Chidamide induces necroptosis via regulation of c-FLIP_L expression in Jurkat and HUT-78 cells

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Abstract. T-cell acute lymphoblastic leukemia (T-ALL) is a hematopoietic malignancy, which is associated with a poor prognosis. It is difficult to achieve complete remission or long-term survival with conventional chemotherapy, partly due to decreased apoptosis. However, necroptosis can serve as an alternative pathway to induce cell death. The present study investigated whether the selective histone deacetylase (HDAC) inhibitor chidamide exerted a therapeutic effect on T-ALL and explored the underlying mechanism. The results revealed that HDAC expression was increased in Jurkat and HUT-78 cells treated compared with the control cell line (H9), and was accompanied by elevated cellular Fas-associated death domain-like interleukin-1β converting enzyme inhibitory protein long form (c-FLIP_L) levels. Chidamide treatment (2 µmol/l) also induced mitochondrial dysfunction, necroptosis and apoptosis in T-ALL cells in vitro. Furthermore, necroptosis was increased when apoptosis was blocked in T-ALL cells. Additionally, chidamide (2 µmol/l) downregulated c-FLIP_L, HDAC1 and HDAC3 expression, and increased receptor-interacting protein kinase 3 expression and the phosphorylation of mixed lineage kinase domain-like pseudokinase in Jurkat and HUT-78 cells. The results obtained in the present study revealed that chidamide may induce necroptosis via regulation of c-FLIP_L expression when apoptosis is inhibited in Jurkat and HUT-78 cells.

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a type of malignant hematopoietic tumor caused by malignant transformation of T lymphocytes. T-ALL has a poor prognosis and it is difficult to achieve complete remission after recurrence through chemotherapy alone (1). Therefore, there is a requirement for novel therapeutic agents. Abnormal proliferation, disordered differentiation and inhibition of apoptosis are implicated in the pathogenesis and recurrence of leukemia. Cellular Fas-associated death domain-like interleukin-1β converting enzyme inhibitory protein long form (c-FLIP_L) is an important anti-apoptotic protein, which has been reported as an important factor affecting apoptosis sensitivity and has been demonstrated to exert a significant inhibitory effect on apoptosis (2). A previous study revealed that c-FLIP_L is highly expressed in leukemia (3). It has been reported that c-FLIP_L is highly expressed in bone marrow mononuclear cells obtained from patients with T-ALL and is associated with the malignancy and prognosis of T-ALL.

Histone deacetylase (HDAC) inhibitors (HDACis) comprise an important class of epigenetic drugs with high efficacy and low toxicity, which are becoming valuable antitumor drugs. It has been reported that HDACis successfully inhibit proliferation and increase apoptosis in various malignant tumor cells by decreasing c-FliPl transcription and translation (4). Chidamide is a novel HDACi that has been independently researched and developed in China, which has been approved by the US Food and Drug Administration. Zheng et al (5) reported that patients with peripheral T-cell lymphoma exhibited increased c-FLIP_L expression, and that HDACis inhibited the expression of c-FLIP_L and increased apoptosis in T-cell lymphoma (5).

Necroptosis is a novel type of cell death that exhibits characteristics of apoptosis and necrosis, and is regulated by a series of signaling molecules independent of caspases (6). Furthermore, necroptosis is inhibited by the specific inhibitor necrostatin-1 (Nec-1) (7). Necroptosis has attracted increasing attention due to its close association with inflammation, ischemia-reperfusion injury, and tumor occurrence and development (6,8). In general, activation of death receptors induces apoptosis; however, cytokine-mediated apoptosis can also initiate necroptosis if apoptosis is inhibited or blocked (9). Certain leukemia cell lines (such as Jurkat cells) have congenital caspase function deficiency; hence, necroptosis is more likely to occur in these cell lines (10). The present study investigated the necroptosis-inducing effects of chidamide on Jurkat and HUT-78 T-cell leukemia cells and explored the underlying mechanism.
Materials and methods

Cell culture. Jurkat, HUT-78 and H9 cells (Shanghai Cell Bank of Chinese Academy of Sciences) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (both from Gibco; Thermo Fisher Scientific, Inc.), 100 units penicillin and 100 µg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO₂.

Reverse-transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from Jurkat, HUT-78 and H9 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using a Super MMLV RT kit (Takara Bio, Inc.), according to the manufacturer's instructions. The reaction was carried out at 37°C for 15 min, 85°C for 5 sec and then terminated at 4°C. For qPCR, the reaction solution was prepared on ice in five replicate wells per cell type. The 20-µl reaction mixture consisted of 10 µl 2X SYBR Premix Ex Taq II (Takara Bio, Inc.), 0.8 µl forward primer (10 µmol/l), 0.8 µl reverse primer (10 µmol/l), 0.4 µl ROX Reference Dye or Dye II (50X), 6 µl cDNA solution and 6 µl dH₂O. The primer sequences were as follows: c-FLIPI, forward, 5'-CTAAGTCATAGTCCGCTA-3' and reverse, 5'-CTAAGTCATAGTCCGCTAAGCA-3'. The amplification conditions were as follows: Pre-denaturation 100°C for 34 sec. The mRNA expression levels of c-FLIP amplification conditions were as follows: Pre-denaturation 100°C for 34 sec. The mRNA expression levels of c-FLIP

Western blotting. Cells were harvested and lysed in lysis buffer (Beyotime institute of Biotechnology). Lysates were centrifuged at 16,099.2 x g for 1 min at 4°C and the supernatants were collected. Total cellular protein was extracted by using lysis buffer (Wanleibio) and the protein concentration was determined by the bicinchoninic acid assay. In total, 20 µl protein extracts were separated by 10 or 12% polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (EMD Millipore). The membranes were blocked in 5% skim milk solution and incubated with primary antibodies against HDAC1 (ABclonal, Inc.; cat. no. A0238; 1:500), HDAC3 (ABclonal, Inc.; cat. no. A2603; 1:500), c-FLIPₐ (ProteinTech Group, Inc.; cat. no. 10394-1-AP; 1:800), mixed lineage kinase domain-like pseudokinase (MLKL) (Cell Signaling Technology, Inc.; cat. no. 14993S; 1:1000), phosphorylated (p)-MLKL (Cell Signaling Technology, Inc.; cat. no. 91689S; 1:1000), receptor-interacting protein kinase (RIP) 3 (BD Biosciences; cat. no. 610458; 1:500), caspase-3 (ABclonal Inc.; cat. no. A11021; 1:500), caspase-8 (ProteinTech Group, Inc.; cat. no. 13423-1-AP; 1:500), RIP1 (ProteinTech Group, Inc.; cat. no. 17519-1-AP; 1:1000), cyclin-dependent kinase inhibitor 1A (p21) (Wanleibio; cat. no. WL0362; 1:400), proliferating cell nuclear antigen (PCNA; Wanleibio; cat. no. WL02208; 1:500), β-actin (Wanleibio; cat. no. WL01845; 1:5,000) and GAPDH (ProteinTech Group, Inc.; cat. no. 60004-1-Ig; 1:10,000) at 4°C overnight. Following primary antibody incubation, the membrane was incubated with a secondary antibody (Wanleibio; cat. no. WLA023; 1:5,000) at 37°C for 45 min. Protein bands were visualized using enhanced chemiluminescence (Wanleibio; cat. no. WLA003).

Cell treatment. Jurkat and HUT-78 cells were divided into five groups: i) Control group, untreated cells cultured at 37°C; ii) chidamide-treated group [cells were treated with 2 µmol/l chidamide (Sigma-Aldrich; Merck KGaA) at 37°C for 24 h]; iii) Z-VAD-FMK-treated group [following pretreatment with 50 µmol/l Z-VAD-FMK (Abcam) for 30 min, cells were treated with 2 µmol/l chidamide at 37°C for 24 h]; iv) Nec-1-treated group [following pretreatment with 20 µmol/l Nec-1 (Abcam) for 1 h, cells were treated with 2 µmol/l chidamide at 37°C for 24 h]; and v) Z-VAD-FMK and Nec-1-treated group (following pretreatment with 50 µmol/l Z-VAD-FMK for 30 min and 20 µmol/l Nec-1 for 1 h sequentially, cells were treated with 2 µmol/l chidamide at 37°C for 24 h).

Apoptosis analysis. Annexin V-FITC/propidium iodide (PI) staining kit (BD Biosciences) was used to detect apoptosis in Jurkat and HUT-78 cells. Cells were collected by centrifugation at 71.552 x g for 5 min at 4°C. The supernatant was removed and the cells were resuspended in 500 µl PBS. The cells were then incubated with 5 µl Annexin V-FITC and PI staining reagent in the dark for 15 min at room temperature. Living cells are negative to Annexin V-FITC and PI, early apoptotic cells are positive to Annexin V-FITC and negative to PI (Annexin V⁺PI), late apoptotic cells and necrotic cells are double positive to Annexin V-FITC and PI (Annexin V⁺PI⁺). Necroptosis and apoptosis were subsequently analyzed using a flow cytometer (NovoCyte; ACEA Biosciences, Inc.) and quantified by NovoExpress 1.2.5 (ACEA Biosciences, Inc.).

Transmission electron microscopy. Jurkat and HUT-78 cells were fixed using 2.5% glutaraldehyde (Servicebio) at 4°C for 24 h, dehydrated in a graded series of ethanol, embedded in epoxy resin and sectioned into ultra-thin sections (60-80 nm). The sections were subsequently stained with 2% uranyl acetate and lead citrate at room temperature for 15 min. Finally, features of necroptosis were visualized using transmission electron microscopy (JEOL; cat. no. JEM-1400) and analyzed by JEOL TEM Center Ver.1.7.15 (JEOL, Ltd.).

Mitochondrial function assay. JC-1 staining solution (Beyotime Institute of Biotechnology) was used to determine the mitochondrial membrane potential of treated Jurkat and HUT-78 cells, according to the manufacturer's protocol. The change in mitochondrial membrane potential was analyzed using a flow cytometer (NovoCyte) and quantified by NovoExpress (Novoexpress 1.2.5). In order to assess the production of reactive oxygen species (ROS), Jurkat and HUT-78 cells were treated with DCFH-DA solution at 37°C for 20 min according to the manufacturer's instructions (KeyGEN BioTECH) and were subsequently quantified using a flow cytometer (NovoCyte) and quantified by NovoExpress (Novoexpress 1.2.5).

Patient samples. A total of 75 patients with T-ALL (46 men and 29 women; mean age, 20 years) were recruited from Shengjing Hospital of China Medical University (Shenyang,
According to the fluorescence intensities of Chidamide induces necroptosis and apoptosis in Jurkat and in regulation of the cell cycle, in T-cell leukemia cell lines. and regulated cell cycle arrest, as p21 and PCNA are involved data indicated that chidamide inhibited histone deacetylation SPSS software (version 17; SPSS, Inc.) was used to perform comparison test. P<0.05 was considered to indicate a statisti­cal analysis. Data are expressed as the mean ± SD. all experiments were repeated three times (n=3). Two sample groups were compared using an unpaired Student’s t-test. Data from three or more groups were analyzed by one-way analysis of variance followed by Tukey’s multiple Student’s t-test. Data from three or more groups were analyzed by one-way analysis of variance followed by Tukey’s multiple test. P<0.05 was considered to indicate a statistically significant difference.

Results

**c-FLIP<sub>L</sub> is highly expressed in Jurkat and HUT-78 cells.** The mRNA expression levels of c-FLIP<sub>L</sub> were significantly increased in Jurkat (4.94±0.14; n=5) and HUT-78 cells (4.15±0.17; n=5) compared with human T lymphocyte H9 cells (0.39±0.03; n=5; Fig. 1; P<0.05).

Chidamide inhibits histone deacetylation in Jurkat and HUT-78 cell lines. As shown in Fig. 2, A, the expression levels of HDAC1 and HDAC3 were increased in Jurkat and HUT-78 cells, indicating that HDAC levels were elevated in T-cell leukemia cell lines. Treatment with 2 µmol/l chidamide markedly reduced the expression levels of HDAC1 and HDAC3 in Jurkat and HUT-78 cells (Fig. 2B). Additionally, p21 and PCNA levels were increased and decreased, respectively, in 2 µmol/l chidamide-treated Jurkat and HUT-78 cells (Fig. 2C). These data indicated that chidamide inhibited histone deacetylation and regulated cell cycle arrest, as p21 and PCNA are involved in regulation of the cell cycle, in T-cell leukemia cell lines.

Chidamide induces necroptosis and apoptosis in Jurkat and HUT-78 cell lines. According to the fluorescence intensities of Annexin V and PI staining, Annexin V<sup>−</sup>/PI<sup>+</sup> cells were defined as late apoptotic and necrotic cells, whereas Annexin V<sup>+</sup>/PI<sup>−</sup> cells were categorized as early apoptotic cells (12). As shown in Fig. 3A, cell death was markedly increased in Jurkat cells compared with in control cells following treatment with 2 µmol/l chidamide for 24 h (early apoptotic cells, 62.13%; necrotic and late apoptotic cells, 26.87%); the proportion of early apoptotic cells was higher. Pretreatment of cells with 50 µmol/l Z-VD-FMK (pan-caspase inhibitor) for 30 min followed by the addition of chidamide (2 µmol/l) significantly increased necroptosis and late apoptosis (93.39%). The proportion of necrotic and late apoptotic cells induced by chidamide (2 µmol/l) was significantly reduced (1.71%) following Nec-1 treatment (20 µmol/l) for 1 h. Compared with the control group, Chidamide-induced cell death was not significantly different following treatment with Z-VAD-FMK and Nec-1. HUT-78 cell death was also significantly increased following treatment with 2 µmol/l chidamide, and a high proportion of early apoptotic cells was observed compared with the control group (early apoptotic cells, 67.57%; necrototic and late apoptotic cells, 21.33%; Fig. 3B). Pretreatment with Z-VAD-FMK (50 µmol/l) significantly increased the proportion of necrotic and late apoptotic cells (62.95%). However, Nec-1 treatment (20 µmol/l) significantly reduced the proportion of necrotic and late apoptotic cells (0.14%). Following pretreatment with Z-VAD-FMK (50 µmol/l) and Nec-1 (20 µmol/l), chidamide-induced necroptosis/late apoptosis and early apoptosis was reduced to 0.13 and 0.11%, respectively. As shown in Fig. 3C and D, membraneanalysis, mitochondrial swelling and organelle disappearance were observed in 2 µmol/l chidamide-treated cells. In addition, representa­tive apoptotic and necrotic features could be reversed by Z-VAD-FMK (50 µmol/l) and Nec-1 (20 µmol/l) pretreatment. These results suggested that chidamide induced necroptosis in Jurkat and HUT-78 cell lines.

Chidamide aggravates mitochondrial dysfunction in Jurkat and HUT-78 cells. The effects of chidamide on mitochondrial function in Jurkat and HUT-78 cells were investigated. JC-1 fluorescent dye was used to detect alterations in mitochondri­al membrane potential following chidamide treatment (2 µmol/l). Chidamide induced mitochondrial dysfunction, which could be alleviated by single or combined pretreat­ment with Z-VAD-FMK (50 µmol/l) and Nec-1 (20 µmol/l) (Fig. 4A and B; P<0.05). ROS generation was also analyzed, and the results were consistent with the results of mitochondri­al function detection mentioned above (Fig. 4C and D; P<0.05). The present results suggested that chidamide induced mitochondrial dysfunction in Jurkat and HUT-78 cells.

Chidamide downregulates c-FLIP<sub>L</sub> expression in Jurkat and HUT-78 cells. The mRNA expression levels of c-FLIP<sub>L</sub> were significantly decreased in Jurkat and HUT-78 cells following chidamide treatment (2 µmol/l; Fig. 5A; P<0.05). In addition, the protein expression levels of c-FLIP<sub>L</sub> were decreased in Jurkat and HUT-78 cell lines following treatment with chidamide (2 µmol/l; Fig. 5B), indicating that chidamide
downregulated c-FLIP<sub>L</sub> mRNA and protein expression in Jurkat and HUT-78 cells.

**Effects of chidamide on caspase activation and RIP3/MLKL signaling.** Chidamide induced apoptosis of Jurkat and HUT-78 cells. The expression levels of caspase-3 and caspase-8 were markedly reduced following Z-VAD-FMK pretreatment (50 µmol/l), indicating that chidamide-induced apoptosis was inhibited by caspase inhibition. Z-VAD-FMK pretreatment (50 µmol/l) also increased the expression of the key necroptosis proteins RIP1 and RIP3, and enhanced phosphorylation of MLKL (Fig. 6). These results indicated that chidamide induced necroptosis via the RIP3/MLKL signaling pathway when apoptosis was inhibited.

**High levels of c-FLIP<sub>L</sub> predict poor prognosis in patients with T-ALL.** As shown in Table I, the mRNA expression levels of c-FLIP<sub>L</sub> were not associated with a specific age group or sex.
However, according to the National Comprehensive Cancer Network Guidelines for diagnosing and treating pediatric acute lymphoblastic leukemia (13), high c-FLIPL mRNA expression was closely associated with dangerous degree, complete remission rate, levels of hydroxybutyrate and lactate dehydrogenase, leukocyte counts, CD45 expression and silver (mouse homolog) like-TAL bHLH transcription factor 1 erythroid differentiation factor gene fusion.

Discussion

Aberrant apoptosis and proliferation are important mechanisms underlying the pathogenesis and recurrence of T-ALL. The majority of chemotherapeutic drugs used in leukemia induce apoptosis of cancer cells, which are initially sensitive to apoptosis inducers but may develop resistance following prolonged treatment. Additionally, hyper-malignant T-ALL cells demonstrate primary drug resistance. A previous study has revealed that multidrug resistance in leukemia cells caused by P-glycoprotein, multidrug resistance associated protein 1, ATP binding cassette subfamily G member 2 (Junior blood group), BCL2 apoptosis regulator and BCL2 like 1 may be overcome by inducing necroptosis (14).

C-FLIPL is an apoptosis-inhibiting protein widely found in viruses, eukaryotes and mammals, which was first identified in malignant melanoma by Irmler et al (15) in 1997. Similar to the function of pro-caspase-8, c-FLIPL binds to Fas-associated protein with death domain and competes with caspase-8 for a binding site on the death-inducing signaling complex. This leads to incomplete activation of caspase-8, thereby inhibiting apoptosis mediated by death receptors, such as Fas cell surface death receptor, tumor necrosis factor-related apoptosis-inducing ligand receptor and tumor necrosis factor receptor 1. A recent study has demonstrated that c-FLIPL expression is significantly increased in certain solid tumors and is associated with advanced disease progression (16). c-FLIPL expression decreases during disease remission, but remains high in drug-resistant tumor cells not undergoing apoptosis. However, c-FLIPL expression is downregulated by c-FLIPL inhibitors, which in turn induce apoptosis (17). High c-FLIPL expression is associated with a poor prognosis in patients with leukemia (18), which is consistent with a previous clinical investigation (4). Therefore, it is important to investigate gene-targeting drugs that can specifically modulate the expression levels of c-FLIPL. The results of the current study revealed that Jurkat and HUT-78 cells expressed increased levels of c-FLIPL compared with control cells. Therefore, the
effect of inducing necroptosis in Jurkat and HUT-78 cells by downregulating c-FLIP<sub>L</sub> expression was investigated.

Epigenetic alterations affect gene transcription through the chemical modification of DNA without altering the DNA sequence (19). Histone modifications, including phosphorylation, methylation, acetylation and ribosylation, represent important epigenetic mechanisms that serve an important role in the regulation of gene expression (20). Acetylation by histone acetyltransferase promotes gene expression and HDAC gene expression can lead to silencing of tumor suppressor genes (21). As a key enzyme regulating gene expression, HDAC has an important role in the occurrence and development of hematological malignancies via the promotion of cell proliferation and inhibition of cell apoptosis (22). There are 18 types of HDACs in mammals, which are divided into four groups: Class I, IIa, IIb and IV, according to their structure and
function. Class I HDACs, including HDAC1, HDAC2, HDAC3 and HDAC8, are mainly distributed in the nucleus and regulate histone acetylation levels. Recent studies have demonstrated that class I HDACs serve an important role in regulating differentiation, proliferation and apoptosis (23-25). HDACis target and inhibit HDACs to increase histone acetylation levels and trigger chromatin remodeling, thereby regulating the expression levels of key proto-oncogenes or tumor suppressor genes. Furthermore, HDACis may potentially inhibit proliferation, and induce differentiation and apoptosis. HDACis have exhibited efficacy in the treatment of hematopoietic malignancies and four HDACis, including vorinostat, romidepsin, panobinostat and belinostat, have been approved by the US Food and Drug Administration for clinical use (26).

Chidamide is a benzamide HDACi developed in China, which selectively inhibits HDAC class I subtypes 1, 2 and 3, and class IIb subtype 10. Numerous in vitro experiments have demonstrated the effects of chidamide on various tumors (27-29). The results of the present study revealed that Jurkat and HUT-78 cells expressed high levels of HDAC1 and HDAC3, which were significantly decreased following treatment with chidamide. Additionally, chidamide regulated cell cycle arrest, as p21 and PCNA are involved in regulation of the cell cycle, and induced mitochondrial dysfunction and necroptosis by downregulating c-FliPl transcription and translation in Jurkat and HUT-78 cells.

Degterev et al (30) proposed the term necroptosis in 2005 and revealed that the small molecule Nec-1 acted specifically on RIP1 without affecting apoptosis. Necroptosis is defined as cell death mediated by death receptors, which is specifically inhibited by Nec-1, but not the apoptosis inhibitor Z-VAD-FMK. The current study revealed that the proportions of Jurkat and HUT-78 cells undergoing necroptosis and late apoptosis were significantly increased following pretreatment with the apoptosis inhibitor Z-VAD-FMK. Furthermore, treatment with the necroptosis-specific inhibitor Nec-1 significantly reduced the proportion of cells undergoing necroptosis induced by chidamide. Chidamide-induced cell death was also significantly inhibited in Jurkat and HUT-78 cells following pretreatment with both Z-VAD-FMK and Nec-1. Similarly, membrandolysis, mitochondrial swelling and organelle disappearance were observed following chidamide treatment. However, representative apoptotic and necroptotic features, and mitochondrial dysfunction were alleviated by Z-VAD-FMK and Nec-1 pretreatment.

The expression levels of caspase-3 and caspase-8 were markedly decreased after Z-VAD-FMK treatment in chidamide-treated cells. Chidamide increased the expression of the key necroptosis proteins RIP1 and RIP3 and phosphorylation of MLKL in Jurkat and HUT-78 cells, when apoptosis was inhibited. The RIP3 gene is located on human chromosome 2q33, which is subject to multiple gene mutations and chromosomal damage, particularly in nasopharyngeal carcinoma and leukemia (31). Additionally, RIP3 is a key molecule that regulates the transformation between apoptosis and necrosis, and RIP3-deficient cells are only able to undergo apoptosis. Downregulation of RIP3 prevents necroptosis without affecting apoptosis (32). Tumor necrosis factor-α, which is considered to be a specific factor controlling necroptosis, does not induce necroptosis in mouse embryonic fibroblasts lacking the RIP3 gene but with wild type RIP1 expression (33). In addition, RIP3 expression is positively correlated with necroptosis and its expression level is a key factor determining the ability of cells to undergo necroptosis (34,35). MLKL is an important downstream effector of RIP3, and serves as an

![Figure 5. Chidamide downregulates c-FLIP₃ expression in Jurkat and HUT-78 cells. (A) c-FLIP₃ mRNA and (B) c-FLIP₃ protein expression levels were markedly decreased in Jurkat and HUT-78 cells following treatment with chidamide. Data are presented as the mean ± SD. *P<0.05 vs. the indicated group.](image)

![Figure 6. Effects of chidamide on caspase activation and RIP3/MLKL signaling. Western blot analysis of caspase-8, caspase-3, RIP1, RIP3 and p-MLKL in chidamide-treated (A) Jurkat and (B) HUT-78 cells. MLKL, mixed lineage kinase domain like pseudokinase; p, phosphorylated; RIP, receptor-interacting protein kinase.](image)
important switch for RIP3-mediated necroptosis (36,37). In
the present study, Z-VAD-FMK pretreatment followed by
chidamide treatment increased RIP3 expression and MLKL
phosphorylation in Jurkat and HUT-78 cells, indicating that
chidamide induced necroptosis when apoptosis was inhibited
in T-ALL cells.

In conclusion, the present study revealed that Jurkat and
HUT‑78 cells expressed high levels of c‑FLIPl. The HDACi
chidamide induced necroptosis in T-ALL cells by suppressing
the transcription and translation of c-FLIPl, which may serve
as a novel target for the treatment of T-ALL.

Acknowledgements

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Funding

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Table I. Clinical features of patients with T-ALL in the present study.

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c-FLIPl, cellular Fas-associated death domain-like interleukin-1β converting enzyme inhibitory protein long form; CR, complete remission; HBDH, hydroxybutyrate dehydrogenase; LDH, lactate dehydrogenase.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WY designed and directed the study. ZC was the major contributor in writing the manuscript and analyzed the data. HG, HL, BZ and MG participated in performing the experiments. BW participated in writing and was involved in the design of the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was in accordance with the Declaration of Helsinki and was approved by The Ethics Committee of
References


