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Original Article

Targeting SLUG sensitizes leukemia cells to ADR-induced apoptosis

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Abstract: Background and Aims: Slug is an E-cadherin repressor and a suppressor of PUMA (p53 upregulated modulator of apoptosis) and it has recently been demonstrated that Slug plays an important role in controlling apoptosis. In this study, we examined whether Slug's ability to silence expression suppresses the growth of leukemia HL-60 cells and/or sensitizes leukemia HL-60 cells to adriamycin (ADR) through induction of apoptosis. Methods: SLUG siRNA was transfected into the HL-60 and HL-60^{ADR} cell lines (an adriamycin resistant cell line). The stably SLUG siRNA transfected HL-60 and HL-60^{ADR} cells was transiently transfected with PUMA siRNA. The mRNA and protein expression of SLUG and PUMA were determined by Quantitative real-time RT-PCR and Western blot assay. The effects of SLUG siRNA alone or combined with ADR or PUMA siRNA on growth and apoptosis in HL-60 and HL-60^{ADR} cells was detected by MTT, ELISA and terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay. Results: The results showed that SLUG was less expressed in the HL-60 cells, and high expressed in the HL-60^{ADR} cells. Obvious down-regulation of SLUG mRNA and protein levels and up-regulation of PUMA mRNA and protein levels after SLUG siRNA transfection was showed in the HL-60^{ADR} cells. Treatment with ADR induced SLUG mRNA and protein in the HL-60 cells. Significant positive correlation was observed between basal SLUG mRNA and protein and ADR sensitivity. SLUG gene silencing by SLUG siRNA transfection inhibited growth and induced apoptosis, and increased ADR killing of the HL-60 and HL-60^{ADR} cell lines. After the SLUG siRNA transfected HL-60 and HL-60^{ADR} cells was transiently transfected with PUMA siRNA, did not increase ADR killing of the HL-60 and HL-60^{ADR} cell lines. Conclusion: SLUG level positively correlated with sensitivity to ADR. SLUG siRNA could effectively reduce SLUG expression and induce PUMA expression and restore the drug sensitivity of resistant leukemic cells to conventional chemotherapeutic agents.

Keywords: Myeloid leukemia, cytarabine, SLUG, PUMA

Introduction

Acute myeloid leukemia (AML) accounts for one-fourth of acute leukemia in children, but is responsible for more than half of the leukemia deaths in this patient population [1]. Resistance to cytarabine (ara-C)-based chemotherapy is a major cause of treatment failure in this disease [2, 3]. Therefore, further research is warranted into developing therapeutic strategies for the treatment of this disease.

Apoptosis, or programmed cell death, is a biological process essential for the regular development and maintenance of tissue homeostasis [4]. Disturbance in the regulation of apoptosis machinery contributes to the development of tumor and subsequent multi-drug resistance [4]. As the majority of cytotoxic drugs mainly kill malignant cells by the activation of apoptosis, recent anti-cancer approaches are focusing their efforts on specifically targeting the mediators involved within the respective apoptotic pathways [5].

The human Slug gene belongs to the highly conserved Slug/Snail family of transcription repressors, master regulators of neural crest cell specification and melanocyte migration during development in vertebrates [6, 7]. Previous studies have demonstrated that the overexpression of Slug can be found in many kinds of cancer [8]. Moreover, Slug activates multiple signal intermediates, such as E-cadherin, which

are key factors that influence the events of tumor invasion and metastasis [9]. In addition, Slug is a component of leukemic progenitor resistance to imatinib mesylate (IM) driven by Bcr-Abl point mutations and, in particular, by T315I. Slug over-expression associated with p210 Bcr-Abl TK either in the wild type (wt) or mutated conformation results in a significant reduction of E-cadherin, the substrate of Beta catenin at cell membranes [10].

PUMA is a downstream target of p53 and a BH3-only B-cell CLL/lymphoma-2 (Bcl-2) family member. It is induced by p53 following exposure to DNA-damaging agents, such as γ-irradiation and commonly used chemotherapeutic drugs [11-13]. It is also activated by a variety of nongenotoxic stimuli independent of p53, such as serum starvation, kinase inhibitors, glucocorticoids, endoplasmic reticulum stress, and ischemia/reperfusion [14]. The pro-apoptotic function of PUMA is mediated by its interactions with anti-apoptotic Bcl-2 family members, which lead to mitochondrial dysfunction and caspase activation [15].

K. Hemavathy et al. has reported that SLUG promotes survival and hinders cell death by directly repressing PUMA in melanoma cells [16]. Slug is transcriptionally induced by p53 upon irradiation and then protects the damaged cell from apoptosis by directly repressing PUMA [17]. The aim of the present study was to investigate and define the ability of SLUG siRNA to increase the susceptibility of HL-60 cells to ADR. Then we investigated the hypothesis that SLUG siRNA sensitizes ADR -induced apoptosis through upragulation of PUMA.

Materials and methods

Cell culture

HL-60 and HL-60^{ADR} cell lines (an adriamycin resistant cell line) were obtained from the American Type Culture Collection (ATCC; Shanghai, China). The cell lines were grown in RPMI 1640 (Invitrogen) supplemented with 10% FBS. HL-60^{ADR} was dissolved in DMSO was added. Cells were used in the exponential growth phase in all the experiments.

Drug exposure

Cells were exposed to ADR for 2 h to reproduce the clinical conditions of leukemia treatment. Taking into account that the LD-50 level is 5.02 μM for ADR, we tested 2.5- and 5.02-μM concentrations for ADR. Evaluation of the cytotoxic effect was performed 48 h after the end of drug exposure.

siRNA transfections

Three different double strand siRNA oligonucleotides (Invitrogen, Shanghai, China) were utilized alone or pooled together. A validated medium GC scramble (SCR) double strand siRNA oligonucleotide (Invitrogen) was used as control for transfection. The siRNA oligonucleotide showing the highest efficiency of SLUG mRNA knocking-down in HL-60 and HL-60^{ADR} cell lines was utilized for the experiments reported in the manuscript. Just before transfection, the cells were cultivated in RPMI-1640 medium free of serum and antibiotics. siRNA transfection (at a final concentration of 40 nM in all experiments) was performed using Lipofectamine™2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. Briefly, siRNAs and lipofectamine (4 μl/ml of transfection medium) were diluted in Opti-MEM I Reduced Serum Medium (Invitrogen) separately and incubated for 10 min at room temperature. The diluted solutions were then mixed and incubated for 20 min at room temperature. Subsequently, the mixtures were added to each well containing cells and medium. Moreover, the treated cells with only the transfection reagent were considered as a blank control. The cell culture plates were then incubated for 6 h at 37°C in a CO_2 incubator.The total incubation time before drug treatment was 72 h at 37°C.

The SLUG siRNA transfected cells was selected with G418 (600 ug/mL) for 14-21 days to acquired the stable

HL-60 and HL-60^{ADR}/SLUG siRNA clones. To further investigate the relationship between SLUG, PUMA expression and ADR sensitivity, stable SLUG siRNA transfected cells was transiently transfected with PUMA siRNA for 48 h before drug treatment.

Quantitative real-time RT-PCR

Real-time RT-PCR for SLUG and PUMA and housekeeping gene GAPDH was done using

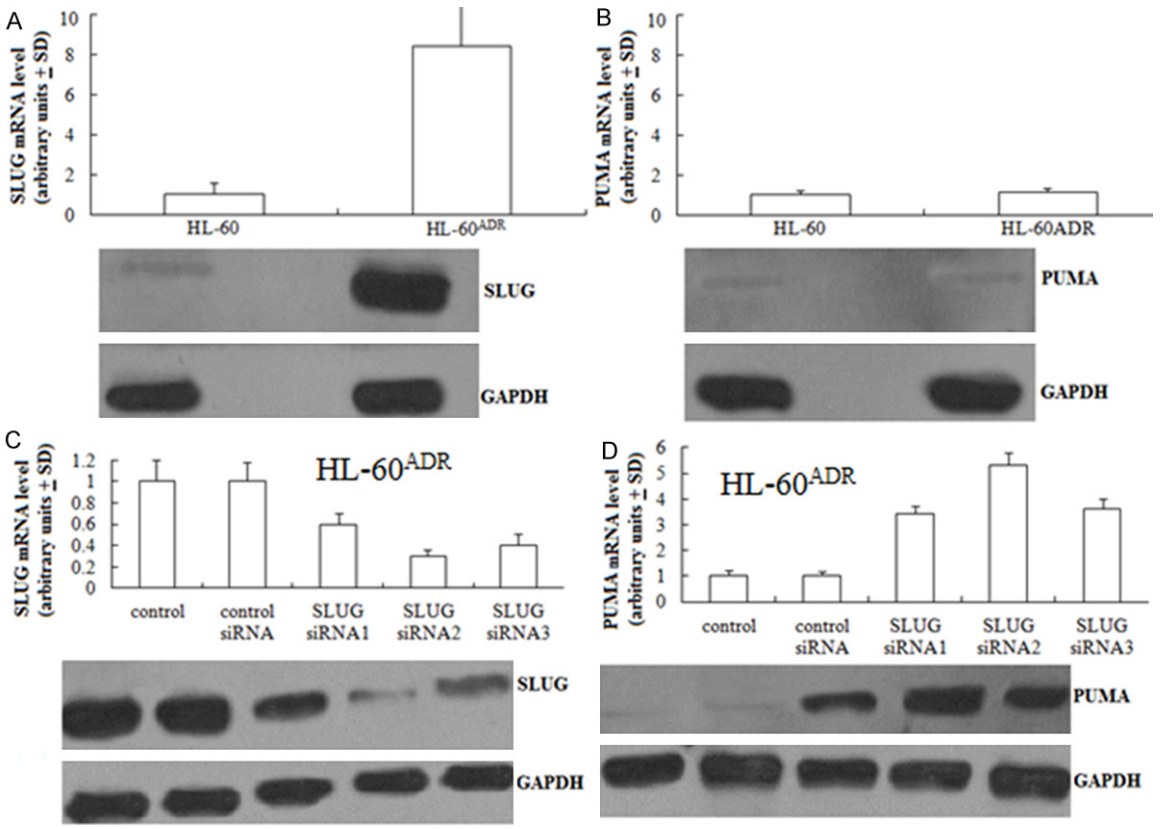

Figure 1. SLUG and PUMA expressed in HL-60 and HL-60^{ADR} cell lines. A. Real time PCR analysis of SLUG mRNA level (arbitrary units; ± SD, standard deviation of 3 replicates, upper part) and Western blot (WB) analysis of SLUG and *GAPDH* (lower part). B. Real time PCR analysis of PUMA mRNA level (arbitrary units; ± SD, standard deviation of 3 replicates, upper part) and Western blot (WB) analysis of PUMA and *GAPDH* (lower part). C. HL-60^{ADR} cells administered with either SLUG-specific (SLUG) or control siRNA. Real time PCR analysis of mRNA levels, normalized over control mRNA level (arbitrary units; ± SD, standard deviation of 3 replicates, upper part).WB analysis of SLUG and *GAPDH* (lower part). D. HL-60^{ADR} cells administered with either SLUG-specific (SLUG) or control siRNA. Real time PCR analysis of PUMA mRNA levels, normalized over control mRNA level (arbitrary units; ± SD, standard deviation of 3 replicates, upper part). WB analysis of PUMA and *GAPDH* (lower part).

iScript One-Step RT-PCR kit with SYBR Green (Bio-Rad), according to the manufacturer's instructions. The primer sequences used were as follows: forward 5′-AGA TGCATA TTC GGA CCC ACA-3′ and reverse 5′-CCT CAT GTT TGT GCA GGA GAG-3′ for *Slug*; forward 5′-CAG ACT GTG AAT CCT GTG CT-3′ and reverse 5′-ACA GTA TCT TAC AGG CTG CC-3′ for *PUMA*; forward primer 5′-CCTGGCACCCAGCACAAT-3′ , reverse primer 5′-GGG CCGGACTCGTCATCG-3′ for GAPDH.

Western blot assay

For protein extraction, cells were homogenized on ice in lysis buffer (50 mM Tris-HCL, PH7.5, 150 mM NaCl, 1% NP-40, 0.25% Na-desoxycholate, 5 mM EDTA, 1 mM NaF, 25 mM Na_3VO_4, 0.1 mM PMSF and 2 mg/ml Aprotinin) and cellular debris was pelleted at 13 000 g for 10 min at 4°C. Equal amount of protein (80 μg/well) were separated by 8% or 10% SDS-PAGE and transferred onto PVDF membranes. After blocked with 2% fat-free milk (MRP1), the membranes were incubated with antibody against human SLUG (1:1000 dilutions; Abcam, UK) or PUMA (1:400 dilutions; Abcam, UK) or GAPDH (1:5 000 dilutions; Sigma, USA) at 4°C overnight. The bound antibodies were detected using horseradish peroxidase (HRP)-conjugated IgG and visualized with enhanced chemiluminescence (ECL) detection reagents (Thermo scientific, USA).

Cell proliferation assay (MTT)

The method used was similar to that previously described [18]. After 48 hours the drugs and

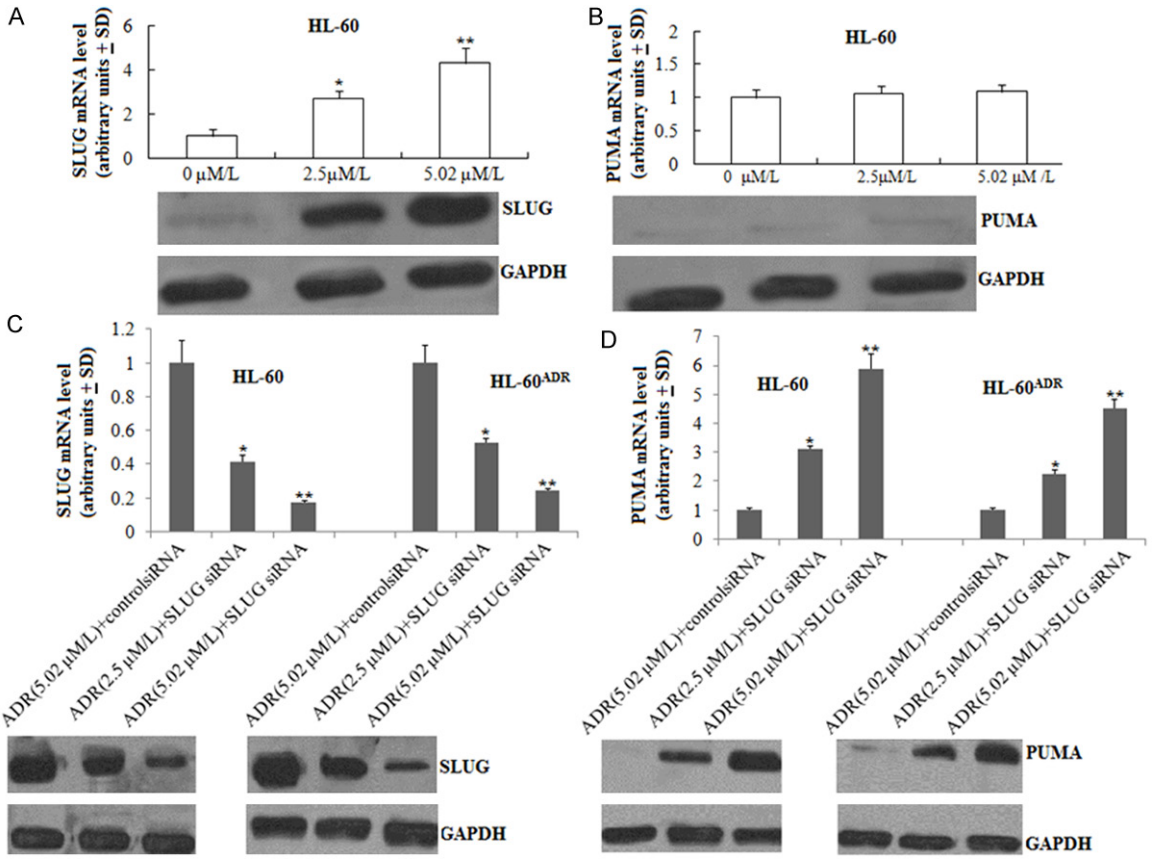

Figure 2. SLUG and PUMA expressed in HL-60 and HL-60^{ADR} cells treated with ADR. A. HL-60 cells were treated with ADR (2.5 μM/L and 5.02 μM/L) for 48 h. Real time PCR analysis of SLUG mRNA level (arbitrary units; ± SD, standard deviation of 3 replicates, upper part) and Western blot (WB) analysis of SLUG and *GAPDH* (lower part). B. Real time PCR analysis of PUMA mRNA level (arbitrary units; ± SD, standard deviation of 3 replicates, upper part) and Western blot (WB) analysis of PUMA and *GAPDH* (lower part). C. HL-60 and HL-60^{ADR} cells were treated with SLUG siRNA combined with ADR (2.5 μM/L and 5.02 μM/L) for 48 h. Real time PCR analysis of SLUG mRNA level (arbitrary units; ± SD, standard deviation of 3 replicates, upper part) and Western blot (WB) analysis of SLUG and *GAPDH* (lower part). D. Real time PCR analysis of PUMA mRNA level (arbitrary units; ± SD, standard deviation of 3 replicates, upper part) and Western blot (WB) analysis of PUMA and *GAPDH* (lower part). Vs control, $^{*}P<0.05$; $^{**}P<0.01$.

medium were removed from the wells by flicking and 50 μl of MTT solution (2 mg MTT ml-1 Hanks balanced salt solution without phenol red) was added to all wells. The microtitre plates were re-incubated for 4 hours and any formazan crystals formed were dissolved in acid/alcohol (0.04 N HCl in isopropanol). The plates were read at 570 nm (reference 690 nm) on an Anthos 2001 plate reader.

Terminal Deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) assay

Cells in different groups were cultured on chamber slides for 24 h. Apoptosis of the cells was evaluated on the basis of the Terminal Deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) assay using the Dead End Fluorometric TUNEL System (Promega, Madison, WI, USA) according to the manufacturer's instructions. All assays were performed in quadruplicate.

Annexin V staining

The Annexin V assays were performed according to the manufacture's protocol (PharMingen). Briefly, the cultured cells were collected, washed with binding buffer, and incubated in 200 μL of a binding buffer containing 5 μL of Annexin-V-FITC. The nuclei were counterstained with PI. The percentage of apoptotic cells was determined using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA).

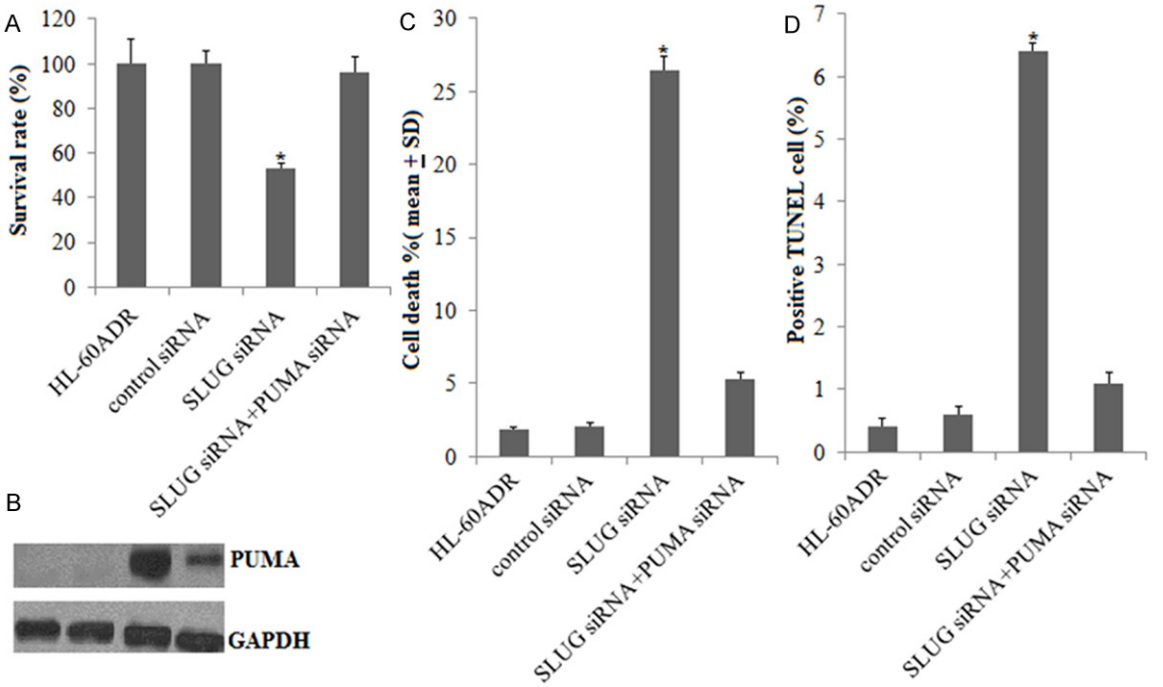

Figure 3. Effect of SLUG /PUMA signal on HL-60^{ADR} cells survival and apoptotic death. HL-60^{ADR} cells were transfected with control siRNA or SLUG siRNA alone or combined with PUMA siRNA transfection. A. Cell viability was determined by MTT assay. B. Western blot was used to detected PUMA expression in 4 groups. C. Annexin V Staining was used to detected apoptotic death. D. TUNEL was used to detected cell apoptosis. The results are expressed as mean ± SD (n=3); $^{*}P<0.05$ versus other 3 groups.

Statistical analysis

Statistics were conducted by SPSS 11.0 software. All data were expressed as mean ± standard error. One-way ANOVA was used for comparisons between groups; the t-test was used to compare the mean values of the samples from different groups; A value of $P<0.05$ was considered statistically significant.

Results

SLUG and PUMA expression

SLUG mRNA and PUMA mRNA and protein levels were evaluated in HL-60 and HL-60^{ADR} cell lines. HL-60^{ADR} cell lines expressed rich levels of SLUG mRNA and protein and HL-60 cell lines expressed undetectable SLUG mRNA and protein (**Figure 1A**). Less PUMA mRNA and protein was detected in HL-60 and HL-60^{ADR} cell lines (**Figure 1B**).

When the HL-60^{ADR} cell lines were exposed to three RNA double strand SLUG-specific short interfering oligonucleotides (SLUG siRNA), a downregulation of the SLUG mRNA and protein was observed with respect to exposure to an appropriate oligonucleotide control siRNA transfected HL-60^{ADR} cell lines (**Figure 1C**). Moreover, we observed that treatment with SLUG siRNA upregulated of PUMA mRNA and protein levels with respect to control siRNA in the HL-60^{ADR} cell lines (**Figure 1D**). Because SLUG siRNA2 has the highest effency for targeting SLUG, we selected SLUG siRNA2 for further study.

ADR treatment upregulates SLUG, but not PUMA

HL-60 and HL-60^{ADR} cell lines were exposed to ADR (2.5 μM/L and 5.02 μM/L) for 48 h. SLUG mRNA and protein was significantly increased in HL-60 cell lines (**Figure 2A**). No upregulation or down-regulation of PUMA mRNA or protein expression was present in HL-60 cell lines that were exposed to ADR (**Figure 2B**).

However, no upregulation of SLUG mRNA or protein expression was present in HL-60^{ADR} cell lines that were exposed to ADR (data not

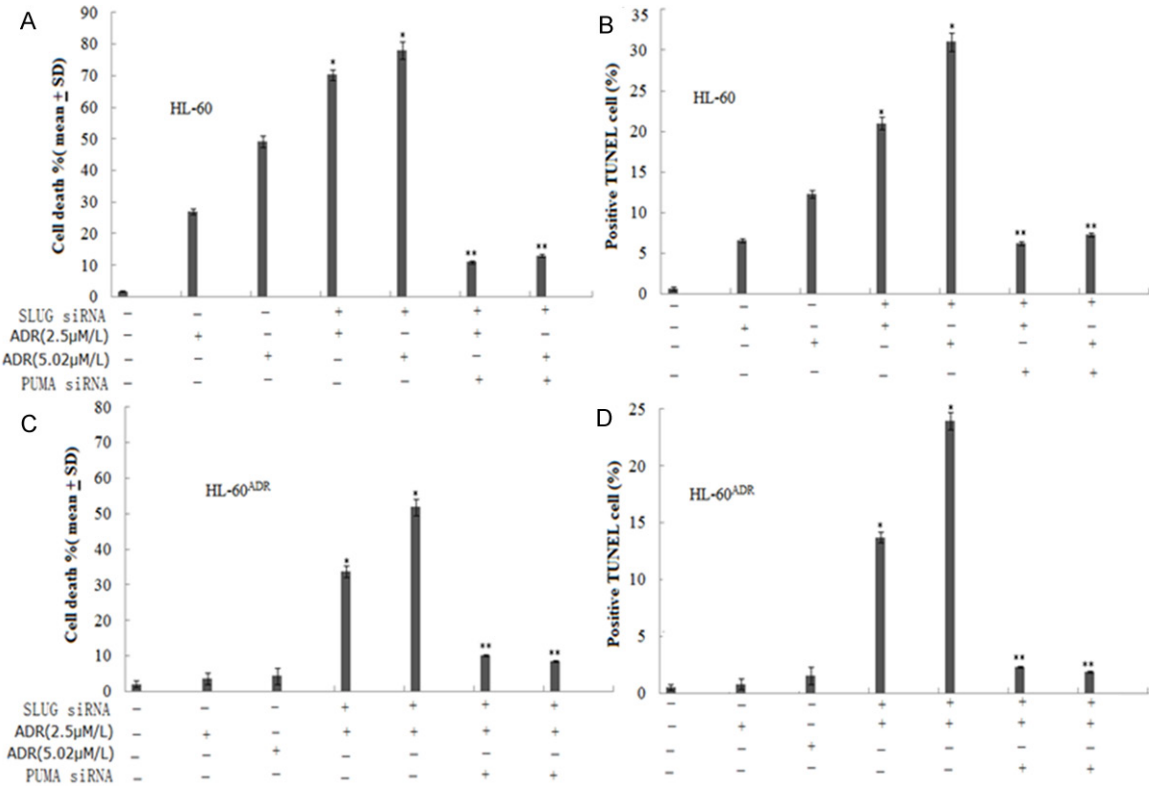

Figure 4. Effect of SLUG siRNA on ADR-induced apoptosis in HL-60 and HL-60^{ADR} cells. HL-60 or HL-60^{ADR} cells were treated with ADR or/and transfected with SLUG siRNA or combined with PUMA siRNA transfection. A. Annexin V Staining was used to detected apoptotic death in HL-60 cells. B. TUNEL was used to detected cell apoptosis in HL-60 cells. C. Annexin V Staining was used to detected apoptotic death in HL-60^{ADR} cells. D. TUNEL was used to detected cell apoptosis in HL-60^{ADR} cells. $^{*}P<0.05$, $^{**}P<0.01$.

shown). No upregulation or down-regulation of PUMA mRNA or protein expression was present in HL-60^{ADR} cell lines that were exposed to ADR (data not shown).

We observed that treatment with SLUG siRNA combined with ADR downregulated of SLUG mRNA and protein levels with respect to control siRNA in the HL-60 and HL-60^{ADR} cell lines (**Figure 2C**). Conversely, the PUMA siRNA and protein was significantly increased (**Figure 2D**).

SLUG siRNA-induced HL-60^{ADR} cell growth inhibition by PUMA upregulation

We next examined the growth inhibitory effects of SLUG siRNA using the MTT assay in HL-60^{ADR} cell lines.

Transfection with SLUG siRNA in HL-60^{ADR} cells for 72 h significantly inhibited SLUG expression (**Figure 1C**) and increased PUMA expression (**Figure 1D**), followed by the cell growth inhibition (**Figure 3A**). However, when SLUG siRNA transfected HL-60^{ADR} cells was transiently transfected with PUMA siRNA for 48 h, PUMA expression was inhibited (**Figure 3B**), and cell growth inhibition was not observed in SLUG siRNA transfected HL-60^{ADR} cells (**Figure 3A**).

SLUG siRNA-induced HL-60^{ADR} cell apoptosis by PUMA upregulation

SLUG siRNA was transiently transfected into the HL-60^{ADR} cells for 72 h. After transfection, the degree of apoptosis was measured by ELISA assay. We found that SLUG siRNA induced apoptosis in HL-60^{ADR} cells (**Figure 3C**). To confirm this result, we also used TUNEL methods to detect apoptosis: TUNEL assay also showed that SLUG siRNA induced apoptosis in HL-60^{ADR} cells (**Figure 3D**). However, when SLUG siRNA transfected HL-60^{ADR} cells was transiently transfected with PUMA siRNA for 48 h, cell apoptosis was not observed in SLUG siRNA transfected HL-60^{ADR} cells by ELISA assay (**Figure 3C**) and TUNEL assay (**Figure 3D**).

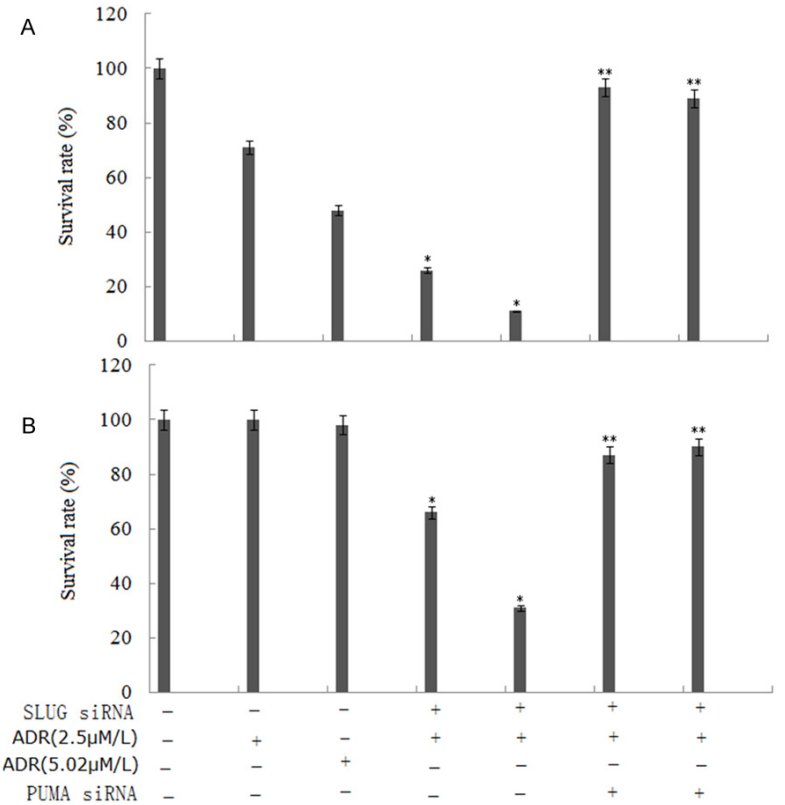

Figure 5. Effect of SLUG siRNA on ADR-induced growth inhibition in HL-60 and HL-60ADR cells. HL-60 or HL-60ADR cells were treated with ADR or/and transfected with SLUG siRNA or combined with PUMA siRNA transfection. A. Cell viability was determined by MTT assay in HL-60 cells. B. Cell viability was determined by MTT assay in HL-60ADR cells. *P<0.05, **P<0.01.

SLUG siRNA augments ADR-induced apoptosis by PUMA upregulation in HL-60 and HL-60^{ADR} cells

To further define the roles of SLUG in ADR sensitivities in HL-60 and HL-60^{ADR} cell lines, SLUG siRNA was transfected in HL-60 and HL-60^{ADR} cells. HL-60 and HL-60^{ADR} cell lines were exposed to ADR (2.5 μM/L and 5.02 μM/L) for 48 h. ADR treatment resulted in markedly increased apoptosis in HL-60 cells in a dose-dependant manner by ELISA assay (**Figure 4A**) and TUNEL assay (**Figure 4B**), whereas no important changes were observed in HL-60^{ADR} cell lines treated with ADR by ELISA assay (**Figure 4C**) and TUNEL assay (**Figure 4D**).

Furthermore, when SLUG siRNA transfected HL-60 and HL-60^{ADR} cells was exposed to ADR (2.5 μM/L and 5.02 μM/L) for 48 h, cell apoptosis was markedly increased in both HL-60 and HL-60^{ADR} cell lines (**Figure 4A-D**).

However, when SLUG siRNA transfected HL-60 and HL-60^{ADR} cells was transiently transfected with PUMA siRNA for 48 h, then exposed to ADR (2.5 μM/L and 5.02 μM/L) for 48 h, cell apoptosis was not markedly increased in both HL-60 and HL-60^{ADR} cell lines (**Figure 4A-D**). These results demonstrate that inhibition of SLUG can upregulate PUMA, significantly enhance ADR sensitivities in HL-60 and HL-60^{ADR} cell lines.

SLUG siRNA augments ADR-induced growth inhibition by PUMA upregulation in HL-60 and HL-60^{ADR} cells

HL-60 and HL-60^{ADR} cell lines were exposed to ADR (2.5 μM/L and 5.02 μM/L) for 48 h. ADR treatment resulted in significant cell growth inhibition in HL-60 cells by MTT assay (**Figure 5A**), whereas no important changes were observed in HL-60^{ADR} cell lines treated with ADR (**Figure 5B**).

Furthermore, when SLUG siRNA transfected HL-60 and HL-60^{ADR} cells was exposed to ADR (2.5 μM/L and 5.02 μM/L) for 48 h, cell viability was markedly decreased in both HL-60 and HL-60^{ADR} cell lines (**Figure 5A**, **5B**).

However, when SLUG siRNA transfected HL-60 and HL-60^{ADR} cells was transiently transfected with PUMA siRNA for 48 h, then exposed to ADR (2.5 μM/L and 5.02 μM/L) for 48 h, cell viability was not markedly changed in both HL-60 and HL-60^{ADR} cell lines (**Figure 5A**, **5B**). These results demonstrate that inhibition of SLUG can upregulate PUMA, significantly inhibited cell viability in HL-60 and HL-60^{ADR} cell lines.

Discussion

Leukemic cancers arise from genetic alterations in normal hematopoietic stem or progenitor cells, leading to impaired regulation of proliferation, differentiation, and apoptosis as well as survival of malignant cells. The front-line therapy in leukemia is chemo (drug) therapy

[19, 20], including broad-spectrum cytotoxic agents against fast-proliferating cells and small-molecule inhibitors targeting specific signal transduction pathways, so called molecular therapies [21].

Leukemic cells generally respond well to drug therapy at the onset of treatment, but the drugs lose their effectiveness over a period of six-twelve months in a significant fraction of patients. It is now well recognized that the resistance to broad-spectrum drugs is inevitable, but recent evidence also indicated that even the most advanced molecular drugs can lose their efficacy [22].

The human SLUG gene belongs to the highly conserved Slug/Snail family of transcription repressors, which are master regulators of neural crest cell specification and melanocyte migration during development in vertebrates [23, 24]. It has found that SLUG is aberrantly expressed in a number of cancers, specifically t(17:9) leukemic cells [25] and leukemic stem cells [26]. A definitive role for Slug in the development of mesenchymal tumors (leukemias: acute B-cell lymphoblastic leukemia and acute myeloid leukemia; and sarcomas) has been demonstrated in mice carrying a tetracycline-repressible Slug transgene [27]. Importantly, as the postnatal expression of Slug and the effects of Slug deletion are similar in humans and mice, it may be valid to extrapolate from the role of Slug in the protection of blood progenitors from IR in mice, to its importance in mesenchymal human cancers. Drawing on the findings of Wu et al., [28] it will be fascinating to establish whether cancers with deregulated Slug expression carry wild-type p53. It is tempting to speculate that in these tumors, Slug confers resistance to p53-mediated death via Puma suppression. Accordingly, Slug may protect certain tumor types from p53-induced apoptosis in response to oncogenic stress, or to genotoxic anticancer treatments.

Cha et al. has found that Slug is overexpressed in RA ST and that suppression of SLUG gene facilitates apoptosis of FLS by increasing Puma transactivation [29]. Zhang et al. has found that knockdown of SLUG could upregulate PUMA which contributes to the radiosensitivity of cholangiocarcinomas [30]. Kim et al. has reported Slug inhibition by shRNA sensitized tumor cells to apoptosis by DNA damage, resulting in caspase-3 and PARP cleavage. The pro-survival effect of Slug was found to be caused by direct repression of the proapoptotic gene, Puma (Bbc3), by Slug [31]. Mancini et al. has recently reported that SLUG contributes to apoptosis resistance of leukemic progenitors through the repression of pro-apoptotic PUMA. It has a central role involved in prolonged survival and IM resistance of CML progenitors [29].

Whether a high level of SLUG gene is directly associated with resistance to ADR treatment in human leukemia cells remains controversial. Our study did show a positive relationship between ADR sensitivity and the level of SLUG in the leukemia cell line. In the HL-60 cell lines, which has low SLUG protein level, the ADR LD50 was 5.02 μmol/L; in the HL-60^{ADR} cell lines, which has high SLUG protein level, the ADR LD50 was over 5.02 μmol/L; Because ADR treatment increased the SLUG expression in HL-60 cells analyzed, we therefore suggested that endogenous and exogenous SLUG level did correlate with sensitivity to ADR in leukemia cell line in vitro.

Here, we investigated the mechanism by which SLUG siRNA elicits its biological effects on LH-60 and HL-60^{ADR} cells. In this study, we used SLUG less expressed LH-60 cells and ADR resistant LH-60 cells HL-60^{ADR}. In the present study we showed that SLUG siRNA was capable of inducing significant growth inhibition in the HL-60^{ADR} cells as detected by the MTT assay. Moreover, SLUG siRNA also induced apoptotic cell death in HL-60^{ADR} cells, suggesting that blocking SLUG is sufficient to trigger apoptosis in ADR resistant HL-60^{ADR} cells overexpressing the SLUG protein. The mechanism by which SLUG siRNA induced an increase in apoptosis involves the upregulation of PUMA.

SLUG siRNA increases both the susceptibility of HL-60 and HL-60^{ADR} cell lines to the cytotoxic action of ADR. Drug concentrations were based on *in vivo* peak plasma levels, and the exposure time was chosen on the basis of each drug's plasmatic half-life. We found that, in the presence of SLUG siRNA, ADR induced cell death at a concentration corresponding to half of its peak plasma level, which is not active in control siRNA cells. The mechanism by which SLUG

siRNA induced an increase in ADR induced apoptosis involves the upregulation of PUMA.

In summary, we presented experimental evidence, that endogenous and exogenous SLUG level was positively correlated with sensitivity to ADR in leukemia cell line. It also strongly supports the antitumor effects of SLUG siRNA in leukemia cells overexpressing SLUG in vitro. Furthermore, SLUG siRNA promotes drug-induced cell death. However, further mechanistic studies could be useful to fully support our strategy for the treatment of patients with leukemia.

Disclosure of conflict of interest

None.

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