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Original Article

TANK-binding kinase 1 inhibitor GSK8612 enhances daunorubicin sensitivity in acute myeloid leukemia cells via the AKT-CDK2 pathway

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Abstract: Purpose: It has been established in previous studies that TANK-binding kinase 1 (TBK1) is upregulated in malignant tumors and is therefore associated with poor prognosis. However, the role of TBK1 in acute myeloid leukemia (AML) remains unclear. In this study, we investigated the expression levels and the function of TBK1 in AML. Methods: First, TBK1 expression was detected and analyzed using Western blot and qRT-PCR. Then, GSK8612, a novel TBK1 inhibitor, and TBK1-specific siRNA (si-TBK1) were used to inhibit TBK1 function and expression. The effects of TBK1 inhibition on AML were investigated first through a cell counting kit (CCK-8) assay, followed by trypan blue staining to assess cell apoptosis and cell cycle progression *in vitro*. Finally, the signaling pathway activities in HL-60 and Kasumi-1 cells and patients' mononuclear cells (MNCs) were explored using western blot. Results: We found a significantly higher TBK1 expression in AML patients with poor prognoses. GSK8612 successfully inhibited TBK1 expression, resulting in the increased sensitivity of AML cells to daunorubicin. Mechanistically, TBK1 inhibition (by GSK8612 and si-TBK1) regulated cyclin-dependent kinase 2 (CDK2) levels in AML cells via the AKT pathway. Moreover, it was observed that the inhibition of protein kinase B (AKT) activity also resulted in the increased sensitivity of AML cell lines to daunorubicin, validating the relationship between TBK1 and the AKT-CDK2 pathway. Similar results were obtained in MNCs from patients with AML. Conclusion: TBK1 is a potential prognostic factor for AML, and its inhibition may improve the sensitivity of AML cells to daunorubicin. This regulatory effect is predicted to involve the TBK1-AKT-CDK2 pathway.

Keywords: Acute myeloid leukemia, resistance, apoptosis, daunorubicin, GSK8612, TBK1, CDK2

Introduction

Among the different kinds of adult leukemia, AML has the highest morbidity and mortality rates. Currently, the primary treatment for AML is still chemotherapy, including daunorubicin and cytarabine treatment [1]. However, more than half of patients fail to achieve remission due to intrinsic and acquired anticancer drug resistance [2, 3]. Therefore, it is necessary to elucidate the molecular mechanisms underlying chemotherapy resistance to develop more effective treatment regimens.

TBK1, a Ser/Thr kinase, plays an important role in innate immunity. Apart from its well-established role in the coordination of innate immune signaling, further studies are gradually unraveling the diverse functions of TBK1, including the regulation of inflammatory responses, autophagy, and the cell cycle [4-6]. In particular, TBK1 inhibition may inhibit the mitotic process, resulting in aberrations in mitotic structures and reduced cell viability [7]. A growing body of literature has shown that TBK1 is overexpressed in various cancers and may be considered one of the driving factors of cancer progression [8]. However, the specific role of TBK1 in AML biology and its molecular mechanisms remain unknown. For this reason, this study aimed to investigate the role of TBK1 in AML and validate

Table 1. Characteristics of acute myeloid leukemia patients and healthy donors

	Health donor	Complete remission	Relapse/ refractory	*P* value
Number of samples	20	22	22	
Sex				0.561
Female	8 (40.0%)	9 (40.9%)	12 (54.5%)	
Male	12 (60.0%)	13 (59.1%)	10 (45.5%)	
Age (years)				0.358
Median	35.5	38	44.5	
Range	(18-57)	(19-65)	(6-71)	

Differences between groups were determined using the one-way ANOVA, with P<0.05 considered as statistically significant.

whether TBK1 inhibition is associated with AML prognosis.

In order to study the role of TBK1 in AML, GSK8612, a new type of TBK1 inhibitor, was used. This inhibitor exhibits high selectivity and efficiency and is reported to bind strongly with mouse, rat, and human proteins in the blood, with an average pK_d of 8.0 against TBK1 [9].

TBK1 has been reported to be associated with the AKT pathway, particularly in regulating osteoclast differentiation and function [10]. Additionally, it has been observed that the AKT pathway is abnormally activated in most patients with AML and plays a role in AML chemotherapy resistance [11]. Since the AKT pathway is known to influence cancer cell growth and survival, we inferred that TBK1 might affect AML prognosis via the AKT pathway.

Previous studies have also shown that the AKT pathway may ultimately affect CDK2, which is highly expressed in anthracycline-resistant AML cells [12]. CDK2 is a known regulator of cell cycle progression, particularly in the G0/G1 to the S phase. Moreover, CDK2 expression has been observed to be positively correlated with cell proliferation.

In this study, we investigated the role of TBK1 in AML by analyzing the effects of TBK1 inhibition in bone marrow samples. Then, we explored possible pathways that may be involved in these functions.

Materials and methods

Patients and donors

Bone marrow samples were obtained from 44 patients with AML (M3 patients were excluded) at the Affiliated Hospital of Guiyang Medical University (Guiyang, Guizhou Province, China) from February 2019 to January 2020. Their clinical characteristics are summarized in **Table 1**. All experiments were approved by the ethics committee, and all the patients and healthy donors (HDs) involved have provided signed consent forms (ethical number: Guizhou Medical University, 2019-123).

Inclusion criteria: Patients newly diagnosed with AML; Patients with basically normal functions of important organs; Patients with no previous history of other tumor diseases. Complete remission (CR): bone marrow blasts <5% after receiving 2 courses of intensive chemotherapy. Relapse/refractory (R/R): refractory: patients who failed to achieve CR after receiving at least 2 courses of intensive chemotherapy; relapse: bone marrow blasts ≥5% after CR.

Exclusion criteria: Patients not newly diagnosed with AML; AML patients had experienced radiotherapy or chemotherapy; Patients with other complex and serious diseases; Patients with no support for follow-up treatment. Patients wiht AML-M3 (The chemotherapy regimen of AML-M3 is quite different from other AML, and the cure rate of AML-M3 is significantly higher than that of other AML).

Samples were collected at the time of initial diagnosis. We stored samples at -80°C and followed up the treatment outcomes. In this study, Percoll density gradient centrifugation was adapted to isolate mononuclear cells from bone marrow samples, with primary AML cells included. Meanwhile, following G-CSF mobilization, primary normal hematopoietic cells were isolated from the peripheral blood stem cells (PBSC) donated by HDs for allogeneic stem cell transplantation and the treatment outcomes were followed up.

Sample preparation

GSK8612 and Akti-1/2 were obtained from Selleck Chemicals (Houston, Texas, USA), daunorubicin from Solarbio (Beijing, China), and anti-AKT, anti-P-Akt and anti-β-actin antibodies from Cell Signaling Technology (Danvers, MA, USA). Samples were prepared by staining with different antibodies against TBK1, P-TBK1,

CDK2, Bcl-2, and cleaved-caspase-3 from Santa (Heidelberg, Germany).

Cell culture and maintenance

Obtained from the Hematology Laboratory of Affiliated Hospital of Guiyang Medical University, the human myeloid leukemia cell lines Kasumi-1, HL-60 and THP-1 were incubated at 37°C and 5% CO_2 in RPMI-1640 medium with 10% FBS (Gibco BRL, MD, USA), penicillin (100 units/mL) and streptomycin (100 mg/mL) (Carlsbad, CA, USA).

Cell viability assay

Cells were inoculated in a 96-well plate and incubated overnight before treatment with 100 nmol daunorubicin plus 0, 1, 2 and 4 μM GSK8612 for 24 hours, and control cells were without any treatment. Next, a CCK-8 assay was performed to analyze cell viability.

Apoptosis assay

AML cells were inoculated at 3×10^5 cells/well in culture plates and incubated overnight before treatment with daunorubicin (100 nmol) and GSK8612 (0, 1, 2 and 4 μM) for 24 hours. The number of dead cells was assessed via trypan blue staining. And the rate of cell apoptosis was measured through propidium iodide (PI) and annexin V staining according to the manufacture's instructions (7sea biotech, Shanghai, China). Microscopy images were assessed under a microscope (Zeiss, Germany). Data acquired were further analyzed via the Cell Quest software (BD Biosciences, CA, USA).

RNA extraction and quantification

First, RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Then, qRT-PCR was performed using the RNeasy Kit (Qiagen, Hilden, Germany) and the Omniscript Reverse Transcription Kit (Qiagen).

Primers listed were provided by Airui Technology Corporation (Guiyang, China) and iQ SYBR Green Supermix (Bio-Rad, Singapore): β-actin-F 5'-GAGACCTTCAACACCCCAGC-3', β-actin-R 5'-ATGTCACGCACGATTTCCC-3', TBK1-F 5'-TGGGTGGAATGAATCATCTACGA-3', TBK1-R 5'-GCTGCACCAAAATCTGTGAGT-3', CDK2-F 5'-TGAGATTGACCAGCTCTTCCGG-3', and CDK2-R 5'-ATCTTCATCCAGGGGAGGTACAAC-3'.

Western blotting

Cells from various groups were first collected and washed (ice-cold PBS), followed by ultrasonic lysis in RIPA buffer for protein extraction. Afterward, the lysates were transferred into EP tubes and centrifuged for 10 mins at 12,000×g and 4°C to obtain the supernatant. Then, the supernatant was mixed with the loading buffer, heated until boiling and stored at -80°C. Separation was performed through PAGE (polyacrylamide gel electrophoresis) in accordance with the standard protocol, followed by the transfer to PVDF membranes (Millipore Corporation, Milford, MA, USA) for western blot analysis. Consecutively, the membranes were blocked using Tris buffer with 5% skimmed milk for 2 h and washed. The primary antibodies were incubated with the membranes for 2 h. Anti-AKT, anti-P-Akt and anti-β-actin were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-TBK1, anti-P-TBK1, anti-CDK2, anti-Bcl-2 and anti-cleaved-caspase3 were purchased from Santa (Heidelberg, Germany). Anti-β-actin was diluted using a ratio of 1:2000, others were of 1:1000. After washing, secondary antibodies were added, followed by incubation at 37°C for 45 mins. The protein bands were visualized via chemiluminescence (7sea Biotech, Shanghai, China) and analyzed using ImageJ software (National Institutes of Health, USA). The expression levels of related proteins were quantified and normalized against β-actin.

Cell cycle analysis

One day after GSK8612 treatment (0, 1, 2, and 4 μM), the AML cells were fixated using 70% ice-cold ethanol overnight at 4°C. After washing with PBS, a mixture of RNase and PI was added, followed by a 30-min incubation at 37°C in the dark. Next, data were acquired through flow cytometry (FCM) (FACScan, BD, Franklin Lakes, NJ, USA) and analyzed using the Cell Quest Pro software (BD Biosciences) to assess cell cycle progression.

siRNA transfection

In this study, we used siRNA against TBK1 (si-TBK1) to downregulate the expression of

endogenous TBK1. The siRNA sequence (si-TBK1: 5'-GGUAUAGUGCACCGUGAUA-3'), designed and synthesized by Transheep Biotech (Shanghai, China), was used to transfect the cells, following the manufacturer's protocol. Scrambled siRNA (si-NC: 5'-UUCUCCGAACGUGUCACGUTT-3') served as the negative control for this experiment. Gene expression for both the treatment and the control setup was then examined 48 h post-transfection.

Statistical analysis

Data analysis was performed using GraphPad Prism 8.0 software. Results were presented as mean ± standard deviation (SD). Differences between groups were determined using the t-test or one-way ANOVA, with $P<0.05$ considered as statistically significant.

Results

Poor prognosis in patients with AML was associated with high TBK1 expression

In this study, the transcription levels of TBK1 in the bone marrow of 44 patients with AML were analyzed through qRT-PCR. The same number of samples were collected from each group for optimization, and 20 HDs were included in a control group. Detailed information about the patient representation is presented in **Table 1**. Our results showed that the mRNA expression of TBK1 was significantly higher in AML patients than in the HDs. Compared with the HDs and complete remission (CR) group of patients, the TBK1 mRNA expression level in the relapse/refractory (R/R) group were the highest (**Figure 1A**). Similar results were also observed in the analysis of TBK1 protein expression levels (**Figure 1B**). In addition to this, it was also found that the expression of TBK1 in the Kasumi-1, HL-60 and THP-1 cell lines was significantly higher than that in the HDs cells. However, compared with HL-60 and Kasumi-1, the expression of TBK1 in THP-1 was lower (**Figure 1C**).

Inhibition of TBK1 increased the sensitivity of AML cells to daunorubicin

Due to our great interest in elucidating the role of TBK1 in patients with AML, we used the new TBK1 inhibitor GSK8612 for further analysis. At the protein level, GSK8612 was observed to significantly reduce the phosphorylation of TBK1 in HL-60 and Kasumi-1 cells. Furthermore, it was also observed that GSK8612 concentration was negatively correlated with P-TBK1 expression in the same cell lines (**Figure 2A**). Our data also showed that decreased TBK1 phosphorylation leads to increased sensitivity of AML cell lines (HL-60, Kasumi-1) to daunorubicin, an anthracycline chemotherapeutic drug, indicating a positive correlation between TBK1 inhibition and daunorubicin sensitivity. Moreover, we found that, compared with daunorubicin alone, the increase in number of dead cells caused by the TBK1 inhibitor combined with daunorubicin was not significant in THP-1 (**Figure 2B**), possibly due to the low basal expression of TBK1 in THP-1 cells (**Figure 1C**). Hence, in order to further examine the changes in cell proliferation and apoptosis induced by daunorubicin and GSK8612, CCK-8 and apoptosis assays were therefore conducted (**Figure 2C**, **2D**). These findings were verified at the protein level through western blot. It was observed that GSK8612 concentration was positively correlated with apoptosis-related cleaved-caspase-3 protein expression but negatively correlated with anti-apoptosis-related Bcl-2 protein expression (**Figure 2E**).

Inhibition of TBK1 phosphorylation resulted in cell cycle arrest at the G0/G1 phase

As previously observed in this study, the sensitivity of AML cells to daunorubicin may be improved by GSK8612 in a dose-dependent manner. However, the mechanisms underlying this phenomenon warrants further study. It has been observed in previous studies that cell cycle arrest is correlated with chemotherapy resistance. Results showed that many inhibitor-treated HL-60 and Kasumi-1 cells were arrested in the G0/G1 phase. Thus, it was inferred that the inhibition of TBK1 phosphorylation might lead to cell cycle arrest in the G0/G1 phase, indicating that inhibiting TBK1 activity in AML cells may, in turn, suppress cell proliferation and improve cell sensitivity towards chemotherapeutic drugs (**Figure 3**).

TBK1 enhanced the sensitivity of AML cells to daunorubicin by regulating the AKT-CDK2 pathway

To further explore the signaling pathway associated with cell cycle arrest, TBK1 knockdown

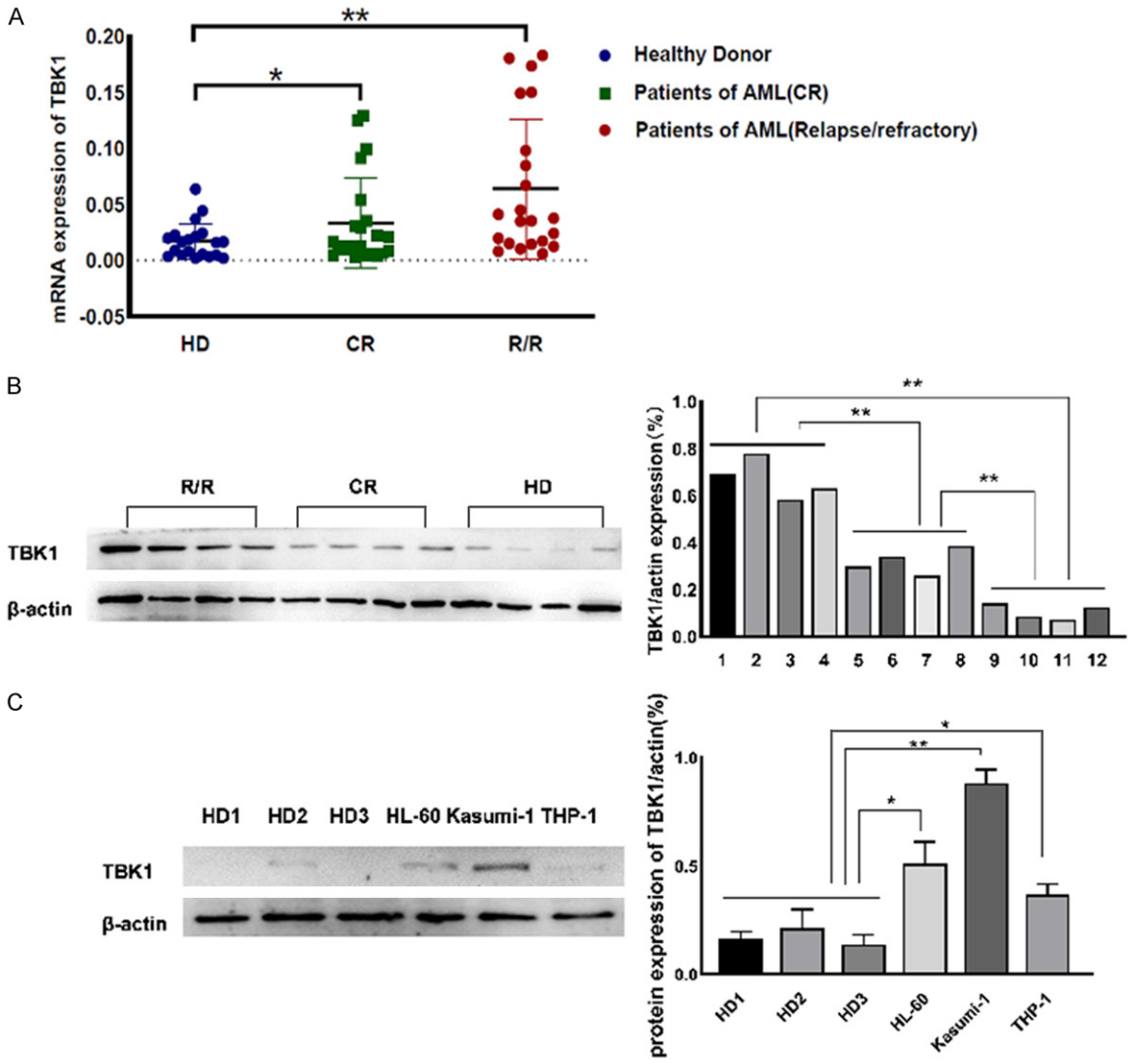
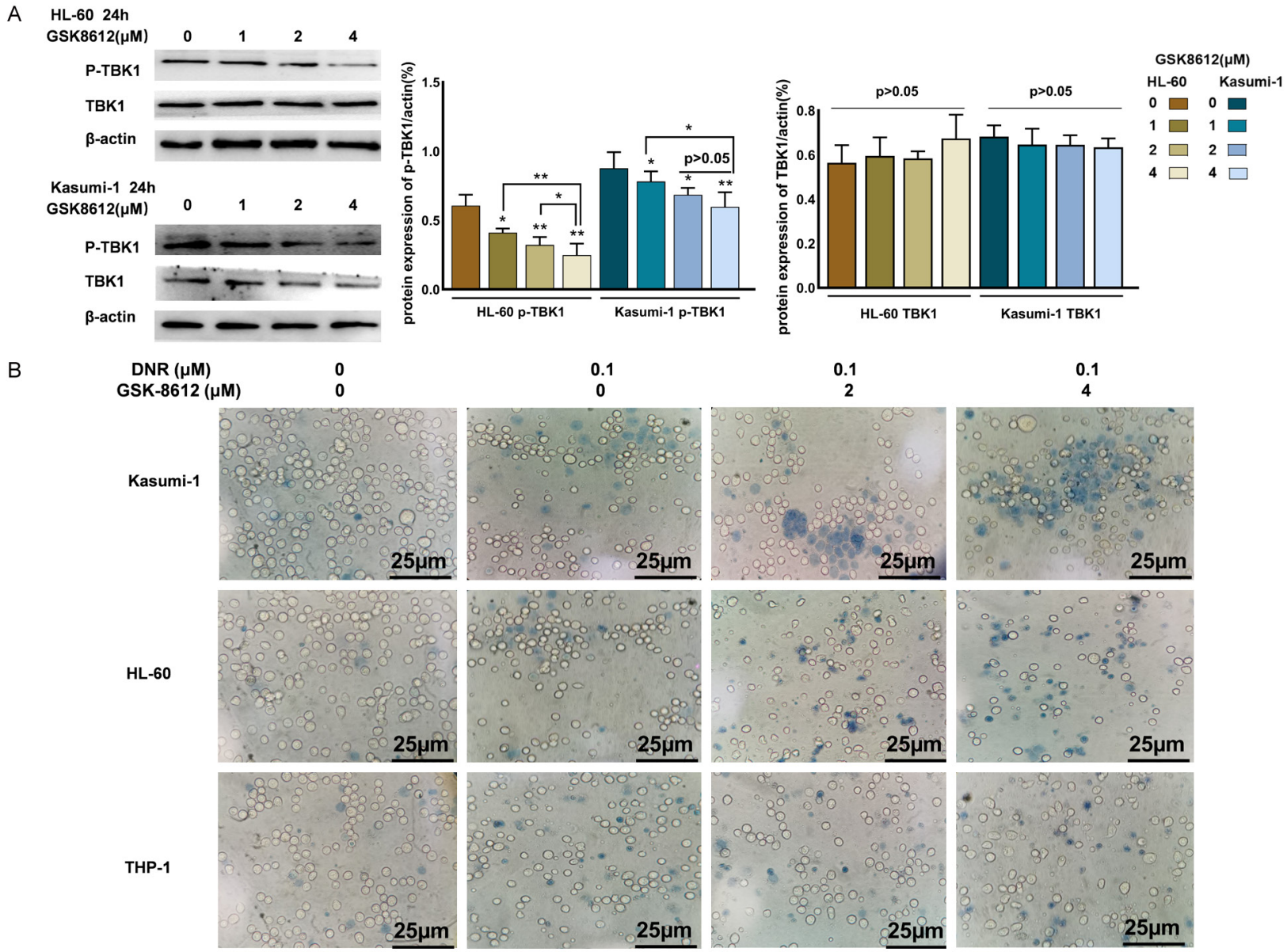
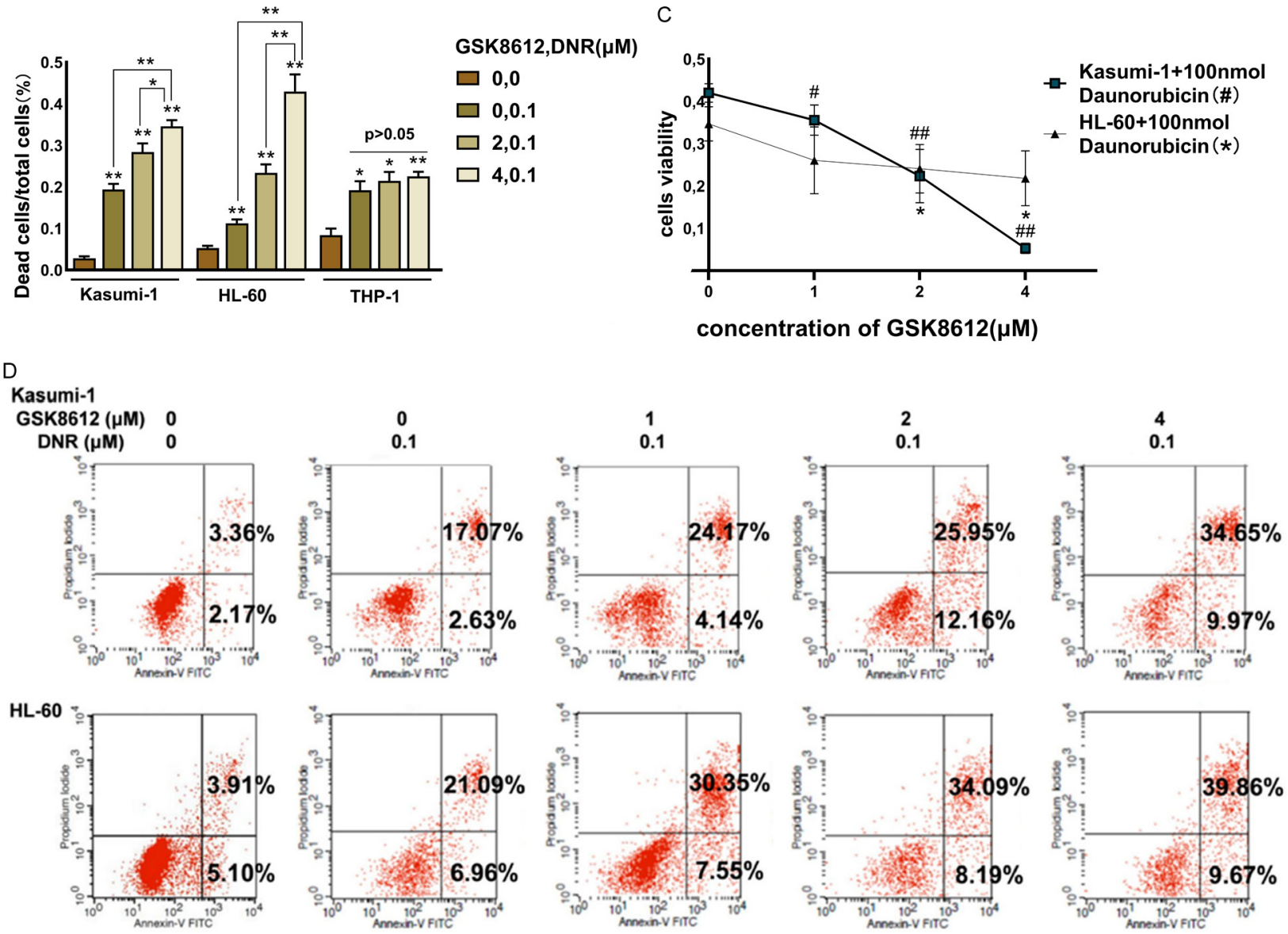


Figure 1. The differential expression of TBK1 in AML patients and AML cell lines. A. TBK1 transcription levels of patients with AML in the R/R and CR were higher than HDs groups. Results were shown as mean ± SD. Statistical analysis was performed by the student's t test. B. Western blotting was performed to detect TBK1 protein expression in AML patients in each group. C. Protein expression of TBK1 in HDs cells (control) was lower than that in THP-1, Kasumi-1, and HL-60 cell lines. β-actin was used as an internal reference. (*P<0.05, **P<0.01).

was performed by using siRNAs that target the TBK1 gene. Kasumi-1 and HL-60 cells were treated with si-TBK1, and si-NC was added as a negative control to exclude possible effects brought by transfection or RNA incorporation (**Figure 4**). After 48 hours of incubation, it was found that si-TBK1 significantly inhibited the mRNA expression of TBK1, whereas si-NC had little effect on TBK1 expression (**Figure 4A**). Next, in order to investigate the downstream genes of TBK1, the expression of DFFA, BID, YWHAZ and CDK2 genes was detected and analyzed in TBK1-knockdown samples (Supplementary Figure 1). Findings revealed a positive association between TBK1 and CDK2 expression, both at the protein and mRNA levels (**Figure 4A**, **4B**). The inhibitory effects of GSK8612 on P-TBK1, P-AKT, and CDK2 were observed to be concentration-dependent, while the total expression of AKT was not significantly different in the control and the TBK1-silenced samples (**Figure 4C**). Therefore, it is hypothesized that CDK2 may be regulated by AKT in AML cells. To verify the upstream and downstream effects of this pathway, Akti-1/2, an AKT inhibitor, was added to inhibit P-AKT. As


A
HL-60 24h
GSK8612(μM) 0 1 2 4
P-TBK1
TBK1
β-actin
Kasumi-1 24h
GSK8612(μM) 0 1 2 4
P-TBK1
TBK1
β-actin
protein expression of p-TBK1/actin(%)
HL-60 p-TBK1
Kasumi-1 p-TBK1
p>0.05
protein expression of TBK1/actin(%)
HL-60 TBK1
Kasumi-1 TBK1
p>0.05
p>0.05
GSK8612(μM)
HL-60 Kasumi-1
B
DNR (μM) 0 0.1 0.1 0.1
GSK-8612 (μM) 0 0 2 4
Kasumi-1
HL-60
THP-1
25μm


Dead cells/total cells(%)
0.5
0.4
0.3
0.2
0.1
0.0
Kasumi-1
HL-60
THP-1
p>0.05
GSK8612,DNR(μM)
0,0
0,0.1
2,0.1
4,0.1
C
cells viability
0,5
0,4
0,3
0,2
0,1
0
1
2
4
concentration of GSK8612(μM)
Kasumi-1+100nmol Daunorubicin(#)
HL-60+100nmol Daunorubicin(*)
D
Kasumi-1
GSK8612 (μM) 0 0 1 2 4
DNR (μM) 0 0.1 0.1 0.1 0.1
3.36%
2.17%
17.07%
2.63%
24.17%
4.14%
25.95%
12.16%
34.65%
9.97%
HL-60
3.91%
5.10%
21.09%
6.96%
30.35%
7.55%
34.09%
8.19%
39.86%
9.67%
Propidium Iodide
Annexin-V FITC

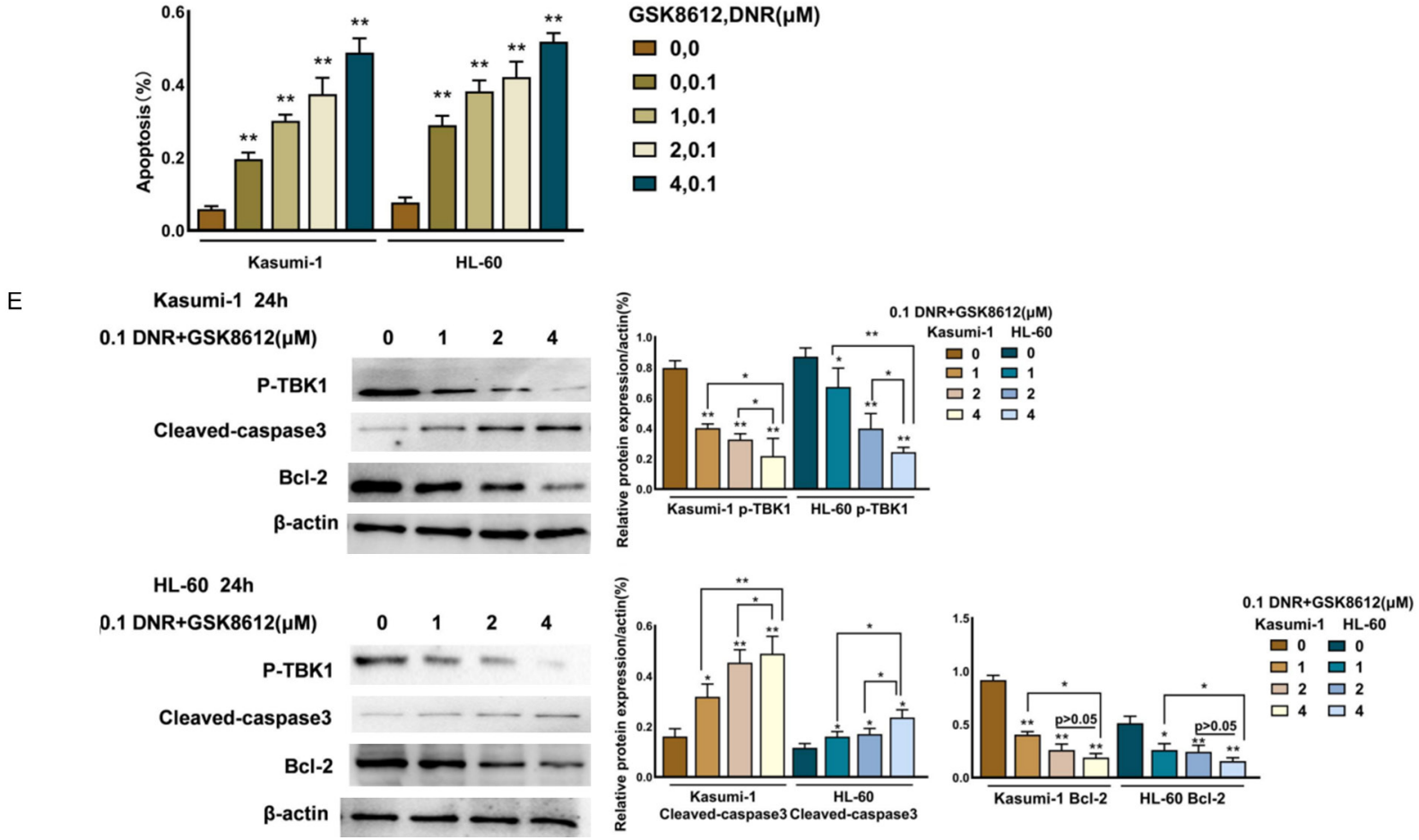


Figure 2. TBK1 inhibition lowered the resistance of AML cells to daunorubicin. A. GSK8612 dose-dependently inhibited TBK1 phosphorylation in AML cells. There was no significant difference in TBK1. B. Number of dead cells with 0.1 μM daunorubicin and 0, 1, 2, and 4 μM GSK8612 in HL-60 and Kasumi-1 cells. Magnification of microscopic figure is 400×. The scale bar of magnification is 400-fold. C. CCK-8 assay was conducted to analyze the cell viability. The result indicated that p-TBK1 down-regulation could enhance the daunorubicin-induced cell lethality. D. FCM was conducted to assess apoptosis progression. The result indicated that down-regulation of TBK1 could significantly attenuate the daunorubicin-induced apoptotic rate. E. The changes in protein level of TBK1, cleaved-caspase-3, and Bcl-2 were determined after treating with DNR plus GSK8612 compared with control groups. All experiments were performed in three trials, and the data were expressed as the mean ± SD. *P<0.05, **P<0.01, $^{\#}$P<0.05, $^{\#\#}$P<0.01. Statistical analysis was performed by the student's t test. β-actin was used as the loading control.

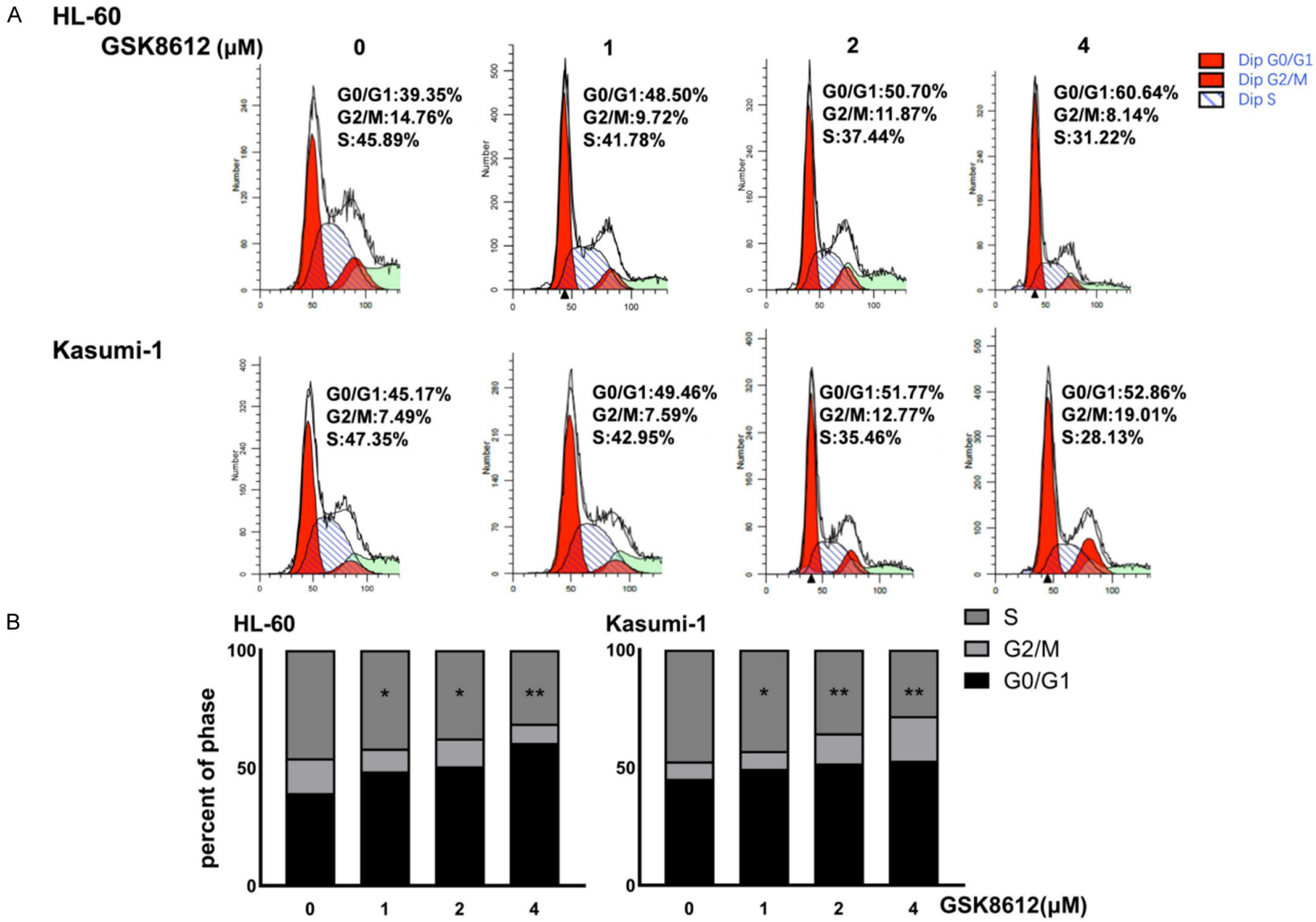
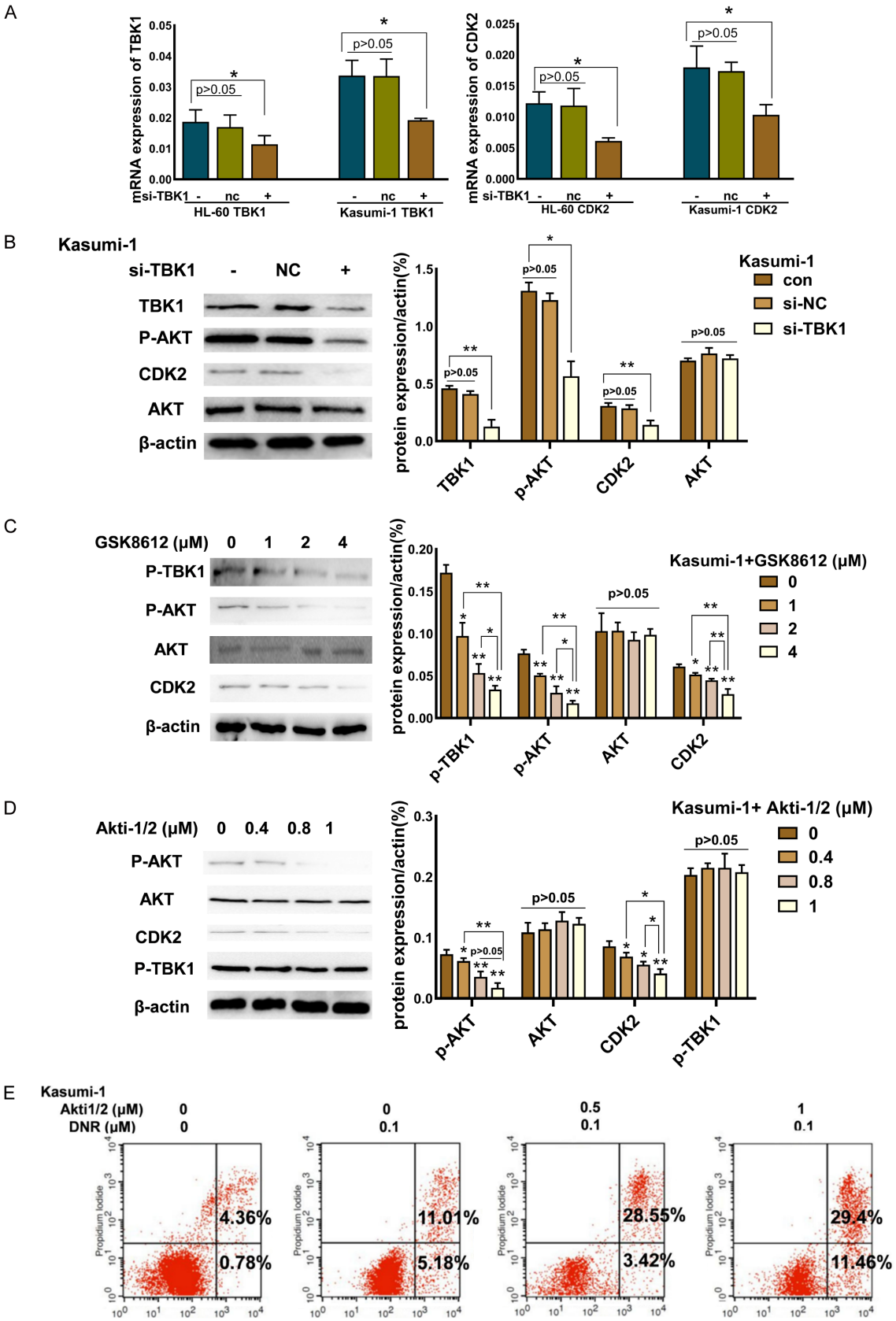


Figure 3. Inhibition of TBK1 phosphorylation in AML cells led to cell cycle arrest in the G0/G1 phase. A. Cell cycle progression in HL-60 and Kasumi-1 cells 24 hours post-GSK8612 treatment (0, 1, 2, and 4 μM). B. The number of cells in the S phases was compared with the control group. Data were shown as mean ± SD. Statistical analysis was performed by the student's t test. All experiments were run in three trials. $^{*}P<0.05$, $^{**}P<0.01$.


A
mRNA expression of TBK1
si-TBK1 - nc +
HL-60 TBK1
Kasumi-1 TBK1
mRNA expression of CDK2
HL-60 CDK2
Kasumi-1 CDK2
p>0.05
B
Kasumi-1
si-TBK1 - NC +
TBK1
P-AKT
CDK2
AKT
β-actin
protein expression/actin(%)
Kasumi-1
con
si-NC
si-TBK1
C
GSK8612 (μM) 0 1 2 4
P-TBK1
P-AKT
AKT
CDK2
β-actin
Kasumi-1+GSK8612 (μM)
D
Akti-1/2 (μM) 0 0.4 0.8 1
Kasumi-1+ Akti-1/2 (μM)
E
Kasumi-1
Akti1/2 (μM) 0 0 0.5 1
DNR (μM) 0 0.1 0.1 0.1
Propidium Iodide
4.36%
0.78%
11.01%
5.18%
28.55%
3.42%
29.4%
11.46%

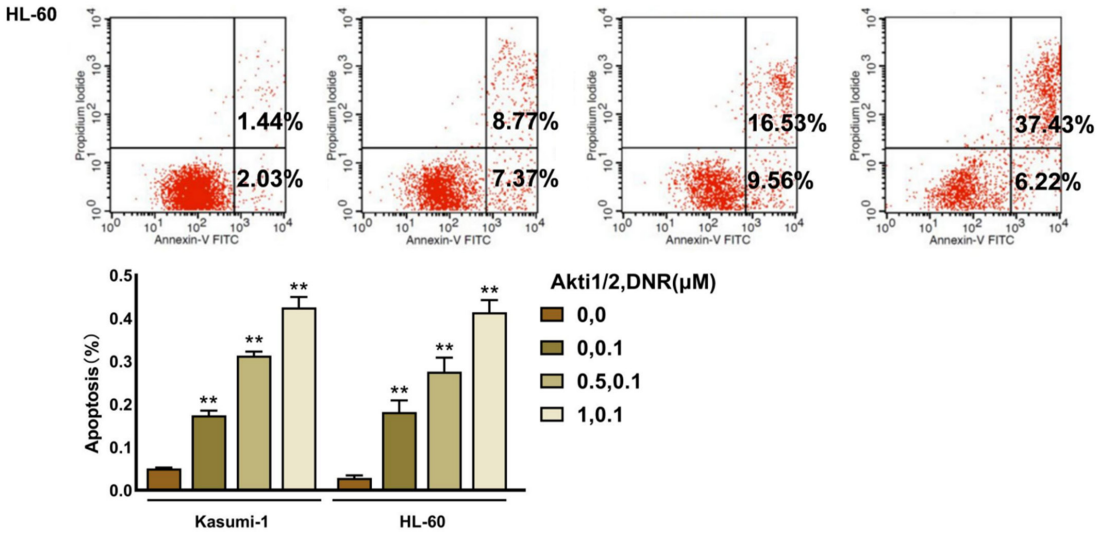


Figure 4. TBK1-AKT-CDK2 pathway in AML cell lines. A. si-TBK1 significantly lowered the expression of TBK1 and CDK2 at the mRNA level in HL-60 and Kasumi-1 cells. B. Protein levels of TBK1, AKT, P-AKT, and CDK2 were assessed through Western blotting after si-TBK1 treatment. C. Protein levels after treatment with GSK8612 (0, 1.0, 2.0 and 4.0 μM). Western blot analysis showed that the expression levels of p-TBK1, p-AKT and CDK2 proteins were decreased and the expression levels of AKT were not significantly different from the control group. D. Akti-1/2 reduced the expression of P-AKT and CDK2, while p-TBK1 expression did not change significantly after treatment with inhibitor. E. Treatment with Akti-1/2 plus daunorubicin resulted in a higher rate of AML cell apoptosis compared to treatment with daunorubicin alone. All experiments were performed in three trials. $^{*}P<0.05$, $^{**}P<0.01$. Statistical analysis was performed by the student's t test.

observed, the expression levels of CDK2 decreased with decreasing P-AKT expression, while there was no significant difference in terms of P-TBK1 expression in the control and AKT-inhibited samples (**Figure 4D**). Therefore, it is suggested that TBK1 regulates CDK2 through the AKT signaling pathway. To further confirm whether TBK1 promotes daunorubicin resistance through the AKT-CDK2 pathway, we also used Akti-1/2 in our apoptosis experiments. It was observed in this study that the inhibition of the AKT pathway increased the rates of daunorubicin-induced AML cell apoptosis (**Figure 4E**). Hence, it is inferred that TBK1 inhibition enhances AML sensitivity to daunorubicin through the AKT-CDK2 pathway.

GSK8612 inhibited TBK1-AKT-CDK2 expression in AML-afflicted MNCs

To verify whether AML patients exhibit similar patterns, the same experiments were performed using MNCs from patients with AML (**Figure 5**). Similarly, GSK8612 effectively inhibited TBK1 in the MNCs of five patients with AML (**Figure 5A**). Furthermore, we also found that inhibiting TBK1 activity in MNCs inhibited CDK2 expression through the AKT pathway (**Figure 5B**, **5C**). Therefore, we conclude that GSK8612 may inhibit TBK1 activity and improve AML cell sensitivity to daunorubicin through the TBK1-AKT-CDK2 pathway.

Discussion

AML is an aggressive cancer that affects thousands of people every year. Despite the efforts in the clinical management of AML, prognosis remains poor, with high mortality and recurrence rates possibly due to chemotherapy resistance. Therefore, detailing the mechanisms underlying drug resistance in AML and developing new therapeutic regimens may be the key to reversing drug resistance in AML patients and improving the efficacy of chemotherapy.

TBK1 is overexpressed in multiple malignancies such as breast, colon, lung, and pancreatic cancers, as well as in hepatocellular carcinoma (HCC) [13-16]. In HCC, TBK1 overexpression is markedly correlated with clinical stage and pathological grade [17]. Similarly, TBK1 expression is strongly linked to the prognosis

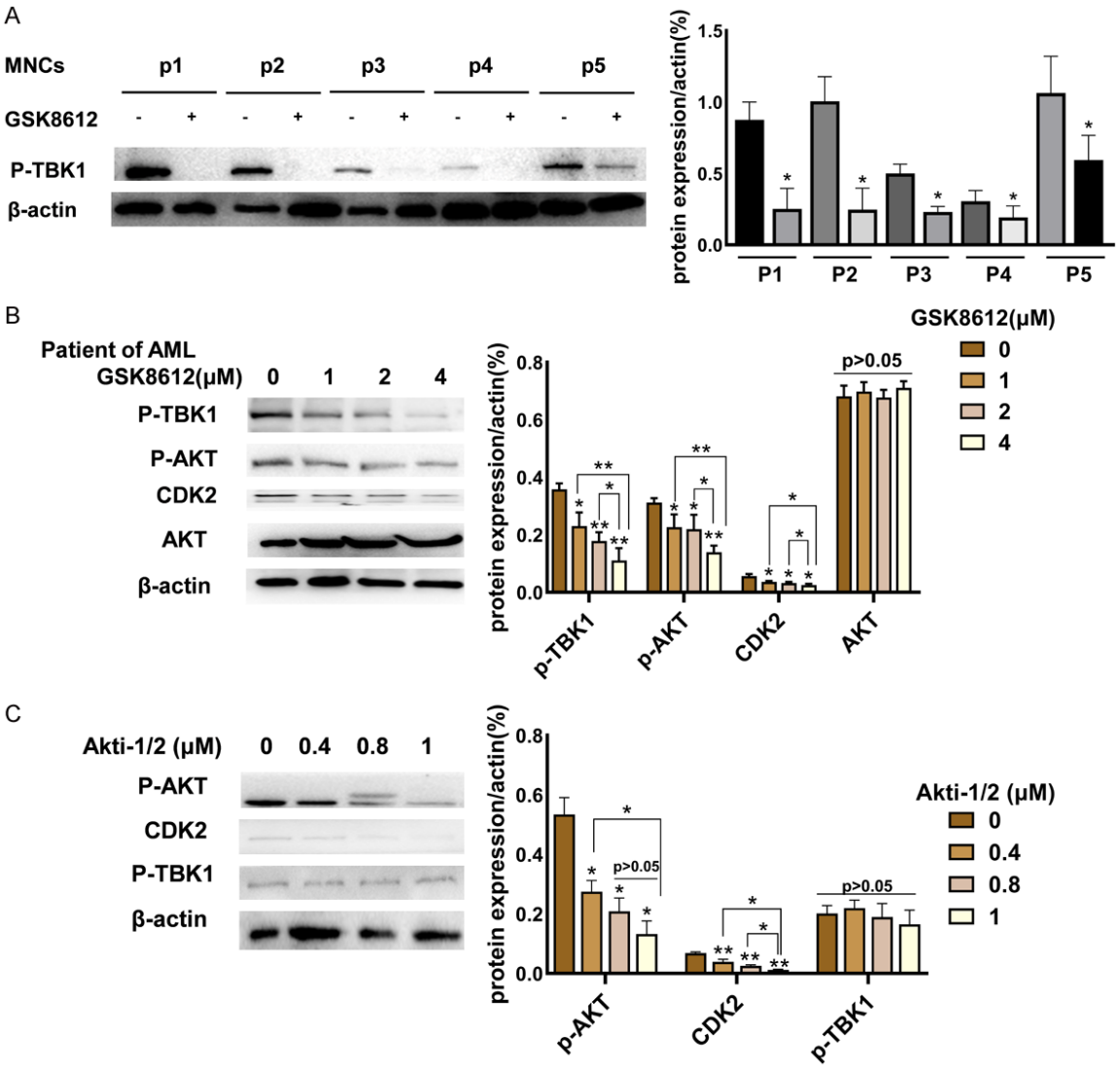


Figure 5. The TBK1-AKT-CDK2 pathway in the MNCs of patients with AML. A. GSK8612 (4.0 μM) downregulated P-TBK1 protein expression in the MNCs of patients with AML. B, C. Similarly, it was also found that the TBK1-AKT-CDK2 pathway was involved in the MNCs of patients with AML. All experiments were performed thrice. *P<0.05, **P<0.01. Statistical analysis was performed by the student's t test.

of esophageal carcinoma [18]. However, despite the ongoing efforts to examine the role of TBK1 in different cancers, its mechanisms and functions in AML progression are not yet well characterized. This study showed that the TBK1 expression was significantly higher in patients with relapsed or refractory AML than that in patients with CR, indicating that TBK1 may be a prognostic factor for AML. Furthermore, a significantly negative correlation was also observed between TBK1 activity and the sensitivity of AML cells to daunorubicin.

TBK1 is associated with NuMa and CEP170, both of which are essential for mitosis [19]. Therefore, we propose that TBK1 inhibition will, in turn, inhibit mitosis, resulting in reduced cancer cell proliferation. Our findings revealed that GSK8612 inhibited TBK1 in a dose-dependent manner and induced the cycle arrest of AML cells.

CDK2 is a well-known cell cycle-related protein. Researchers have recently discovered the diverse functions of CDK2, especially in cancer. Previous studies have demonstrated a close association between CDK2 overexpression and poor prognosis of malignant tumors, including breast, glioma, kidney, thyroid adrenal cortex, and prostate cancers [20, 21].

Moreover, it has been observed that CDK2 expression is related to the drug resistance of tumors to all-trans-retinoic acid, palbociclib, tamoxifen, and daunorubicin [22-24]. Our study found a positive correlation between CDK2 expression and TBK1 phosphorylation and that the inhibition of the TBK1-CDK2 signaling pathway may enhance the sensitivity of AML cells to daunorubicin.

In addition, the activation of the AKT pathway, a key pathway related to tumor cell survival [25, 26], was found to be positively correlated with TBK1 and CDK2 expression. By using the AKT inhibitor Akti-1/2, it has been demonstrated that the TBK1-AKT-CDK2 pathway is involved in AML, particularly by enhancing sensitivity to daunorubicin. Similar results were also obtained in examining the effects of TBK1 inhibition in MNCs from patients with AML.

Nevertheless, the elucidation of mechanisms by which TBK1 inhibition increases the sensitivity of AML cells to daunorubicin warrants further studies. This study found a correlation between TBK1 and AML resistance to daunorubicin, revealed the relationship between CDK2 and TBK1, and further explored the molecular mechanisms underlying AML resistance to daunorubicin. Moreover, we found a signaling pathway that promotes the resistance of AML cells to daunorubicin as observed in patients and may catalyze the development of new targeted therapies that address daunorubicin resistance in AML patients. In later experiments, lentivirus transfection and gene knockout mice could be used to further explore the functions of TBK1.

Conclusion

There is an association between high TBK1 expression and poor AML prognosis. TBK1 inhibition downregulates CDK2 expression through the AKT pathway, leading to AML cell cycle arrest. Consequently, this leads to the inhibition of AML cell proliferation, and enhanced sensitivity to daunorubicin (Supplementary Figure 2). This study sheds light on the possible role and mechanism of TBK1 in the observed drug resistance in patients with AML and confirms that TBK1 is a potential target for AML treatment. Furthermore, we have provided a possible target for the future investigation of AML prognosis and the reversal of chemotherapy resistance in patients with AML.

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Disclosure of conflict of interest

None.

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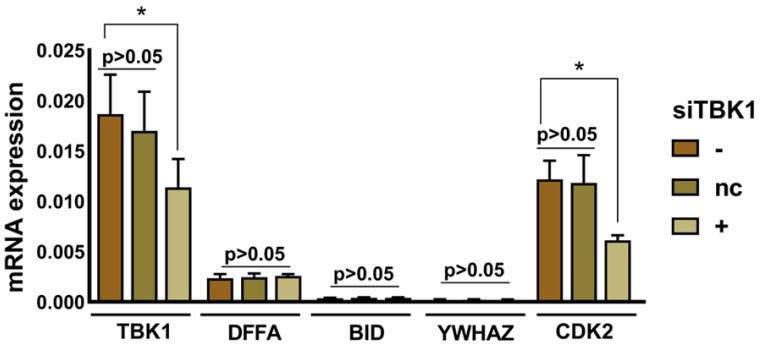
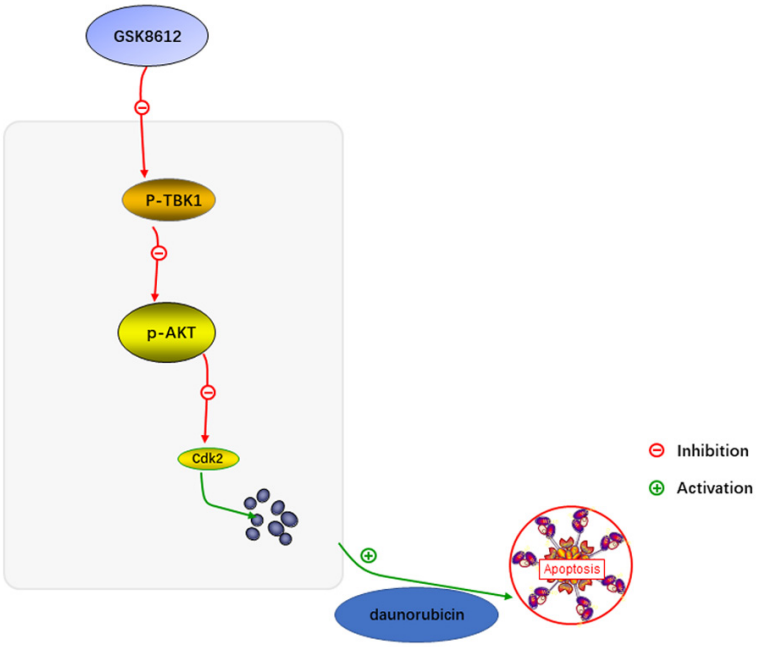


Supplementary Figure 1. The mRNA expression level of TBK1, DFFA, BID, YWHAZ and CDK2 in HL-60 cells after si-TBK1 treatment. *P<0.05, **P<0.01. Statistical analysis was performed by the student's t test.



Supplementary Figure 2. Mechanism schematic diagram.