Luks-pv Induces Apoptosis Through Methyltransferase Set8 in Human Acute Myeloid Leukaemia Cells

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Abstract

Background We previously reported that LukS-PV induces apoptosis in human acute myeloid leukaemia (AML) cells. Furthermore, SET8 is a member of the SET domain-containing methyltransferase family and overexpressed in numerous tumours, including AML and indicated a poor prognosis. However, it is unclear whether LukS-PV induce apoptosis via SET8. In current study, we aimed to investigate the regulatory mechanisms of SET8 and effects on AML cells under the LukS-PV.

Methods Flow cytometry was performed to detect apoptosis in AML primary blasts/cell lines treated with LukS-PV and invasion of AML cells *in vivo*. The expression of SET8 was quantified through reverse-transcription PCR and western blotting. Chromatin-immunoprecipitation (ChIP) sequencing and bioinformatic methods was performed to explore transcriptional target genes which regulated by SET8-H4K20me1 axis. All experiments were performed in triplicate, and all statistical analyses were conducted using SPSS 16.0.

Results In this research, we found that LukS-PV induce cell apoptosis *in vitro* and inhibit cell invasion *in vivo*. Our results further confirmed SET8 and H4K20me1 were downregulated in LukS-PV-treated AML cells. Furthermore, we confirmed that LukS-PV induced apoptosis via downregulating SET8/H4K20me1. Finally, Genome-wide analysis identified PIK3CB is the target gene for cell apoptosis mediated by SET8/H4K20me1. In a nutshell, LukS-PV induces apoptosis via the PIK3CB/PI3K/AKT/FOXO1 signal pathway by targeting SET8.

Conclusions The present results indicate that LukS-PV induces apoptosis in AML cells by downregulating SET8 and regulating downstream molecular targets, suggesting that SET8 is a potential target for AML therapy using LukS-PV for anti-leukaemia treatment.

Introduction

Acute myeloid leukaemia (AML) is a heterogeneous clonal disorder characterized by immature myeloid cell proliferation and bone marrow failure and a short course[24]. Treatments for AML primarily include radiotherapy, chemotherapy, and hematopoietic stem cell transplantation; however, the 5-year survival rate remains low[17, 32]. Thus, it is important to identify more targeted therapies for AML.

In recent years, bacterial toxins have received increasing attention for the development of novel anticancer drugs owing to their specificity and cytotoxicity in target cells, and some bacterial toxin-containing anticancer drugs have entered clinical trials[19, 31]. Pantone-Valentine leucocidin (PVL) is an extracellular toxin produced by *Staphylococcus aureus* and comprises two subunits, LukS-PV and LukF-PV[1]. Our previous study reported that LukS-PV induces apoptosis in AML cells mediated by C5aR and inhibit AML cell proliferation *in vitro* and *in vivo*, and have no toxic side effects[2, 25, 26, 37].

SET8 (also known as PR-Set7/9, SETD8, and KMT5A) is a member of the SET domain-containing methyltransferase family, and its activity is specific to H4K20me1[18]. SET8 is overexpressed in

numerous tumours including hepatocellular carcinoma, gastric cancer, small-cell lung cancer, breast cancer, ovarian cancer and AML[13, 27, 29, 34, 38]. High SET8 levels are also associated with poor survival in AML patients. Thus, SET8 is considered therapeutic targets and should be further studied in AML.

In this study, we investigated the effects of LukS-PV through downregulate SET8 on apoptosis of AML cells and further explored its molecular mechanism of action.

Materials And Methods

Cell culture and Reagent

Human acute leukaemia cell lines HL-60 and NB-4 were obtained from the Shanghai Institute for Biological Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (FBS, HyClone, Logan, UT, USA) and 1% penicillin/streptomycin in incubator at 37 °C with 5% CO₂. The medium was changed every 2–3 d. PIK3CB inhibitor GSK2636771 was purchased from MedChemExpress(MCE, Shang Hai, China).

Total RNA extraction of peripheral blood from AML patient and normal people

AML patients were diagnosed in accordance with clinical and laboratory criteria. Add five times the volume of erythrocyte lysate to fresh peripheral blood, place it on a shaker for 15–20 minutes. Then, centrifuge for 5 minutes, 1000 rpm/min, discard the supernatant, wash bottom cells twice with PBS, and re-lyse according to the lysis. Total RNA was extracted using TRIzol (Invitrogen, USA) in accordance with the manufacturer's instructions. This study was approved by the Ethics Committee and Institutional Review Board of University of Science and Technology of China, Anhui, China (Approval number: 2019-N(H)-101).

Separation and culturing of primary bone marrow cells

AML patients were diagnosed in accordance with clinical and laboratory criteria. Primary AML cells were harvested from the bone marrow samples of AML patients. Fresh bone marrow mononuclear cells were isolated through Ficoll density-gradient centrifugation and resuspended in RPMI-1640 medium supplemented with 10% FBS in an incubator. The concentrations of LukS-PV incubate with primary AML cells 24 h for following experiments. This study was approved by the Ethics Committee and Institutional Review Board of University of Science and Technology of China, Anhui, China (Approval number: 2019-N(H)-101).

Recombinant LukS-PV production and purification

The pET28a vector (Roche Diagnostics Corp, Switzerland) was used to generate six recombinant His-tagged LukS-PV proteins. The LukS-PV sequence was amplified from PVL-positive *S. aureus* isolates.

PCR products were digested with XhoI and BamHI (Promega, Madison, WI, USA) and ligated into the pET28a vector. Recombinant LukS-PV was purified as previously described[21].

RNA isolation and quantitative real-time RT-PCR

Total RNA was extracted using TRIzol (Invitrogen, USA) in accordance with the manufacturer's instructions. Reverse transcription was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). All quantitative real-time PCR (qRT-pCR) assays were carried out using a StepOnePlus RT-PCR system (Applied Biosystems, Carlsbad, CA, USA). Relative expression levels were quantified using the comparative Ct method. Gene-specific primer sequences were the following: SET8: 5'-ACTTACGGATTTCTACCCTGTC-3' and 5'-CGATGAGGTCAATCTTCATTCC-3'; PIK3CB: 5'-ATCGCTCTGGCCTCATTGAAGTTG-3' and 5'-ATGGCTCGGTCCAGGTCATCC-3'.

Lentiviral transduction

The lentiviral vector used for SET8 silencing and overexpression (Hanbio, Shanghai, China) was transduced into HL-60 and NB-4 cells. As controls, lentiviral vectors containing short hairpin RNA sequences targeting a non-mammalian gene were used. After 48 h of transduction, the cells were screened using puromycin and cultured.

Flow cytometric analysis

To assess apoptosis, cells were harvested through centrifugation at 1000 rpm for 5 min, washed twice in cold PBS, resuspended in 500 µl staining buffer, and co-stained with Annexin V-PE and 7AAD (eBioscience, CA, USA) at room temperature for 15 min in the dark, and then analysed using a FACS Calibur flow cytometer (BD Biosciences, NJ, USA) in 1 h. For proliferation assay, cells were pre-treated with the kFlour647 Click-iT Flow detection kit (KeyGEN BioTECH, Jiangsu, China) in accordance with the manufacturer's instructions. The data were analysed by the FCS Express software.

Western blotting

Cells were lysed by RIPA with 1% PMSF (Beyotime, Shanghai, China) on ice for 30–60 min and then centrifuged at 12000 rpm for 5 min and the pellet was discarded. The protein samples and SDS were boiled for 15 min and the proteins were separated through SDS-PAGE and electro-transferred onto a 0.45-µm nitrocellulose membrane (Millipore, Bedford, MA, USA). The membranes were blocked with Protein Free Rapid Blocking Buffer (EpiZyme, Jiangsu, China) and subsequently probed with primary antibodies. The anti-human SET8(Rabbit), PIK3CB(Rabbit), FOXO1(Rabbit), AKT(Rabbit), p-AKT(Mouse), BAK(Rabbit), Bcl2(Rabbit), H4K20me1(Rabbit), GAPDH(Mouse) and H4(Rabbit) antibodies were purchased from Cell Signaling Technology(Danvers, MA). Thereafter, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody for 1.5 h at room temperature. Immunoreactive bands were visualized using an enhanced chemiluminescence detection system.

Chromatin immunoprecipitation (ChIP)-sequencing assay and ChIP-PCR

Approximately 4×10^6 HL-60 cells were fixed with 1% formaldehyde and then subjected to ChIP with an ChIP grade anti-H4K20me1 antibody (Abcam, ShangHai, China) using the Cell Signaling SimpleChIP enzymatic ChIP kit (Cell Signaling Technology, Danvers, MA) in accordance with the manufacturer's instructions. Input and H4K20me1-immunoprecipitated chromatin samples were sequenced at GeneSky (SuZhou, China). The Gene-specific primer sequences used for ChIP-PCR was the following: PIK3CB: 5'-GGAAGAGCGGAATCTCTCGG-3' and 5'-GCACGGCCTTTCCTAACTCT-3'. The PCR reaction program was the following: 1).initial denaturation:95°C*3 min; 2).Denatruue:95°C*15sec; 3)Anneal and Extension:60°C*60sec; 4)Repeat step 2) and 3) for a total of 40 cycles. The %Input = $2\% * 2^{(CT_{\text{Input}} - CT_{\text{IP sample}})}$.

Xenograft mice assay

Male BALB/c nude mice(4 weeks old) were obtained from nanjing China GemPharmatech. Ltd and maintained in a specific pathogen-free facility at the Lab Animal Center of Anhui Medical University. Mice were injected intraperitoneally (i.p.) with cyclophosphamide (CTX, 100 mg/kg body weight) on each of 3 successive days and then randomized into 3 groups, including the normal control(5 mice), HL-60 group(10 mice) and NB4 group(10 mice). Mice in the normal control group received only PBS. The HL-60 group and NB4 group were injected 5×10^6 HL-60 or NB4 cells per mice via tail vein and then randomized into PBS group(5 mice) and LukS-PV group(5 mice) respectively. Then LukS-PV group mice were treat with LukS-PV (300 ug/kg body weight per mice) via tail vein for 3 successive days. After 30 days, mice were sacrificed and their spleens and peripheral blood samples were collected for next experiments. CD33, a myeloid lineage-specific antigen, is a sialoadhesin family member that is normally expressed on precursor myeloid cells and it can be used as a specific marker to observe leukemic cell proliferation and infiltration in a mouse leukemia model[7]. Hence, we used CD33-PE(BD, cat:555450) to assess invasion of AML cell in vivo via FCM. This study was approved by the Ethics Committee and Institutional Review Board of University of Science and Technology of China, Anhui, China (Approval number: 2019-N(H)-101).

Statistical analysis

All data are expressed as mean $\pm$ standard deviation (SD) values, and all experiments were performed in triplicate. Statistical analyses were performed using t-tests or one-way ANOVA. All statistical analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

Results

LukS-PV induce cell apoptosis in vitro and inhibit cell invasion in vivo

We randomly isolated bone marrow samples from 4 AML patients for in vitro culture and treated them with LukS-PV at different concentrations to detect apoptosis via flow cytometry, these results showed LukS-PV induces apoptosis in a dose-dependent manner in primary AML blasts (Fig. 1A). In order to further study the anti-leukemia activity of Luks-PV *in vivo*, we injected AML cell lines (HL-60 and NB4) into

the tail vein of nude mice and treat with LukS-PV. The results showed that the spleen index of treatment group was lower than untreated group (Fig. 1B). Besides, FCM analysis showed the percentage of AML cells(CD33⁺ cells) in peripheral blood and spleen was lower in treatment group compare to untreated group (Fig. 1C-D). These results indicated that LukS-PV not only induce AML cell apoptosis *in vitro*, but also inhibit cells invasion *in vivo*.

SET8 is upregulated in AML cells

We first analyze AML patient peripheral blood RNA-seq data from TCGA database and normal people peripheral blood RNA-seq data from GTEx database[33] to explore the expression of SET8 between AML patient and normal people. The analysis results showed that the expression of SET8 mRNA in AML patients was significantly higher than normal people, and indicated a poor prognosis (Fig. 2A-B). Then, we sampled peripheral blood from 20 AML patients and 20 healthy controls and quantified SET8 expression in peripheral blood leukocytes of the two groups to verified results of database. Consequently, RT-PCR and western blotting revealed that SET8 was significantly upregulated in AML patients than in the healthy controls (Fig. 2C-D), especially in M3 AML (Fig. 2E). These results indicated that SET8 involved in the leukaemia pathogenesis and maybe a potential therapeutic target in AML.

LukS-PV can downregulate SET8 and H4K20me1

Since SET8 was upregulated in leukaemia cells and is associated with leukaemia pathogenesis, concurrent with a previous study reporting that LukS-PV induces apoptosis in AML cells. To investigate whether LukS-PV exerts anti-leukaemia effects by regulating SET8, we first treated HL-60 and NB-4 cells with different concentrations of LukS-PV and quantified SET8 expression via RT-PCR and western blotting. Consequently, SET8 gene and protein were significantly downregulated after exposure with LukS-PV (Fig. 3A-B). Further, we assessed SET8 gene and protein expression levels in LukS-PV-treated HL-60 and NB-4 cells at different timepoints, the results showed SET8 gene and protein were significantly downregulated after exposure with LukS-PV (Fig. 3C-D). These results indicate that LukS-PV downregulates SET8 in a dose- and time-dependent manner.

As SET8 is a member of the SET domain-containing methyltransferase family that specifically catalyses monomethylation of histone H4 Lys-20 (H4K20me1), so we also treated HL-60 and NB-4 cells with different concentrations of LukS-PV and quantified H4K20me1 expression via western blotting. Consequently, H4K20me1 levels were significantly lower in than those in control cells (Fig. 3E). Similarly, we determined H4K20me1 levels in LukS-PV-treated HL-60 and NB-4 cells at different timepoints, and our results were consistent with SET8 downregulation, and H4K20me1 levels were reduced (Fig. 3F). These results indicate that LukS-PV can downregulate H4K20me1 through SET8 in a dose- and time-dependent manner.

LukS-PV induces apoptosis in AML cells by downregulating SET8/H4K20me1

To determine whether LukS-PV exerts anti-leukaemia effects by downregulating SET8, we first transfected HL-60 and NB4 cells with siRNAs to silence or overexpress SET8, and then quantified SET8 and H4K20me1 expression by RT-PCR and western blotting (Fig. 4A-B). Then, we assessed apoptosis via flow cytometry, the results showed both early and late apoptosis were significantly increased in set8 knockdown AML cell lines (Fig. 4C-D).

Furthermore, we overexpressed or knocked down SET8 in AML cells and then treated the cells with 2.0 μ M LukS-PV, followed by the assessment of apoptosis via flow cytometry. Consequently, the LukS-PV-treated cells displayed a significantly higher extent of apoptosis than the PBS control group, and this effect was further enhanced in SET8 knockdown cells but markedly alleviated in SET8-overexpressing cells. In other words, the effect of LukS-PV on apoptosis can be reversed by SET8 overexpression (Fig. 4E-F). In accordance, LukS-PV was able to decrease the levels of SET8, H4K20me1, Bcl-2 and increase protein levels of Bax, and this effect was further enhanced in SET8 knockdown cells but markedly alleviated in SET8-overexpressing cells (Fig. 4G-H). These results indicated that LukS-PV induced cell apoptosis in AML cells by downregulating SET8 expression.

PIK3CB is the target gene for cell apoptosis mediated by SET8 /H4K20me1

To further explore the molecular mechanism that LukS-PV induces apoptosis by downregulating SET8 in AML cells, we speculated whether it functions by regulating downstream target genes through H4K20me1. To verify this speculation, target genes regulated by LukS-PV-mediated H4K20me1 were determined through analysis of genome-wide transcriptional targets of LukS-PV through ChIP-seq. ChIP experiments were first performed with HL-60 cells with antibodies against H4K20me1 after LukS-PV treatment. Thereafter, H4K20me1-associated DNA sequences in LukS-PV-treated cells were amplified under nonbiased conditions, labelled, and sequenced. Through HiSeq2000 with a p-value cut-off of 10^{-5} , we identified 2450 H4K20me1-specific binding peaks, of which 731 were upregulated and 1719 were downregulated (Supplement Table S1).

Since SET8 promotes the transcription of target genes through H4K20me1, and LukS-PV downregulates SET8, we focused on the downregulated peaks related genes binding by LukS-PV-mediated H4K20me1. GO-based biological process analysis indicated these genes to be significantly enriched in transcription coactivator activity, magnesium ion binding, phosphatidylinositol bisphosphate binding, potassium ion transmembrane transporter activity and so on (Fig. 5A). Furthermore, these genes were then classified into various cellular signalling pathways, using the Molecule Annotation System software with a p-value cut-off of 10^{-3} . These signalling pathways included protein export, axonal guidance, leukocyte trans-endothelial migration, and cAMP and Wnt pathways, which are critical for cell growth, survival, and apoptosis (Fig. 5B). As our previous study illuminated that LukS-PV induces apoptosis in AML cell through C5aR, and considering Yoshihiro Maeda et al. reported that C5aR plays a crucial role in invasion of metastatic renal cell carcinoma via PI3K pathways[35], we identified a potential gene *PIK3CB* as a target for LukS-PV induces cell apoptosis mediated by SET8 /H4K20me1 (Fig. 5C). Further, we verified

this finding through ChIP-PCR in HL-60 and NB4- cells. Accordingly, upon LukS-PV treatment, the binding of H4K20me1 at the *PIK3CB* promoter was significantly reduced (Fig. 5D). Finally, we treated HL-60 and NB4 with LukS-PV at different concentrations, and PIK3CB gene and protein expression levels were further reduced in a dose-dependent manner on qRT-PCR and western blotting (Fig. 5E-F).

PIK3CB was identified as a downstream target of LukS-PV induces apoptosis by downregulating SET8/H4K20me1, hence, we speculated whether it plays a role in cell apoptosis. To investigate the biological function of PIK3CB, we tested its effect on apoptosis through inhibition with GSK2636771 (a PIK3CB inhibitor) in SET8-overexpressing cells. The flow cytometry results showed GSK2636771 induced apoptosis in control SET8-expressing cells; however, this effect was alleviated in SET8-overexpressing cells in both HL-60 and NB-4 cells (Fig. 5G-H). Together, our results indicate that LukS-PV induces cell apoptosis through downregulate expression of the target gene PIK3CB mediated by SET8 /H4K20me1 in AML cells.

LukS-PV induces apoptosis via the PIK3CB/PI3K/AKT/FOXO1 signal pathway by targeting SET8

It was reported that PIK3CB inhibits transcription factor FOXO1 by regulating AKT phosphorylation, and then inhibits apoptosis by regulating apoptotic proteins Bax and Bcl2. Therefore, we further verified this molecular mechanism by western blotting, and the results were in line with our expectations. We found that 2.0 μ M LukS-PV treated cells had lower levels of PIK3CB, pAKT and anti-apoptotic Bcl2 but higher levels of FOXO1 and pro-apoptotic Bax than PBS-treated control cells in both HL-60 and NB4 cells. These effects were further enhanced in SET8 knockdown cells but markedly alleviated in overexpressed SET8 cells (Fig. 6A).

Similarly, we determined the levels of associated proteins in primary AML blasts via western blotting. In accordance, the result showed that 2.0 μ M LukS-PV significantly decreased the levels of SET8, H4K20me1, PIK3CB, pAKT and anti-apoptotic Bcl2 but increased the levels of FOXO1 and pro-apoptotic Bax in comparison with the PBS-treated control group (Fig. 6B). Together, our results indicate that LukS-PV induces apoptosis via the PIK3CB/PI3K/AKT/FOXO1 signal pathway by targeting SET8 in primary AML blasts.

In conclusion, this study demonstrated that LukS-PV decreased SET8 expression and regulated the PI3K/AKT/FOXO1 signal pathway mediated by SET8, thereby inducing apoptosis in AML cells (Fig. 6C).

Discussion

Bacterial toxins reportedly have specific cytotoxic effects on target cells including tumour cells, and they have received increasing attention in the development of anti-tumour drugs. LukS-PV is the S component of PVL secreted by *S. aureus*. We previously reported that LukS-PV induces apoptosis in AML cells mediated by C5aR and inhibit AML cell proliferation in vitro and in vivo and have no obvious side effects

on mice[2, 25, 26, 37]. However, the precise mechanisms underlying histone modification regulation by LukS-PV exerts its anti-leukaemia effects in AML cells have remained poorly understood.

AML is complex disease with a diverse genetic landscape. Recent studies have revealed that epigenetic dysregulation plays an important role in leukaemia pathogenesis[6, 11]. Further histone-modifying enzymes reportedly contribute to leukaemia pathogenesis. For example, MLL1 is a member of the SET family of methyltransferases and has been reported affect stem cell differentiation in leukaemia patients[10]. SETD2 is a histone methyltransferase that regulates leukaemia pathogenesis[30, 39]. This phenomenon is precisely based on the reversibility of epigenetic modifications may facilitate targeted leukaemia therapy[12]. For example, azacitidine and decitabine are DNA methyltransferase inhibitors approved for clinically treating AML[8, 15]. EZH2 and LSD1 are reportedly involved in leukaemia pathogenesis, and these targeted drugs have entered clinical trials[14, 22]. In summary, histone modifications are potentially promising for targeted therapy for leukaemia.

SET8 is a member of the SET domain-containing methyltransferase family specifically targeting H4K20me1[18]. SET8 is involved in vital cellular processes, including transcriptional regulation[5, 20], S-phase progression[28], genome replication and stability[4, 16], and DNA repair[36]. Aberrant SET8 expression has been reported in numerous tumours. High SET8 levels are also associated with poor survival in cancer patients. Zhou et al reported that SET8 and H4K20me1 levels were higher in AML cells than in control cells, and that SET8 can regulate the Wnt signalling pathway and is associated with leukaemia pathogenesis[38]. Our observation showed that SET8 is downregulate targeted by LukS-PV revealed a mechanism underlying SET8 regulation, thus underscoring the importance of SET8 regulation in AML.

Previous studies have reported that SET8 is a specific monomethylation catalytic enzyme for H4K20me1[18]. Therefore, we further found that LukS-PV reduced H4K20me1 levels by downregulating SET8 in a dose- and time-dependent manner. To further assess the downstream mechanism of LukS-PV in exerting anti-leukaemia effects through the SET8-H4K20me1 axis, we performed ChIP-seq to identify H4K20me1 downstream target genes. Finally, a potential target gene PIK3CB was identified by classifying target genes, and we tested its effect on apoptosis through PIK3CB inhibitor GSK2636771. Together, our study indicate that LukS-PV induces cell apoptosis through downregulate expression of the target gene PIK3CB mediated by SET8 in AML cells.

The phosphatidylinositol 3-kinase (PI3K) pathway plays pivotal roles in cell growth, proliferation, and survival by integrating extracellular growth signals[3]. PI3K proteins fall into three classes, of which class IA are heterodimeric lipid kinases, consisting of a p85 regulatory subunit and a p110 catalytic subunit. The p110 catalytic subunits are p110 α , β , and δ . PIK3CB (PI3-kinase p110 β) is a member of the PI3K pathway, and hyperactivation of this pathway contributes to cancer progression in humans[9]. Akt, a serine/threonine protein kinase, is one of the best-characterized targets of the PI3K pathway. Recent studies have reported numerous Akt substrates including BAD, CREB, GSK-3 β , mTOR, FOXO1, and PTEN[23]. Initiation of these signalling cascades regulates cellular proliferation, motility, and survival in

cancer cells. The present ChIP-seq experiments and bioinformatics analysis revealed that PIK3CB is the transcriptional target gene for LukS-PV-mediated H4K20me1. Furthermore, our results show that LukS-PV decreases AKT phosphorylation and increases FOXO1 levels, thus inducing apoptosis by downregulating anti-apoptotic protein Bcl2 and upregulating pro-apoptotic protein Bax in HL-60 and NB-4 cells.

In conclusion, these results show that LukS-PV induces apoptosis in AML cells via the PI3K/AKT/FOXO1 signal transduction pathway by targeting SET8, implying that SET8 is a potential target for LukS-PV-mediated treatment of AML.

Abbreviations

AML

Acute myeloid leukaemia; FCM:Flow cytometry; ChIP:Chromatin immunoprecipitation; SET-NC:SET8 negative control; SET8-OE:SET8 overexpress; SET8-SiRNA:SET8 small interfere RNA

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee and Institutional Review Board of University of Science and Technology of China, Anhui, China (Approval number: 2019-N(H)-101). Written informed consent was obtained from individual or guardian participants.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no conflict of Interest.

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Author Contributions

LF.X and L.S designed the study and wrote the paper; LF.X ,PS.D, F.M, Yy.W and Xe.L performed assay; P.Q, Kd.S and Wj.Ch performed the Statistical analysis. Yy.D and XL.M reviewed the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

LukS-PV induces apoptosis via the PIK3CB/PI3K/AKT/FOXO1 signal pathway by targeting SET8 (A) Levels of PIK3CB, FOXO1, AKT, apoptosis-associated (Bax/Bcl2) proteins, and AKT phosphorylation in HL-60 and NB4 cells were assessed through western blotting. (B) Expression levels of SET8, H4K20me1, PIK3CB, FOXO1, AKT, apoptosis-associated (Bax/Bcl2), proteins, and AKT phosphorylation in primary AML blasts were assessed through western blotting. (C) Proposed mechanism of action of LukS-PV in

acute myeloid leukaemia cells. (SET8-OE: SET8 over expression; SET8-NC: SET8 negative control; SET8-SiRNA: SET8 small interfere RNA;* : p<0.05; ** : p<0.01; *** : P<0.001).

Figure 2

PIK3CB is the target gene for cell apoptosis mediated by SET8 /H4K20me1 (A) GO analysis of downregulated peaks related genes binding by LukS-PV-mediated H4K20me1 through chromatin immunoprecipitation and sequencing (ChIP-seq) analysis. (B) Functional groups in downregulated peaks

related genes binding by LukS-PV-mediated H4K20me1. (C) The binding of LukS-PV and PBS on target gene PIK3CB. (D) The binding of H4K20me1 at the PIK3CB promoter was significantly reduced upon LukS-PV treatment via quantitative ChIP (qChIP) analysis. Data are presented as fold-change relative to the control with PBS as a negative control. (E, F) HL-60 and NB-4 cells were treated with LukS-PV at different concentrations for 24 h, and PIK3CB gene and protein expression levels were assessed via quantitative real-time PCR and western blotting. (G, H) Apoptosis was inhibited upon SET8 overexpression but induced upon treatment of PIK3CB inhibitor GSK2636771 in HL-60 (G) and NB4 (H) cells. (* : $p < 0.05$; ** : $p < 0.01$; *** : $P < 0.001$).

Figure 3

LukS-PV induces apoptosis in AML cells by downregulating SET8/H4K20me1 (A)–(B) Relative expression of SET8 and H4K20me1 in cells transduced with a lentiviral vector (HL-60 and NB-4) determined through quantitative real-time PCR and western blotting. (C)–(D) Flow cytometric analysis of Annexin V-PE/7-AAD staining shows that the knockdown of SET8 induced apoptosis in HL-60 and NB4 cells. (E)–(F) SET8 knockdown induced apoptosis and SET8 overexpression inhibited apoptosis in HL-60

(E) and NB4 (F) cells treated with LukS-PV. (G) Expression of SET8 and apoptosis-associated proteins in HL-60 and NB-4 cells was assessed through western blotting. (SET8-OE: SET8 over expression; SET8-NC: SET8 negative control; SET8-SiRNA: SET8 small interfere RNA;* : p<0.05; ** : p<0.01; *** : P<0.001).

Figure 4

LukS-PV can downregulate SET8 and H4K20me1 (A) HL-60 and (B)NB4 cells were treated with LukS-PV at different concentrations, and SET8 mRNA and protein expression levels were determined through

quantitative real-time PCR and western blotting. (C) HL-60 and (D) NB4 cells were treated with 2.0 μ M LukS-PV at different timepoints, and SET8 mRNA and protein expression levels were determined through quantitative real-time PCR and western blotting. (E) HL-60 and NB-4 cells were treated with LukS-PV at different concentrations for 24 h, and H4K20me1 expression was assessed through western blotting. (F) HL-60 and NB-4 cells were treated with 2.0 μ M LukS-PV at different timepoints, and H4K20me1 expression was assessed through western blotting. (* : $p<0.05$; ** : $p<0.01$; *** : $P<0.001$).

Figure 5

SET8 is upregulated in AML cells (A) –(B) TCGA and GTEx database analyzed expression of SET8 between AML patient and normal people. (C) SET8 mRNA expression in AML patients. (D) SET8 protein expression in AML patients. (E) Expression of SET8 mRNA in types of AML.

Figure 6

LukS-PV induce cell apoptosis in vitro and inhibit cell invasion in vivo (A) Flow cytometric analysis shows that treatment of LukS-PV induces apoptosis in a dose- dependent manner in primary AML blasts. (B)

The Spleen volume (left) and Spleen index((spleen weight/body weight)*% , right) of mice. (C) The percentage of CD33+ cell in peripheral blood. (D) The percentage of CD33+ cell in spleen.

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