SIRT1 downregulation enhances chemosensitivity and survival of adult T-cell leukemia-lymphoma cells by reducing DNA double-strand repair

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Abstract. Most chemotherapy drugs used for the treatment of adult T-cell leukemia-lymphoma (ATL) cause cell death directly by inducing DNA damage, which can be repaired via several DNA repair pathways. Enhanced activity of DNA damage repair systems contributes to ATL resistance to chemotherapies. Targeting DNA repair pathways is a promising strategy for the sensitization of ATL cells to chemotherapeutic drugs. In the present study, inhibition of SIRT1 deacetylase by shRNA sensitized Jurkat cells to etoposide by reducing the activity of non-homologous end joining (NHEJ) and homologous recombination (HR). Silencing of SIRT1 deacetylase by shRNA resulted in enhanced apoptosis and cell cycle arrest, while reduced colony formation of Jurkat cells after etoposide treatment was accompanied by elevated acetylation of FOXO1. Furthermore, inhibition of SIRT1 led to decreased activity of DNA damage repair by NHEJ and HR, accompanied by increased Ku70 acetylation. Furthermore, SIRT1 downregulation prolonged the survival time of Jurkat-xenografted mice. These results suggested that SIRT1 promotes DNA double-strand repair pathways in Jurkat cells by deacetylating Ku70, and increases cell proliferation by deacetylating FOXO1. The results suggest that SIRT1 is a potential target for the development of combinatorial treatment for ATL.

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Introduction

Adult T-cell leukemia-lymphoma (ATL), an aggressive peripheral T-cell neoplasm, results from long-term infection with human T-cell leukemia virus-1 (HTLV-1) and is associated with a poor prognosis (1,2). DNA-damaging chemotherapy drugs, such as etoposide and anthracyclines, are the first-line treatment for ATL. However, drug resistance is a challenge for the management of ATL patients (3,4). Most cancer chemotherapeutic agents induce cell death by producing DNA strand breaks and DNA replication fork collapse (5). However, cells have evolved complicated DNA integrity surveillance systems including DNA damage response (DDR) and repair networks, which jointly function to maximize survival and minimize the gene mutation rate (6). Accumulating evidence has shown that enhanced DNA damage repair plays a critical role in the resistance of cancer cells to chemotherapy (7). Therefore, targeting DNA repair pathways is a promising strategy to overcome ATL resistance to chemotherapy.

Most DNA-damaging chemotherapeutic agents directly or indirectly cause DNA double-strand breaks (DSBs), which are highly lethal lesions that kill cells by inactivating essential genes or, in metazoans, by triggering apoptosis (8,9). Although replication-associated DSBs are repaired by homologous recombination (HR) and related replication repair pathways, DSBs are mainly repaired by non-homologous end joining (NHEJ), which comprises the Ku70 and Ku80 heterodimer, DNA-dependent protein kinase (DNA-PKcs), DNA ligase IV and scaffold Xrcc4. The Ku70 and Ku80 heterodimer recognizes and binds to DNA ends, and activates DNA-PKcs by stabilizing its interaction with the DNA ends, while DNA ligase IV and the scaffold Xrcc4 complex are involved in the final ligation step (10). Deregulation of the NHEJ pathway affects the efficiency of DNA-damaging drugs, thereby leading to escape and survival of leukemia cells following chemotherapy.

SIRT1 is a multifaceted, NAD^+-dependent protein deacetylase, and a key regulator of lifespan mediated by caloric restriction (11). SIRT1 also plays an important role in anti-aging and tumorigenesis (12). One of the mechanisms by which SIRT1 prolongs lifespan and suppresses tumorigenesis

is the modification of numerous DNA damage repair proteins, including Ku70 (13), FOXOs (14,15), Nijmegen breakage syndrome protein (NBSP) (16), WRN protein (17), xeroderma pigmentosum C protein (18) and NF-κB (19). Recent findings have shown that SIRT1 plays a key role in leukemogenesis and resistance to leukemia treatment presumably by facilitating DNA repair in leukemia cells (20-22). SIRT1 has been shown to be consistently overexpressed in primary ATL samples and the pharmacologic inhibition or shRNA-mediated knockdown of SIRT1-induced apoptosis in ATL (23). However, the underlying molecular mechanism and whether inhibition of SIRT1 sensitizes ATL cells to DNA damage drugs remain to be determined.

In the present study, we knocked down SIRT1 by shRNA in Jurkat cells, one of the most representative cell lines of ATL, and assessed apoptosis, cell cycle arrest and proliferation following etoposide treatment in cell culture, and leukemia blast and the survival time of Jurkat-xenografted mice. We further assayed DNA damage repair capacity and examined the underlying mechanism after SIRT1 silencing in response to etoposide treatment.

Materials and methods

Cell lines and peripheral blood mononuclear cells. HL-60, THP-1, HEL, Daudi, Karpas 299, K562, Namalwa, Su-DHL-4, Jurkat and 293T/17 cell lines were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). The lentivirus packaging cell line 293T/17 was cultured in Dulbecco's modified Eagle's medium (DMEM) and the remaining cells were maintained in RPMI-1640 medium and Iscove's modified Dulbecco's medium (IMDM). The above media were supplemented with 100 U/ml of penicillin, 0.1 mg/ml of streptomycin, 2 mM L-glutamine and 10% fetal bovine serum (FBS). The cells were maintained at 37˚C in a humidified atmosphere of 5% CO_2. The cells in the logarithmic phase were used for subsequent experiments. Four peripheral blood (PB) samples were obtained from four healthy volunteers. Written informed consent for participation in the present study was obtained, and laboratory experiments were approved by the ethics committee of the Tongji Hospital of Tongji University (Shanghai, China). Peripheral blood mononuclear cells (PBMCs) were obtained from peripheral blood by separation on the Lymphoprep (Axis-Shield, Oslo, Norway) density gradient with centrifugation at 400 x g for 30 min. The fresh PBMCs were used for reverse-transcriptase PCR (RT-PCR).

Reagents. Etoposide (Sigma-Alrich, St. Louis, MO, USA), also known as VP16, was used at the concentration of 20 μM to treat cells seeded at $1x10^5$/ml in 6-well plates. After incubation for 4 h, cells were washed twice in PBS and maintained in fully supplemented RPMI-1640 medium for 48 h before further analysis. Primary antibodies against SIRT1, Ku70 (Abcam, Cambridge, UK), phospho-Histone H2AX (Ser139), acetylated-lysine, GAPDH (Cell Signaling Technology, Danvers, MA, USA), acetylated FOXO1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were used. Horseradish peroxidase-conjugated secondary antibodies were purchased from Cell Signaling Technology.

Gene knockdown. The shRNA targeting SIRT1 (sequence as 5'-GAAGTGCCTCAGATATTAA-3') and negative control (sequence as 5'-TTCTCCGAACGTGTCACGT-3') were cloned into pLVX-shRNA1 vector (Clontech Laboratories) and designated as shSIRT1 and SCR. Lentiviral particles were produced by co-transfection of 293T/17 cells with shSIRT1 or SCR and the lentiviral packaging plasmid at a ratio of 4:3:2 using the calcium phosphate precipitation method. Transduction was carried out in the presence of 5 μg/ml polybrene. Following transduction, 1.5 μg/ml puromycin (Sigma-Aldrich) was added for positive selection. Subsequent experiments were performed 72 h after transduction.

Measurement of DNA repair capacity. Plasmids containing NHEJ, HR reporter cassettes and pDsRed-N1 as the internal controls were kindly provided by Dr Zhiyong Mao from the School of Life Science and Technology of Tongji University (Shanghai, China). NHEJ or HR reporter cassettes containing plasmids were first linearized by I-*Sce*I restriction enzymes and purified using the Qiagen QIAEX II purification kit (20021; Qiagen, Valencia, CA, USA). A 0.5 μg of the NHEJ reporter construct, or 2 μg of the HR reporter constructs, and 0.1 μg of pDsRed-N1 were transfected into exponentially growing cells. Transfections were performed using the Amaxa Nucleofector (Walkersville, MD, USA). Jurkat cells were transfected using Cell Line Nucleofector kit V (Amaxa VPA-1003) and program X001. Cells were analyzed by FACS 72 h after the transfection.

RNA extraction and qPCR. Total RNA was extracted from cell lines and PBMCs using TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. First-strand cDNA was synthesized in 10 μl reaction volume using PrimeScript RT Master Mix (Takara, Dalian, China). Relative mRNA levels of target gene and β-actin were detected by RT-qPCR in Applied Biosystems 7500 Fast Real-Time PCR systems (Life Technologies) with SYBR Premix Ex Taq™ (Takara). The primers were used: SIRT1, forward 5'-ATACCCAGAACATAGACAC GCT-3' and reverse 5'-CGT ACAGCTTCACAGTCAACTT-3'; β-actin, forward 5'-GAA CGGTGAAGGTGACAGCAG-3' and reverse 5'-GTGGAC TTGGGAGAGGACTGG-3'. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method, where $\Delta Ct = (Ct_{target\ gene} - Ct_{\beta\text{-}actin})$.

Immunoblotting and immunoprecipitation. The cells were lysed in Cell Lysis Buffer (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 1 mM PMSF (Beyotime Institute of Biotechnology, Nantong, China). Cell lysates were incubated on ice for 30 min and the supernatants were collected for immunoblotting by centrifuging at 13,300 rpm for 30 min at 4˚C. BCA assay (Beyotime Institute of Biotechnology) was used to determine the protein concentration. The proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Beyotime Institute of Biotechnology). The separated proteins were electrophoretically transferred to PVDF membranes (Millipore, Billerica, MA, USA). After blocking in 5% non-fat milk in Tris-buffered saline/Tween-20 (TBST) for 1 h, the membranes were incubated independently with the primary antibodies against specific proteins in 5% BSA TBST overnight at 4˚C. For the detection of acetylated Ku70, the cell

lysates were first immunoprecipitated by anti-Ku70 antibody overnight at 4˚C with gentle rotation. The immunoprecipitants were then incubated with 30 μl protein G agarose beads (Santa Cruz Biotechnology, Inc.) for 4 h and resolved by SDS-PAGE after four washes with lysis buffer. The immunoprecipitated samples were subjected to immunoblot analysis using an anti-acetylated antibody. The membranes were incubated with an anti-rabbit or anti-mouse IgG, HRP-linked antibody (Cell Signaling Technology) for 2 h at room temperature and detected with ECL Plus (Millipore) followed by LAS-4000 scanning (Fujifilm, Tokyo, Japan).

Apoptosis and cell cycle analysis. Apoptosis was analyzed by staining with Annexin V-FITC and propidium iodide (BD Biosciences Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Flow cytometry was then performed within 1 h. Data was analyzed through FlowJo software (Tree Star, Ashland, OR, USA). For the cell cycle analysis, the cells were collected and washed twice with PBS. Subsequently, the cells were fixed with cold ethanol and incubated at -20˚C for 2 h. After centrifugation, the cells were treated with PI/RNase staining buffer (BD Biosciences), and then incubated for 15 min at room temperature followed by flow cytometry.

Soft agar colony formation assay. For the clonogenic assay, a standard two-layer soft agar culture was performed with bottom 0.6% agarose and top 0.3% agarose. The cells were seeded at 10^5/ml in 24-well plates with soft agar as described above, and colonies were scored after 7 days.

Xenotransplantation assays. Jurkat cells ($1x10^6$) transduced with vectors expressing a shRNA directed against SIRT1 or scrambled shRNA were transplanted via tail vein injection into sub-lethally irradiated (2.5 Gy) NOD-SCID mice (Shanghai SLAC Laboratory Animal, Co., Ltd., Shanghai, China). The survival time was subsequently recorded. Twenty days after transplantation, three mice from each group were euthanized, and bone marrow and spleen were collected for subsequent experiments.

Statistical analyses. Unless otherwise specified, data are presented as mean ± SD. For data analysis, unpaired two-tailed Student's t-test or Mann-Whitney U test were performed. For the animal studies, Kaplan-Meier survival analysis was performed and survival was calculated using the log-rank test. Statistical computations were performed using Prism 6.0 (GraphPad Software).

Results

SIRT1 is overexpressed in Jurkat cells. We assessed the relative SIRT1 mRNA levels in several cell lines and 4 PBMC samples of the healthy human controls. As shown in Fig. 1A, Karpas 299, K562, Namalwa, Su-DHL-4 and Jurkat demonstrated a significantly higher expression of SIRT1 than in the normal controls. The Jurkat cell line showed the highest SIRT1 mRNA level. Consistent with the mRNA expression levels, the immunoblot analysis revealed that Jurkat cells manifested markedly higher SIRT1 protein levels than the remaining four cell lines (Fig. 1B and C). Subsequently, Jurkat cells were selected for subsequent experiments.

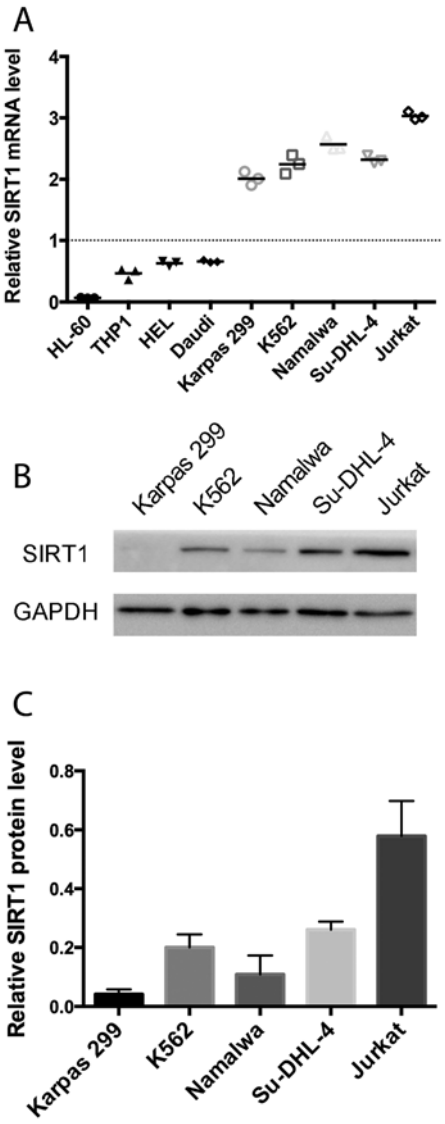

Figure 1. SIRT1 expression levels in the cell lines and normal control PBMCs. (A) SIRT1 mRNA levels of HL-60, THP-1, HEL, Daudi, Karpas 299, K562, Namalwa, Su-DHL-4 and Jurkat were analyzed by RT-qPCR relative to β-actin as the internal control. Horizontal bars indicate the mean percentages of mRNA expression. Dotted line is the mean of SIRT1 mRNA levels of 4 normal control PBMCs. (B) Protein expression levels of SIRT1 and GAPDH in the cell lysates of Karpas 299, K562, Namalwa, Su-DHL-4 and Jurkat were determined by immunoblotting. (C) SIRT1 protein expression was quantified by densitometric analysis and normalized to GAPDH expression. The normalized SIRT1 expression levels of Karpas 299, K562, Namalwa, Su-DHL-4 and Jurkat are shown.

shRNA-mediated knockdown of SIRT1 sensitizes Jurkat cells to etoposide. To investigate the role of SIRT1 in response to DNA damage of Jurkat cells, we downregulated SIRT1 by shRNA and analyzed the apoptosis, cell cycle distribution and colony formation ability of Jurkat cells following etoposide treatment. The shRNA against SIRT1 specifically decreased SIRT1 protein level in Jurkat cells (Fig. 2A). Compared with the control shRNA, SIRT1 shRNA did not apparently alter cell apoptosis under normal growth conditions, but significantly enhanced the induction of apoptosis by etoposide in Jurkat

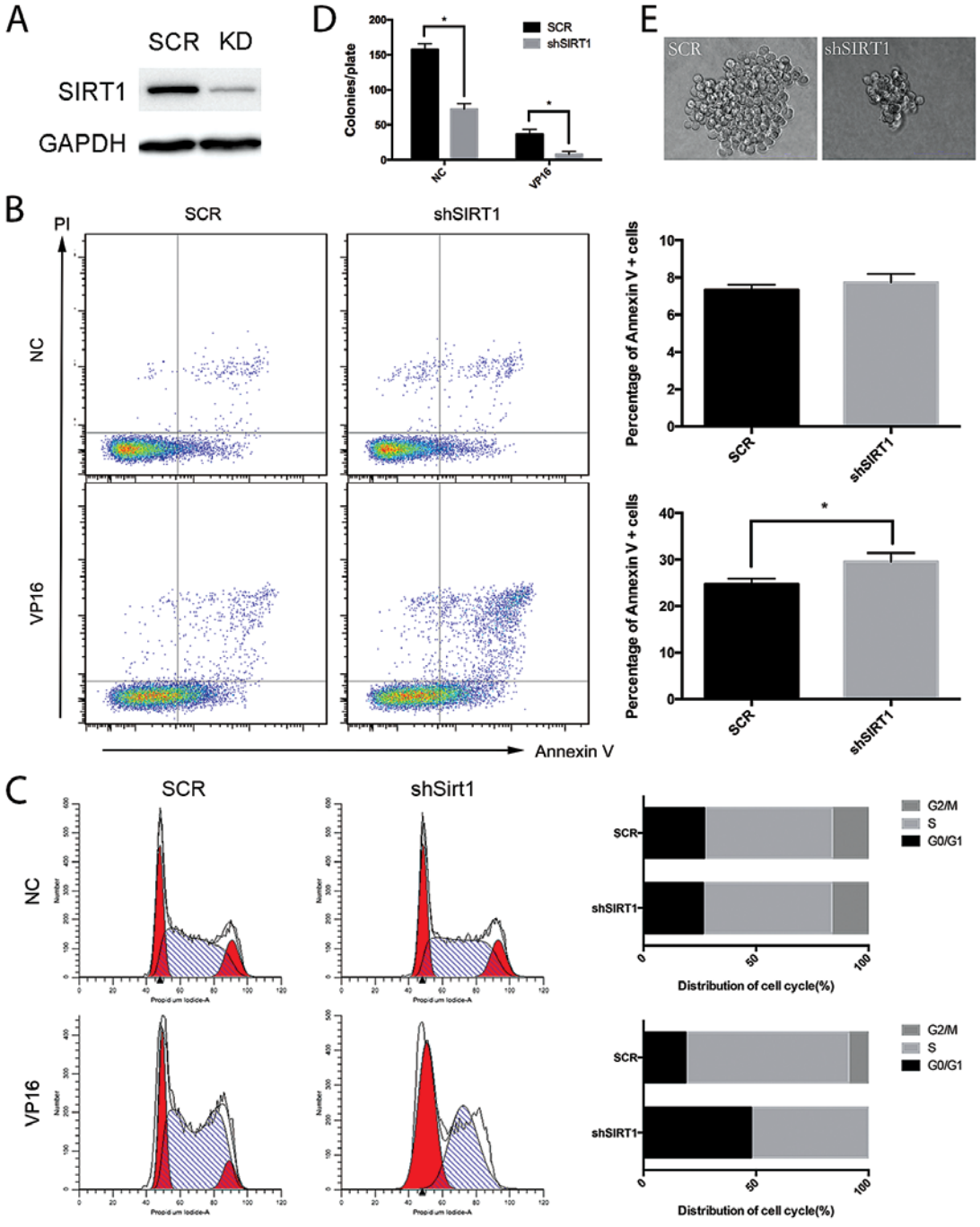

Figure 2. SIRT1 silencing by shRNA-sensitized Jurkat cells to DNA damage-inducing drug etoposide. (A) Protein expression levels of SIRT1 and GAPDH in cell lysates of three different groups of Jurkat cells, including normal Jurkat cells (WT) and Jurkat cells transduced with lentiviral vectors expressing SCR or with shSIRT1 clones. (B) Analysis of apoptosis and Jurkat cells infected with lentivirus-expressing SCR or shSIRT1 following etoposide treatment (VP16) or control (NC). Flow cytometric analysis results (left panel) and the percentage of Annexin V-positive cells (right panel) are presented. (C) Cell cycle distribution of Jurkat cells transduced with SCR or shSIRT1 were detected following treatment with etoposide (VP16) or control (NC). Flow cytometric analysis results (left panel) and statistical analysis of the mean values (right panel) . (D) Colony formation assay was conducted using shSIRT1- or SCR-transduced Jurkat cells with (VP16) or without (NC) etoposide treatment. (E) Images showing colony size are presented. Unless otherwise specified, data are presented as mean values ± SD of three independent experiments. (*P<0.05; unpaired Student's t-test).

cells (Fig. 2B). Similarly, the cell cycle analysis revealed that SIRT1 shRNA did not alter apoptosis under normal growth conditions. However, SIRT1 knockdown increased the cell population at G0/G1 phase with a marked reduction of cells in the G2/M phase following treatment with etoposide (Fig. 2C). Furthermore, the shRNA-mediated knockdown of SIRT1 decreased the colony-forming potential of Jurkat cells by 2-fold under normal growth conditions, and further suppressed the colony-forming potential of Jurkat cells by 4-fold following etoposide treatment (Fig. 2D). In addition, the colony size of Jurkat cells with SIRT1 shRNA was much smaller than that of cells with control shRNA (Fig. 2E). Taken together, these

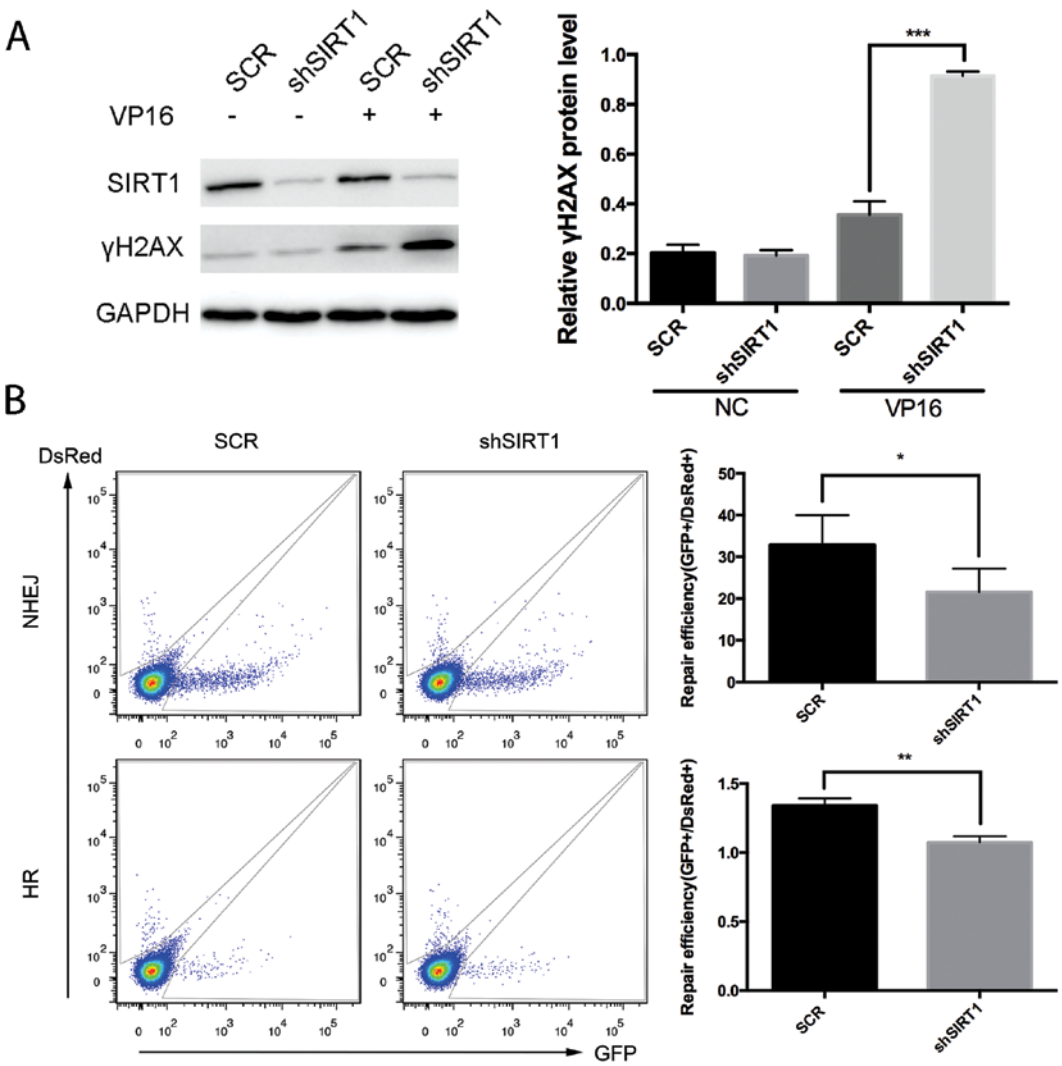

Figure 3. Inhibition of SIRT1 impairs DNA repair in Jurkat cells. (A) Western blot analysis of Jurkat cells expressing non-silencing shRNA (SCR) or shRNA-targeting SIRT1 (shSIRT1) with etoposide (VP16) treatment or control probed with γH2AX, and GAPDH-specific antibodies. γH2AX protein expression was quantified by densitometric analysis and normalized to GAPDH expression, presented in the right panel. (B) Analysis of HR and NHEJ in normal (SCR) and SIRT1-knockdown (shSIRT1) Jurkat cells. Flow cytometric analysis results (left panel) show the gating for the analysis of GFP$^+$ and DsRed$^+$ cells using cells transfected with GFP or DsRed expression vectors and cells transfected with a negative control plasmid to exclude auto-fluorescent cells. The ratio of GFP$^+$ to DsRed$^+$ cells (right panel), which was used as a measure of repair efficiency, is also presented. Experiments were repeated at least three times (*P<0.05, **P<0.01, ***P<0.001; unpaired Student's t-test).

results demonstrated that the shRNA-mediated knockdown of SIRT1 reduced the cell viability, and increased the induction of apoptosis and G0/G1 arrest by etoposide in Jurkat cells.

Silencing of SIRT1 results in impairs DNA repair by HR and NHEJ in Jurkat cells. Previous results suggested that SIRT1 increases cell survival under stressful conditions including hydrogen peroxide, anticancer drugs (24), and ionizing radiation (25). The impaired cell viability and increased sensitivity to etoposide following SIRT1 knockdown suggests that SIRT1 may promote DNA damage repair. To test this hypothesis, we analyzed the levels of DSBs in Jurkat cells after SIRT1 knockdown with etoposide treatment. The phosphorylation of H2AX (γH2AX), a marker of DSBs, increased in response to the etoposide treatment in Jurkat cells; whereas, the level of γH2AX was significantly higher in Jurkat cells transduced with SIRT1 shRNA than that with control shRNA (Fig. 3A), indicating that SIRT1 was required for the repair of DSBs in Jurkat cells. DSBs are repaired by NHEJ and HR. To monitor the efficiency of NHEJ and HR in a quantitative manner, we used DNA repair reporter plasmids containing fluorescent reporter constructs in which a functional GFP gene was reconstituted following the activity of HR or NHEJ event. The results showed that inhibition of SIRT1 by shRNA reduced the efficiency of NHEJ by 25% and HR by 50% (Fig. 3B), suggesting that SIRT1 was required for both HR and NHEJ.

SIRT1 knockdown by shRNA leads to the acetylation of p53, FOXO1 and Ku70 in Jurkat cells. SIRT1 has been shown to deacetylate various proteins involved in DNA damage response, including p53, FOXO1 and Ku70 (26). We therefore determined the acetylation levels of p53, FOXO1 and Ku70 in Jurkat cells after SIRT1 knockdown with or without etoposide treatment. Etoposide treatment resulted in the increased acetylation level of FOXO1. SIRT1 knockdown led to the elevated acetylation of FOXO1 under normal growth conditions and

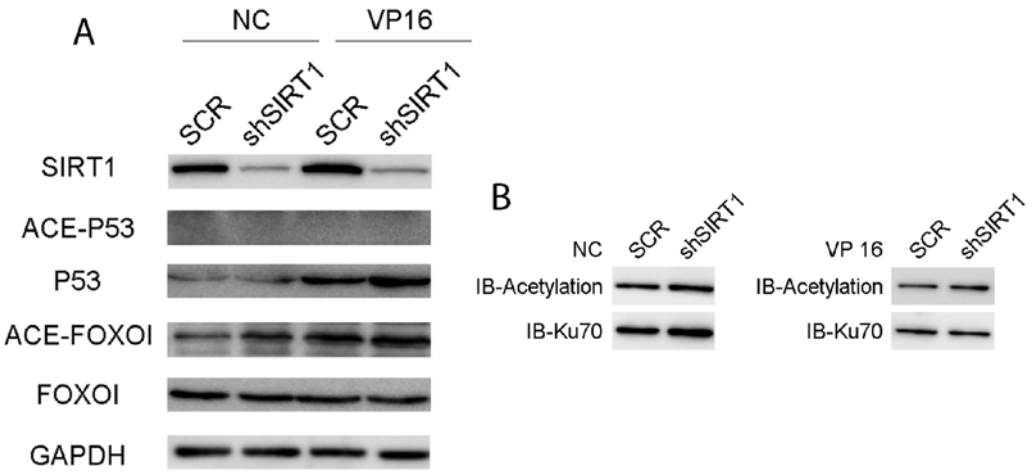

Figure 4. SIRT1 regulated the acetylation level of Ku70 and FOXO1. Jurkat cells were transduced with lentiviral vectors expressing SCR or with shSIRT1 clones; 48 h after transduction, cells were treated with (VP16) or without (NC) etoposide. (A) The cell lystes were detected for the total and acetylated expression of p53 and FOXO1. (B) The cell lystes were first immunoprecipitated using antibody against Ku70 and then immunoblotted for the detection of the acetylation level. Experiments were repeated at least 3 times.

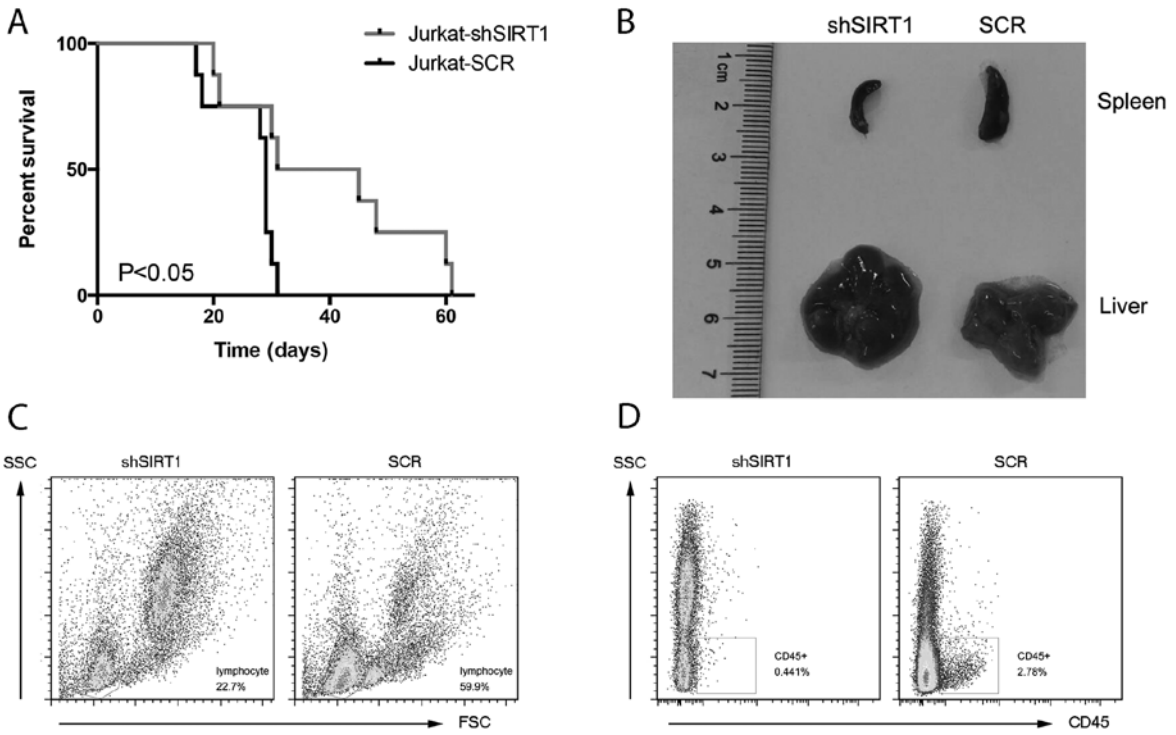

Figure 5. Role of SIRT1 in Jurkat-xenografted mice. (A) Kaplan-Meier survival plots of mice injected with Jurkat cells expressing non-silencing shRNA (SCR) or shRNA targeting SIRT1 (shSIRT1); SCR (n=8), shSIRT1 (n=8). After 20 days, the transplanted mice were euthanized and (B) spleen and liver size determined; flow cytometry results of (C) total bone marrow cells and (D) human CD45-positive cells were detected. Experiments were repeated at least three times (unpaired Student's t-test).

slightly enhanced FOXO1 acetylation by etoposide (Fig. 4A). Total p53 protein level was significantly elevated by SIRT1 knockdown after the etoposide treatment, but the acetylation level of p53 was undetectable in Jurkat cells. Similarly, SIRT1 shRNA increased the acetylation level of Ku70 under both normal growth conditions and etoposide treatment (Fig. 4B). Ku70 is a core component of the NHEJ repair pathway and FOXO transcription factors regulate the expression of genes related to cell cycle, DNA repair and apoptosis in response to DNA damage and oxidative stress. The findings suggested that SIRT1 inhibition impairs the viability of Jurkat cells by reducing DNA repair efficiency via elevation of the acetylation levels of Ku70 and FOXO1.

SIRT1 silencing prolongs the survival time of Jurkat-xenografted mice. We determined whether inhibition of SIRT1 induces the regression of leukemic blasts *in vivo*. The results showed that the lifespan of the shSIRT1 group was 38

days, while that of the SCR group was ~29 days (Fig. 5A). The difference in survival time was statistically significant ($P<0.05$). On day 20 after the Jurkat cell injection, three mice from each group were sacrificed for histopathological analyses. Compared with the mice injected with Jurkat cells transfected with SIRT1 shRNA, the mice injected with Jurkat cells transfected with control shRNA presented larger splenic size and pallor of liver (Fig. 5B). Furthermore, the bone marrow FACS assay showed that the SIRT1 shRNA group had a lower percentage of lymphocytes (Fig. 5C) and human $CD45^+$ cells (Fig. 5D) compared with that of the control siRNA group. These results indicated that SIRT1 inhibition delayed the leukemic blasts of Jurkat-xenografted mice.

Discussion

In the present study, we have demonstrated that SIRT1 was overexpressed in Jurkat cells and SIRT1 silencing by shRNA reducing the cell viability, and increasing the induction of apoptosis and G0/G1 arrest by etoposide in Jurkat cells. Additionally, SIRT1 downregulation resulted in impaired DNA repair capacity, accompanied by reduced activity of the HR and NHEJ in Jurkat cells. Further analyses showed that SIRT1 knockdown by shRNA elevated the acetylation of FOXO1 and Ku70 in Jurkat cells. Notably, we found that SIRT1 silencing prolonged the survival time and delayed leukemic blasts in Jurkat-xenografted mice. Our findings suggest that targeting SIRT1 is a promising strategy for the sensitization of ATL to DNA damage-based chemotherapies.

SIRT1, a well-known longevity factor, is a NAD(+)-dependent protein deacetylase that is involved in a wide variety of cell processes from cancer to aging (11). SIRT1 is also consistently upregulated in malignant cells or tissues from patients with glioblastoma, prostate, colorectal or skin cancers (27). In the present study, SIRT1 demonstrated the highest expression level in Jurkat cells, a typical ATL cell line, suggesting that the deregulation of SIRT1 may contribute to the anticancer drug resistance of Jurkat cells.

Etoposide, targeting DNA topoisomerase II (TOP2), is widely used as an anticancer drug, which increases the TOP2 cleavage complex and thus TOP2-mediated chromosome DNA breakage (28,29). In the present study, etoposide treatment was administered at a concentration of 20 μM for 4 h (30), and further experiments were performed 48 h later, to induce DNA damage and provide adequate time for DNA repair in Jurkat cells. Our results showed that SIRT1 knockdown enhanced etoposide-induced γH2AX (31), suggesting that SIRT1 was required for the repair of etoposide-induced DNA damage. NHEJ for direct DSBs (32) and HR for replication-associated DSBs (33,34), are two core DNA repair mechanisms for DSB lesions. The results showed that SIRT1 knockdown leads to impaired capacity of the NHEJ and HR pathways.

The homologous recombination repair pathway is essential during the proliferative stages of development and during somatic cell renewal in adults to protect against cell death and mutagenic outcomes from DNA damage. HR is a crucial DNA repair pathway in mammalian cells. RAD51, as a core protein of HR, catalyses the defining biochemical step of HR (35). SIRT1 deacetylates the HR repair factor Nijmegen breakage syndrome (NBS) (16), a component of MRE11-RAD50-NBS1 (MRN) complex, and regulates the recruitment of NBS1 and RAD51 to DNA damage foci for repair (36). In the present study, we have shown that SIRT1 silencing reduced the HR by 50% in Jurkat cells. These findings indicate that SIRT1 was required for efficient HR repair pathway.

Ku70 and Ku80 heterodimer is essential for NHEJ (10). Both biochemical and *in vivo* systems have shown that incompatible end joining can occur without Ku, but the joining is much less efficient (32). SIRT1 protein physically interacts with Ku70 (13), and this interaction may modulate Ku70 protein activity by controlling its acetylation status. In this study, acetylated Ku70 levels, which represent inactive Ku70 status, increased and the repair efficiency of the NHEJ pathway decreased in Jurkat cells following SIRT1 knockdown. Notably, it was reported that SIRT1 regulated NBS1 and RAD51, and inhibition of either NBS1 or Rad51 resulted in impaired NHEJ repair (37). Therefore, SIRT1 may be involved in the NHEJ repair pathway by modulating various proteins, including acetylation levels of Ku70 protein in ATL.

In the present study, we found that inhibition of SIRT1 rendered Jurkat cells more sensitive to etoposide treatment, as demonstrated by the higher level of apoptosis, G0/G1 phase arrest and impaired colony-formation capacity. Our results suggest that impaired DNA repair by SIRT1 inhibition may lead to accumulation of lesions in cells following treatment with DNA damage-inducing drugs, which in turn induces apoptosis and cell cycle arrest. SIRT1 has been shown to deacetylate various proteins involved in the DNA damage response, including p53 (24,25), FOXO (15), NF-κB (19) and Ku70. Of these, acetylated p53 and active status were reported to induce apoptosis and cell cycle arrest. Our results showed a significant elevation of total p53, however, the expression level of p53 was too low for us to detect the acetylation level. The FOXO1 acetylation level was elevated after SIRT1 knockdown in Jurkat cells. FOXO1 regulates cell proliferation and differentiation (38). Acetylated FOXO1, the inactive form, may therefore contribute to reduced colony formation of cultured Jurkat cells and longer survival time of Jurkat-xenografted mice by affecting cell viability and proliferation.

In conclusion, the present study provides evidence that SIRT1 inhibition induces the acetylation of various substrate proteins, affects DNA damage repair efficiency in ATL cells, renders ATL cells more sensitive to DNA damage drugs and prolongs the survival time of xenografted mice. The findings suggest that SIRT1 is a novel target for the development of ATL treatment.

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