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Original Paper

KU70 Inhibition Impairs Both Non-Homologous End Joining and Homologous Recombination DNA Damage Repair Through SHP-1 Induced Dephosphorylation of SIRT1 in Adult T-Cell Leukemia-Lymphoma Cells

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Key Words
Ku70 • SHP-1 • SIRT1 • DNA damage repair • Non-homologous end joining Homologous recombination

Abstract
Background/Aims: Adult T-cell leukemia-lymphoma (ATL) is an aggressive disease which is highly resistant to chemotherapy. Studies show that enhanced ability of DNA damage repair (DDR) in cancer cells plays a key role in chemotherapy resistance. Here, we suggest that defect in DDR related genes might be a promising target to destroy the genome stability of tumor cells. ***Methods:*** Since KU70 is highly expressed in Jurkat cells, one of the most representative cell lines of ATL, we knocked down KU70 by shRNA and analyzed the impact of KU70 deficiency in Jurkat cells as well as in NOD-SCID animal models by western blot, immunofluorescence, flow cytometry and measuring DNA repair efficiency. ***Results:*** It is observed that silencing of KU70 resulted in accumulated DNA damage and impaired DDR in Jurkat cells, resulting in more apoptosis, decreased cell proliferation and cell cycle arrest. DNA damage leads to DNA double-strand breaks (DSBs), which are processed by either non-homologous end joining(NHEJ) or homologous recombination(HR). In our study, both NHEJ and HR are impaired because of KU70 defect, accompanied with increased protein level of SHP-1, a dephosphorylation enzyme. In turn, SHP-1 led to dephosphorylation of SIRT1, which

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further impaired HR repair efficiency. Moreover, KU70 deficiency prolonged survival of Jurkat-xenografted mice. ***Conclusion:*** These findings suggest that targeting KU70 is a promising target for ATL and might overcome the existing difficulties in chemotherapy.

Introduction

Adult T-cell leukemia-lymphoma (ATL) is an aggressive malignancy caused by human T-lymphotropic virus type I (HTLV-1), resulting in quite poor prognosis compared with other non-Hodgkin lymphomas [1, 2]. Clinically, although there are efficient chemotherapy choices such as etoposide and anthracyclines, the first-line DNA damage drugs, patients still have high chance of tumor recurrence because of chemo-resistance of cancer cells. There is growing evidence that enhanced ability of DNA damage repair (DDR) in cancer cells plays a key role in chemotherapy resistance [3-8].

DDR can be processed by two major pathways: homologous recombination(HR) and non-homologous end-joining(NHEJ). HR appears the predominant mechanism of DDR in yeast, which is operative only in the S/G2 phases of the cell cycle. By contrast, NHEJ works in all phases of the cell cycle by binding together the broken DNA ends and is considered to be the major repair pathway of DSBs in mammalian cells [9, 10].

Sirtuin-1 (SIRT1), a class III histone deacetylase (HDAC), has been reported to mediate anti-aging effects and prevents metabolic syndrome-associated disease [11-14]. However, the function of SIRT1 in cancer is contradictory. It is reported that SIRT1 works as a tumor suppressor in solid tumor by its function in P53 deacetylation [15, 16]. In addition, several SIRT1 regulators are reported to induce growth arrest and apoptosis, combined with deacetylation of STAT3 and NF-KB, and reduction of c-Myc protein levels [17, 18]. On the contrary, increased expression of SIRT1 has also been reported in CML and AML patients and its activity is modulated via the ATM-DBC1-SIRT1 axis in a FLT3-ITD-dependent manner [19, 20].

The importance of SIRT1 also lies in its role in maintaining genome stability through DNA damage repair. It is demonstrated that SIRT1 regulates HR repair capacity through its interaction with WRN [21]. Besides, SIRT1 is reported to physically complex with KU70, leading to increase of NHEJ repair capacity [22, 23].

Ku proteins are the critical NHEJ factors including XRCC6/KU70 (70kda) and XRCC5/KU80 (80kda), and can form a heterodimer binding to DNA double-strand break ends to participate in the NHEJ pathway of DNA repair. Cell lines lacking DNA-dependent protein kinase catalytic subunits (DNA-PKcs) or DNA-targeting heterodimer (KU70 and KU80) are more sensitive to DNA damage [24-27].

To our knowledge, KU70 has been considered to be downstream of SIRT1 [22, 23], but the role of KU70 in ATL is still not clear.

In this study, we knocked down KU70 by shRNA in Jurkat cells, one of the most representative cell lines of ATL, and assessed apoptosis, cell cycle, cell proliferation following etoposide treatment *in vitro*, as well as leukemia blast and survival of Jurkat-xenografted mice *in vivo*. We further analyzed DNA damage repair capacity and interestingly found that both NHEJ and HR repair efficiency were impaired after KU70 silencing. Moreover, we observed enhanced expression of SHP-1, a member of the protein tyrosine phosphatase (PTP)family. Increased SHP-1 protein level further led to dephosphorylation of SIRT1. In addition, we found that KU70 deficiency led to over-expression of pro-apoptotic genes like BAX, indicating its role in regulating apoptosis. In all, these data suggest that KU70 defect in ATL cells increases their sensitivity to chemotherapy and may work as a novel target to ATL treatment.

Materials and Methods

Cell lines and cell culture

KARPAS, NAMALWA, HEL, RAJI, THP1, SU-DHL-1, DAUDI, SU-DHL-4, HL-60, K562, U937, 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 maintained in DMEM and the rest cells were cultured in RPMI-1640 medium and IMDM. The mentioned medium was supplemented with 10% fetal bovine serum(FBS). The cells were maintained at 37°C in a humidified atmosphere of 5% CO2. Three peripheral blood(PB) samples from our healthy volunteers were used as control. Peripheral blood mononuclear cells(PBMC) were obtained from peripheral blood by separation on the lymphoprep density gradient with centrifugation at 400x g for 30min. 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 1 h, cells were washed twice in PBS and maintained in fully supplemented RPMI-1640 medium for 48 h before further analysis. Primary antibodies against KU70, SIRT1, ATM, SHP-1, phosphorylated SIRT1, ATM, RAD51, KI67, BAX, BCL2(Abcam, Cambridge, UK), phospho-Histone H2AX (Ser139), GAPDH (Cell Signaling Technology, Danvers, MA, USA), P53 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were used.

Protein extraction and western blotting

Western blotting experiments were performed using whole cell extracts following standard techniques. The quantitative analysis was performed using Quantity One Analysis Software(Bio-Rad). To analyze each antibody, experiments were performed for at least three independent repeats.

Apoptosis and cell cycle analysis

Apoptosis was analyzed by staining with Annexin V and propidium iodide (BD Biosciences Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Flow cytometry analysis was then performed within 1 h. The cell apoptotic ratio was detected by a FACScan cytometer (BD Biosciences, San Jose, CA, USA) and was analyzed by Flowjo software. For the cell cycle analysis, the cells were collected and washed twice with PBS followed by ethanol fixation at -20°C for 2 h. The cells were then incubated with PI/RNase staining buffer (BD Biosciences) for 15 min at room temperature. The sample was detected on a FACScan cytometer and was analyzed by ModFit software.

Soft agar colony formation assay

For the colony formation assay, a standard two-layer soft agar culture, namely a 0.6% agarose bottom layer and a 0.3% agarose top layer, was used. The cells were seeded at 10^5/ml in 24-well plates with soft agar as described above. After 7 days, colonies were analyzed for colony number and size and representative images were captured.

Immunofluorescence staining

Cells grown on coverslips were fixed in 4% paraformaldehyde for 20 minutes, then permeabilized with 0.1% Triton X-100 in PBS for 30 minutes at room temperature. After washing three times with PBS, samples were blocked in PBS containing 5% BSA for 1 hour. Rabbit anti-human γH2AX (Abcam; 1:1000) antibody was diluted in 3% donkey serum in PBS for 2 hours at room temperature. After washing three times with PBS and incubating for one hour with donkey anti-rabbit secondary antibody (CF543; 1:1000), cell nuclei were stained with DAPI. After washing three times with PBS, slides were mounted and observed under fluorescence microscope. The cells were observed in three randomly selected fields.

Measurements of DNA repair capacity

DNA repair capacity measurement was performed as previously described [28]. Briefly, Plasmids containing NHEJ or HR reporter cassettes were linearized by I-SceI restriction enzymes and purified using Qiagen Qiaex II purification kit (20021; Qiagen, Valencia, CA). 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 flow cytometry 72 h after the transfection.

Animal model

For *in vivo* tumor growth studies, 10^7 Jurkat cells of scramble group or KU70 knockdown group in a volume of 100 μl were injected subcutaneously into the lower flanks of NOD/SCID mice. Twenty days after engraftment, three mice from each group were euthanized for analysis of spleen and liver. All the animal experiments were approved and conducted under the Guideline for Animal Care at Tongji University School of Medicine.

Statistical analysis

All quantitative data are presented as mean ± SEM. For data analysis, unpaired two-tailed Student's t-test or Mann-Whitney U test were used. For Kaplan-Meier survival analysis, the log-rank test was used. Statistical computations were performed using Prism 6.0 (Graph Pad Software). A p value less than 0.05 was considered statistically significant.

Results

Jurkat cells are enriched for KU70

We measured the relative KU70 mRNA levels in several cell lines and 3 PBMC samples of healthy human as control. According to qPCR results, the KU70 mRNA expression in Jurkat cell line was approximately 1.5±0.12 times that of normalized control (Fig. 1A, P<0.01). Furthermore, we chose five cell lines that has higher expression of KU70, namely K562, U937, HL-60, DAUDI and Jurkat cells, and conducted Western Blot to measure relative protein level of KU70 in these cells (Fig. 1B). Results showed that Jurkat cell line has the highest KU70 protein expression (Fig. 1C). As a result, Jurkat cells were selected for subsequent experiments.

KU70 knockdown Jurkat cells are functionally defective

We constructed a KU70 shRNA plasmid to down regulate its expression in Jurkat cell line. Knock down efficiency was confirmed by Western Blot (Fig. 3G). To investigate the role of KU70 in response to DNA damage, we treated both scramble Jurkat cells and KU70 knockdown Jurkat cells with 20 μM VP16 for 1 hour and analyzed the apoptosis, cell cycle distribution and colony formation ability of

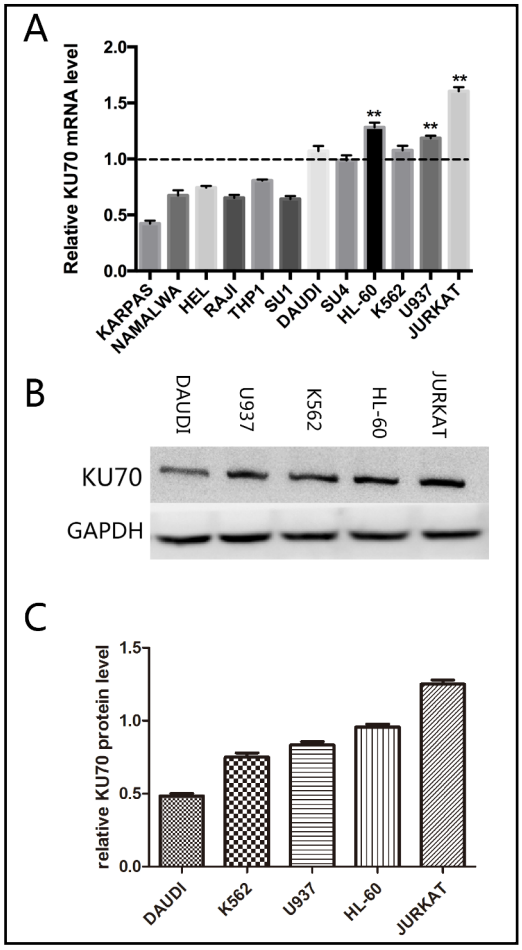

Fig. 1. Jurkat cells are enriched for KU70.(A) KU70 mRNA levels of KARPAS, NAMALWA, HEL, RAJI, THP1, SU-DHL-1, DAUDI, SU-DHL-4, HL-60, K562, U937 and JURKAT cells were analyzed by RT-PCR relative to β-actin as the internal control. **P<0.01(B) Representative western blot analysis of KU70 protein level in K562, U937, HL-60, Kasumi, Jurkat cells.(C) Quantification of KU70 protein level by densitometric analysis and normalized to GAPDH expression.

these two groups. The KU70 knockdown Jurkat cells induced double apoptosis rate than scramble Jurkat cells(5.12% compared to 2.38%, P < 0.001), and were more fragile to VP16 treatment(14.25% compared to 12.81%, P < 0.05, Fig. 2A-B). As shown in Fig. 2C-E, the proportion of G0/G1 phase cells in the KU70 knockdown Jurkat cells was 8.25±0.23% versus 5.89±0.48% in the scramble Jurkat cells (P < 0.01). After etoposide treatment, proportions of G0/G1 phase cells were 12.30±0.45% in the KD group and 8.68±0.52% in the SCR group, respectively (P < 0.001, Fig. 2D-E). These results indicate that silencing of KU70 resulted in cell cycle arrest at G0/G1 phase under normal growth conditions and in response to etoposide treatment. To explore the role of KU70 in regulating Jurkat cell proliferation, we then examined the colony formation ability with soft agar. The KU70 knockdown group only formed 19 colonies compared to 50 colonies in scramble group and 55 in wild type control. After VP16 treatment, KU70 knockdown Jurkat cells were almost unable to form valid colonies (Fig. 2F-G); indicating that silencing of KU70 results in loss of cell viability(P < 0.0001). We then conducted immunofluorescence dyeing of Ki67, a marker associated with cell proliferation. Results show that KU70 knockdown Jurkat cells expressed the least positive foci both before and after VP16 treatment, while scramble Jurkat cells and wild type Jurkat cells exhibited remarkable foci of Ki67(P < 0.05, Fig. 2H).

Silencing of KU70 results in impaired DNA repair in Jurkat cells

The impaired cell viability and increased sensitivity to VP16 following KU70 knockdown suggests that KU70 may take part in DNA damage repair. We conducted western blot to detect γH2AX expression, a marker of DSBs, in KU70 knockdown Jurkat cells and its control group. Although the three groups had almost the same γH2AX expression before VP16 treatment, KU70 knockdown Jurkat cells expressed relatively higher γH2AX protein level in response to VP16 induced DNA damage(P < 0.01, Fig. 3A). Immunofluorescence staining of γH2AX also demonstrated that the KU70 knockdown Jurkat cells had the most γH2AX foci following etoposide treatment(P < 0.05, Fig. 3B), indicating that KU70 defect lead to impaired DNA damage repair in Jurkat cells. DNA damage repair (DDR) can be processed by two major pathways, namely homologous recombination (HR) and nonhomologous end-joining (NHEJ) [9]. To test the efficiency of NHEJ and HR in a quantitative manner, we used DNA repair reporter plasmids containing fluorescent reporter constructs as previously reported [28]. KU70 knockdown Jurkat cells and its control group were transfected with plasmids containing GFP-based reporter constructs through electro-transfer. Results showed that NHEJ repair efficiency rate of KU70 knockdown Jurkat cells were impaired to 32.8% of KU70 scramble Jurkat cells(P<0.05, Fig. 3D-E). This result is in line with its known function in regulating NHEJ repair efficiency [24, 26]. In addition, KU70 knockdown also reduced the efficiency of HR repair to 36.6% compared with scramble Jurkat cells(P<0.05, Fig. 3F). Further more, the relative protein level of RAD51, a key gene in HR repair pathway, was down regulated in KU70 knockdown Jurkat cells before(P < 0.01) and after(P < 0.001) etoposide treatment confirmed by western blot (Fig. 3G-H).

KU70 silencing reduces the tumorigenesis ability of Jurkat cells

We constructed subcutaneous tumor model by using NOD/SCID mice. 10^7 Jurkat cells of scramble group and KU70 knockdown group were respectively injected into each mouse subcutaneously. The median survival time of scramble group was only 19 days, while more than half of KU70 knockdown group survived more than 40 days (Fig. 4A). The difference in survival time was statistically significant(P < 0.01). In addition, the KU70 knockdown mice showed smaller tumor size and were less aggressive compared with mice that injected with KU70 scramble Jurkat cells (Fig. 4B).

KU70 regulates phosphorylation of SIRT1 and ATM by SHP-1 in Jurkat cells

We constructed a KU70 knockdown cell line and a KU70 overexpression cell line in Jurkat cells to explore the molecular mechanism of KU70 in regulating DNA damage repair. Western blot results show that KU70 knockdown lead to decreased p-SIRT1 level (P<0.01)

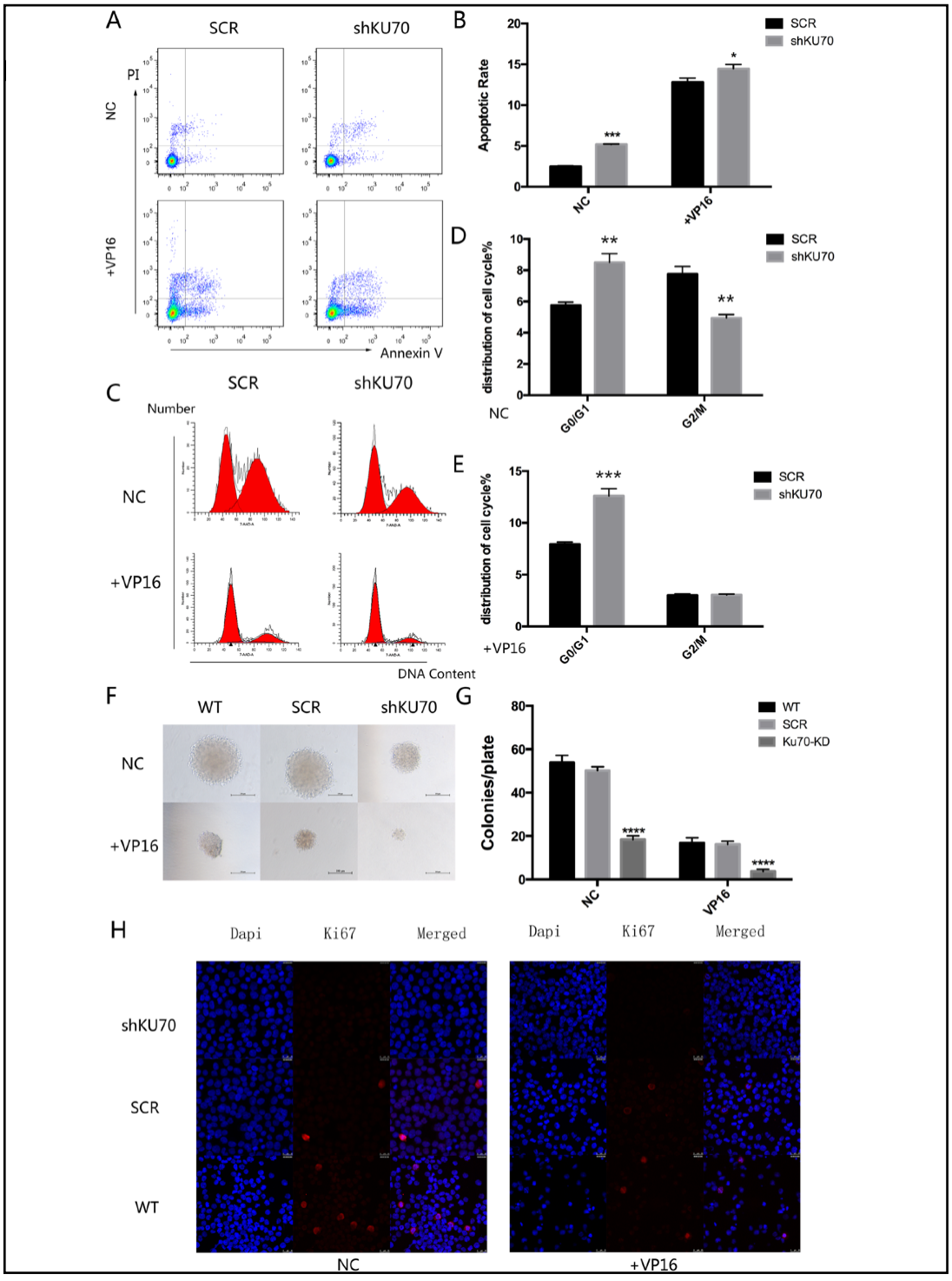

Fig. 2. KU70 knockdown Jurkat cells are functionally defective.(A-B) Analysis of apoptosis of Jurkat cells infected with lentivirus-expressing SCR or shKU70 following etoposide(VP16) treatment or control. Flow cytometric analysis results and the percentage of Annexin-V-positive cells are presented. *P<0.05, ***P<0.001(C-E) Cell cycle distribution of SCR or shKU70 Jurkat cells were detected following treatment of etoposide or control. Flow cytometric analysis results and statistical analysis of the mean values. **P<0.01, ***P<0.001(F-G) Colony formation assay was conducted using SCR or shKU70 Jurkat cells with or without etoposide treatment. Images showing colony size and statistical analysis are presented. ****P<0.0001(H) Immunofluorescence dyeing of Ki67 of SCR or shKU70 Jurkat cells with or without etoposide treatment.

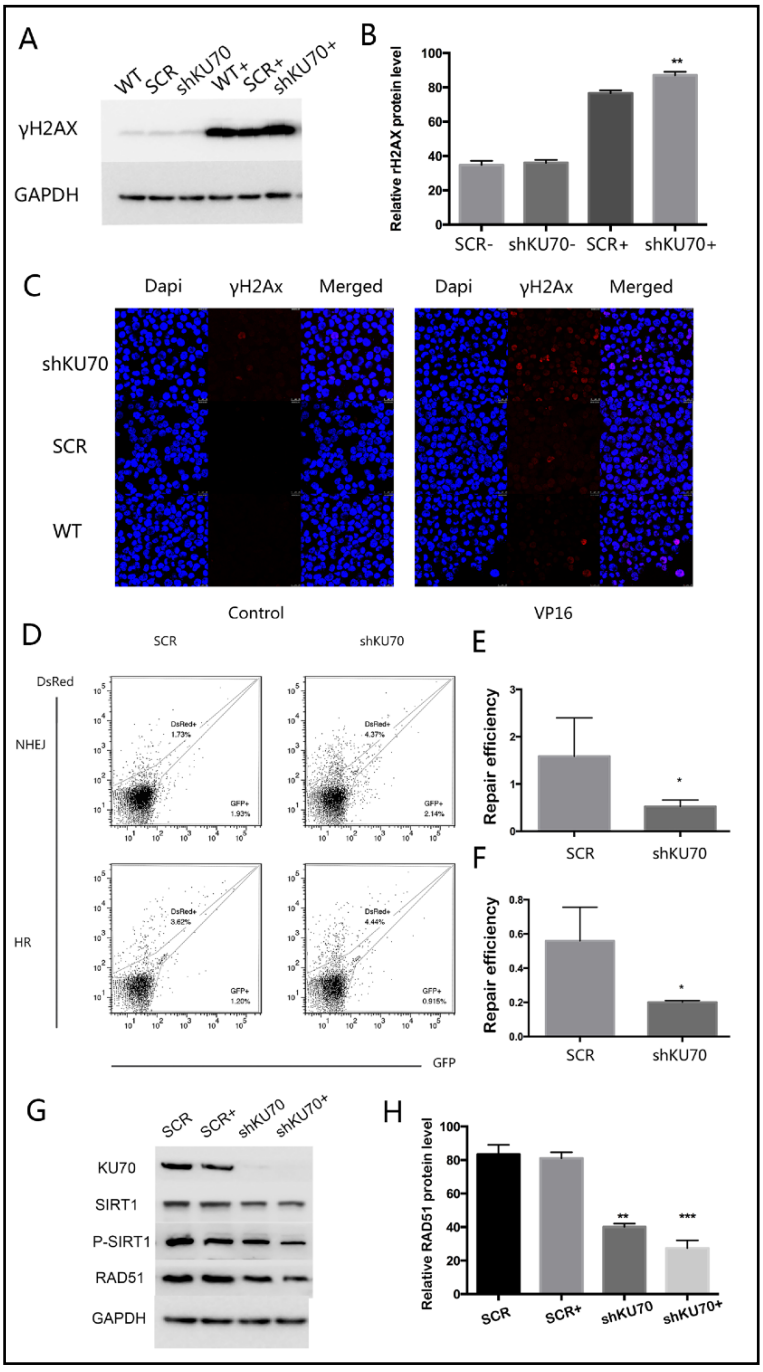

Fig. 3. Silencing of KU70 results in impaired DNA repair in Jurkat cells.(A) Representative western blot analysis of γH2AX protein level in SCR or shKU70 Jurkat cells with or without etoposide treatment. (B) Quantification of γH2AX protein level by densitometric analysis and normalized to GAPDH expression. **P<0.01. (C) Immunofluorescence dyeing of γH2AX of SCR or shKU70 Jurkat cells with or without etoposide treatment. (D-F) Analysis of HR and NHEJ repair efficiency in SCR or shKU70 Jurkat cells. Flow cytometric analysis results 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. The ratio of GFP+ to DsRed+ indicates repair efficiency. Experiments were repeated at least three times. *P<0.05.(G) Representative western blot analysis in SCR or shKU70 Jurkat cells with or without etoposide treatment. (H) Quantification of RAD51 protein level by densitometric analysis and normalized to GAPDH expression. **P<0.01, ***P<0.001.

and decreased p-ATM level (P<0.01) along with enhanced expression of SHP-1(P<0.01), a member of the protein tyrosine phosphatase (PTP)family. By contrast, KU70 over expression give rise to p-SIRT1 (P<0.001) and p-ATM (P<0.01) protein level, combined with decreased expression of SHP-1(P < 0.05, Fig. 5A, 5C). Thus we further constructed a SHP-1 knockdown Jurkat cell line and found that SHP-1 deficiency lead to increased protein level of p-SIRT1 (P<0.05) and p-ATM(P < 0.05, Fig. 5B, 5D) without influencing the protein level of SIRT1 and ATM. Taken together, KU70 knockdown led to dephosphorylation of SIRT1 and ATM through up regulation of SHP-1.

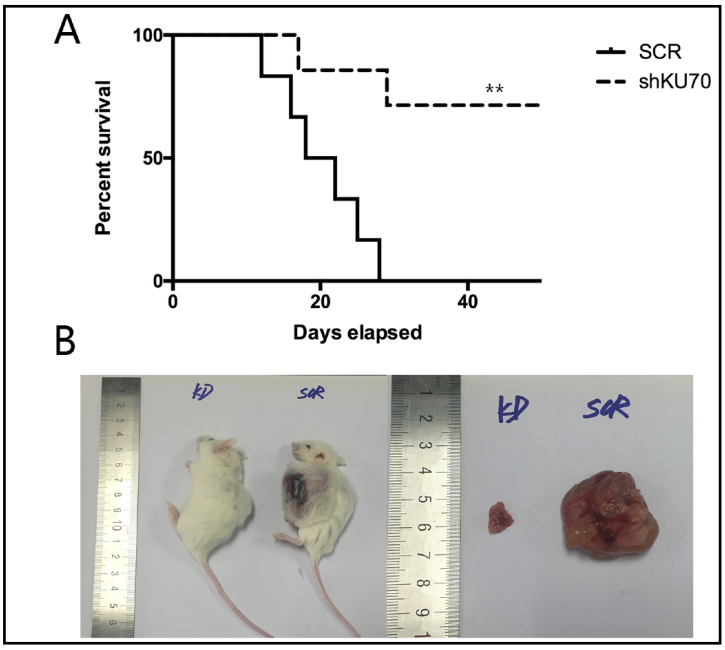

Fig. 4. Role of KU70 in Jurkat-xenografted mice. (A) Kaplan-Meier survival curve of mice injected with SCR Jurkat cells and shKU70 Jurkat cells respectively. SCR(n=10), shKU70(n=10). **P<0.01.(B) After 20 days, the transplanted mice were euthanized and tumor was peeled off.

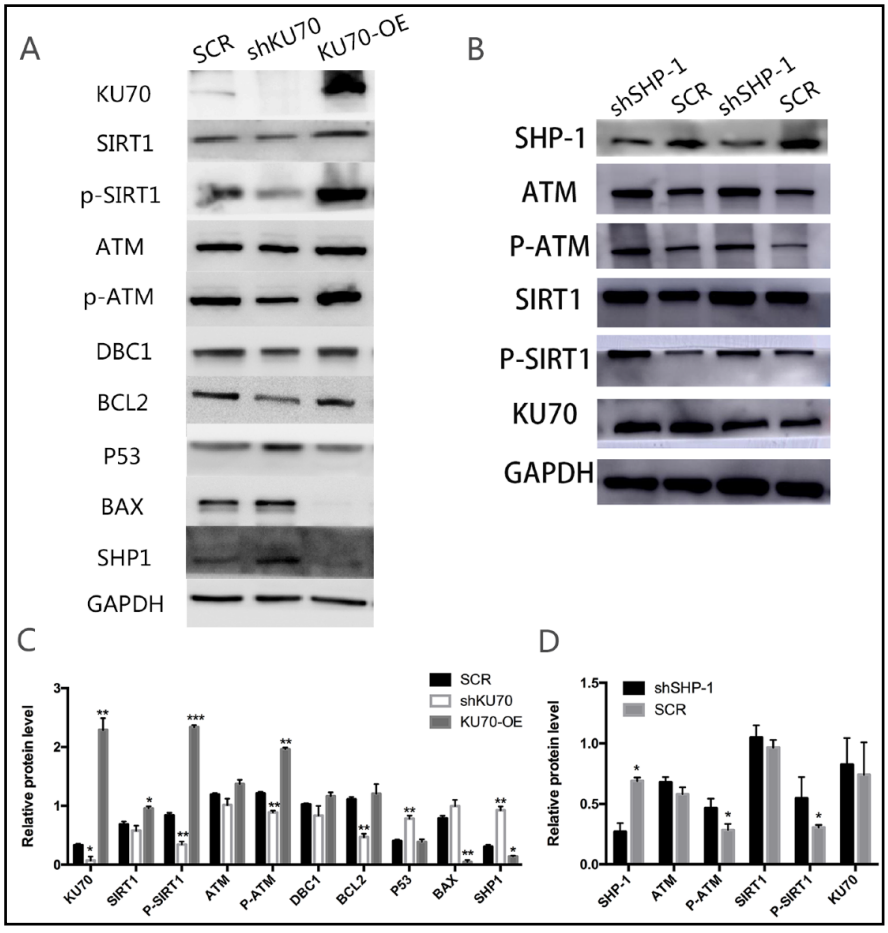

Fig. 5. KU70 regulates phosphorylation of SIRT1 and ATM by SHP-1.(A) Representative western blot analysis in KU70 knockdown/over-expression/scramble cells.(B) Representative western blot analysis in SHP-1 knockdown/scramble cells. (C) Quantification of respective protein level by densitometric analysis and normalized to GAPDH expression in KU70 knockdown/over-expression/scramble cells. *P<0.05, **P<0.01, ***P<0.001.(D) Quantification of respective protein level by densitometric analysis and normalized to GAPDH expression in SHP-1 knockdown/scramble cells. *P<0.05.

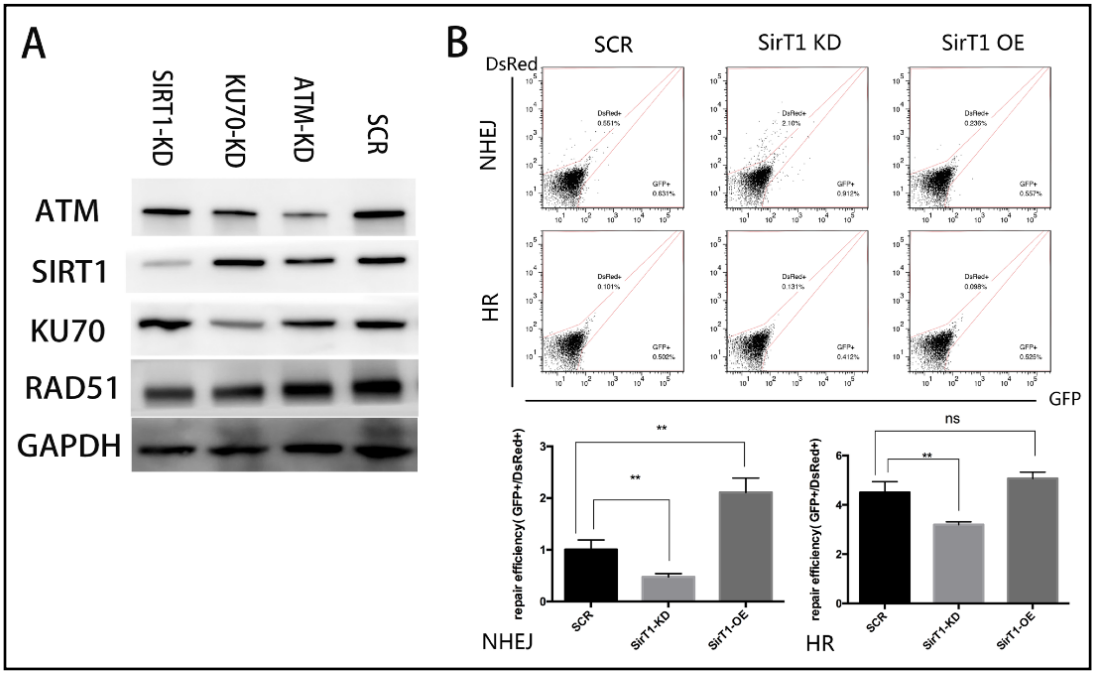

Fig. 6. KU70 defect leads to impaired HR repair efficiency through down regulation of SIRT1(A) Representative western blot analysis of RAD51 protein level in ATM, SIRT1, KU70 knockdown cell lines. (B) Analysis of HR and NHEJ repair efficiency in SIRT1 knockdown/SIRT1 over-expression/SCR cell lines. **P<0.01.

KU70 defect leads to impaired HR repair efficiency through down regulation of SIRT1

Reports show that ATM, SIRT1 and KU70 can modulate HR repair efficiency respectively in different system [21, 29-32]. Thus, we respectively constructed ATM, SIRT1, KU70 knockdown cell line and SCR cell line in Jurkat cells. We examined RAD51 protein level in these cells by Western Blot and found that SIRT1 knockdown lead to the most obvious drop of RAD51 expression in Jurkat cells($P < 0.05$, Fig. 6A). Moreover, we constructed a SIRT1 knockdown cell line, a SIRT1 over-expression cell line and SCR cell line in Jurkat cells and examined the efficiency of HR and NHEJ in these three cell lines. Results show that SIRT1 knockdown impaired NHEJ efficiency to 48.2% and impaired HR efficiency to 72.5%($P < 0.01$, Fig. 6B). Taken together, we conclude that KU70 defect leads to impaired HR repair efficiency through down regulation of SIRT1 in Jurkat cells.

KU70 regulates apoptosis-related genes

Since enhanced apoptosis rate was detected in KU70 knockdown Jurkat cells (Fig. 2A), we further found that KU70 knockdown gave rise to P53 protein level ($P<0.01$) but reduced protein level of BCL2 ($P<0.01$), while KU70 over expression led to dropped BAX protein level($P < 0.01$, Fig. 5A), indicating that KU70 defect remains Jurkat cells in a pro-apoptosis status.

Discussion

ATL is an aggressive disease and is highly resistant to chemotherapy, resulting in poor prognosis and high rate of reoccurrence. Several studies has made efforts to work out the pathogenesis of ATL [33, 34], however, it remains one of the most lethal malignancy due to ineffective therapy.

In this present study, we have demonstrated that KU70 expression was upregulated in Jurkat cells, the typical cell line of ATL. KU70 downregulation by shRNA led to increased

apoptosis, cell cycle arrest and reduced cell proliferation in Jurkat cells. Moreover, KU70 defect promoted etoposide-induced DNA damage in Jurkat cells accompanied with reduced NHEJ and HR repair efficiency. In addition, KU70 defect led to enhanced SHP-1 protein level, a member of the protein tyrosine phosphatase family, which in turn down regulated phosphorylation status of SIRT1 (Fig. 7). Furthermore, our study showed that KU70 defect influenced the expression of P53, BCL2 and BAX, remaining Jurkat cells in a pro-apoptosis status. Most importantly, we found that KU70 silencing prolonged the survival time and impaired the tumorigenesis ability in Jurkat-xenografted mice.

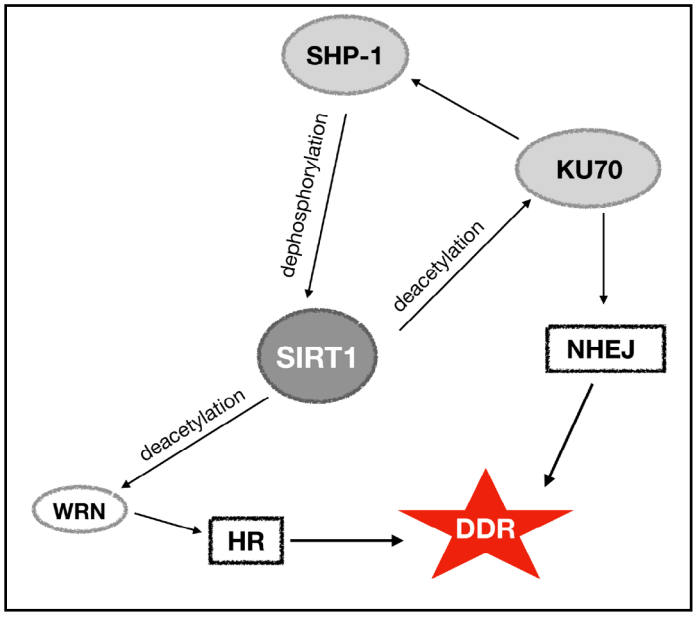

Fig. 7. Comparative summary of the studied proteins in Jurkat cells.

KU70, a well-known NHEJ factor, is key to maintain DNA stability. Recent studies indicate that NHEJ is the main DSB repair pathway in human cells and KU proteins are the critical NHEJ factors. The lack of KU proteins often leads to an accumulation of DNA damage [23, 24, 26, 27].

Such efforts have been made in identifying the importance of KU70 in several systems and disease including regulating the growth of pancreatic β-cells [35], maintaing the integrity of testicular cells [36] and protecting from hepatocellular tumorigenesis [37]. Paradoxically, the level of KU70 is also increased in many types of tumor, suggesting that tumors may rely on KU70 for survival [38, 39]. To date, KU70 specific inhibitors have not been identified.

Notably, we found that KU70 disruption results in impaired DNA repair in Jurkat cells, impairing not only NHEJ repair efficiency but also HR repair efficiency. KU70 was reported as a key regulator in NHEJ repair pathway, but few was known about its role in HR repair pathway. Our western blot confirmed that KU70 defect led to downregulated RAD51 protein level, the key HR factor. Studies show that the release of the MRN complex and Ku from DNA ends by Mre11 nuclease activity and Ctp1 is a critical step required to expose ssDNA for RPA localization and ensuing HR repair [40]. HR repair pathway is generally restricted to S and G2 phase since it utilizes sister-chromatid sequences as the template to conduct faithful repair. By contrast, NHEJ repair pathway can operate in any phase of the cell cycle [41]. It suggests that KU70 has great potential in mediating DNA damage repair.

DNA damage is closely related to human disease, aging and cancer. DNA damage repair mechanisms are important to maintain genomic integrity as well as tumor proliferation. Unlike solid tumor, the most prevalent treatment for hematological malignancy is chemotherapy and radiotherapy that target at generating DNA damage. Up regulation of DDR genes can provide leukemia cells with escape mechanisms to the DDR anticancer barrier and induce chemotherapy resistance [42-44]. Conversely, modulation of DDR also influences genomic instability within tumor cells, especially after chemotherapy. Defect in DDR related genes in tumor cells leads to impaired DNA repair, triggering apoptosis in tumor cells. Recent studies have demonstrated that DDR is important for the maintenance of tumor genome integrity, and dysregulation of DDR sensitizes tumor cells to chemotherapy [45-48]. Thus, inhibition of DDR pathway including HR and NHEJ via chemotherapeutic compounds selectively kill tumor cells that evolve to escape DDR and checkpoint signaling.

In sum, our study demonstrates the specific role of KU70, the key NHEJ factor, in ATL cells and Jurkat-xenografted mice model. These findings suggest that KU70 might be a novel target to ATL and might help to overcome chemo-resistance.

Conclusion

In summary, Jurkat cells are enriched for KU70, the key NHEJ factor. KU70 defect Jurkat cells are functionally compromised, resulting in increased apoptosis, decreased proliferation, cell cycle arrest and impaired DNA damage repair ability, including NHEJ and HR repair pathway. KU70 silencing leads to enhanced SHP-1 protein level, a member of the protein tyrosine phosphatase family. This enzyme dephosphorylates SIRT1 and this effect further impaired HR repair efficiency. Taken together, KU70 might be a novel target to ATL and provides new avenues for chemo-resistance management.

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Disclosure Statement

The authors declare that there is no conflict of interests regarding the publication of this article.

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