DNMT3A R882H mutation drives daunorubicin resistance in acute myeloid leukemia via regulating NRF2/NQO1 pathway

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Abstract

Background: DNA methyltransferase 3A (DNMT3A) often mutate on arginine 882 (DNMT3A^{R882}) in acute myeloid leukemia (AML). AML patients with DNMT3A R882 mutation are usually resistant to daunorubicin treatment; however, the associated mechanism is still unclear. Therefore, it is urgent to investigate daunorubicin resistance in AML patients with DNMT3A R882 mutant.

Method: AML cell lines with DNMT3A-wild type (DNMT3A-WT), and DNMT3A-Arg882His (DNMT3A-R882H) mutation were constructed to investigate the role of DNMT3A R882H mutation on cell proliferation, apoptosis and cells' sensitivity to Danunorubin. Bioinformatics was used to analyze the role of nuclear factor-E2-related factor (NRF2) in AML patients with DNMT3A R882 mutation. The regulatory mechanism of DNMT3A R882H mutation on NRF2 was studied by Bisulfite Sequencing and CO-IP. NRF2 inhibitor Brusatol (Bru) was used to explore the role of NRF2 in AML cells carried DNMT3A R882H mutation.

Results: AML cells with a DNMT3A R882H mutation showed high proliferative and anti-apoptotic activities. In addition, mutant cells were less sensitive to daunorubicin and had a higher NRF2 expression compared with those in WT cells. Furthermore, the NRF2/NQO1 pathway was activated in mutant cells in response to daunorubicin treatment. DNMT3A R882H mutation regulated the expression of NRF2 via influencing protein stability rather than decreasing methylation of NRF2 promoter. Also, NRF2/NQO1 pathway inhibition improved mutant cells' sensitivity to daunorubicin significantly.

Conclusion: Our findings identified NRF2 as an important player in the regulation of cell apoptosis through which helps mediate chemoresistance to daunorubicin in AML cells with DNMT3A R882H mutation. Targeting NRF2 might be a novel therapeutic approach to treat AML patients with a DNMT3A R882H mutation.

Keywords: AML, DNMT3A R882H mutation, Daunorubicin, Chemoresistance, NRF2

Introduction

Acute myeloid leukemia (AML) is a type of malignant blood disease characterized by a rapid development and treatment inefficiency, recurrence and multidrug resistance in AML still remain to be resolved [1]. The tumor suppressor, DNA methyltransferase 3A (DNMT3A) [2], is involved in regulating DNA methylation. Studies have confirmed that about 20% AML patients are

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accompanied with DNMT3A mutations [3]. DNMT3A mutations contain several point mutations, R882H and R882C mutations being the most common mutations [4]. Recent studies identified that DNMT3A R882 mutation is associated with reoccurrence, incomplete response rate, and low average survival time in AML patients. This makes DNMT3A R882 mutation a dependent prognosis factor in AML patients [5–8].

Till now, researchers have explored xenogeneic hematopoietic stem cell transplantation [9] and chemotherapy [10, 11] in the treatment of AML patients with DNMT3A R882 mutation. Some studies suggested that only a high concentration of daunorubicin can obviously increase the survival rate for those patients [12] and promote cell apoptosis in AML cells with DNMT3A R882H mutation [13]. These observations indicated that DNMT3A R882H mutation may mediate drug resistance to daunorubicin. Despite the effective therapeutic effect of high concentration of daunorubicin, its side effects should not be ignored. Studies have shown that high concentration of daunorubicin often causes adverse reactions such as infection, hemorrhage, suppression of bone marrow function, and cardiac toxicity [14]. Therefore, it is important to investigate the resistance mechanism of mutant cells to daunorubicin to improve its curative effect as well as reduce the adverse drug reaction and toxic effect.

Nuclear factor-E2-related factor (NRF2) is an important transcription factor which can be activated by drug treatment, toxic substances, carcinogens, and oxidative stress [15, 16] to prevent chemical and radiation induced tumorigenesis [17–20]. However, the hyperactivation of the NRF2 pathway can protect cancer cells from oxidative stress, chemotherapeutic agents, and radiotherapy. These events were observed in different types of cancer such as breast cancer, lung cancer, colon cancer, and pancreatic cancer [21–24]. Brusatol, which is extracted from Brucea javanica, is a unique inhibitor of NRF2 through reducing its expression [25]. Previous studies have shown that brusatol has great effects on different cancers [26–28] and that NRF2 could mediate drug resistance in AML cells [29–31]. Therefore, we hypothesized that NRF2 may play a role in mediating drug resistance to daunorubicin in AML with DNMT3A R882H mutation.

Experimentally, our results demonstrated that DNMT3A R882H obviously increases tumor cells' proliferation and restricts cell apoptosis. In addition, mutant cells showed a low sensitivity to daunorubicin treatment compared with that in wild-type cells. After investigating the mechanism, we found that the expression of NRF2 and its downstream NQO1 (NAD (P) H: quinone oxidoreductase 1) were significantly increased in mutant cells. Moreover, DNMT3A R882H mutation may affect the sensitivity of the AML cells to daunorubicin via activating the NRF2 /NQO1 pathway.

Collectively, we identified that NRF2 mediates drug resistance to daunorubicin by regulating cell growth and apoptosis in AML cells with DNMT3A R882H mutation. Thus, the inhibition of NRF2 may have a guiding significance for the treatment of AML patients.

Results

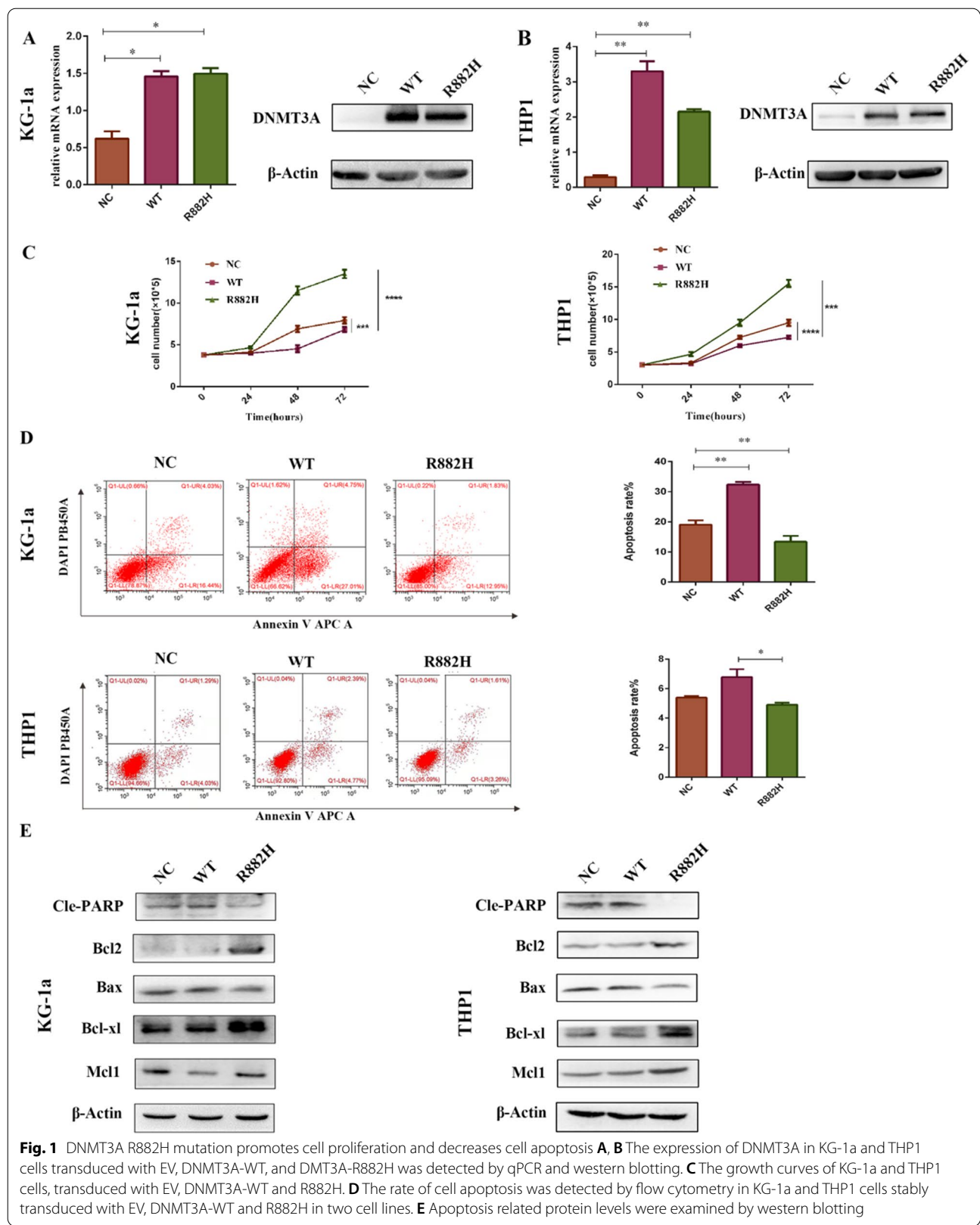### DNMT3A-R882H influences cell proliferation and apoptosis

To explore the role of DNMT3A R882H mutation in AML cells, we generated DNMT3A R882H mutant, wild-type (WT) and empty vector (EV) in KG-1a and THP1 cell lines (Fig. 1A, B). Cell counting results showed that cell proliferation was significantly enhanced in cells with DNMT3A-R882H mutant compared with that of EV and WT cells (Fig. 1C). Moreover, flow cytometry results suggested that the cells carrying the DNMT3A-R882H mutation have a reduced apoptosis rate compared with that of EV or DNMT3A-WT expressing cells (Fig. 1D). In addition, western blot results demonstrated that the presence of DNMT3A-R882H mutation can decrease the expression of pro-apoptotic proteins (cl-PARP, Bax) while increase the expression of anti-apoptotic proteins (Bcl2, Bcl-xl, Mcl1) (Fig. 1E).

DNMT3A R882H mutation is associated with poor sensitivity to daunorubicin

To better explore whether chemoresistance to daunorubicin is mediated by DNMT3A R882H mutation, we detected cell apoptosis rate by flow cytometry, with and without daunorubicin treatment. We found that the mutant cells exhibited a lower apoptosis rate compared with that of WT-expressing cells (Fig. 2A, B). The CCK-8 data (Fig. 2C, D) showed that DNMT3A R882H mutation reduced sensitivity to daunorubicin. Moreover, DNMT3A R882H mutant strongly suppressed cleaved PARP and Bax in response to different concentrations of daunorubicin (0, 0.2, 0.4, and 0.8 μM). However, western blot results showed that the inhibition effects of daunorubicin on anti-apoptotic proteins (Bcl2, Bcl-xl, Mcl1) was not as effective in DNMT3A mutant cells compared with that in cells transduced with DNMT3A-WT (Fig. 2E, F). Also, the same results were observed in mutant cells after treatment with DNR (0.4 μM) and detectedat different times (Fig. 2G, H).

The NRF2/NQO1 pathway is activated in DNMT3A-R882H mutant cells

To explore whether NRF2 plays a role in AML of patients with the DNMT3A-R882 mutation, we detected the differential expression of NRF2 in AML patients with DNMT3A-WT and DNMT3A-mutation using the Beat AML

Fig. 1 DNMT3A R882H mutation promotes cell proliferation and decreases cell apoptosis **A**, **B** The expression of DNMT3A in KG-1a and THP1 cells transduced with EV, DNMT3A-WT, and DMT3A-R882H was detected by qPCR and western blotting. **C** The growth curves of KG-1a and THP1 cells, transduced with EV, DNMT3A-WT and R882H. **D** The rate of cell apoptosis was detected by flow cytometry in KG-1a and THP1 cells stably transduced with EV, DNMT3A-WT and R882H in two cell lines. **E** Apoptosis related protein levels were examined by western blotting

database and found that the level of NRF2 was higher in AML patients with the DNMT3A R882 mutation (Fig. 3A). The results of Gene Set Enrichment Analysis (GSEA) also showed that the NRF2 pathway was enriched in DNMT3A R882 mutant group of AML patients (Fig. 3B). Kaplan–Meier analysis showed that patients with the DNMT3A-R882 mutation and high NRF2 expression have a shorter overall survival (Fig. 3C). To further determine whether DNMT3A R882H can regulate the expression of NRF2 in AML cells, NRF2 mRNA and protein expressions and its downstream target NQO1 were detected. The results (Fig. 3D, E) demonstrated that NRF2 and NQO1 expressions increased in mutant cells compared with those of WT and EV cells. NRF2 usually translocates into the nucleus and regulate oxidative stress related genes and detoxifying enzymes, and therefore, we also detected the expression of NRF2 in the nucleus. We observed that nuclear NRF2 had a higher expression in KG-1a mutant cells (Fig. 3F, G). The results of immunofluorescence also suggested that the expression of NRF2 was increased in mutant cells compared with that in DNMT3A-NC and DNMT3A-WT cells (Fig. 3H). In addition, we found that with the increased concentration of daunorubicin, the expression of NRF2 and NQO1 gradually increased in KG-1a and THP1 cells expressing the DNMT3A R882H mutant and the increment in mutant cells was much higher than that in cells transduced with the WT (Fig. 3I, J). Similarly, the increase rates of NRF2 and NQO1 were higher in mutant cells after treatment with DNR (0.4 μM) at different times (Fig. 3K, L).

DNMT3A R882H mutation regulates the expression of NRF2 in a DNA-methylation-independent way

To identify the mechanism by how the DNMT3A R882H mutation regulates the expression of NRF2, we detected the methylation level in the CPG islands of the NRF2 promoter in KG-1a cells expressing DNMT3A NC, WT, and R882H. Nevertheless, we observed no significant difference in methylation level of these cells (Fig. 4A). Then we treated KG-1a WT and mutant cells with CHX to investigate whether DNMT3A R882H mutation regulates NRF2 by influencing protein stability. Our data proved that DNMT3A R882H mutation reduced NRF2 degradation by increasing the half-life of NRF2 (Fig. 4B). Furthermore, the IP results showed that DNMT3A interacted with NRF2 in both WT and R882H cells (Fig. 4C).

NRF2 inhibition increases the antitumor effect of daunorubicin in DNMT3A R882H mutant cells

To investigate the role of NRF2 in mutant cells, brusatol (Bru), a unique inhibitor of Nrf2 pathway, was used to treat cells expressing DNMT3A R882H mutation. Interestingly, DNMT3A-R882H mutant cells showed a higher apoptosis rate after brusatol treatment compared with that in DNMT3A WT cells, (Fig. 5A, B). We also found that brusatol can suppress the expression of NRF2 and NQO1 in DNMT3A-mutant cells (Fig. 5C). Furthermore, the treatment of brusatol inhibited cell proliferation and markedly triggered cell apoptosis in mutant cells (Fig. 5D, E). After treatment with DNR (0.4 μM) and Bru (40 nM), the NRF2/NQO1 pathway was significantly inhibited (Fig. 5F). Cell viability was also significantly decreased in the combined treatment group compared with that of DNR or Bru treatment alone group (Fig. 5G, H, J).

Discussion

DNMT3A mutation happened in a variety of cancers such as colorectal cancer, lung cancer, chronic myelocytic leukemia, but is mainly with AML [32–34] and usually occur with other types of mutations, such as FLT3, NPM1, C/EBP alpha, resulting in synergistic effects in AML [35–37]. Being the most common mutation, DNMT3A R882H mutation can not only lower methylation enzymatic activity [38, 39] but can also co-act with other proteins to influence immune activity, cell apoptosis, proliferation, and DNA damage repair to regulate AML [40–42]. Researchers also found that Twist1, mTOR pathway, CDK1, and Dot1l [43–46] which might be the potential therapeutic targets of AML patients carry DNMT3A mutation. Reports have shown that the loss of DNMT3A or its mutant can cause an abnormal proliferation of hematopoietic stem cells, which then transform into preleukemic stem cells that are resistant to chemo-drugs and responsible for relapse in AML patients [47]. To better understand the role of DNMT3A R882H mutation in leukemia stem cells, the AML LSC-like cell line, KG1a, with the expression profile, CD34+ and CD38−, was selected as our research objects [48]. Researchers have pointed out that AML patients with a DNMT3A mutation tend to be M4/M5 subtype [49], and therefore, we have also chosen the human acute monocytic leukemia cell line, THP1, for our studies. Our study confirmed that the DNMT3A R882H mutation did promoted cell growth and

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Fig. 2 DNMT3A R882H mutation confers resistance to daunorubicin. **A**, **B** KG-1a and THP1 cells stably expressing DNMT3A-WT and the mutant were exposed to DMSO and DNR (0.4 μM) for 36 h and used for apoptosis analysis. **C**, **D** Sensitivity to DNR in KG-1a and THP1 cells expressing DNMT3A-WT and the mutant **E**, **F** Analysis of apoptosis related proteins in KG-1a and THP1 cells expressing DNMT3A mut and wild-type and treated with different concentrations of daunorubicin for 36 h. **G**, **H** Analysis of apoptosis related proteins in KG-1a and THP1 cells expressing DNMT3A mut and wild-type at different time points (0, 6, 12, 24, 48 h) after daunorubicin (0.4 μM) treatment

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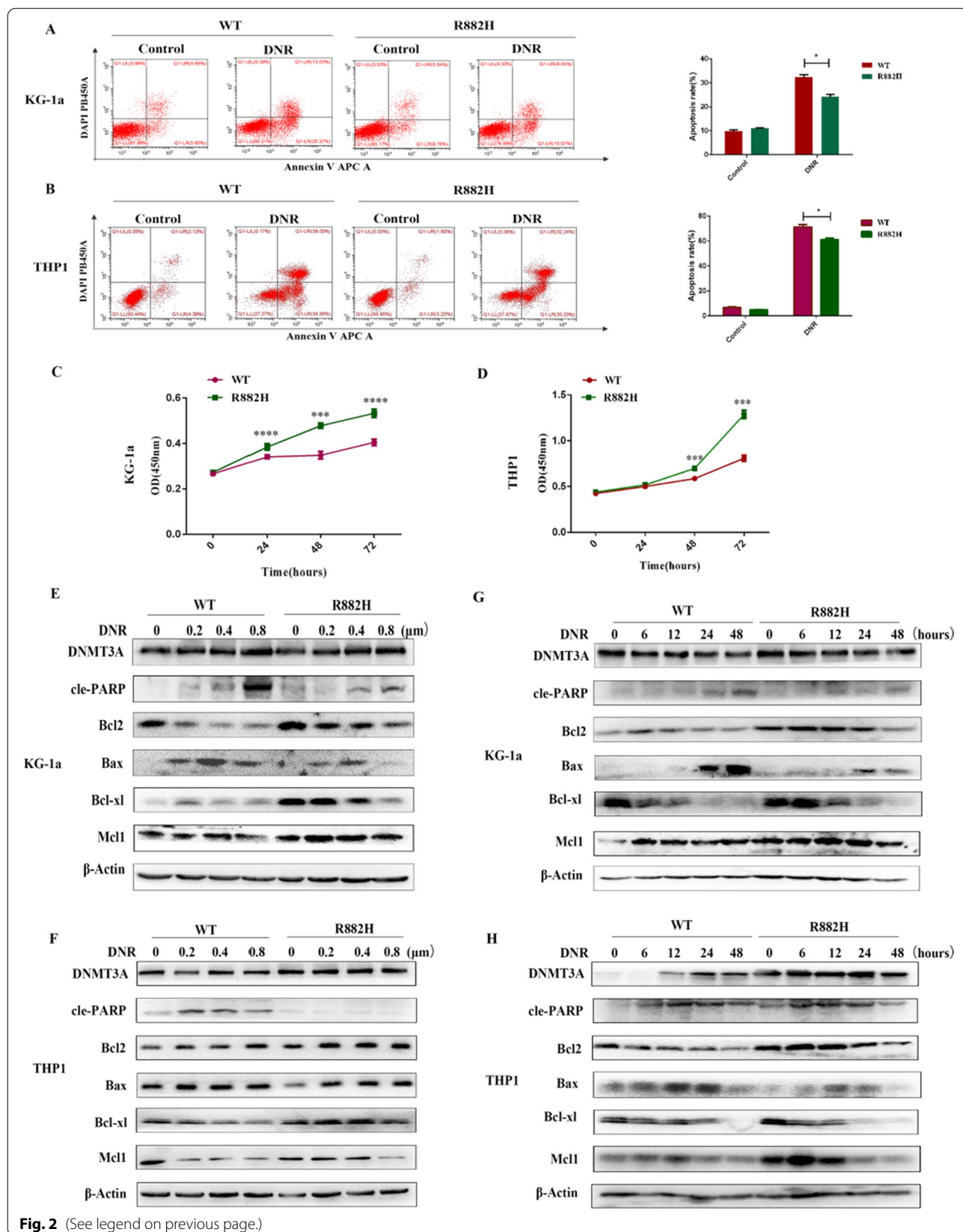
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decreases cell apoptosis in mutant KG-1a and THP1 cells. DNR is an anthracycline drug that has two main mechanisms in cells, including inhibition of the nucleic acids synthesis and effects on the oxidoreductase system [50–52] which is effective in treating nonlymphocytic leukemia and acute lymphocytic leukemia [53–55]. Though DNR showed great effects in AML, toxicities associated with caused by daunorubicin, including cytopenias, hepatotoxicity, and cardiotoxicity [56–58] are major challenges. Our results identified that AML cells with a DNMT3A R882H mutation were not as sensitive to DNR as that of DNMT3A WT cells, which was in line with previous studies that confirmed that a high-dose daunorubicin improves the outcome of AML with a DNMT3A mutation [12, 13, 59].Then exploring how DNMT3A R882H mutation influence AML cells' sensitivity to DNR is of great importance to improve the prognosis of AML patients after DNR treatment.
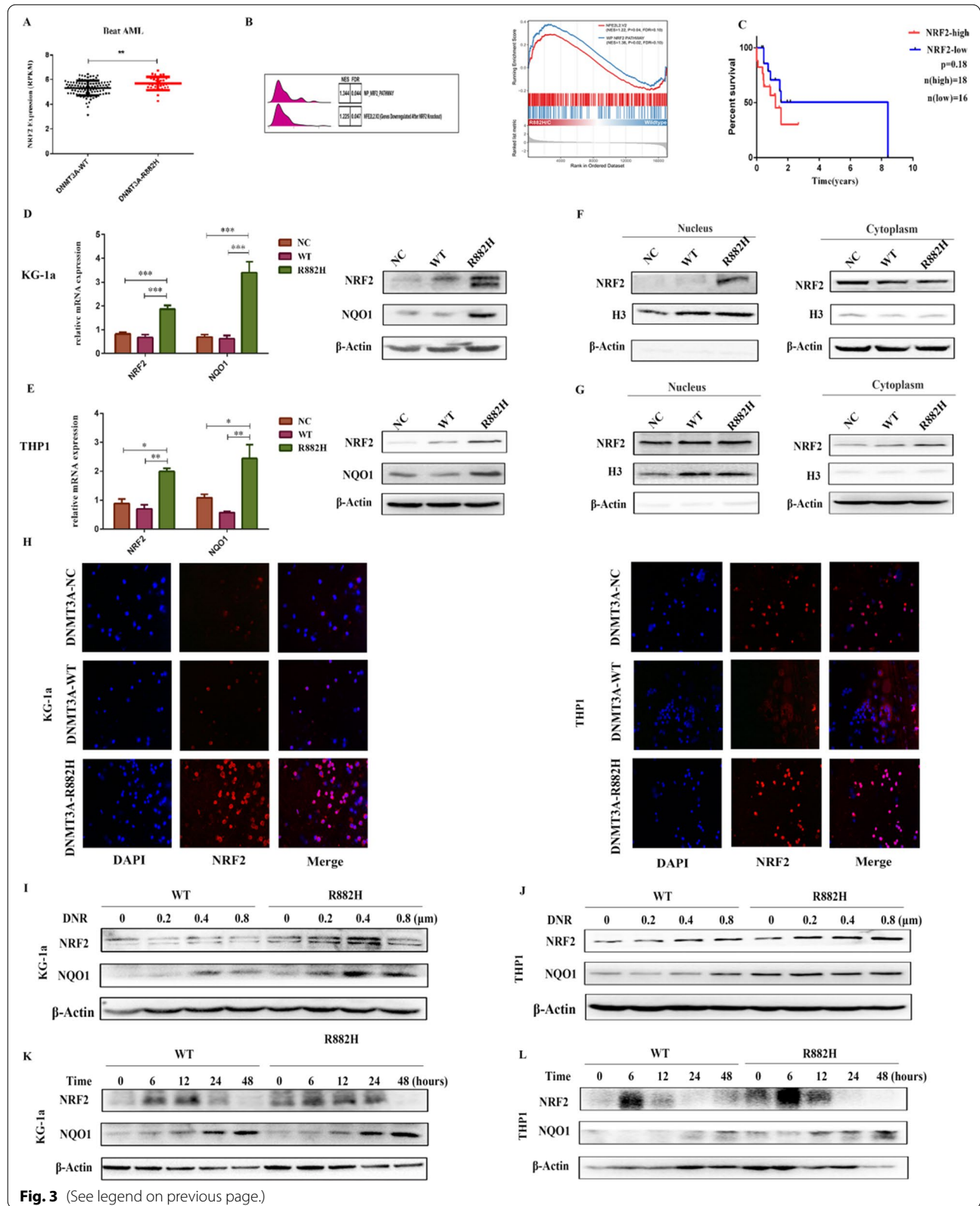
NRF2, first identified in 1994, is an important transcription factor which plays a role in cellular stability [60]. Studies have confirmed that the excessive activation of NRF2 can protect cells from apoptosis mediated by drug resistance in different cancers such as ovarian cancer, multiple myeloma, NSCLC tumors, colorectal cancer, and neck cancer [61–65]. Rabindranath Bera et al. pointed out that cells with a DNMT3A R882 mutation have a lower level of ROS when compared with that in WT cells, suggesting a potential role of DNMT3A R882H mutation in the regulation of the redox system in cells [42]. Therefore, we supposed that NRF2 may play a role in DNMT3A R882H related DNR-resistance. Researchers confirmed that NQO1 is related to anthracyclines metabolism [66, 67], and thus, we also investigated the role of NQO1 in our study. We found that NRF2 and NQO1 were increased in both in KG-1a and THP1 DNMT3A R882H mutant cells. We also observed that the expression of nuclear NRF2 was increased in KG-1a mutant cells. However, we did not notice similar results in THP1 cells, which may be explained by cell heterogeneity. Moreover, it was surprising to find that NRF2 expression was also increased in mutant cells after treatment with different concentrations of DNR, suggesting that DNR can also activate NRF2 and NQO1 in mutant cells. This increase may also explain the insensitivity of AML

cells with DNMT3A R882H mutation to DNR. Given that NRF2 may help DNMT3A R882H mutant cells show low sensitivity to DNR, we treated mutant KG-1a and THP1 cell with brusatol, a specific inhibitor of NRF2. We observed that mutant cells were more sensitive to brusatol treatment, suggesting that the cells with the DNMT3A R882H mutation may be dependent on NRF2. Then we identified that brusatol did inhibit the NRF2/NQO1 pathway and decreased cell growth and promoted cell apoptosis. Moreover, the combination of DNR and brusatol not only reduced the expression of NRF2 and NQO1 but also dramatically promoted cell apoptosis. The great effects of this combination meant that NRF2 inhibition may help improve mutant cells' sensitivity to DNR. Given that DNMT3A may regulate gene expression by influencing CpG methylation status of promoter regions, we detected the methylation of CpG islands of NRF2 promoter. However, the methylation status was not significantly different between DNMT3A-NC, DNMT3A-WT, and DNMT3A-R882H cells, suggesting that DNMT3A R882H may regulate NRF2 in a DNA methylation-independent way. Then we found that the stability of NRF2 increases in DNMT3A R882H mutant cells which indicated that there may be a posttranslational modification in DNMT3A R882H mutant cells that influences the expression of NRF2.
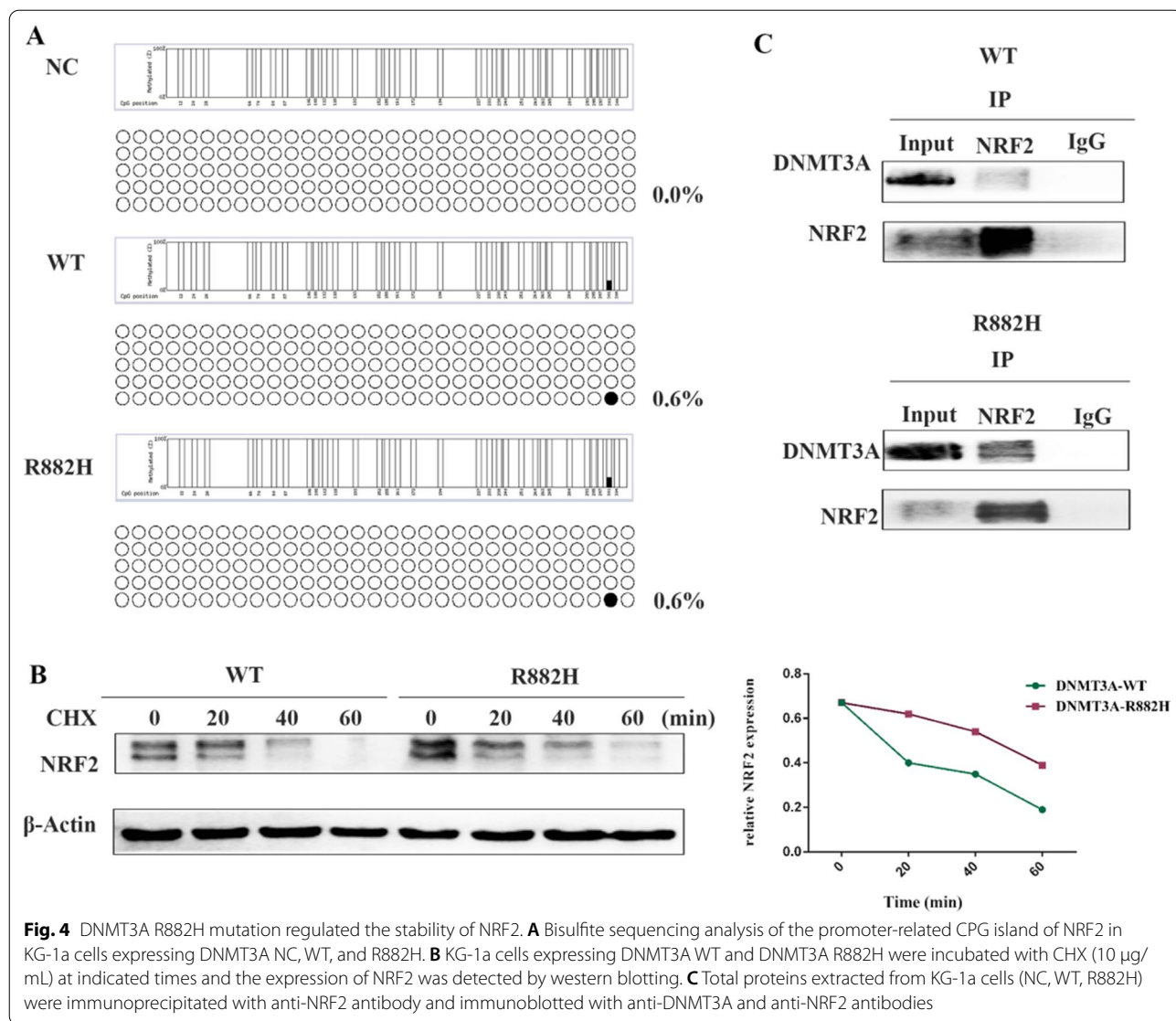
A study reported that AML patients with DNMT3A R882 mutation were resistant to venetoclax, a specific inhibitor of Bcl-2 [68]. Researchers have confirmed that the drug resistance to venetoclax is associated with the expression of Mcl-1 and Bcl-xl [69]. In our study, we found that AML cells with DNMT3A R882H had higher Bcl2, Bcl-xl and Mcl1 expressions compared with those in WT cells. In addition, the expression levels of the three proteins were higher in mutant cells compared with those in WT cells even when these cells were treated with DNR. These effects may also explain why AML patients with DNMT3A R882 mutation have a low sensitivity to venetoclax. Our research found that NRF2 inhibition can reduce the level of Bcl2, Bcl-xl, and Mcl-1, then in our future work, we will also explore whether NRF2 plays a role in venetoclax resistance to help improve the effects of venetoclax in AML patients with DNMT3A R882 mutation.

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Fig. 3 NRF2 is significantly augmented in AML cells expressing the DNMT3A R882H mutation. **A** NRF2 mRNA expression in samples of AML with the DNMT3A-R882 mutation compared with that in samples of AML with DNMT3A -WT. **B** GSEA analysis was used to detect NRF2 pathway enrichment in AML patients with the DNMT3A R882 mutation **C** The Beat AML database was used to analyze survival rate based on NRF2 expression. **D**, **E** The expression of NRF2 and NQO1 mRNA expression in WT and mutant KG-1a and THP1 cells were detected by qPCR and western blotting. **F**, **G** The expression of NRF2 in the nucleus and cytoplasm of KG-1a and THP1 cells expressing NC, WT and R882H was evaluated by western blot. **H** Cytoplasmic localization of NRF2 in KG1 and THP1 cells expressing DNMT3A WT and R882H. Immunostaining with anti-NRF2 (red) and DAPI (blue). **I**, **J** Immunoblot of NRF2 and NQO1 expressions in DNMT3A WT and mutant KG-1a and THP1 cells after treated with different concentrations of DNR for 24 h. **K**, **L** Immunoblot of NRF2 and NQO1 expressions in DNMT3A WT and mutant KG-1a cells and THP1 cells after treated with 0.4 μM DNR and harvested at different times

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Fig. 3 (See legend on previous page.)

Fig. 4 DNMT3A R882H mutation regulated the stability of NRF2. **A** Bisulfite sequencing analysis of the promoter-related CPG island of NRF2 in KG-1a cells expressing DNMT3A NC, WT, and R882H. **B** KG-1a cells expressing DNMT3A WT and DNMT3A R882H were incubated with CHX (10 μg/mL) at indicated times and the expression of NRF2 was detected by western blotting. **C** Total proteins extracted from KG-1a cells (NC, WT, R882H) were immunoprecipitated with anti-NRF2 antibody and immunoblotted with anti-DNMT3A and anti-NRF2 antibodies
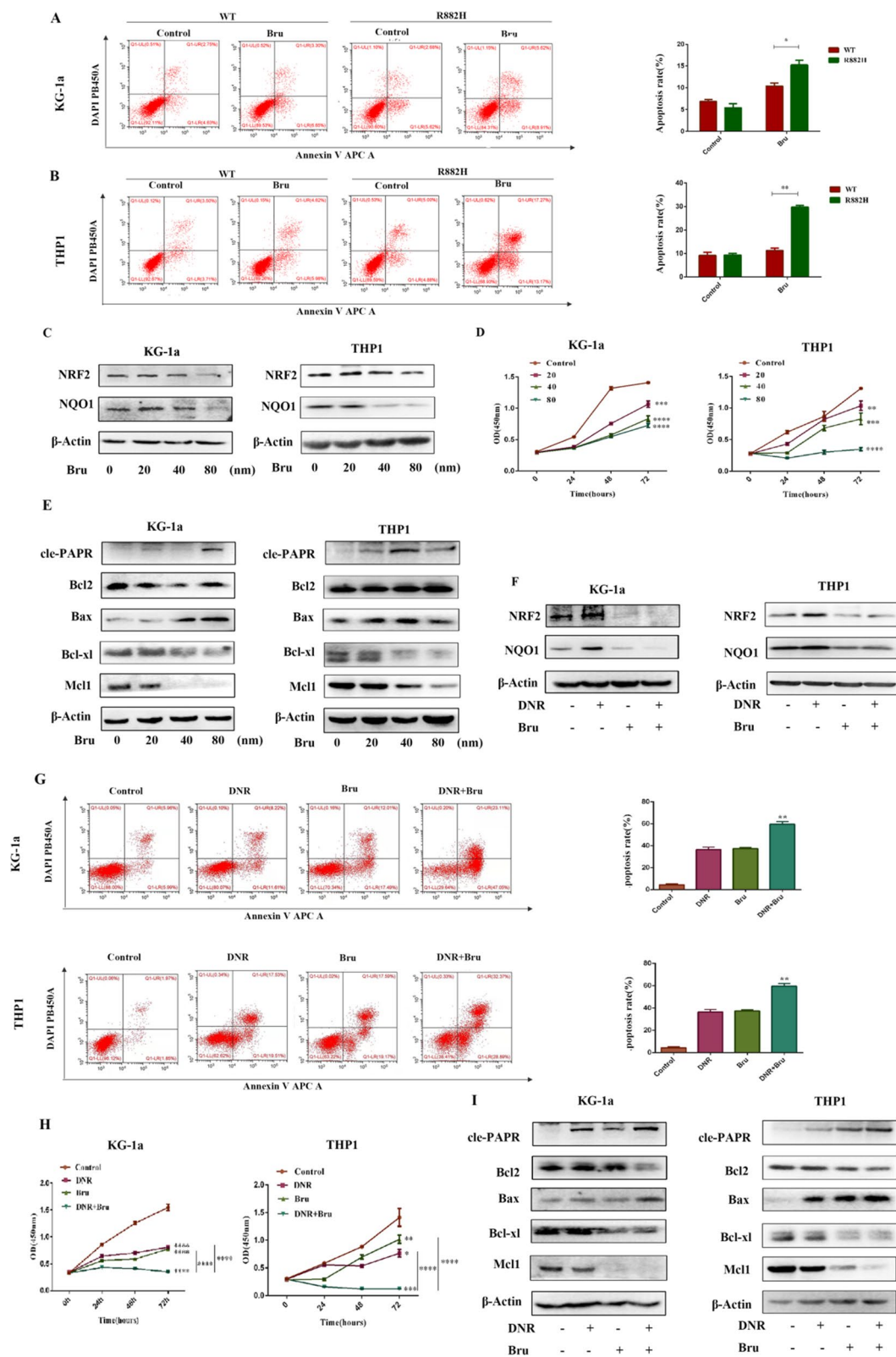
Certainly, our research has some limitations. Firstly, in the analysis of the survival rate of AML with DNMT3A R882 mutation, based on NRF2 expression, the number of AML cases in the database was too small to provide meaningful statistical results. We may collect a larger amount of clinical data to investigate whether NRF2 can work as a new biomarker to predict the prognosis of AML patients with DNMT3A R882 mutation in follow-on research. Secondly, we confirmed that DNMT3A R882H can influence the expression of NRF2 in a methylation-independent way, which suggests that DNMT3A R882H mutation not only influences DNA methylation of NRF2 promoter. Thirdly, we proved that the DNMT3A R882H mutation may regulate the stability of NRF2, and in our future work, we will investigate how DNMT3A R882H mutation affects the expression of NRF2 to better understand the role of DNMT3A R882H mutation. Furthermore, all experiments were carried in vitro which may not be enough to study how the DNMT3A R882H mutation mediates drug resistance

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Fig. 5 Inhibition of NRF2 improves sensitivity to DNR in DNMT3A R882H mutant cells. **A**, **B** Apoptosis rate was measured by flow cytometry in KG-1a and THP1 cells expressing WT and R882H after treatment with 40 nM brusatol. **C–E** Mutant KG-1a and THP1 cells were exposed to different concentrations of brusatol for 24 h to detect the expressions of NRF2, NQO1 and apoptosis-related proteins. **F** The expressions of NRF2, NQO1 and apoptosis related proteins were detected by Western blotting after treatment of KG-1a and THP1 mutant cells with DNR and brusatol. **G–I** The role of brusatol or DNR-brusatol combination on cell viability and cell apoptosis in KG-1a and THP1 mutant cells

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to DNR via regulating NRF2. Thus, this phenomenon should be studied in vivo by generating DNMT3A R878H heterozygous mice models (a mice model mimics DNMT3A R882H in humans) [70] to study the relationship between DNMT3A R882H mutation and NRF2 and investigate the drug resistance to DNR.

In summary, we find that NRF2 can be activated by the DNMT3A R882H mutation and that it mediates chemoresistance to DNR. Additionally, our study suggests that NRF2 can reduce mutant cells' sensitivity to DNR by regulating cell proliferation and apoptosis. The mechanism is illustrated in the schematic diagram (Fig. 6). Targeting NRF2 can be helpful in making the DNR treatment more effective in AML patients with DNMT3A R882H mutation and minimizing side effects meanwhile.

Materials and methods
Cell lines and culture
KG-1a cell line was available in our own laboratory. The THP1 cell line was purchased from the cell bank of the Chinese Academy of Science (Kunming, China). The cells were cultured in RPMI-1640 medium supplemented with 10% FBS and then incubated at 37 °C under 5% CO_2 atmosphere.

Reagents
Daunorubicin (HY-15648B), the cell counting kit-8 (HY-K0301), Brusatol (HY-19543), Cycloheximide (HY-12320), 2-deoxy-D-glucose (HY-13966) and D-glucose (HY-B0389) were purchased from Med Chem Express (MCE, NJ, USA). RPMI-1640 medium was supplied by Gibco (MA, USA), fetal bovine serum (FBS; 900–108) was purchased from Gemini bio-products (Sacramento, CA, USA). The Nuclear and cytoplasmic protein extraction Kit (P0027) was obtained from Beyotime Biotechnology (China).

### Generation of DNMT3A R882H mutant cell lines
For the study, we generated KG-1a and THP1 DNMT3A R882H mutant cell lines. Briefly, lentiviral vectors expressing wild-type DNMT3A (DNMT3A -WT) and DNMT3A R882H (DNMT3A-mutant) were purchased from Genechem. An amount of 5×10^4 leukemic cells per well were plated into a 24-well plate and infected with the above lentiviruses for 3 days using HitransG (for KG-1a cells) and HistransP (for THP1 cells) (Genechem). The stable expressing cell lines were selected with puromycin.

Cell viability assay
The proliferation rates of cells were monitored using the CCK-8 assay kit. Briefly, the cells were seeded in 96-well culture plates at a density of 5×10^3 cells/well and subsequently treated with indicated reagents. Finally, 10 μl of CCK-8 solution was added into the cell culture medium. Optical density (OD) measurements were carried out after incubation for 2 h. The OD value of each well was recorded at 450 nm using a micro-plate absorbance reader.

Cellular apoptosis detection
For the evaluation of cellular apoptosis, the cells were seeded into 6-well plates at a density of 5×10^5 cells / well and treated with indicated reagents. The apoptosis rate was determined by the Annexin V-FITC Apoptosis Detection Kit (SUNGENE BIOTECH, Tianjing). The flow

Fig. 6 The mechanism of chemoresistance to DNR in AML cells with DNMT3A-R882H mutation
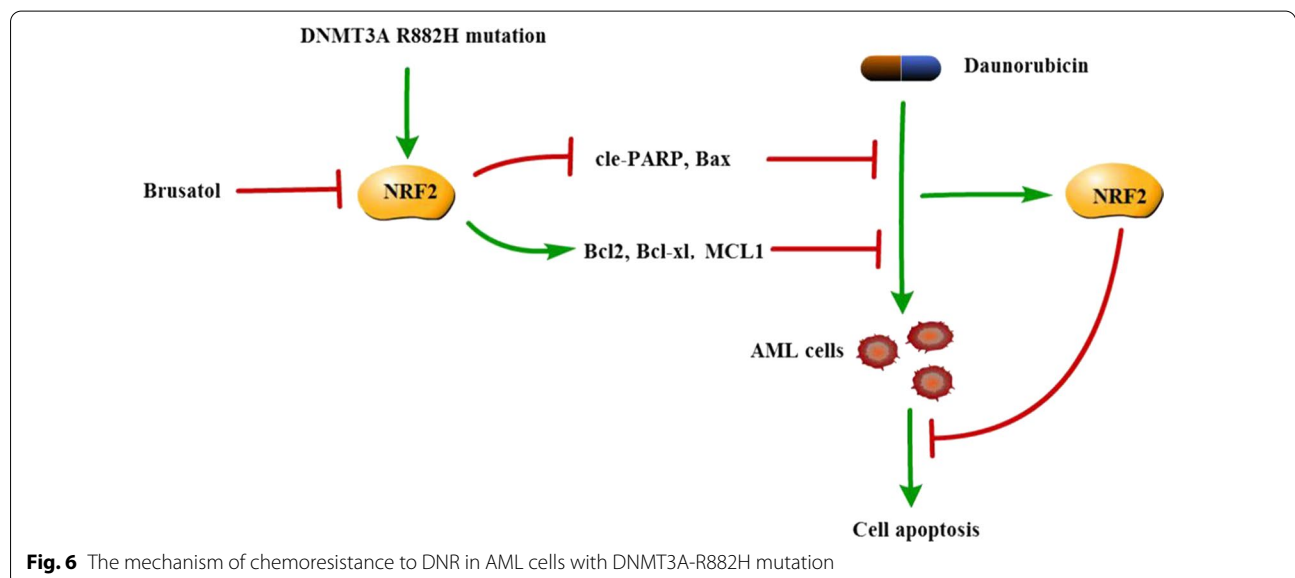
cytometric analysis was performed using a CytoFLEX flow cytometer (Beckman Coulter, USA).

Antibodies and western blot analysis

After indicated treatments, total protein, nuclear and cytoplasm proteins were extracted according to the instructions and the concentration of total protein was determined. The protein extracts were mixed with $5 \times$ loading buffer, heated for 5 min, separated with sodium dodecyl sulfate poly acrylamide gel electrophoresis, and then transferred onto polyvinylidene fluoride membranes. After blocking with a 5% skimmed milk solution, the membranes were incubated with primary antibodies at 4 °C overnight. The next day, the membranes were incubated with secondary antibodies for 1 h at room temperature and then detected with an enhanced chemiluminescence (ECL) Ultra Western HRP Substrate kit (WBULS0100; EMD Millipore, USA) using an ECL visualization system (GE Healthcare, USA). The following antibodies were used for immunoblotting: β-Actin (TA-09) and H3 (bsm-33042 M) were purchased from ORIGENE (Beijing, China) and Bioss (Beijing, China), respectively. DNMT3A (ab188470) was obtained from abcam(Cambridge, MA, USA). NRF2 (16396–1-AP), MCL1 (16225–1-AP), Bcl2 (12789–1-AP), and Cle-PARP (13371–1-AP) were obtained from proteintech group (Rosemont, IL, USA). Bax (A5131), Bcl-xl (A5091), and NQO1 (A5464) were obtained from bimake. IgG for CO-IP was purchased from Cell Signaling Technology (USA).

Immunofluorescence analysis

The cells were collected and washed with cold PBS, blocked in fetal sheep serum (Zhong-Shan Golden Bridge Biotechnology, China) for 30 min, and then incubated with the anti-NRF2 antibody at 4 °C overnight. The next day, the cells were washed with cold PBS and then incubated for 1 h with coralite 594-conjugated goat anti-rabbit secondary antibody (SA00013-4, Proteintech group), and then counterstained with DAPI (Beyotime) for 5 min. Images of the cells were analyzed by inverted fluorescence microscopy. The exposure time for DAPI and red fluorescence were both 150 ms.

Bisulfite sequencing

Genomic DNA samples were isolated from KG-1a ells, that were stably transduced with DNMT3A-NC, DNMT3A-WT, and DNMT3A-R882H mutant, and subjected to bisulfite conversion with the EZ DNA methylation-gold kit (Zymo Research, Beijing) according to the manufacturer's instructions. Bisulfite-converted DNA was amplified by PCR using specific primers. The PCR products were cloned using the Pmd18-T vector (Takara,

Japan) and the CpG methylation pattern was visualized after bisulfite sequencing. The sequencing primers used for the bisulfite sequencing analysis of the NRF2 gene were forward primer: TAATTTTAAATTAGGGAGGYG TAGT and reverse primer: ACAAAAATCCRAACCCTT CC.

Co-IP

Protein A/G Magnetic Beads (MCE), and antibodies (NRF2 and IgG) were added into two tubes respectively, and incubated on an inverted mixer for 2 h to mix the beads with the antibodies. After washing with PBST, the protein extracts were incubated with the beads on an inverted mixer at 4 °C overnight. Next day, the proteins were analyzed with western blotting.

Cycloheximide assay

To investigate protein stability of NRF2 in DNMT3A-WT and DNMT3A-R882H KG-1a cells, these later were treated with 10 μg/mL cycloheximide (CHX; MCE) to block de novo protein synthesis for 0, 20, 40 and 60 min. Then the expression of NRF2 was analyzed by western blot.

Quantitative real-time PCR

PrimeScript ™ RT reagent Kit (Takara, Japan) was used to synthesize cDNA. qRT-PCR was performed on a CFX Connect ™ real-time PCR operating system (Bio-Rad, USA) using the SYBR ® Premix ExTaq ™ II kit (Takara, Japan). The primers were synthesized at Sangon Biotech (Shanghai, China). The sequences of all primers used for qRT-PCR are shown in Table 1.

Statistical analysis

The Beat AML database was used to identify the expression of NRF2 in DNMT3A-WT and AML patients with DNMT3A-R882 mutation (www.vizome.org) [32]. For Gene Set Enrichment Analysis (GSEA), the data was also obtained from the Beat AML database. The overall survival of AML patients with DNMT3A-R882H mutation was divided into high and low NRF2 expression groups using

Table 1 Sequences of all primers used of quantitative real-time PCR

Gene	Forward/reverse	Primer Sequence (5′–3′)
DNMT3A	F	CATCTGCATCTCCTGTGGG
	R	GCAGTTTTGGCACATTCCTC
NRF2	F	TCAGCGACGGAAAGAGTATGA
	R	CCACTGGTTTCTGACTGGATGT
NQO1	F	GAAGAGCACTGATCGTACTGGC
	R	GGATACTGAAAGTTCGCAGGG
β-Actin	F	TGACGTGGACATCCGCAAAG
	R	CTGGAAGGTGGACAGCGAGG

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the Beat AML dataset (n = 42) and evaluated by Cox proportional hazards regression models. The p-value was calculated by the log-rank test and corrected according to the database description.

The statistical difference between the two groups was compared using unpaired Student's t test. The data were presented as mean ± standard deviation (SD). p-value < 0.05 was considered statistically significant. "*", "**", "***" and "****" shown a statistical significance with p-value < 0.05, < 0.01, < 0.001, < 0.0001, respectively. All statistical analyses were carried out using GraphPad Prism 5.0 and the SPSS Statistics software (19.0).

Abbreviations
DNMT3A: DNA methyltransferase 3A; AML: Acute myeloid leukemia; NRF2: Nuclearfactor-E2-related factor; DNMT3A-WT: DNMT3A-wild type; DNMT3A-R882H: DNMT3A -Arg882His; NQO1: NAD (P) H: quinone oxidoreductase 1; Dau: Daunorubicin; Bru: Brusatol; CHX: Cycloheximide.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12964-022-00978-1.

Availability of data and materials
Data and materials will be shared.

Declarations

Ethics approval and consent to participate
Beat AML belongs to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

Consent for publication
Informed consent for publication was obtained from all participants.

Competing interests
The authors declare no competing interests.

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References
1. Shlush LI, Mitchell A. AML evolution from preleukemia to leukemia and relapse. Best Pract Res Clin Haematol. 2015;28(2–3):81–9.
2. Yang L, Rau R, Goodell MA. DNMT3A in haematological malignancies. Nat Rev Cancer. 2015;15(3):152–65.
3. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, Kandoth C, Payton JE, Baty J, Welch J, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med. 2010;363(25):2424–33.
4. Yang L, Rodriguez B, Mayle A, Park HJ, Lin X, Luo M, Jeong M, Curry CV, Kim SB, Ruau D, et al. DNMT3A loss drives enhancer hypomethylation in FLT3-ITD-associated leukemias. Cancer Cell. 2016;29(6):922–34.
5. Marcucci G, Metzeler KH, Schwind S, Becker H, Maharry K, Mrozek K, Radmacher MD, Kohlschmidt J, Nicolet D, Whitman SP, et al. Age-related prognostic impact of different types of DNMT3A mutations in adults with primary cytogenetically normal acute myeloid leukemia. J Clin Oncol. 2012;30(7):742–50.
6. Thol F, Damm F, Ludeking A, Winschel C, Wagner K, Morgan M, Yun H, Gohring G, Schlegelberger B, Hoelzer D, et al. Incidence and prognostic influence of DNMT3A mutations in acute myeloid leukemia. J Clin Oncol. 2011;29(21):2889–96.
7. Renneville A, Boissel N, Nibourel O, Berthon C, Helevaut N, Gardin C, Cayuela JM, Hayette S, Reman O, Contentin N, et al. Prognostic significance of DNA methyltransferase 3A mutations in cytogenetically normal acute myeloid leukemia: a study by the Acute Leukemia French Association. Leukemia. 2012;26(6):1247–54.
8. Ostronoff F, Othus M, Ho PA, Kutny M, Geraghty DE, Petersdorf SH, Godwin JE, Willman CL, Radich JP, Appelbaum FR, et al. Mutations in the DNMT3A exon 23 independently predict poor outcome in older patients with acute myeloid leukemia: a SWOG report. Leukemia. 2013;27(1):238–41.
9. Xu Y, Sun Y, Shen H, Ding L, Yang Z, Qiu H, Sun A, Chen S, Wu D. Allogeneic hematopoietic stem cell transplantation could improve survival of cytogenetically normal adult acute myeloid leukemia patients with DNMT3A mutations. Am J Hematol. 2015;90(11):992–7.
10. Dohner H, Dolnik A, Tang L, Seymour JF, Minden MD, Stone RM, Del Castillo TB, Al-Ali HK, Santini V, Vyas P, et al. Cytogenetics and gene mutations influence survival in older patients with acute myeloid leukemia treated with azacitidine or conventional care. Leukemia. 2018;32(12):2546–57.
11. Ayala R, Rapado I, Onecha E, Martinez-Cuadron D, Carreno-Tarragona G, Bergua JM, Vives S, Algarra JL, Tormo M, Martinez P, et al. The mutational landscape of acute myeloid leukaemia predicts responses and outcomes in elderly patients from the PETHEMA-FLUGAZA phase 3 clinical trial. Cancers. 2021;13(10):2458.
12. Luskin MR, Lee JW, Fernandez HF, Abdel-Wahab O, Bennett JM, Ketterling RP, Lazarus HM, Levine RL, Litzow MR, Paietta EM, et al. Benefit of high-dose daunorubicin in AML induction extends across cytogenetic and molecular groups. Blood. 2016;127(12):1551–8.
13. Guryanova OA, Shank K, Spitzer B, Luciani L, Koche RP, Garrett-Bakelman FE, Ganzel C, Durham BH, Mohanty A, Hoermann G, et al. DNMT3A mutations promote anthracycline resistance in acute myeloid leukemia via impaired nucleosome remodeling. Nat Med. 2016;22(12):1488–95.
14. Maral RJ, Jouanne M. Toxicology of daunorubicin in animals and man. Cancer Treat Rep. 1981;65(Suppl 4):9–18.
15. Ma Q. Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol. 2013;53:401–26.
16. Shanmugam G, Narasimhan M, Tamowski S, Darley-Usmar V, Rajasekaran NS. Constitutive activation of Nrf2 induces a stable reductive state in the mouse myocardium. Redox Biol. 2017;12:937–45.
17. Knatko EV, Ibbotson SH, Zhang Y, Higgins M, Fahey JW, Talalay P, Dawe RS, Ferguson J, Huang JT, Clarke R, et al. Nrf2 activation protects against solar-simulated ultraviolet radiation in mice and humans. Cancer Prev Res. 2015;8(6):475–86.

18. Sekhar KR, Freeman ML. Nrf2 promotes survival following exposure to ionizing radiation. Free Radic Biol Med. 2015;88(Pt B):268–74.

19. Xue J, Yu C, Tang Y, Mo W, Tang Z, Sheng W, Jiao Y, Zhu W, Cao J. NF-E2-related factor 2 (Nrf2) ameliorates radiation-induced skin injury. Front Oncol. 2021;11:680058.

20. Mohs A, Otto T, Schneider KM, Peltzer M, Boekschoten M, Holland CH, Hudert CA, Kalveram L, Wiegand S, Saez-Rodriguez J, et al. Hepatocyte-specific NRF2 activation controls fibrogenesis and carcinogenesis in steatohepatitis. J Hepatol. 2021;74(3):638–48.

21. Wolowczyk C, Neckmann U, Aure MR, Hall M, Johannessen B, Zhao S, Skotheim RI, Andersen SB, Zwiggelaar R, Steigedal TS, et al. NRF2 drives an oxidative stress response predictive of breast cancer. Free Radic Biol Med. 2022;184:170–84.

22. Lignitto L, LeBoeuf SE, Homer H, Jiang S, Askenazi M, Karakousi TR, Pass HI, Bhutkar AJ, Tsirigos A, Ueberheide B, et al. Nrf2 activation promotes lung cancer metastasis by inhibiting the degradation of Bach1. Cell. 2019;178(2):316–29.

23. Zhang Q, Zhang ZY, Du H, Li SZ, Tu R, Jia YF, Zheng Z, Song XM, Du RL, Zhang XD. DUB3 deubiquitinates and stabilizes NRF2 in chemotherapy resistance of colorectal cancer. Cell Death Differ. 2019;26(11):2300–13.

24. Lister A, Nedjadi T, Kitteringham NR, Campbell F, Costello E, Lloyd B, Copple IM, Williams S, Owen A, Neoptolemos JP, et al. Nrf2 is overexpressed in pancreatic cancer: implications for cell proliferation and therapy. Mol Cancer. 2011;10:37.

25. Ren D, Villeneuve NF, Jiang T, Wu T, Lau A, Toppin HA, Zhang DD. Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. Proc Natl Acad Sci U S A. 2011;108(4):1433–8.

26. Liu Y, Lu Y, Celiku O, Li A, Wu Q, Zhou Y, Yang C. Targeting IDH1-mutated malignancies with NRF2 blockade. J Natl Cancer Inst. 2019;111(10):1033–41.

27. Lee JH, Mohan CD, Deivasigamani A, Jung YY, Rangappa S, Basappa S, Chinnathambi A, Alahmadi TA, Alharbi SA, Garg M, et al. Brusatol suppresses STAT3-driven metastasis by downregulating epithelial-mesenchymal transition in hepatocellular carcinoma. J Adv Res. 2020;26:83–94.

28. Oh ET, Kim CW, Kim HG, Lee JS, Park HJ. Brusatol-mediated inhibition of c-Myc increases HIF-1alpha degradation and causes cell death in colorectal cancer under hypoxia. Theranostics. 2017;7(14):3415–31.

29. Li Y, Guo Y, Feng Z, Bergan R, Li B, Qin Y, Zhao L, Zhang Z, Shi M. Involvement of the PI3K/Akt/Nrf2 signaling pathway in resveratrol-mediated reversal of drug resistance in HL-60/ADR cells. Nutr Cancer. 2019;71(6):1007–18.

30. Karathedath S, Rajamani BM, Musheer Aalam SM, Abraham A, Varatharajan S, Krishnamurthy P, Mathews V, Velayudhan SR, Balasubramanian P. Role of NF-E2 related factor 2 (Nrf2) on chemotherapy resistance in acute myeloid leukemia (AML) and the effect of pharmacological inhibition of Nrf2. PLoS ONE. 2017;12(5):e0177227.

31. Liu P, Ma D, Wang P, Pan C, Fang Q, Wang J. Nrf2 overexpression increases risk of high tumor mutation burden in acute myeloid leukemia by inhibiting MSH2. Cell Death Dis. 2021;12(1):20.

32. Tyner JW, Tognon CE, Bottomly D, Wilmot B, Kurtz SE, Savage SL, Long N, Schultz AR, Traer E, Abel M, et al. Functional genomic landscape of acute myeloid leukaemia. Nature. 2018;562(7728):526–31.

33. Li WL, Xiao MS, Zhang DF, Yu D, Yang RX, Li XY, Yao YG. Mutation and expression analysis of the IDH1, IDH2, DNMT3A, and MYD88 genes in colorectal cancer. Gene. 2014;546(2):263–70.

34. Kim MS, Kim YR, Yoo NJ, Lee SH. Mutational analysis of DNMT3A gene in acute leukemias and common solid cancers. APMIS. 2013;121(2):85–94.

35. Cull AH, Mahendru D, Snetsinger B, Good D, Tyryshkin K, Chesney A, Ghorab Z, Reis M, Buckstein R, Wells RA, et al. Overexpression of Arginase 1 is linked to DNMT3A and TET2 mutations in lower-grade myelodysplastic syndromes and chronic myelomonocytic leukemia. Leuk Res. 2018;65:5–13.

36. Kumar D, Mehta A, Panigrahi MK, Nath S, Saikia KK. DNMT3A (R882) mutation features and prognostic effect in acute myeloid leukemia in Coexistent with NPM1 and FLT3 mutations. Hematol Oncol Stem Cell Ther. 2018;11(2):82–9.

37. Loghavi S, Zuo Z, Ravandi F, Kantarjian HM, Bueso-Ramos C, Zhang L, Singh RR, Patel KP, Medeiros LJ, Stingo F, et al. Clinical features of de novo acute myeloid leukemia with concurrent DNMT3A, FLT3 and NPM1 mutations. J Hematol Oncol. 2014;7:74.

38. Russler-Germain DA, Spencer DH, Young MA, Lamprecht TL, Miller CA, Fulton R, Meyer MR, Erdmann-Gilmore P, Townsend RR, Wilson RK, et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. Cancer Cell. 2014;25(4):442–54.

39. Nguyen TV, Yao S, Wang Y, Rolfe A, Selvaraj A, Darman R, Ke J, Warmuth M, Smith PG, Larsen NA, et al. The R882H DNMT3A hot spot mutation stabilizes the formation of large DNMT3A oligomers with low DNA methyltransferase activity. J Biol Chem. 2019;294(45):16966–77.

40. Koya J, Kataoka K, Sato T, Bando M, Kato Y, Tsuruta-Kishino T, Kobayashi H, Narukawa K, Miyoshi H, Shirahige K, et al. DNMT3A R882 mutants interact with polycomb proteins to block haematopoietic stem and leukaemic cell differentiation. Nat Commun. 2016;7:10924.

41. Que Y, Li H, Lin L, Zhu X, Xiao M, Wang Y, Zhu L, Li D. Study on the immune escape mechanism of acute myeloid leukemia with DNMT3A mutation. Front Immunol. 2021;12:653030.

42. Bera R, Chiu MC, Huang YJ, Liang DC, Lee YS, Shih LY. Genetic and epigenetic perturbations by DNMT3A-R882 mutants impaired apoptosis through augmentation of PRDX2 in myeloid leukemia cells. Neoplasia. 2018;20(11):1106–20.

43. Xu J, Zhang W, Yan XJ, Lin XQ, Li W, Mi JQ, Li JM, Zhu J, Chen Z, Chen SJ. DNMT3A mutation leads to leukemic extramedullary infiltration mediated by TWIST1. J Hematol Oncol. 2016;9(1):106.

44. Dai YJ, Wang YY, Huang JY, Xia L, Shi XD, Xu J, Lu J, Su XB, Yang Y, Zhang WN, et al. Conditional knockin of Dnmt3a R878H initiates acute myeloid leukemia with mTOR pathway involvement. Proc Natl Acad Sci U S A. 2017;114(20):5237–42.

45. Yang Y, Dai Y, Yang X, Wu S, Wang Y. DNMT3A mutation-induced CDK1 overexpression promotes leukemogenesis by modulating the interaction between EZH2 and DNMT3A. Biomolecules. 2021;11(6):781.

46. Rau RE, Rodriguez BA, Luo M, Jeong M, Rosen A, Rogers JH, Campbell CT, Daigle SR, Deng L, Song Y, et al. DOT1L as a therapeutic target for the treatment of DNMT3A-mutant acute myeloid leukemia. Blood. 2016;128(7):971–81.

47. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506(7488):328–33.

48. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3(7):730–7.

49. Yang L, Liu Y, Zhu L, Xiao M. DNMT3A R882 mutation is associated with elevated expression of MAFB and M4/M5 immunophenotype of acute myeloid leukemia blasts. Leuk Lymphoma. 2015;56(10):2914–22.

50. Di Marco A, Cassinelli G, Arcamone F. The discovery of daunorubicin. Cancer Treat Rep. 1981;65(Suppl 4):3–8.

51. Di Marco A. Mechanism of action and mechanism of resistance to antineoplastic agents that bind to DNA. Antibiot Chemother. 1971;1978(23):216–27.

52. Al-Aamri HM, Ku H, Irving HR, Tucci J, Meehan-Andrews T, Bradley C. Time dependent response of daunorubicin on cytotoxity, cell cycle and DNA repair in acute lymphoblastic leukaemia. BMC Cancer. 2019;19(1):179.

53. Lancet JE, Uy GL, Newell LF, Lin TL, Ritchie EK, Stuart RK, Strickland SA, Hogge D, Solomon SR, Bixby DL, et al. CPX-351 versus 7+3 cytarabine and daunorubicin chemotherapy in older adults with newly diagnosed high-risk or secondary acute myeloid leukaemia: 5-year results of a randomised, open-label, multicentre, phase 3 trial. Lancet Haematol. 2021;8(7):e481–91.

54. Tulpule A, Rarick MU, Kolitz J, Bernstein J, Myers A, Buchanan LA, Espina BM, Traynor A, Letzer J, Justice GR, et al. Liposomal daunorubicin in the treatment of relapsed or refractory non-Hodgkin's lymphoma. Ann Oncol. 2001;12(4):457–62.

55. Lee SM, Lee WS, Shin HJ, Lee JJ, Sohn SK, Moon JH, Eom HS, Won JH, Lee KH, Lee JH, et al. Escalated daunorubicin dosing as an induction treatment for Philadelphia-negative adult acute lymphoblastic leukemia. Ann Hematol. 2013;92(8):1101–10.

56. Kim M, Williams S. Daunorubicin and cytarabine liposome in newly diagnosed therapy-related acute myeloid leukemia (AML) or aml with myelodysplasia-related changes. Ann Pharmacother. 2018;52(8):792–800.

57. Kell WJ, Burnett AK, Chopra R, Yin JA, Clark RE, Rohatiner A, Culligan D, Hunter A, Prentice AG, Milligan DW. A feasibility study of simultaneous administration of gemtuzumab ozogamicin with intensive chemotherapy in induction and consolidation in younger patients with acute myeloid leukemia. Blood. 2003;102(13):4277–83.
58. Von Hoff DD, Layard M. Risk factors for development of daunorubicin cardiotoxicity. Cancer Treat Rep. 1981;65(Suppl 4):19–23.
59. Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, Van Vlierberghe P, Dolgalev I, Thomas S, Aminova O, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366(12):1079–89.
60. Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. Proc Natl Acad Sci U S A. 1994;91(21):9926–30.
61. Deng X, Lin N, Fu J, Xu L, Luo H, Jin Y, Liu Y, Sun L, Su J. The Nrf2/PGC1alpha pathway regulates antioxidant and proteasomal activity to alter cisplatin sensitivity in ovarian cancer. Oxid Med Cell Longev. 2020;2020:4830418.
62. Riz I, Hawley TS, Marsal JW, Hawley RG. Noncanonical SQSTM1/p62-Nrf2 pathway activation mediates proteasome inhibitor resistance in multiple myeloma cells via redox, metabolic and translational reprogramming. Oncotarget. 2016;7(41):66360–85.
63. Ma CS, Lv QM, Zhang KR, Tang YB, Zhang YF, Shen Y, Lei HM, Zhu L. NRF2-GPX4/SOD2 axis imparts resistance to EGFR-tyrosine kinase inhibitors in non-small-cell lung cancer cells. Acta Pharmacol Sin. 2021;42(4):613–23.
64. O'Cathail SM, Wu CH, Thomas R, Hawkins MA, Maughan TS, Lewis A. NRF2 mediates therapeutic resistance to chemoradiation in colorectal cancer through a metabolic switch. Antioxidants. 2021;10(9):1380.
65. Noman ASM, Parag RR, Rashid MI, Islam S, Rahman MZ, Chowdhury AA, Sultana M, Jerin C, Siddiqua A, Rahman L, et al. Chemotherapeutic resistance of head and neck squamous cell carcinoma is mediated by EpCAM induction driven by IL-6/p62 associated Nrf2-antioxidant pathway activation. Cell Death Dis. 2020;11(8):663.
66. Nohl H, Gille L, Staniek K. The exogenous NADH dehydrogenase of heart mitochondria is the key enzyme responsible for selective cardiotoxicity of anthracyclines. Z Naturforsch C J Biosci. 1998;53(3–4):279–85.
67. Zeekpudsa P, Kukongviriyapan V, Senggunprai L, Sripa B, Prawan A. Suppression of NAD(P)H-quinone oxidoreductase 1 enhanced the susceptibility of cholangiocarcinoma cells to chemotherapeutic agents. J Exp Clin Cancer Res. 2014;33:11.
68. Wang J, Ye X, Fan C, Zhou J, Luo S, Jin J, Chen D, Zheng Y, Wu C, Zhu X, et al. Leukemia cutis with IDH1, DNMT3A and NRAS mutations conferring resistance to venetoclax plus 5-azacytidine in refractory AML. Biomark Res. 2020;8(1):65.
69. Haselager MV, Kielbassa K, Ter Burg J, Bax DJC, Fernandes SM, Borst J, Tam C, Forconi F, Chiodin G, Brown JR, et al. Changes in Bcl-2 members after ibrutinib or venetoclax uncover functional hierarchy in determining resistance to venetoclax in CLL. Blood. 2020;136(25):2918–26.
70. Kim SJ, Zhao H, Hardikar S, Singh AK, Goodell MA, Chen T. A DNMT3A mutation common in AML exhibits dominant-negative effects in murine ES cells. Blood. 2013;122(25):4086–9.

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