malignancies, abnormal A to-I editing of hematopoietic cell phosphatase (PTPN6) gene was associated to AML (26). Whether p54nrb/NONO affects the proliferation of THP1 cells by the nuclear retention of A to I hyper-edited RNA remains unknown.

Cell motility and invasion assay showed that knockdown of p54nrb significantly inhibited the motility and invasion of THP1 cells. p54nrb/NONO shows diverse roles in cell proliferation in different tumor cells and research, but its role in migration was very consistent and obvious. Knockdown of p54nrb significantly inhibited migration of melanoma cell lines (21). Overexpression of p54nrb increased migration of HUVEC and HeLa cells (27). Our results indicated that knockdown of p54nrb strongly decreased the motility and invasion of THP1, HUVEC, SKBR-3 and CNE2 cells (partial data not shown). All these studies showed that p54nrb is a powerful molecule involving in the regulation of cell motility. To date, only a few reports on the mechanism of p54nrb participating in cell motility have been reported. Fibroblast growth factor 1 (FGF1) functions as a modifier of endothelial cell migration and proliferation. Heterogeneous nuclear ribonucleoprotein M (hnRNPM) and p54nrb present in protein complexes bound to the FGF1 promoter and to the mRNA internal ribosome entry site. Knockdown of either p54nrb or hnRNPM blocks endogenous FGF1 induction and myotube formation (28). Angiopoietin-1 (Ang1) regulates angiogenesis as a ligand of Tie 2 receptor tyrosine kinase. p54nrb was the tyrosine phosphorylated protein in Ang-1 induced signaling pathway in HUVEC cells. p54nrb was validated as a molecule involved in cell migration of HUVEC cells, which can be supported by the results obtained from microarray analysis: overexpression of p54nrb significantly upregulated the genes involved in cell motility and structural functions in HUVEC cells (data not shown) (27). *In vivo*, NONO gene-trapped mice showed defective wound repair. Considering the above results, we speculate that p54nrb might be a key molecule involving in cell motility via effecting the expression or function of certain cytoskeleton proteins.

THP1 cells are often used as an inflammatory cell model in the study of inflammation and some leukemia is relative to infection. Therefore, we also analyzed the effect of p54nrb silencing on the release and expression of inflammatory mediator TNF-α in THP1 cells. The results indicated that knockdown of p54nrb had no effect on the expression of TNF-α protein, but significantly inhibited the release of TNF-α from THP1 cells, especially for the release of TNF-α induced by LPS, the mechanism of which is still obscure. The secretion of TNF-α from cells is an exocytosis process which depends on microtubules (29-31). Furthermore, knockdown of p54nrb blocks endogenous FGF1 induction and myotube formation (28). Overexpression of p54nrb significantly upregulated the genes involved in cell motility and structural functions in HUVEC cells (27). Thus, we speculate that knockdown of p54nrb might inhibit the release of TNF-α from THP1 cells via effecting the exocytosis of TNF-α which depends on cytoskeleton protein microtubules. Cell movement and organelle transport all depends on the activities of cytoskeleton proteins. There may

be some connection between $p54^{nrb}$ silencing inhibiting the motility of THP1 cells and the release of TNF-α from THP1 cells. Moreover, the infection of negative control shRNA-containing lentiviruses promoted the migration and the release of TNF-α induced by LPS in THP1 cells. We speculate that increase of the migration and the release of TNF-α in THP-1 cells infected by control vector lentiviruses is a defensive reaction of THP1 cells to viruses.

Based on the present study, it is suggest that $p54^{nrb}$ slightly inhibited THP1 cell proliferation, but significantly promoted migration, invasion and release of TNF-α in THP1 cells. Our and other recent studies indicate that $p54^{nrb}$ may be a powerful molecule involving in the regulation of cell motility, and it may promote metastasis, invasion of tumor cells and angiogenesis. $p54^{nrb}$ is more likely to promote the release of inflammatory mediators and the motility of inflammatory cells. The occurrence of many tumors is closely related to infection and inflammation (32), and we also speculate involvement in tumor processes and inflammation.

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References

1. Emili A, Shales M, McCracken S, Xie W, Tucker PW, Kobayashi R, Blencowe BJ and Ingles CJ: Splicing and transcription-associated proteins PSF and p54nrb/nonO bind to the RNA polymerase II CTD. RNA 8: 1102-1111, 2002.
2. Peng R, Dye BT, Pérez I, Barnard DC, Thompson AB and Patton JG: PSF and p54nrb bind a conserved stem in U5 snRNA. RNA 8: 1334-1347, 2002.
3. Izumi H, McCloskey A, Shinmyozu K and Ohno M: p54nrb/NonO and PSF promote U snRNA nuclear export by accelerating its export complex assembly. Nucleic Acids Res 42: 3998-4007, 2014.
4. Straub T, Knudsen BR and Boege F: PSF/$p54^{nrb}$ stimulates 'jumping' of DNA topoisomerase I between separate DNA helices. Biochemistry 39: 7552-7558, 2000.
5. Salton M, Lerenthal Y, Wang SY, Chen DJ and Shiloh Y: Involvement of Matrin 3 and SFPQ/NONO in the DNA damage response. Cell Cycle 9: 1568-1576, 2010.
6. Li S, Kuhne WW, Kulharya A, Hudson FZ, Ha K, Cao Z and Dynan WS: Involvement of $p54^{nrb}$, a PSF partner protein, in DNA double-strand break repair and radioresistance. Nucleic Acids Res 37: 6746-6753, 2009.
7. Lu JY and Sewer MB: p54nrb/NONO regulates cyclic AMP-dependent glucocorticoid production by modulating phosphodiesterase mRNA splicing and degradation. Mol Cell Biol 35: 1223-1237, 2015.
8. Naganuma T and Hirose T: Paraspeckle formation during the biogenesis of long non-coding RNAs. RNA Biol 10: 456-461, 2013.
9. Souquere S, Beauclair G, Harper F, Fox A and Pierron G: Highly ordered spatial organization of the structural long noncoding NEAT1 RNAs within paraspeckle nuclear bodies. Mol Biol Cell 21: 4020-4027, 2010.
10. Bond CS and Fox AH: Paraspeckles: Nuclear bodies built on long noncoding RNA. J Cell Biol 186: 637-644, 2009.
11. Zhang Z and Carmichael GG: The fate of dsRNA in the nucleus: A $p54^{nrb}$-containing complex mediates the nuclear retention of promiscuously A-to-I edited RNAs. Cell 106: 465-475, 2001.
12. Qi L, Chan TH, Tenen DG and Chen L: RNA editome imbalance in hepatocellular carcinoma. Cancer Res 74: 1301-1306, 2014.
13. Chen LL and Carmichael GG: Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: Functional role of a nuclear noncoding RNA. Mol Cell 35: 467-478, 2009.
14. Weissbach R and Scadden AD: Tudor-SN and ADAR1 are components of cytoplasmic stress granules. RNA 18: 462-471, 2012.
15. Osenberg S, Dominissini D, Rechavi G and Eisenberg E: Widespread cleavage of A-to-I hyperediting substrates. RNA 15: 1632-1639, 2009.
16. Desai SS, Kharade SS, Parekh VI, Iyer S and Agarwal SK: Pro-oncogenic roles of HLXB9 protein in insulinoma cells through interaction with Nono protein and down-regulation of the c-Met inhibitor Cblb (casitas B-lineage lymphoma b). J Biol Chem 290: 25595-25608, 2015.
17. Hof D, Cheung K, Roossien HE, Pruijn GJ and Raats JM: A novel subtractive antibody phage display method to discover disease markers. Mol Cell Proteomics 5: 245-255, 2006.
18. Hallier M, Tavitian A and Moreau-Gachelin F: The transcription factor Spi-1/PU.1 binds RNA and interferes with the RNA-binding protein p54nrb. J Biol Chem 271: 11177-11181, 1996.
19. Hu J, Hong X, Li Z and Lu Q: Acute monocytic leukaemia with t(11;12) (p15;q13) chromosomal changes: A case report and literature review. Oncol Lett 10: 2307-2310, 2015.
20. Hall PA, Levison DA, Woods AL, Yu CC, Kellock DB, Watkins JA, Barnes DM, Gillett CE, Camplejohn R, Dover R, *et al*: Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: An index of cell proliferation with evidence of deregulated expression in some neoplasms. J Pathol 162: 285-294, 1990.
21. Schiffner S, Zimara N, Schmid R and Bosserhoff AK: $p54^{nrb}$ is a new regulator of progression of malignant melanoma. Carcinogenesis 32: 1176-1182, 2011.
22. Kowalska E, Ripperger JA, Hoegger DC, Bruegger P, Buch T, Birchler T, Mueller A, Albrecht U, Contaldo C and Brown SA: NONO couples the circadian clock to the cell cycle. Proc Natl Acad Sci USA 110: 1592-1599, 2013.
23. Dominissini D, Moshitch-Moshkovitz S, Amariglio N and Rechavi G: Adenosine-to-inosine RNA editing meets cancer. Carcinogenesis 32: 1569-1577, 2011.
24. Chan TH, Lin CH, Qi L, Fei J, Li Y, Yong KJ, Liu M, Song Y, Chow RK, Ng VH, *et al*: A disrupted RNA editing balance mediated by ADARs (Adenosine DeAminases that act on RNA) in human hepatocellular carcinoma. Gut 63: 832-843, 2014.
25. Chen L, Li Y, Lin CH, Chan TH, Chow RK, Song Y, Liu M, Yuan YF, Fu L, Kong KL, *et al*: Recoding RNA editing of AZIN1 predisposes to hepatocellular carcinoma. Nat Med 19: 209-216, 2013.
26. Beghini A, Ripamonti CB, Peterlongo P, Roversi G, Cairoli R, Morra E and Larizza L: RNA hyperediting and alternative splicing of hematopoietic cell phosphatase (PTPN6) gene in acute myeloid leukemia. Hum Mol Genet 9: 2297-2304, 2000.
27. Kim YM, Seo J, Kim YH, Jeong J, Joo HJ, Lee DH, Koh GY and Lee KJ: Systemic analysis of tyrosine phosphorylated proteins in angiopoietin-1 induced signaling pathway of endothelial cells. J Proteome Res 6: 3278-3290, 2007.
28. Ainaoui N, Hantelys F, Renaud-Gabardos E, Bunel M, Lopez F, Pujol F, Planes R, Bahraoui E, Pichereaux C, Burlet-Schiltz O, *et al*: Promoter-dependent translation controlled by $p54^{nrb}$ and hnRNPM during myoblast differentiation. PLoS One 10: e0136466, 2015.
29. Yuan T, Liu L, Zhang Y, Wei L, Zhao S, Zheng X, Huang X, Boulanger J, Gueudry C, Lu J, *et al*: Diacylglycerol guides the hopping of clathrin-coated pits along microtubules for exo-endocytosis coupling. Dev Cell 35: 120-130, 2015.
30. Li-Villarreal N, Forbes MM, Loza AJ, Chen J, Ma T, Helde K, Moens CB, Shin J, Sawada A, Hindes AE, *et al*: Dachsous1b cadherin regulates actin and microtubule cytoskeleton during early zebrafish embryogenesis. Development 142: 2704-2718, 2015.
31. Graffe M, Zenisek D and Taraska JW: A marginal band of microtubules transports and organizes mitochondria in retinal bipolar synaptic terminals. J Gen Physiol 146: 109-117, 2015.
32. Grivennikov SI, Greten FR and Karin M: Immunity, inflammation, and cancer. Cell 140: 883-899, 2010.