Knockdown of the Hippo transducer YAP reduces proliferation and promotes apoptosis in the Jurkat leukemia cell

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Abstract. Leukemia and lymphoma are common hematological malignancies in children and young adults, which pose a tremendous threat to the survival of these young patients worldwide, despite availability of various effective treatments. The Hippo pathway is a novel-signaling pathway that regulates organ size, cell proliferation, apoptosis and tumorigenesis. The chief component of this pathway is the transducer yes-associated protein (YAP) which is over-expressed in numerous categories of tumors. However, little is known about the effect of YAP in hematological malignancies. In the present study, YAP expression was screened in several leukemia and lymphoma cell lines, and high YAP expression was demonstrated in Jurkat cells. To further unravel its effect on the biological behavior of Jurkat cells, lentivirus transduced short hairpin RNA (shRNA) technique was used to silence YAP. As expected, the YAP-specific shRNA dramatically inhibited YAP expression at the mRNA and protein levels. Reduced leukemia cell proliferation and increased cell apoptosis were demonstrated in YAP knockdown Jurkat cells. It was also demonstrated that YAP knockdown resulted in deregulated expression of a cluster of downstream genes crucial to cell proliferation or apoptosis, including protein kinase B, B-cell lymphoma 2 (BCL2) and BCL2 like protein 1. Consequently, the results of the present study established that suppression of YAP expression serves an important role in Jurkat cell proliferation and apoptosis, which may serve as a potential therapeutic target.

Introduction

Hematological malignancies, represented by leukemia and lymphoma, are life-threatening diseases associated with a poor prognosis. Although treatment outcome of the patients has improved tremendously in the era of combination chemotherapy, autologous or allogeneic hematopoietic stem cell transplantation, a significant proportion of patients remain refractory to or relapse following treatment and ultimately succumb to the disease. Therefore, numerous strategies have been developed to treat such diseases (1-3). In the past three decades, target-based therapies have established milestones in the treatment of leukemia. Two typical successful examples are the applications of all-trans retinoic acid in acute promyelocytic leukemia (APL) and tyrosine kinase inhibitor in chronic myeloid leukemia (CML) (4,5). These impressive achievements are inspired by the understanding of the precise mechanisms of these two types of leukemia. There is no doubt that further research into the pathogenesis of leukemia leading to novel therapeutic targets will benefit patients enormously (6).

The Hippo signaling pathway is an important modulator involved in the regulatory process of cell proliferation, differentiation and death. It was first identified in the course of identifying tumor suppressor genes in Drosophila, which serves a vital role in controlling organ growth or regeneration (7,8). The Hippo signaling pathway consists of three interactive elements: The upstream sensor molecules, downstream transcriptional components and core kinase parts. The core components of Hippo pathway in mammals include mammalian STE20-Like1 (MST1)/(MST2), protein salvador homolog 1, large tumor suppressor (LATS1/LATS2), MOB1/MOB2, yes-associated protein (YAP) and Tafazzin (TAZ) (9). Classical cascade signaling activation of the Hippo pathway begins from the phosphorylation and activation of MST1/MST2 by upstream molecules. Then, the phosphorylated MST1/2 further activate LATS1 and LATS2 under the help of MOB kinase activator (MOBKL1, MOBKL2, which also experiences activation by MST1/2 (10). Finally, the activated LATS1/2 phosphorylates the transcriptional co-activator YAP and TAZ, binding to 14.3.3 proteins in the cytoplasm, which accelerates the sequestration and degradation of YAP/TAZ (11,12).

Abnormal expression of YAP has been identified in various human tumors, including renal cell carcinoma, pancreatic
cancer, breast cancer, cholangiocarcinoma and medulloblas-
toma (13-17). Several groups have documented the aberrant
expression or genetic abnormalities of the Hippo pathway
in hematological malignancies, especially in acute leukemia
and lymphoproliferative neoplasms (18-22). In the present
study, YAP expression was screened in several leukemia and
lymphoma cell lines, and demonstrated high YAP expres-
sion in Jurkat cells. To elucidate its effect on cell biology of
Jurkat cells, lentivirus mediated short hairpin RNA (shRNA)
technique that targets the silencing of YAP was performed.
As anticipated, YAP expression was significantly suppressed
at the mRNA and protein levels by the YAP-specific shRNA.
Notably, decreased leukemia cell proliferation and enhanced
cell apoptosis were demonstrated in YAP knockdown Jurkat
cell line. Taken together, these results indicate that YAP may
be a potential novel therapeutic target for certain types of
leukemia.

Materials and methods

**Materials.** MTT was purchased from Beyotime Institute
of Biotechnology (Shanghai, China); Dulbecco's modi-
fied Eagle's medium (DMEM) was obtained from Gibco
(Thermo Fisher Scientific, Inc., Waltham, MA, USA); Fetal
bovine serum (FBS) was purchased from Hyclone (GE
Healthcare Life Sciences, Logan, UT, USA); interleukin
(IL)-3, IL-6 and stem cell factor (SCF) were purchased
from R&D Systems, Inc., (Minneapolis, MN, USA); TRIzol
reagent and Lipofect 2000 were purchased from Invitrogen
(Thermo Fisher Scientific, Inc.); SYBR Green PCR Master
Mix was obtained from Takara Bio, Inc., (Otsu, Japan);
plenti6.3/V5-DEST lentivirus vector and Packaging
Mix were obtained from Invitrogen (Thermo Fisher
Scientific, Inc.); RIPA protein lysis buffer was obtained
from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany);
Antibodies for western blotting including anti-YAP, protein
kinase B (AKT1), B-cell lymphoma 2 (BCL2) and BCL2
like protein 1 from Santa Cruz Biotechnology, (Shanghai,
China); Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco;
Hematology (Shanghai, China); cultured in DMEM supple-
mented with 10% heat-inactivated FBS, 100 U/ml IL-3
and 293 cells were obtained from Shanghai Institute of
Cell lines and culture. Jurkat, Daudi, SuDHL-4, NB4, HL60
and 293 cells were obtained from Shanghai Institute of
Hematology (Shanghai, China); Cultured in DMEM supple-
mented with 10% heat-inactivated FBS, 100 U/ml of penicillin
and 100 µg/ml of streptomycin. Hematopoietic stem cells and
progenitor cells (HSC/HPC) were collected from normal
murine bone marrow mononuclear cells (MNC). Briefly, mice
were sacrificed and the bone marrow was extracted under
sterile conditions from femurs and tibias. The bone marrow
suspension was filtered through a 40-µm-cell strainer and
centrifuged for 5 min at 500 x g. Bone marrow mononuclear
cells were washed in DMEM supplemented with 10% FBS
after lysis of red blood cells, centrifuged, resuspended and
cultured in the above medium supplemented with IL-3
(10 ng/ml), IL-6 (10 ng/ml) and SCF (50 ng/ml) cytokine
cocktail. Ethical approval was given for the present study by
ethics committee of Xinhua Hospital (Shanghai, China). All

**Cell transduction.** shRNA loaded by lentivirus was used in
the present study. YAP targeting shRNA was synthesized
and purchased from NOVOBIO (Shanghai Novobio, Co.,
Ltd., Shanghai, China; http://www.novobiosci.com). shRNA
specific sequences for YAP located number 1,522 nt of YAP
mRNA (PL/shRNA-YAP-1522): 5'-CAGCATGATCT
CGAGTCCTTTTCGAAAAGATGCTGATTTGTC-3';
negative control sequences (NC): 5'-TTCCTCGAAGCTG
ACGT-3'. Jurkat cells were cultured in 24-well plates. Cells
were transduced by the lentiviral supernatant harboring the
NC or shRNA-YAP packaged in 293 cells, with 1 µg/ml
polybrene (Shanghai GenePharma Co., Ltd., Shanghai, China)
added to enhance transduction efficacy. Following co-culture
for 24 h, the medium was replenished. Fluorescence density
was observed under a fluorescence microscope.

**Cell proliferation test.** Cell proliferation was measured by
MTT assay. Cells were seeded in 96-well-plates at a density
of 1x10^4 cells/well in 100 µl DMEM medium, cultured in a
CO2 incubator for 24-72 h, washed and replenished with 100 µl
fresh medium. A total of 10 µl MTT stock solution was added
to each well and further incubated at 37°C for 4 h in a CO2
incubator. This was followed by adding 100 µl the SDS-HCl
solution to each well and further incubation at 37°C for 4 h in a
CO2 incubator. Each sample was mixed by pipetting up and
down. Absorbance was read at 570 nm. Cell proliferation rate
at 24, 48 and 72 h were calculated.

**RNA isolation and reverse transcription quantitative
polymerase chain reaction (RT-qPCR)**. In order to quantitatively
analyze mRNA expression profile, RT-qPCR was performed.
Total cell RNA was extracted with TRIzol reagent according
to manufacturer's protocol. Reverse transcription was done
with M-MLV at 25°C for 5 min, 42°C for 60 min and 72°C
for 5 min. cDNA amplification was managed by SYBR-Green
PCR Master Mix kit according to the manufacturer's protocol.
Merlin, protein salvador homolog 1 (Sav1), MST1/2, LATS1/2
and YAP genes of normal murine HSC/HPC were amplified by
different primers. Human YAP, AKT, BCL2, TP53, BCL2L1,
tumor necrosis factor receptor superfamily member 6
(FAS), caspase 8 (CASP8), FAS associated death domain
(FADD), CASP3, BAX, tumor necrosis factor receptor type
1-associated DEATH domain (TRADD) protein genes were
separately amplified using specific artificially synthesized
primers, with the homo-actin house-keeping gene serving as
internal control. Sequences of the primers used in the study
are presented in Table I. Expression values were analyzed
using the comparative Cq (2^ΔΔCq) (23).

**Western blot analysis.** Protein was extracted using RIPA
lysis buffer according to protocol and protein concentration
was quantified by bicinchoninic acid protein assay kit. A
total of 50 µg of protein was loaded in 10% SDS-PAGE
and electrophoresed, then transferred to polyvinylidene difluo-
ride membrane, which was further blocked and incubated
with 5% defatted milk powder for 2 h at room temperature.
This was then incubated with a specific primary antibody
Table I. Primer sequences used in the present study.

A, Mouse

| Number | Gene     | Primer | Primer sequence (5’-3’) |
|--------|----------|--------|-------------------------|
| 1      | Merlin   | IF     | CGGACACTGGGGCTTCCGGAAACCT  |
|        |          | IR     | TCCAAAATCTCCTCTCCTCCTTCT |
| 2      | SAV1     | IF     | GACTGGAACATGAGGAGAGAAAAT  |
|        |          | IR     | TGATGACTCTACTGTTGCCAGACCA  |
| 3      | MST1     | IF     | CAGAGTCAGGGGCGGAGTGTCAACG  |
|        |          | IR     | AATAGTTGCCTTTCAGATCTTTGTC  |
| 4      | MST2     | IF     | TAACAGATACAATGGCAAAACGCA   |
|        |          | IR     | AATTGTGTTGTTGAGGGATTGGGGA  |
| 5      | LATS1    | IF     | ATGCGACAAAAATCTCGGGAAATAG  |
|        |          | IR     | TGGAAGGGAAGGGAGATGTGAGCC   |
| 6      | LATS2    | IF     | CTGTCAGAATATCGTTCGGAAATAG  |
|        |          | IR     | TTGAAAGGTCCAACCTCGGAGGAGG  |
| 7      | YAP1     | IF     | CTTTCTCAAGCGCCTGAGCCCAA    |
|        |          | IR     | AGGACAGACGACTGGCCGGAAGG    |

SAV1, protein salvador homolog 1; MST1, mammalian STE20-Like1; MST2, mammalian STE20-Like2; LATS1, large tumor suppressor homolog 1; LATS2, large tumor suppressor homolog 2; AKT1, protein kinase B; YAP, yes-associated protein; BCL2, B cell lymphoma 2; BCL2L1, BCL2 like protein 1; TRADD, Tumor necrosis factor receptor type 1-associated DEATH domain protein; FAS, tumor necrosis factor receptor superfamily member 6; FADD, FAS associated death domain; CASP, caspase; IR, reverse primer; IF, forwards primer.

B, Human

| Number | Gene     | Primer | Primer sequence (5’-3’) |
|--------|----------|--------|-------------------------|
| 1      | YAP      | IF     | TAGCCCTGCGCTAGCAGCTA    |
|        |          | IR     | TCATGCTTAGTCCACTGTCTCT  |
| 2      | AKT1     | IF     | GTCAATGACACGGACTCCCAT  |
|        |          | IR     | AGCTATTAGTCTCAACTCGCTG  |
| 3      | BCL2     | IF     | GGTGGGCTGATGTTGAGGGA   |
|        |          | IR     | CGTGGATGCAGCTAAGCTATG  |
| 4      | TP53     | IF     | CAGCACTACGAGGAGGTGTT   |
|        |          | IR     | TCATCCAATACACTACACGCG  |
| 5      | BAX      | IF     | CCCAGAGGCTCTTTCTCCGAG  |
|        |          | IR     | CCAGCCCATGATGTTTGATG    |
| 6      | BCL2L1   | IF     | GAGCTTGGTTGTTGACTTTC   |
|        |          | IR     | TCATCTGATGCATGTTCTCC   |
| 7      | TRADD    | IF     | GCTGGTTTGAATGGCAGTACTAG|
|        |          | IR     | CGGACATCATATGCATTTGCC  |
| 8      | FAS      | IF     | TCTGGTCTTACATGTCTTGG   |
|        |          | IR     | CTGGTGCAGCCCTTGAGCTTCT  |
| 9      | CASP8    | IF     | GTGGGGAATGACAATTCGCG   |
|        |          | IR     | TCAAGGTTGCTGGTCAAAAGC  |
| 10     | FADD     | IF     | GCTGGCTCAGCTGCTCATCT   |
|        |          | IR     | ACTGTGGTCTTCCTCTCTCT   |
| 11     | CASP3    | IF     | GAAATTTGGAATTTGATCCTG  |
|        |          | IR     | CTAAAGATCCCTCCTGAAAAA  |
| 12     | Homo-actin | IF  | AGGAGGACATCCCCCAAGGT  |
|        |          | IR     | GGCGACAGACGTATACATT    |

SAV1, protein salvador homolog 1; MST1, mammalian STE20-Like1; MST2, mammalian STE20-Like2; LATS1, large tumor suppressor homolog 1; LATS2, large tumor suppressor homolog 2; AKT1, protein kinase B; YAP, yes-associated protein; BCL2, B cell lymphoma 2; BCL2L1, BCL2 like protein 1; TRADD, Tumor necrosis factor receptor type 1-associated DEATH domain protein; FAS, tumor necrosis factor receptor superfamily member 6; FADD, FAS associated death domain; CASP, caspase; IR, reverse primer; IF, forwards primer.
Cell cycle analysis. For experiments on cell cycle analysis, cells were harvested and resuspended in fresh DMEM medium, centrifuged (250 x g for 5 min at 4°C) and washed with PBS and fixed with cold 70% ethanol added drop wise to the pellet while vortexing. Following fixing for 30 min at 4°C and washing with PBS, the fixed cells were stained with 7-AAD (DNA dye) in the presence of RNase for 30 min at room temperature in the dark room. The samples were then analyzed on a FACsort flow cytometer using CellQuest 1.0 software (BD Biosciences, Franklin Lakes, NJ, USA).

Cell apoptosis analysis. In cell apoptosis experiment, cells were harvested and suspended in fresh DMEM medium, then resuspended in Annexin‑binding buffer following centrifugation (300 x g, 5 min, 4°C) and washed according to the corresponding protocols. Annexin V-FITC and 7-AAD working solution (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China) were added to the cells and incubated in the dark at room temperature for 15 min. Following incubation, Annexin‑binding buffer was added and the samples were kept on ice to be analyzed as soon as possible by flow cytometry. Cell apoptosis was measured and analyzed by CellQuest 1.0 software (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical analysis. Values were expressed as the mean ± standard deviation. Statistical analysis was performed using SPSS software version 13.0 (SPSS, Inc., Chicago, IL, USA). Quantitative data was analyzed by one-way analysis of variance, multiple comparisons between different groups was performed using S-N-K method. P<0.05 was considered to indicate a statistically significance difference. Each experiment was repeated at least three times.

Results

YAP is highly expressed in Jurkat cells. Previously the expression level of several vital components of the Hippo signal transduction pathway has been studied and it was demonstrated that Merlin, Sav1, MST1/2, LATS1/2 and YAP were all expressed in normal murine HSC/HPC (Fig. 1A). This provided the basis for the hypothesis that the Hippo signaling pathway may serve certain important roles in murine HSC/HPC function. Notably, the Hippo signaling pathway did not influence the proliferation and colony formation abilities when YAP was ectopically overexpressed in HSC/HPC cells (data not shown). Therefore hematological malignant cells were focused on in the present study. YAP expression levels were screened in several leukemia and lymphoma cell lines including Jurkat, Daudi, SuDHL-4, NB4 and HL60. Compared with the control, it was demonstrated that YAP is highly expressed in Jurkat cells at the mRNA and protein levels (Fig. 1B-D), Daudi cells also had a relatively high expression level of YAP while SuDHL-4 had lower expression level (Fig. 1B-D). NB4 and HL-60 had expression level comparable to the control (data not shown).

YAP knockdown in Jurkat cells. As YAP was highly expressed in Jurkat cells, the function of YAP in Jurkat cells was further investigated, utilizing YAP-shRNA to knockdown YAP expression in Jurkat cells. Lentivirus harboring YAP-shRNA exhibited a high titer (5.2x10⁸ TU/ml) following successful packaging. The lentivirus titration was measured by calculating multiplicity of infection through sequential dilution (data not shown). Jurkat cells were successfully transduced by lentivirus and Jurkat cells were observed using a fluorescence microscope system or light microscope following transduction (Fig. 2A and B). RT-qPCR results demonstrated that mRNA expression of YAP was dramatically decreased in the YAP knockdown (YAP-shRNA) group compared with the NC group, demonstrating 75% interfering efficacy (P<0.01, Fig. 2C). Correspondingly, the western blotting experiment further demonstrated that YAP protein expression was markedly reduced in these cells (Fig. 2D). All these data demonstrated that YAP was successfully silenced by lentivirus mediated shRNA interference.

Influence of YAP silencing on proliferation of Jurkat cells. Uncontrolled cellular proliferation is the hallmark of leukemia cells. To check whether knockdown of YAP by shRNA elicited a suppressive effect on Jurkat cell proliferation, an MTT assay was utilized to investigate the effect of YAP silencing. Compared with the NC counterpart, the proliferation rate of YAP-shRNA Jurkat cells was affected in a time-dependent manner and this was significant following 3 days (P<0.05; Fig. 3A). Furthermore, the cell cycle pattern was analyzed by flow cytometry (FCM). FCM demonstrated that knockdown of YAP in Jurkat cells boosted the percentage of G1/G0 phase cells from 43.6-50.8% (Fig. 3B-C). Finally, the downstream genes associated with cell proliferation or cell cycle regulation including AKT1 and P53 were investigated. It was demonstrated that AKT1 gene expression, which is crucial to the PI3K-AKT proliferation pathway, was decreased in YAP-shRNA transduced Jurkat cells compared with the NC group (Fig. 3D). These data provided strong evidence that YAP silencing could inhibit Jurkat cell proliferation through G1/G0 arrest.

Effect of YAP knockdown on apoptosis of Jurkat cells. Deregulation of apoptosis is another hallmark of leukemia cells. To investigate the effect of YAP knockdown on Jurkat cell apoptosis. FCM analysis was performed on YAP knockdown Jurkat cells following 7-AAD and Annexin V-FITC double staining. Compared with the NC group, YAP knockdown significantly promoted early apoptosis (8.05% vs. 1.93%)
and late apoptosis rate (27.8% vs. 9.9%) in Jurkat cells (P<0.01; Fig. 4A-C). Furthermore, typical morphological features of apoptosis were also discerned in YAP knockdown Jurkat cells (data not shown). The expression levels of several downstream
apoptosis or anti-apoptosis associated genes including BCL2, FAS, CASP8, FADD, CASP3, BCL2L1, BAX and TRADD were further verified. It was demonstrated that expression levels of BCL-2 and BCL2L1 genes associated with anti-apoptosis were significantly reduced (P<0.05; Fig. 4D and F). While no significant difference was observed in the mRNA expression level of FAS, CASP8, FADD, CASP3, BAX and TRADD between YAP knockdown Jurkat cells and the NC group (Fig. 4E). All these results imply that YAP silencing induced apoptosis in Jurkat cells through deregulating the expression of anti-apoptosis associated proteins.

**Discussion**

Despite rapid development in drugs including targeted therapies, hematological malignancies are still challenging diseases to treat due to high incidence of chemo-refractoriness and relapse. There is an urgent need to investigate novel pathogenesis mechanisms and develop more novel therapies. The Hippo signaling pathway contains a large group of interactive proteins, which participate in regulating cell proliferation, differentiation and apoptosis. Importantly, deregulation of Hippo pathway correlates with pro-oncogenic or anti-oncogenic processes. Function of Hippo
pathway has been clarified in a number of organs or tissues, but its function in the hematopoietic system is still unknown. It is therefore interesting to investigate Hippo pathway in normal hematopoiesis and hematological malignancies.

It is controversial whether the Hippo pathway is involved in the hematological system. Several study groups have documented the deregulated expression profile or epigenetic alterations of Hippo pathway in hematological malignancies.
especially in acute leukemia or lymphoproliferative neoplasms (24,25). In acute myeloid leukemia (AML), overexpression of the LATS2 gene was detected in 32 de novo AML patients, suggesting that LATS2 may be associated with leukemogenesis (18). Another group reported that AML patients with t(8;12) translocation generates the MST2-ETV6 fusion gene, which is a potential oncogene (19). In acute lymphoblastic leukemia (ALL) patients harboring t (12;21) chromosome translocation, a vital upstream component in the Hippo pathway named KIBBRA is demonstrated to be highly methylated and is the main underlying leukemogenesis event in this specific subtype of leukemia (20). The LATS2 gene is also a methylation modification target hotspot in ALL patients; its lower expression due to methylation predicts patients' poor prognosis (21). In addition, in adult T cell or natural killer leukemia/lymphoma, decreased expression of LATS2 gives rise to lower expression of proapoptotic genes or causes chemotherapy resistance (26). The Hippo pathway abnormality is also seen in APL pathogenesis, because YAP is required for APL transcriptional activation (27,28). In mantle cell lymphoma, decreased expression of MOBKL2 and LATS2, important component proteins of the Hippo pathway, has been detected in certain patients and indicates poor outcome (22). MST1 deficiency is highly predisposed to develop T-cell ALL under mutagenic stimulation. Furthermore, MST1(−/−) mice display rapid formation of lymphoma in a p53 knockout model and obvious chromosomal instability has been demonstrated in MST1(−/−) lymphocytes (29). YAP is overexpressed in CML cells. Knockdown of YAP by siRNA or inhibiting the function of YAP using verteporfin (VP) not only inhibits the proliferation, induces the apoptosis of CML cells but also reduces the expression of YAP target genes c-myc and survivin (30).

On the contrary, the ectopic overexpression of YAP in the hematopoietic system, reported by another research group, does not influence HSC function neither during steady state nor in situations of hematopoietic stress (31). The author speculates that the discrepant nature of the different tissues may explain the mechanism of cell over-proliferation or excessive growth in solid tissues, while not in the hematopoietic system. Machado-Neto et al (32) demonstrated that YAP may not correlate with hematological malignancies including AML, ALL or myelodysplastic syndromes.

In the authors' previous study, in order to investigate whether Hippo pathway is associated with haematopoiesis, the expression level of several vital components of the Hippo signal transduction pathways in normal mice was checked, and demonstrated that Merlin, Sav1, MST1/2, LATS1/2 and YAP were all expressed in normal murine HSC/HPC. It was therefore hypothesized that Hippo signal pathway may serve certain important roles in murine HSC/HPC function. Notably, it did not influence proliferation and colony formation abilities when ectopic YAP was overexpressed in HSC/HPC cells, which is consistent with what has been reported by other groups (31). In the present study, hematological malignancies were the main focus. YAP expression levels were screened in several leukemia and lymphoma cell lines and it was demonstrated that YAP was highly expressed in Jurkat cells at the mRNA and protein levels. Considering that YAP has oncogenic features, YAP was silenced by shRNA in Jurkat cells and the consequences were investigated. Growth arrest, inhibition of cell proliferation, apoptosis, and differentiation are the most well-characterized effects of tumor suppressor response, therefore we tested for these aspects of Jurkat cells following silencing YAP.

An MTT assay was conducted to assess cell metabolic activity associated with cell proliferation. It was demonstrated that Jurkat cell proliferation was weakened in YAP knockdown group, compared with the control group. Furthermore, G0/G1 phase cells were increased in YAP knockdown group compared with the control group. Finally, several downstream genes associated with cell proliferation or cell cycle regulation were checked. It is well known that inhibition of the PI3K-AKT pathway could inhibit proliferation of tumor cells. In the present study the expression levels of AKT1 were demonstrated to be decreased in the YAP knockdown group, compared with the control group. Therefore, the results indicated that cell proliferation was inhibited in Jurkat cells following silencing YAP by interfering with the PI3K-AKT pathway.

FCM was also used to analyze apoptosis in the YAP-knockdown Jurkat cells. It was demonstrated that YAP knockdown remarkably increased early and late apoptosis in Jurkat cells. In addition, morphological alterations of apoptotic Jurkat cells including nuclear fragmentation or condensation could be easily seen in the YAP silenced group, but not in the NC or normal untreated group. Finally, the expression level of several downstream genes associated with cell apoptosis was measured, and decreased levels of Bcl-2 and BCL2L1 genes were observed, the two of have anti-apoptotic properties. These results suggested that knockdown of YAP by shRNA enhanced apoptosis through deregulating the expression levels of pro-apoptotic or anti-apoptotic associated genes.

Cottini et al (33) demonstrated a tumor suppressor function for YAP in multiple myeloma, lymphoma and leukemia cell lines. In an experimental model, damaged DNA activates apoptosis by a p53-independent pathway in a process guided by nuclear re-localization of the ABL1 kinase and its interaction with YAP, demonstrating that low YAP1 levels prevent nuclear ABL1-induced apoptosis in these hematological malignancies, supporting the hypothesis that YAP1 is involved in the tumor suppressor process. In contrast, the present study demonstrated that Jurkat cells express a high level of YAP1. Furthermore, knockdown of the YAP1 in the Jurkat cell caused growth arrest and apoptosis, indicating that YAP1 promotes oncogenesis process. Possible explanations for the discordance between these two different studies were analyzed: i) The work of Cottini et al (33) has demonstrated that Jurkat cells express a low level of YAP protein, while the cells of the present study expressed a relatively high level. This dissimilarity may originate from different cellular metabolic conditions, excessive passage, mutation or other clonal evolution. ii) Indeed, the YAP protein possesses dual functions, like a double-edged sword, as a pro-oncogenic or anti-oncogenic molecule. Its double role seems to be dependent on its phosphorylation pattern: Phosphorylation by LATS1/2 at Ser127 promotes YAP sequestration in the cytoplasm and prevents its interaction with TEAD transcription factors, while phosphorylation by c-Abelson murine leukemia viral oncogene (ABL) at Tyr357 upon DNA damage confers a tumor suppressive function to YAP (25,33). Furthermore, It was also demonstrated that YAP...
expression was high in Daudi cells and for the next study YAP will be knocked down in Daudi cells to investigate what kind of role YAP serves in these cells.

In conclusion, the current study initially revealed a high expression level of YAP in Jurkat cells. Lentivirus mediated shRNA decreased YAP expression in Jurkat cells. Silencing of YAP expression by shRNA suppressed the growth and promoted apoptosis of Jurkat cells. These results demonstrated that YAP serves an important role in leukemogenesis, which may be a potential target for the treatment of leukemia in the future.

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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

LM made substantial contributions to the design of the present study. RW and HY performed the experiments, data collection and data analysis. RW and LM wrote the manuscript. JW, XD, LC provided help in conceiving and designing the study. SH made substantial contributions in data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was given for the present study by ethics committee of Xinhua hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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