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Decursin induces FLT3-ITD acute myeloid leukemia cell apoptosis by increasing the expression of the ubiquitin-conjugase UBE2L6

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Abstract

Mutation in the internal tandem duplication sequence of the FLT3 gene (FLT3-ITD) is linked to a poor clinical prognosis in acute myeloid leukemia (AML) patients. FLT3 inhibitors have demonstrated efficacy in improving the prognosis of AML patients with FLT3-ITD. However, the efficacy of FLT3 inhibitors is short-lived, and is often limited by secondary drug resistance when used alone. Recent investigations have provided an innovative approach for treating FLT3-ITD AML by targeting FLT3 protein degradation. Our study revealed that decursin selectively impaired the viability of FLT3-ITD-positive AML cells. Subsequent analysis revealed that decursin preferentially induced cell cycle arrest and apoptosis in FLT3-ITD-positive AML cells through proteasome-mediated FLT3-ITD degradation. Further research revealed that decursin significantly increased the expression of UBE2L6, an e2-conjugating enzyme that degrades FLT3-ITD. Downregulation of UBE2L6 by small hairpin RNA (shRNA) reduced decursin-induced FLT3-ITD-linked apoptosis and degradation. The anti-FLT3-ITD AML effect of decursin was also validated in cell lines and patient-derived mouse models. Moreover, decursin synergistically enhanced venetoclax-induced apoptosis.

Keywords AML, Decursin, Degradation, FLT3-ITD, UBE2L6

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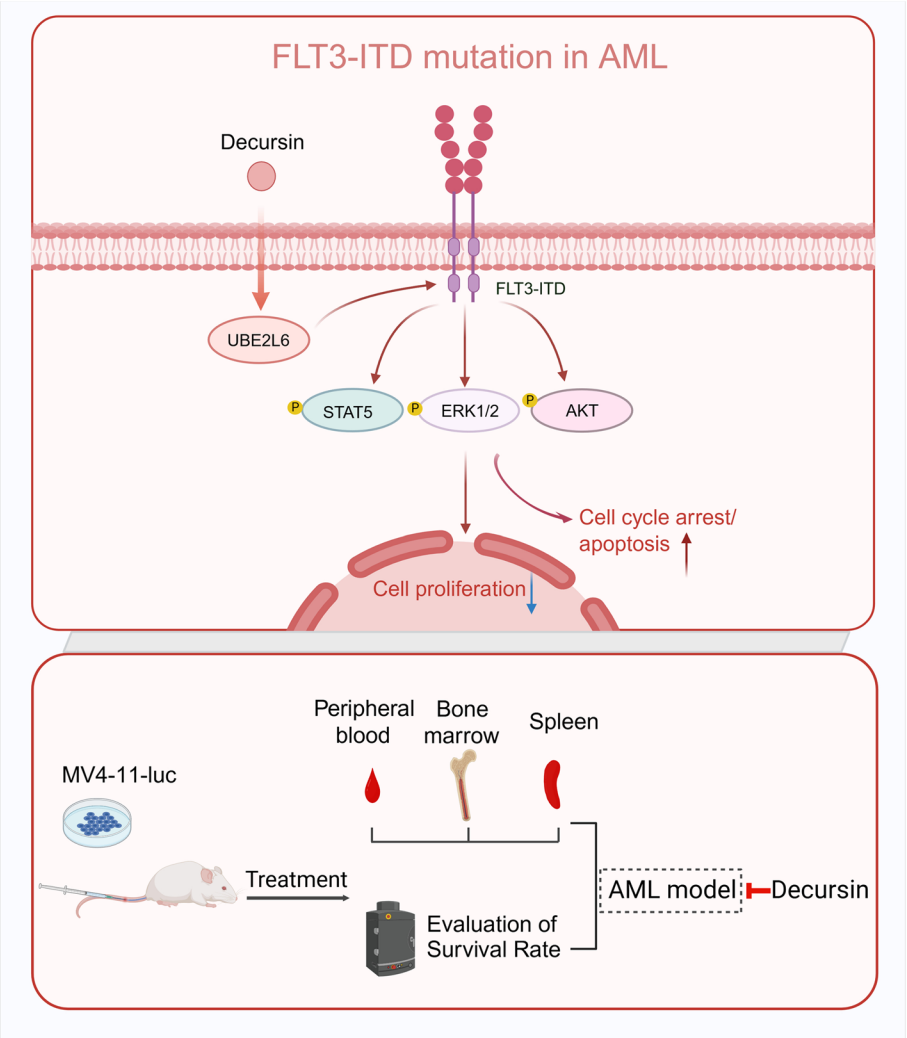
Graphical Abstract



Introduction

Fms-related receptor tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase that is expressed mainly on hematopoietic stem cells (HSCs) and is essential for cell proliferation and differentiation [1, 2]. FLT3 activation mutations are mainly divided into juxtamembrane internal tandem duplication (ITD) mutations and tyrosine kinase domain point (TKD) mutations [3], of which FLT3-ITD is the most predominant form of mutation, with a prevalence of approximately 25% in AML patients [4–6]. FLT3-activating mutations affect multiple downstream signaling molecules, including STAT5, PI3K/AKT, and MAPK/ERK, thereby promoting the uncontrolled proliferation and survival of AML cells [7–9]. AML patients carrying FLT3-ITD have a higher recurrence rate and worse clinical prognosis than patients without mutations [4, 7, 10, 11]. As effective therapeutic targets for FLT3-ITD-positive AML, various FLT3-targeted inhibitors, which are mainly divided into first- and second-generation inhibitors, have been researched and developed [12, 13]. For example, midostaurin and sorafenib, as first-generation FLT3 inhibitors, nonselectively target FLT3 and can effectively inhibit many kinases, including BCR-ABL, RET, and C-KIT [13], causing off-target effects and adverse reactions [14, 15]. Quizartinib, gilteritinib, and crenolanib are classified as second-generation FLT3 inhibitors and exhibit higher specificity for FLT3 than first-generation

drugs do [16]. However, when used as monotherapy, their antileukemic activity is limited and transient, and they are limited by drug resistance caused by secondary mutations or signal compensation [16, 17].

In addition to direct FLT3 kinase inhibition, multiple studies have reported an emerging strategy to target the synthesis and degradation of the FLT3 protein. For example, studies have shown that FLT3 circMYBL2 [18] and miR-29b [19] at the transcriptional and translational levels can directly interfere with the expression level of the FLT3 protein. For protein turnover, there are currently two known main ways to degrade FLT3: the proteasome system and the autophagy lysosomal pathway. USP10 is known to be a deubiquitinase, and inhibiting USP10 activity can significantly increase the proteasomal degradation of FLT3-ITD [20]. UBE2L6, an E2 ubiquitin-conjugating enzyme, also known as UBCH8, binds to the E3 ubiquitin ligase SIAH1, ubiquitinylates FLT3-ITD and induces its destabilization while minimally affecting wild-type FLT3 [21]. Additionally, WP1130, a USP9X inhibitor, has been reported to degrade FLT3-ITD [22]. Bortezomib exerts its antitumor effect by degrading FLT3-ITD through the autophagy–lysosome pathway [23]. Since FLT3-ITD constitutively activates downstream pathways, in addition to reducing FLT3 activity, the strategy of inducing the degradation of the FLT3-ITD protein may also be able to overcome the resistance mechanism caused by kinase domain mutations, unlike FLT3 kinase inhibitors. Therefore, targeting total FLT3 protein degradation is a promising strategy for FLT3-ITD AML patients.

Angelica gigas Nakai root has been used as a supplement to prevent and treat anemia [24]. Decursin, a pyranocoumarin natural product extracted from *Angelica gigas* Nakai root [25], has previously been shown to have multiple therapeutic effects, including anticancer, antiangiogenic, anti-inflammatory, and antioxidant activities [26–29]. Notably, decursin not only has powerful anticancer properties in solid tumors, such as bladder cancer, colorectal cancer, and prostate cancer but also in nonsolid tumors [28, 30–32]. Previous studies have also shown its therapeutic potential in nonsolid tumors. For example, in KBM-5 chronic myeloid leukemia cells, higher concentrations of decursin can downregulate cyclooxygenase-2 (COX-2) and the survival factor-activated apoptosis cascade [33]. Furthermore, decursin can induce apoptosis in multiple myeloma cells by suppressing the STAT3 pathway [34]. However, the potential molecular mechanism of decursin in AML, particularly in patients carrying the FLT3-ITD mutation, is still unclear.

In this study, decursin preferentially triggered apoptosis in FLT3-ITD AML cells and alleviated the leukemia burden in vivo. Mechanistically, decursin degrades FLT3-ITD via the proteasome pathway by increasing UBE2L6 expression, ultimately inhibiting the activation of the FLT3-mediated aberrant pathway. Moreover, we confirmed that the combination of decursin and venetoclax has a synergistic effect in FLT3-ITD-positive cells.

Materials and methods

Reagents and antibodies

Purified decursin (Chroma, Chengdu, China), venetoclax (MedChemExpress, USA), cycloheximide (CHX), MG132 (MedChemExpress, USA), and chloroquine (CQ) (MedChemExpress, USA) were solubilized in dimethyl sulfoxide (DMSO, Oriscience, Chengdu, China). Polyethylene glycol 300 (PEG300) and Tween 80 were obtained from TargetMol (USA). Saline was purchased from Kelun (Shenzhen, China).

Anti-p-STAT5 (13386), anti-p-FLT3 (13858), anti-cyclin E1 (40804), anti-p-AKT (11054), and anti-p-ERK (12082) antibodies were obtained from SAB (Nanjing, China). Antibodies against P–C-MYC (T58) (ab185655), cyclin D1 (ab134175), P–C-MYC (S62) (ab185656), FLT3 (ab245116), caspase 3 (ab32351), C-MYC (ab32072), CDK6 (ab124821), and PIM1 (ab54503) were obtained from Abcam (Cambridge, MA, USA). Antibodies against GAPDH (81640–1-RR), cyclin E2 (11935–1-AP), Lamin B1 (66095–1-Ig), PARP1 (13371–1-AP), β-actin (81115–1-RR), STAT5 (80138–2-RR), MCL-1 (16225–1-AP), AKT (10176–2-AP), ERK1/2 (83533–1-RR), UBE2L6 (17278–1-AP), SIAH1 (83389–4-RR), and CDK4 (11026–1-AP), as well as HRP-conjugated IgG antibodies (SA00001), were from ProteinTech (Wuhan, China).

Cell culture

MV4-11 and MOLM-13 carry the homozygous and heterozygous FLT3-ITD mutations, respectively [35, 36]. The FLT3-ITD-negative cell lines included HL-60 and THP-1. A retrovirus was used to construct Ba/F3 FLT3-ITD and Ba/F3 FLT3-D835Y as described previously [37]. The above cells were maintained in RPMI-1640 medium (Gibco, USA). The parental Ba/F3 cells were maintained under the above culture conditions with 6 ng/mL IL-3 (SinoBiological, Beijing, China). All cell culture media contained 10% fetal bovine serum (FBS, PAN, Germany) and 1% penicillin–streptomycin (Biochannel, Nanjing, China). Cells were cultivated at 37 °C in a humidified incubator with 5% CO2.

Primary cell samples

The study was approved by the Ethics Committee of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (2023KZL-005), in line with the Declaration of Helsinki, and informed consent was obtained from all patients and healthy donors.

Mononuclear cells from bone marrow or peripheral blood were extracted with Ficoll isolation solution (Solarbio, Beijing, China). The patients' clinical characteristics are shown in Supplementary Table S1.

Cell viability assay

AML cells were incubated in 96-well plates and subjected to treatment with decursin and venetoclax. Following a specific period of treatment, add CCK8 reagent (Oriscience, Chengdu, China) to each well. After incubation, the absorbance was assessed at a wavelength of 450 nm utilizing an enzyme labeling instrument (Biobase, Jinan, China).

Transfection

Lentiviruses expressing short hairpin RNA (shRNA) against the UBE2L6 gene were synthesized using the following target sequences: shUBE2L6-1, 5′-CTGTTCAGAAAGAATGCCGAA-3′; shUBE2L6-2, 5′-GCTGGTGAATAGACCGAATAT-3′; shUBE2L6-3, 5′-GATCAAATTCACAACCAAGAT-3′; negative control sequence: sh-NC, 5′-TTCTCCGAACGTGTCACGT-3′. The above sequences were subsequently cloned and inserted into the GV493 (pFU-GW-016) vector (Genechem, Shanghai, China), which has a BsmBI site, and the recombinant vector was detected by DNA sequencing. Lentiviruses were produced, and the cells were infected as described previously [38].

Cell cycle and apoptosis detection

AML cells were incubated in six-well plates with decursin for 24 h. Cell cycle and apoptosis kits (Beyotime, Shanghai, China) were utilized for flow detection, as described previously [38].

Colony formation assay

A sterile solution of 3% agarose (Sangon, Shanghai, China) was prepared. It was diluted to 0.3% and 0.6% in RPMI-1640 medium and placed in the upper and lower layers of a six-well plate, respectively. AML cells (1×10^3) were resuspended in the upper layer with decursin or medium. The medium was added every 2 days and maintained for 10 days until leukemia cell spheres formed. Finally, the cells were stained with nitroblue tetrazolium (NBT; Aladdin, Shanghai, China) and photographed.

Proteomic analysis

MV4-11 cells were lysed with an appropriate amount of cell lysis buffer after treatment with decursin. After being fully lysed by ultrasonication on ice the protein lysate was subsequently digested with trypsin (Promega, USA), CaCl2 (Sigma, USA), and TEAB (Aladdin, Shanghai, China) separately. The supernatant was obtained after centrifugation and passed through a C18 desalting column (Waters, USA). The filtrate was then freeze-dried. Finally, the sample was resuspended in TEAB buffer and labeled with TMT reagents (Thermo, USA). The sample was then desalted and freeze-dried again. LC–MS/MS was used for protein quantification of the prepared samples. Proteome Discoverer (Thermo, USA) was used to process the data, and the protein quantification results were statistically analyzed via a t test.

RT–qPCR

An RNA purification kit (Promega, USA) was used to isolate RNA. Reverse transcription was then performed using cDNA synthesis kit (CWBIO, Taizhou, China). The reaction was detected on a Gene 9660 sequence detection system (Bioer, Hangzhou, China) using superfast SYBR mix (CWBIO, Taizhou, China). The primers used were as follows: GAPDH, forward-TGGTCACCAGGGCTGCTTTTA, reverse-TTCCCGTTCTCAGCCTTGACG; FLT3, forward-TCTGCTTCCATCACACTGCAA, reverse-ATGGGTGGAGGGGCAGCCTT; USP10, forward-GAGGGCACAGCTACCAACG, reverse-AGGGGAGATATGGCGGGAG; USP9X, forward-TCGGAGGGAATGACAACCAG, reverse-GGAGTTGCCGGGGAATTTTCA; UBE2L6, forward-GGACGAGAACGGACAGATTT, reverse-GGCTCCCTGATATTCGGTCTATT; and SIAH1, forward-AGCCGTCAGACTGCTACAG, reverse-AAAAGACTCGCCAAGTCATTGT.

Western blot

The immunoblotting protocols were performed in accordance with previously described methods [38]. AML cells treated with decursin and/or venetoclax were lysed with RIPA buffer (Solarbio, Beijing, China). After quantification via a Bradford Assay Kit (Oriscience, Chengdu, China), the samples were boiled in loading buffer (CWBIO, Taizhou, China) and subsequently subjected to SDS–PAGE. And the expression levels of different proteins were detected with specific antibodies Finally, the bands of the target proteins were detected with an electrochemiluminescence (ECL) kit (Oriscience, Chengdu, China) using a GelView 6000Pro SE Chemiluminescence Imaging System (Bltlux, Guangzhou, China).

Mouse xenograft model and in vivo imaging assays

The Ethics Committee of Chengdu University of Traditional Chinese Medicine approved the protocols for animal research (No. 2024120). MV4-11-GFP/Luc stable cell lines were constructed by infecting MV4-11 with lentivirus (Fenghbio, Changsha, China). Subsequently, 6–8-week-old female M-NSG mice (Model Organisms, Shanghai, China) weighing 18–22 g were injected with 2×10^6 cells to generate the AML xenograft model. After injection with D-luciferin-K (Fluorescence, Beijing,

China), the mice were anesthetized with isoflurane gas to monitor the growth of leukemia weekly via the IVIS Lumina in vivo imaging system (PerkinElmer, USA). Ten days after transplantation, the mice received intraperitoneal injections of 30 mg/kg decursin or vehicle control (2% DMSO, 40% PEG300, 5% Tween-80, and 53% saline) daily for 28 days ($n=8$). During this period, to assess the burden of leukemia, 3 mice in each group were sacrificed following 14 days of treatment with decursin or vehicle. Five additional mice were utilized for survival analysis.

NSG mice were tail-vein injected with 2×10^6 mononuclear cells from FLT3-ITD patients to generate patient-derived xenograft (PDX) models. Model construction was confirmed by the percentage of CD45-positive cells. The mice received an intraperitoneal injection of 30 mg/kg decursin or vehicle control daily. After 28 days, 3 mice in each group were sacrificed, and the percentage of CD45-positive cells was detected. Five additional mice were utilized for survival analysis.

Statistical analysis

All experimental data were analyzed using GraphPad Prism 9 and are presented as the means ± SDs. The significance of differences between groups was assessed using Student's t test, and a p value less than 0.05 was considered statistically significant ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

Results

Decursin preferentially inhibits the proliferation of FLT3-ITD-positive AML cells

AML cell lines were treated with decursin to evaluate its inhibitory effect (Fig. 1A). Interestingly, decursin preferentially impaired the viability of FLT3-ITD AML cells (MV4-11 and MOLM-13) (Fig. 1B), with IC50 values of 26.81 μM and 33.66 μM, respectively (Fig. 1D). Nevertheless, it had almost no effect on the viability of FLT3-ITD-negative cells (HL-60 and THP-1) (Fig. 1C). On the basis of this observation, we selected FLT3-ITD-negative Ba/F3 cells and obtained stable FLT3-positive mutant-transfected strains by transducing Ba/F3 cells with FLT3-ITD or FLT3-D835Y. Our findings indicated that Ba/F3 FLT3-ITD and Ba/F3 FLT3-D835Y cells, when deprived of IL-3, exhibited heightened sensitivity to decursin compared with the parental Ba/F3 cells, which were maintained in IL-3 (Fig. 1E, Fig. S1). Moreover, the decursin-induced

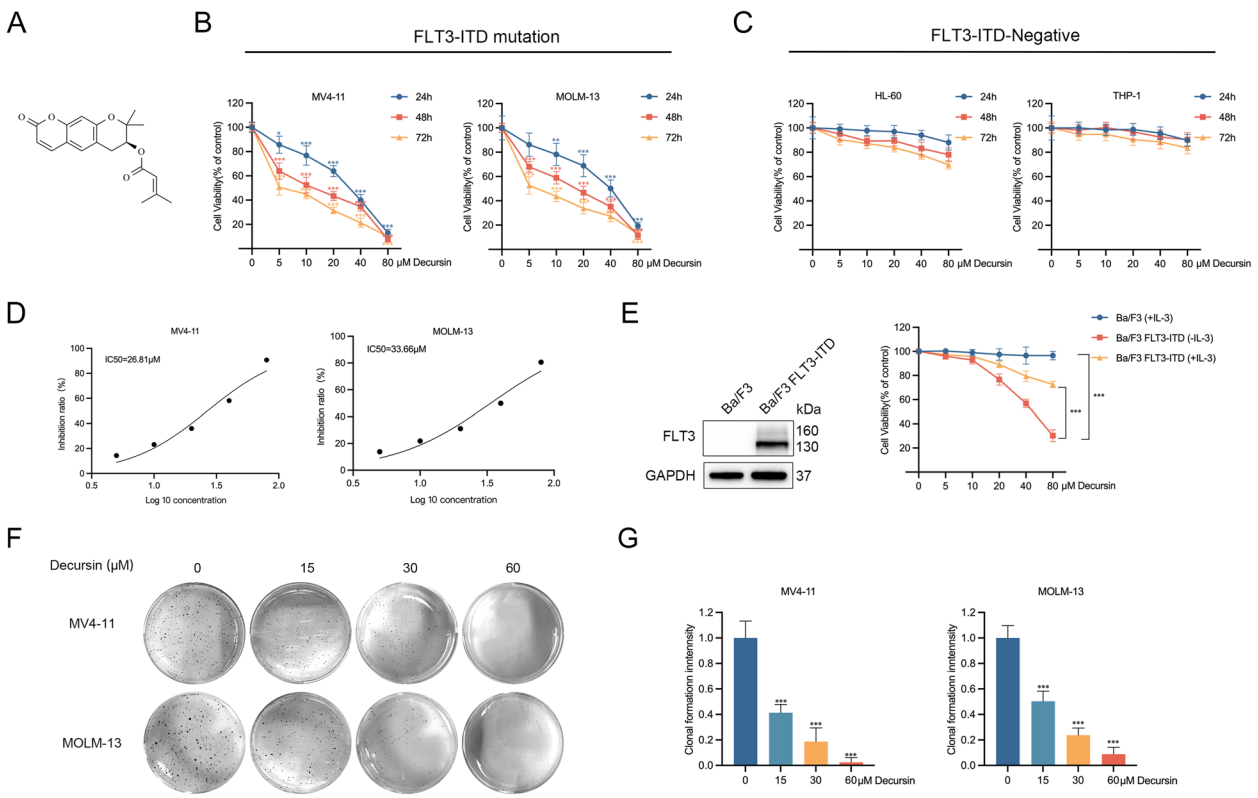


Fig. 1 Decursin preferentially inhibits the proliferation of FLT3-ITD AML cells. **A** The structural formula of decursin. **B**–**E** Assessment of viability of AML cells after treatment with decursin. **F**, **G** Colony formation rate of MV4-11 and MOLM-13 after treatment with decursin. ($n=3$, $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$)

decrease in cell viability was partially prevented by exposing Ba/F3 FLT3-ITD cells to IL-3 (Fig. 1E). These results suggest that the cytotoxic effect of decursin is associated with loss of FLT3 function. In addition, at the same concentration, decursin had a stronger toxic effect on Ba/F3 FLT3-ITD cells than on Ba/F3 D835Y cells, and given lacking the FLT3-D835Y AML cell lines, the subsequent studies focused on the FLT3-ITD mutation.

To further determine the effect of decursin on the proliferation ability of FLT3-ITD AML cells, a smaller number of MV4-11 and MOLM-13 cells were resuspended in agarose gel and exposed to decursin in vitro for several days for additional observation. Decursin suppressed the clonogenic ability of FLT3-ITD AML cells (Fig. 1F and G). These results indicate that decursin has preferential cytotoxicity to FLT3-ITD AML cells.

Decursin induces cell cycle arrest in FLT3-ITD AML cells

Next, we analyzed the intracellular DNA content to investigate the impact of decursin on the cell cycle. Decursin could markedly induce cell cycle arrest at the G0/G1 phase but minimally affected FLT3-negative cells (Fig. 2A). After 24 h of exposure to decursin, a reduction in cyclin E2, cyclin E1, cyclin D1, and cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), together with an increase in P21, was observed in FLT3-ITD AML cells (Fig. 2B and C), further indicating the induction of cell cycle arrest. Consistent with the flow cytometry results, no such effect was observed in the FLT3-ITD-negative cell line HL-60 (Fig. 2D).

Decursin promotes apoptosis in FLT3-ITD AML cells

We tested the apoptotic effect of decursin in AML cells after treatment with decursin. In MV4-11 and MOLM-13 cells, decursin dose-dependently induced apoptosis (Fig. 3A). However, for FLT3-ITD-negative cells, high concentrations of decursin only slightly induced apoptosis in HL-60 and not in THP-1 (Fig. 3A), which was consistent with the observed cell viability. We subsequently evaluated the levels of apoptotic markers. Decursin reduced the expression of caspase3 and PARP1 and increased the cleaved forms of caspase3 and PARP1 in MV4-11 and MOLM-13 (Fig. 3B and C). There was almost no effect on HL-60 cells (Fig. 3D).

Decursin decreases the FLT3-ITD protein level

To explore the mechanism of action of decursin, proteomic analysis was used to compare protein level alterations in MV4-11 before and after decursin treatment. We used an average fold change (FC) > 1.2 as a criterion to identify differentially expressed proteins. Compared with the control, decursin treatment significantly increased 122 different proteins and decreased 95 different proteins (Fig. 4A and B). Following the preferential cytotoxicity of decursin to FLT3-ITD-positive AML cells, subsequent studies indicated a notable decrease in FLT3 levels (Fig. 4C). To validate the proteomics results, we assessed the expression of total and phosphorylated FLT3, STAT5, AKT, and ERK following treatment with decursin. The results demonstrated that decursin decreased FLT3 and P-FLT3 levels while also reducing the phosphorylation of downstream FLT3 proteins, specifically P-STAT5, P-ERK, and P-AKT, along with downstream targets, including C-MYC and PIM1 (Fig. 4E and F). Meanwhile, decursin also decreased the expression of P–C-MYC (S62) (Fig. S2). Nonetheless, for FLT3-ITD-negative HL-60 cells, the influence of varying concentrations of decursin on the FLT3 pathway were inconsistent (Fig. 4G). Therefore, the proliferation suppression of FLT3-ITD-positive cells by decursin is linked to a reduction in FLT3-ITD level and its downstream pathway inhibition.

Decursin degrades FLT3-ITD via the proteasome pathway

We next analyzed the FLT3 mRNA level via RT–qPCR to explore the underlying mechanism of the decursin-induced decrease in FLT3-ITD. However, decursin failed to downregulate the FLT3 mRNA level (Fig. 5A and B), which suggests that decursin may regulate FLT3-ITD expression at the posttranscriptional level. We subsequently investigated the mechanism underlying the effects of decursin on FLT3-ITD. Interestingly, MG132, but not CQ, suppressed the induction of FLT3-ITD degradation by decursin (Fig. 5C and D), indicating that this process was mediated via the proteasome pathway. To further confirm this result, we examined the effect of decursin on FLT3-ITD protein turnover after treating MV4-11 and MOLM-13 with CHX in the presence or absence of MG132. Compared with the control, decursin treatment significantly accelerated FLT3-ITD protein turnover, indicating that decursin reduced FLT3-ITD stability (Fig. 5E and F). MG132 reduced decursin-induced FLT3-ITD turnover (Fig. 5E and F), suggesting that decursin is degraded through the proteasome pathway to accelerate FLT3-ITD protein turnover. We then investigated the mechanism by which decursin degrades FLT3-ITD. Gene set enrichment analysis (GSEA) revealed that decursin significantly upregulated the ubiquitin–proteasome pathway (NES = 1.67, $P < 0.01$) (Fig. 4D).

Decursin-induced apoptosis occurs due to an increase in UBE2L6

Unfortunately, in proteomics, the signals of some low-abundance proteins are not easily captured by mass spectrometers. To identify the upstream factor that causes the FLT3-ITD degradation, we examined the mRNA levels of USP10, USP9X, UBE2L6, and SIAH1

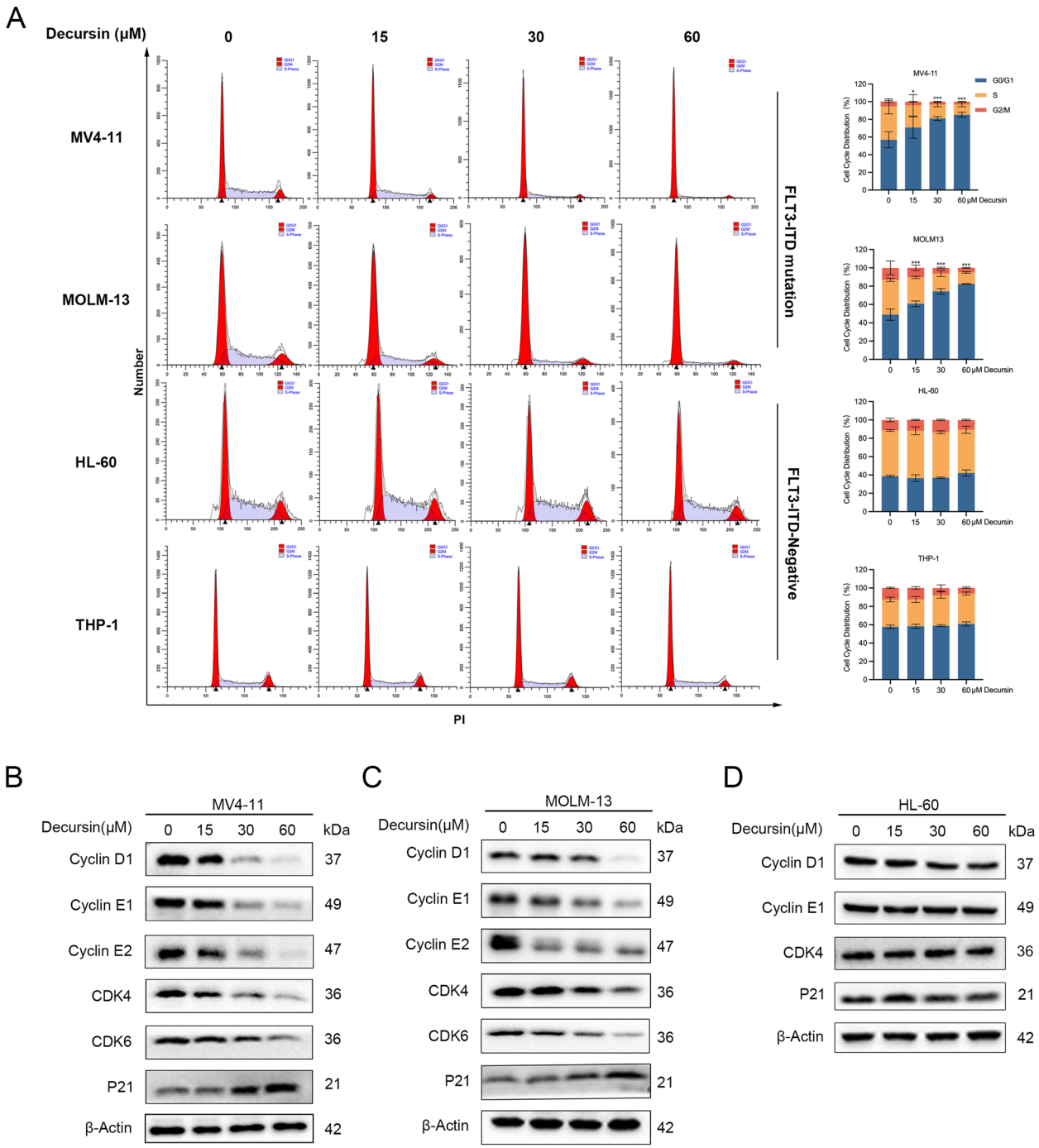


Fig. 2 Decursin induces cell cycle arrest in FLT3-ITD AML cells. **A** Cell cycle distribution of AML cells after treatment with decursin. **B**-**D** Following treatment with decursin, the expression of cyclin E2, cyclin E1, cyclin D1, CDK4, CDK6, and P21 was tested. ($n = 3$, $^{*}p < 0.05$, $^{***}p < 0.001$)

after treatment with decursin. Notably, decursin treatment increased UBE2L6 mRNA levels by approximately 6–eightfold (Fig. 6A). This finding was also substantiated at the protein level (Fig. 6B). Since the E3 ligase SIAH1 also plays a critical role in degrading FLT3-ITD [21], we subsequently assessed whether decursin regulates the expression of SIAH1. Interestingly, decursin induced only a modest upregulation of SIAH1 expression (Fig. 6A and B). AML patient data from the TCGA database were analyzed, revealing that individuals

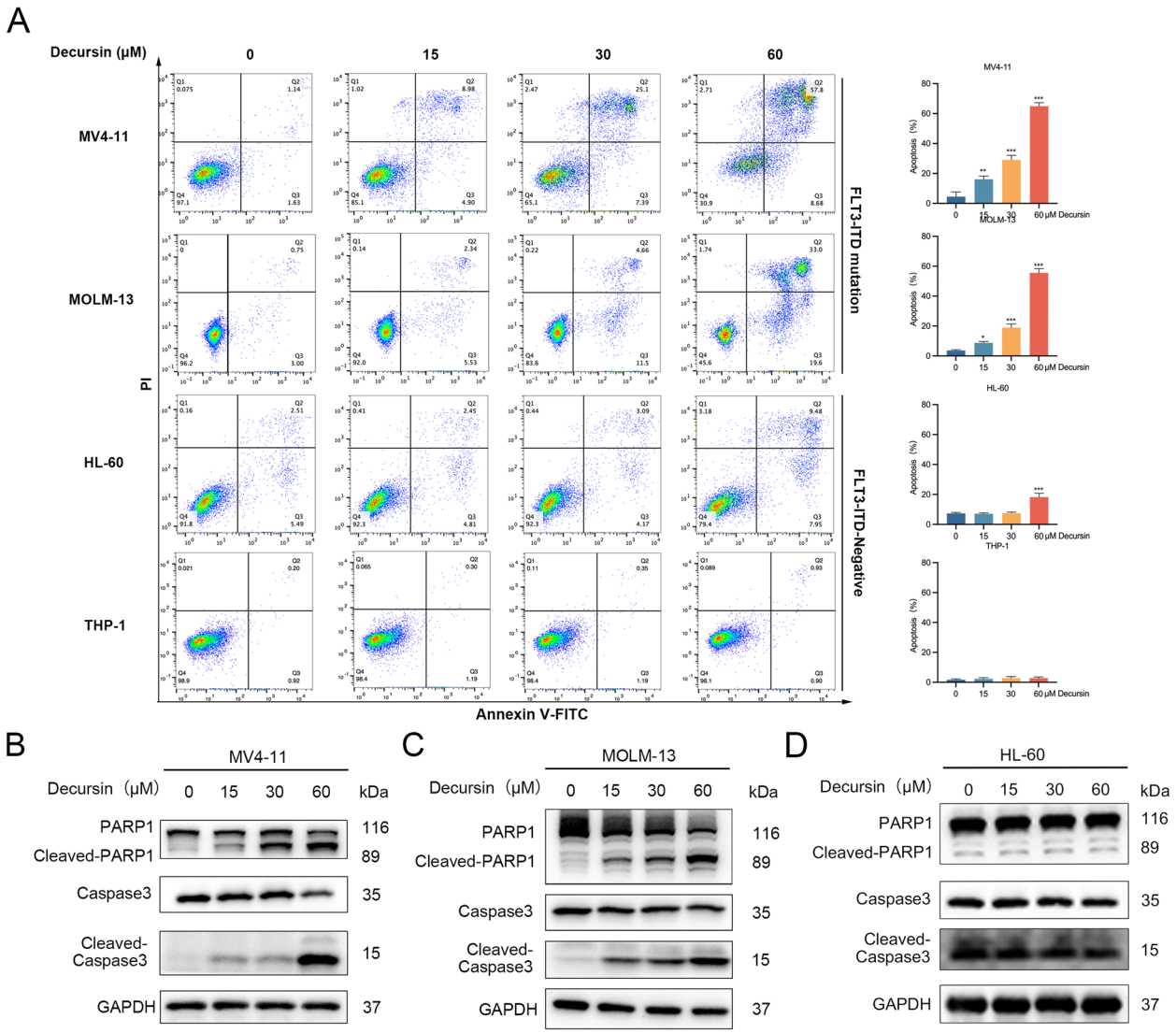


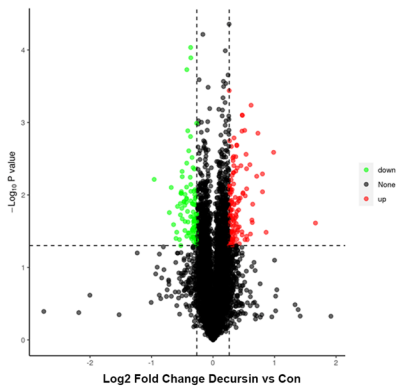
Fig. 3 Decursin promotes apoptosis in FLT3-ITD AML cells. **A** The percentage of apoptotic AML cells treated with decursin. **B**-**D** After AML cells were exposed to decursin, the expression of PARP1, Cleaved-PARP1, Caspase3, and Cleaved-Caspase3 was detected. ($n = 3$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

with the FLT3-ITD mutation presented lower UBE2L6 expression (Fig. 6C). This finding aligned with the results obtained in the cell lines (Fig. 6D). We subsequently knocked down UBE2L6 using shRNA to evaluate its role in decursin-induced growth inhibition and apoptosis (Fig. 6E). MV4-11 and MOLM-13 in which UBE2L6 was stably knocked down were subsequently treated with decursin. As expected, UBE2L6 knockdown effectively prevented decursin-induced proliferation inhibition and apoptosis (Fig. 6F-I). In addition, decursin-induced FLT3-ITD degradation and downstream pathway inhibition were partially relieved by UBE2L6 knockdown (Fig. 6J and K).

Decursin is effective against AML in vivo

NSG mice were injected with MV4-11 expressing green fluorescent protein (GFP) and luciferase (Luc) genes (Fig. 7A). Ten days after transplantation, the mice were treated daily with decursin (Fig. 7B). Decursin suppressed AML progression in mice (Fig. 7C). All the mice in the vehicle control group died 37 days after transplantation, whereas those in the decursin group died 43 days after transplantation. Compared with that

A



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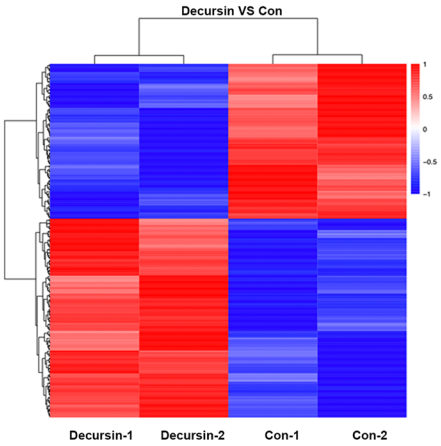

C

Genecard	Fold Change (Decursin/Con)
CDT1	0.61
ORMDL2	0.62
RAP1B	0.64
CHCHD8	0.66
TRIAP1	0.66
RCL1	0.67
COA5	0.68
TLN2	0.69
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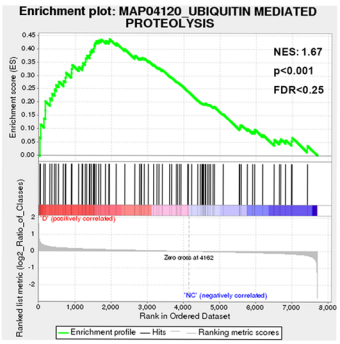
FLT3	0.79

D



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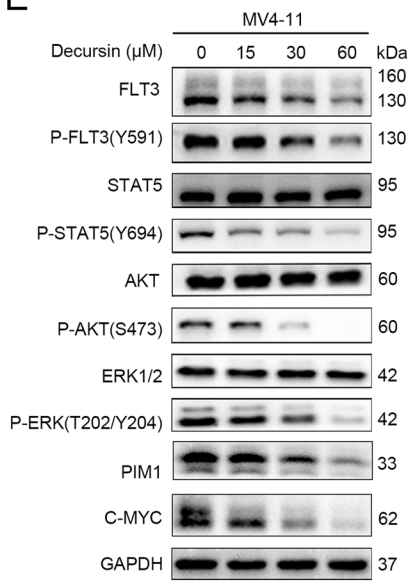

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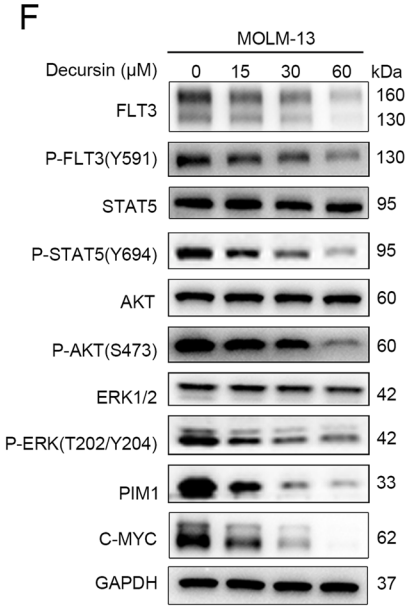

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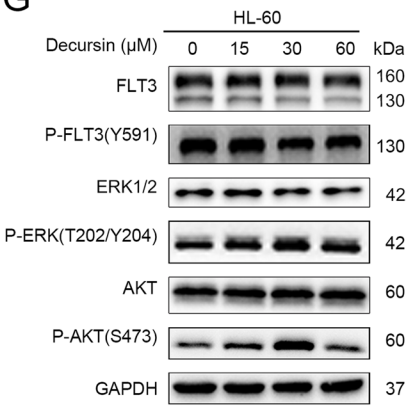

Fig. 4 Decursin decreases the FLT3-ITD protein level. **A** Volcano plots were analyzed for the bases with more than 1.2-fold upregulation (red plot) and downregulation (green plot) of decursin. **B** Heatmap of differential proteins between treatment group and control group. **C** Significantly increased proteins following decursin treatment. **D** GSEA of proteomic data from MV4-11 cells treated with decursin before and after delivery. The figure shows the GSEA enrichment plot of the relevant proteins in the ubiquitin–proteasome pathway after decursin treatment ($n=2$). **E**–**G** Expression of C-MYC, UBE2L6, and PIM1, as well as total and phosphorylated FLT3, STAT5, AKT, and ERK was assessed. ($n=3$)

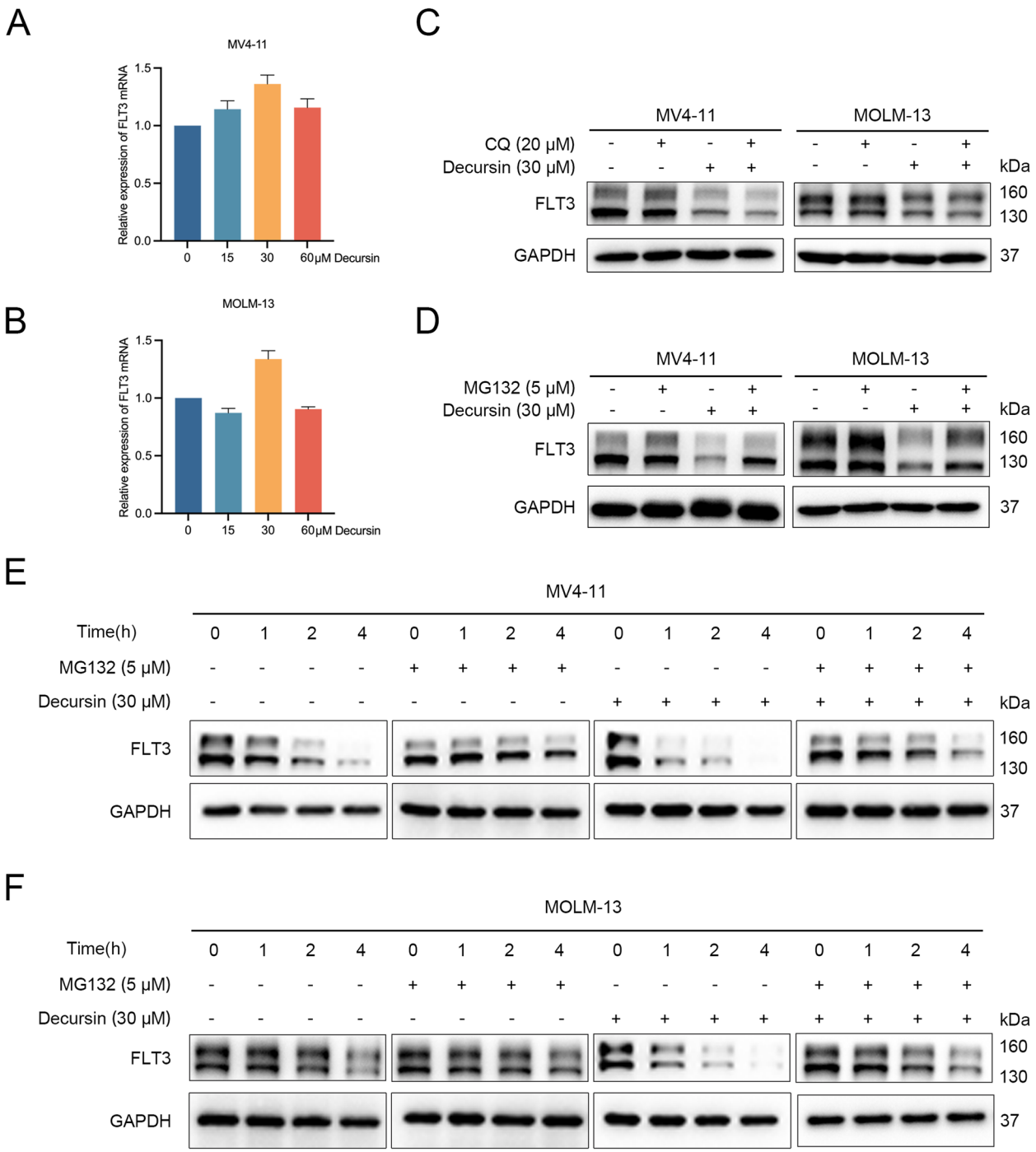

Fig. 5 Decursin degrades FLT3-ITD via the proteasome pathway. **A**, **B** Following treatment with decursin, the mRNA levels of FLT3 were assessed. **C** Expression of FLT3 was assessed after treatment with decursin and CQ. **D** Expression of FLT3 was assessed after treatment with decursin and MG132. **E**, **F** Expression of FLT3 was measured at specific times after treatment with cycloheximide (CHX 100 μg/ml) with or without MG132

of vehicle-treated mice, the survival time of decursin-treated mice was markedly extended ($P=0.0374$) (Fig. 7D). To assess the leukemia burden, following a 14-day treatment with either decursin or vehicle, three mice from each group were sacrificed. The positive rates of GFP and CD45 expression in peripheral blood, spleen, and bone marrow cells were analyzed. Concurrently, we also observed the bone marrow under

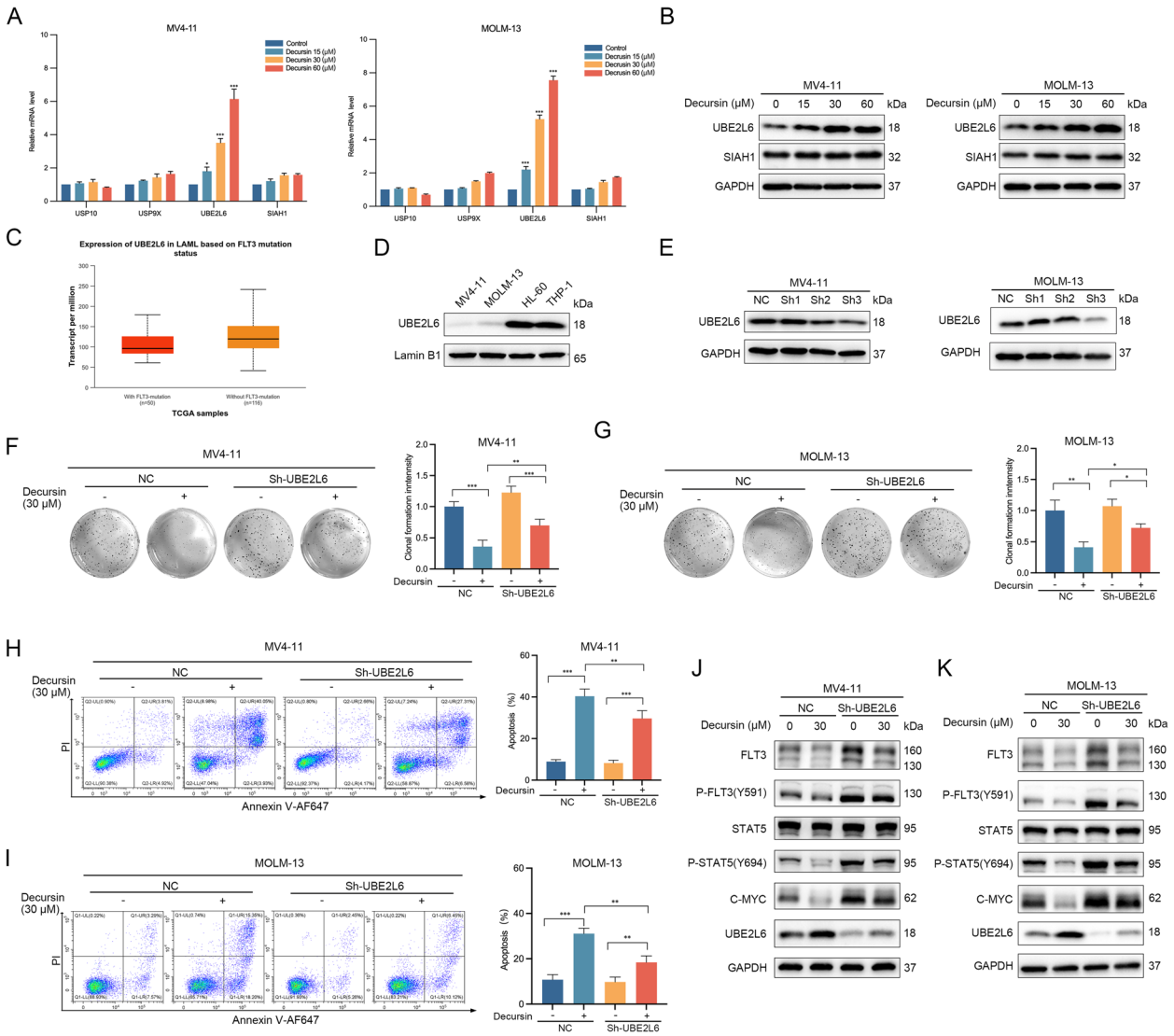

Fig. 6 Decursin-induced apoptosis occurs due to an increase in UBE2L6. **A** mRNA levels of USP10, USP9X, UBE2L6, and SIAH1 relative to those of GAPDH. **B** After treatment with decursin, the levels of UBE2L6 and SIAH1 were evaluated using Western blotting. **C** The levels of UBE2L6 in FLT3-positive ($n=50$) and FLT3-negative ($n=116$) AML patients from the TCGA database were analyzed using UALCAN (https://ualcan.path.uab.edu/index.html). LAML: TCGA project code for acute myeloid leukemia (AML). **D** Expression of UBE2L6 was tested. **E** MV4-11 and MOLM-13 were transfected with a lentiviral vector carrying a negative control or containing three small hairpin RNAs (shRNAs) for UBE2L6. **F**-**I** Colony formation rates and apoptosis rates were assessed in the negative control or UBE2L6-knockdown groups treated with decursin. **J**, **K** Expression of C-MYC and UBE2L6, as well as total and phosphorylated FLT3 and STAT5, in the negative control or UBE2L6-knockdown groups treated with decursin. ($n=3$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$)

a fluorescence microscope. In all the tissues analyzed, the percentage of GFP- and CD45-positive cells was markedly lower in the treated mice than in the control mice (Fig. 7E–H). Similarly, fluorescence microscopy revealed that decursin treatment reduced the distribution of MV4-11-GFP in the bone marrow (Fig. 7I and J). These findings suggest that decursin has an antileukemic effect in vivo.

Effects of decursin in primary AML cells

On the basis of the above antileukemic effects of decursin in FLT3-ITD cell lines, we evaluated the activity of decursin in patient AML cells. Consistent with the results derived from the cell lines, significant apoptosis was assessed in FLT3-ITD patient primary samples after treatment with decursin (Fig. 8A). In the same concentration range, wild-type primary cells were not sensitive to

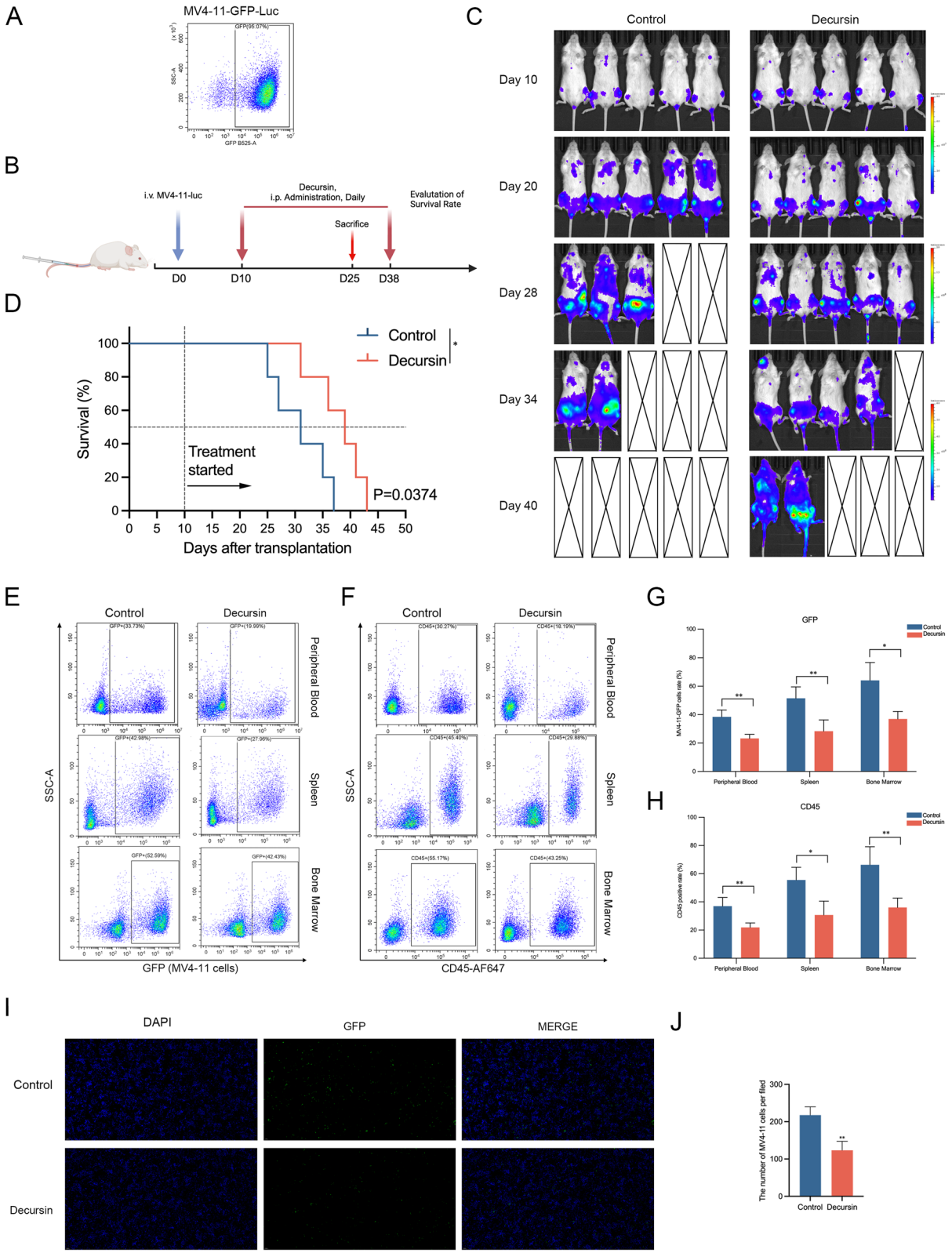

Fig. 7 Decursin is effective against AML in vivo. **A** Positive rate of MV4-11-GFP cells. **B** Schematic diagram of the MV4-11 AML model. **C** Leukemia progression was monitored by imaging with a Xenogen In Vivo Imaging System (IVIS). **D** The Kaplan–Meier method was utilized to assess animal survival ($n=5$). **E**–**H** After 14 days of drug treatment, 3 mice were sacrificed from each group, and the positive rates of GFP and CD45 were quantitatively detected. **I**, **J** The distribution of MV4-11-GFP cells in the bone marrow ($n=3$ $^{*}p<0.05$, $^{**}p<0.01$)

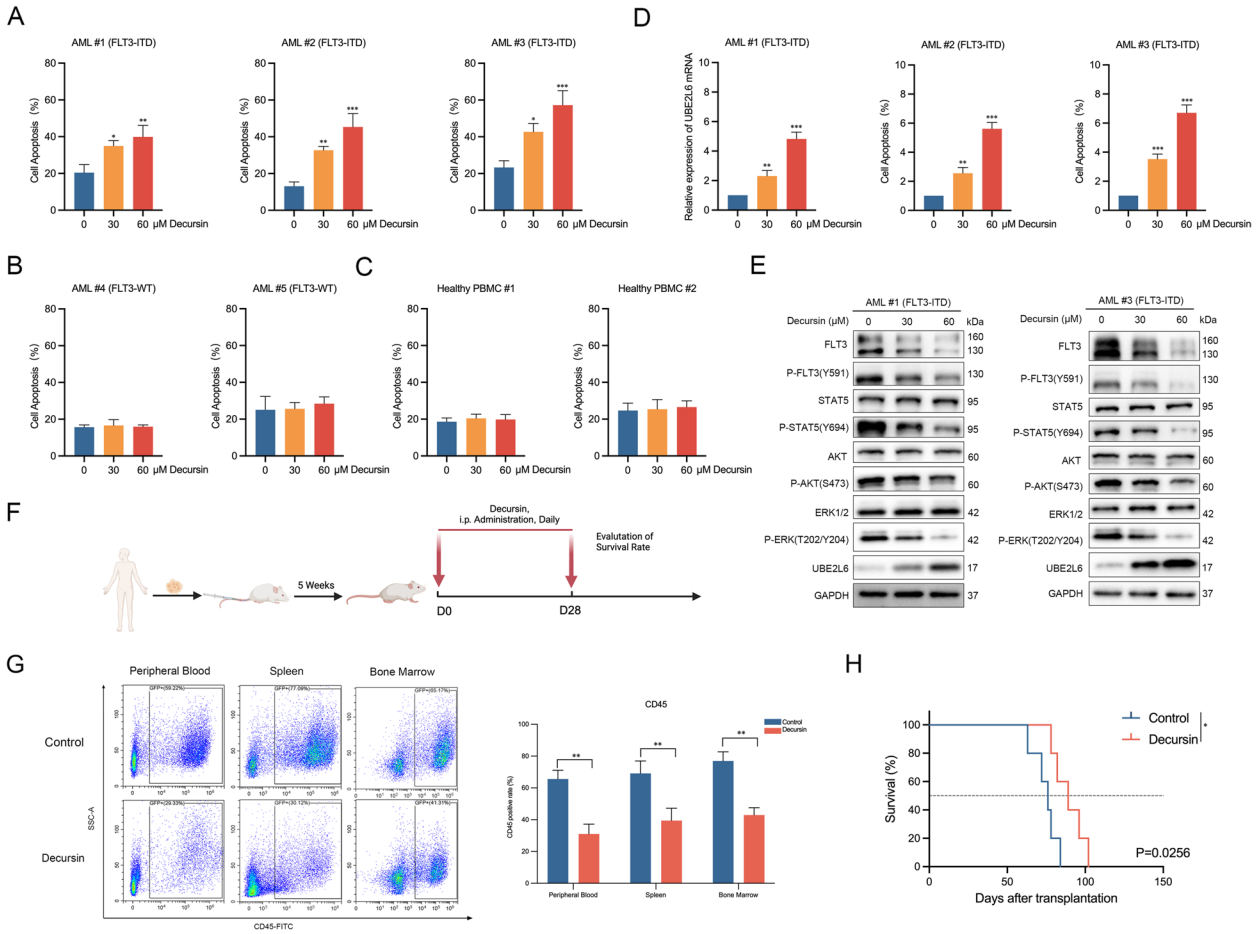

Fig. 8 Effects of decursin in primary AML cells. **A**-**C** Apoptosis rates induced by decursin treatment for 24 h in patient and healthy human samples. **D** Expression of UBE2L6 mRNA levels were assessed. **E** Total and phosphorylated FLT3, STAT5, AKT, and ERK, as well as UBE2L6 in FLT3-ITD primary cells were assessed. **F** Schematic diagram of the PDX model. **G** CD45-positive rates were quantitatively detected ($n=3$). **H** The Kaplan–Meier method was utilized to assess animal survival. ($n=5$) (*$P<0.05$, **$P<0.01$)

the induction of apoptosis by decursin and did not negatively affect healthy human PBMCs, confirming the safety of decursin (Fig. 8B and C). It should be noted, however, that decursin induced apoptosis in the FLT3-ITD patient cells at a less pronounced rate than it did in the cell lines (MV4-11 and MOLM-13), which may be due to the complex genetic background of the patients. We subsequently observed that in FLT3-ITD primary cells, decursin effectively induced UBE2L6 elevation and inhibited the FLT3-ITD downstream pathway (Fig. 8D and E), suggesting that the antileukemic activity of decursin is associated with the degradation of FLT3. To predict the efficacy of decursin in patients, we constructed a FLT3-ITD PDX model (Fig. 8F). Mice with successful transplantation were sacrificed 28 days after drug treatment, and the decursin-treated group showed significantly lower CD45+cell positivity compared with the control group (Fig. 8G). In a subsequent survival analysis, the decursin-treated mice had a significantly greater survival rate than did the control group (Fig. 8H). These results confirmed the effectiveness of decursin in preclinical AML models.

Decursin synergistically enhances venetoclax-induced apoptosis

The above results confirm that decursin can effectively inhibit the FLT3-ITD pathway. Currently, studies have confirmed the synergistic effect of simultaneous inhibition of FLT3 and BCL-2 in FLT3-ITD AML [39]. Therefore, we also assessed the combined effects of decursin and BCL-2 inhibitor venetoclax. As shown in Fig. 9A and B, compared with monotherapy, cotreatment with decursin and venetoclax synergistically inhibited the proliferation of MV4-11 and MOLM-13. After determining the optimal concentration for treatment in MV4-11 and MOLM-13 based on the highest zip score in Fig. 9B,

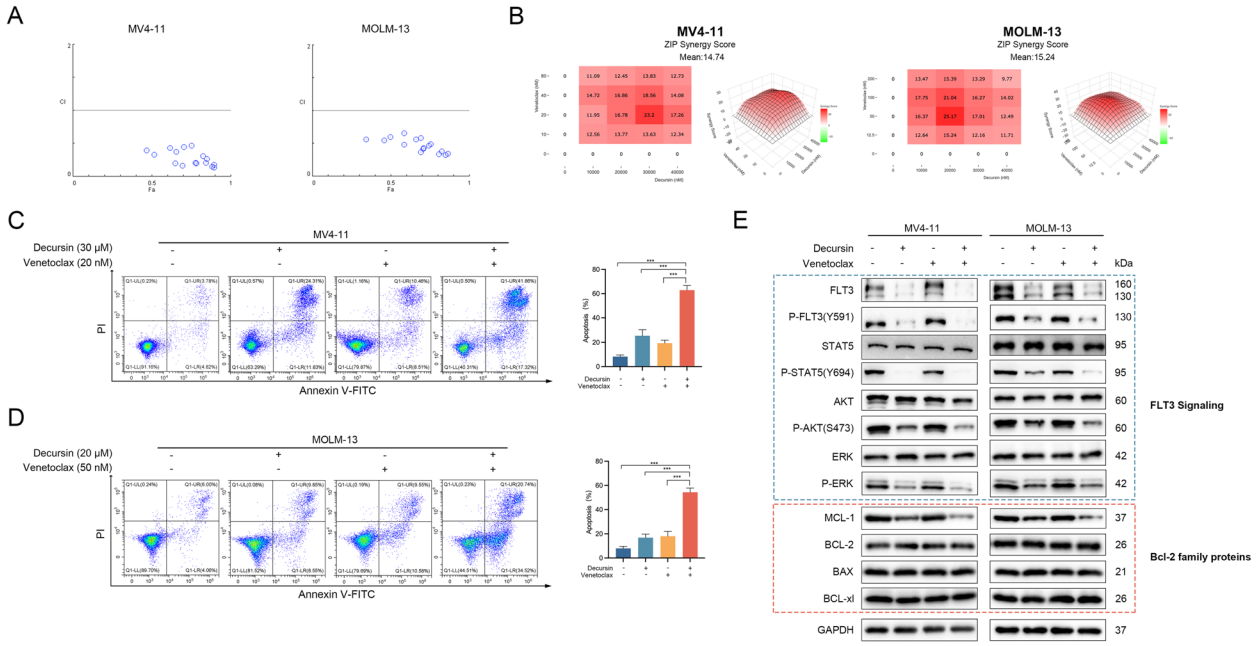

Fig. 9 Decursin synergistically enhances venetoclax-induced apoptosis. **A** Visualized chart from the CompuSyn report showing the combination index (CI) of venetoclax and decursin. CI values. **B** The concentration-response matrix (left) and ZIP synergy score (right) for decursin and venetoclax at different concentrations in MV4-11 and MOLM13. **C, D** Apoptosis rates of FLT3-ITD-positive cells treated with decursin and/or venetoclax for 24 h. **E** Expression of MCL-1, BCL-2, BCL-xl, and BAX, as well as total and phosphorylated FLT3, STAT5, AKT, and ERK were assessed. ($n = 3$, ***$P<0.001$)

we evaluated the effect of the drug combination on the apoptosis of FLT3-ITD-positive cells. Compared with decursin or venetoclax, the combination treatment significantly increased the percentage of apoptotic MV4-11 and MOLM-13 (Fig. 9C and D).

Anti-apoptotic protein MCL-1 is associated with venetoclax resistance [40, 41]. Therefore, to explore the synergistic mechanism of decursin and venetoclax, we assayed the FLT3 pathway as well as the BCL-2 family proteins MCL-1, BCL-xl, BCL-2, and BAX. Phosphorylation of FLT3 and downstream STAT5, AKT, and ERK was inhibited by decursin alone and maintained in the co-treatment group. The expression of BCL-xl, BCL-2, and BAX was barely affected by the single agent or combination groups, however, MCL-1 was significantly downregulated by decursin alone or in combination treatment. The FLT3-ITD mutation causes specific activation of STAT5, which in turn upregulates the expression of MCL-1. Previous studies have shown that FLT3 inhibitors in combination with venetoclax synergistically promote apoptosis by decreasing MCL-1 levels [40, 42]. Similarly, the above results suggest that decursin, as a FLT3 degrader, also synergistically potentiates venetoclax-induced apoptosis, which may be associated with MCL-1 downregulation induced by inhibiting STAT5 activation. It should be emphasized that apoptotic regulation involves dynamic interplay between multiple BCL-2 family members and pro-survival signaling pathways. While these findings provide initial mechanistic insights, the complete molecular mechanisms of decursin and venetoclax synergism require systematic interrogation to fully elucidate.

Discussion

FLT3-ITD mutation significantly affects the survival rate of AML patients [43]. Currently, a variety of FLT3 inhibitors are used to treat FLT3-ITD AML patients. Nevertheless, as monotherapies, FLT3 inhibitors face the challenges of short-lived efficacy and acquired resistance [44]. Here, we found that the natural compound decursin decreases the level of the FLT3-ITD and suppresses the downstream abnormally activated pathway by increasing UBE2L6 expression. Furthermore, decursin, a potent FLT3-ITD degrader, can synergize with venetoclax to enhance the apoptosis of FLT3-ITD AML cells.

FLT3-ITD mutations stimulate constitutive activation of tyrosine kinase component [7], significantly affecting the overall survival rate of AML patients [43]. Most clinically used FLT3 inhibitors directly inhibit kinase activity. However, frequently occurring resistance to FLT3 inhibitors has limited this treatment approach [45]. Unlike FLT3 inhibitors, decursin targets the degradation of cancer proteins, which might overcome the drug resistance mechanism of the more important inhibitor–midpoint mutation. Decursin is also expected

to overcome the drug resistance of FLT3 inhibitors to some extent [20] and represents a new treatment strategy for FLT3-ITD AML [46]. For example, PROTACs based on quinzatinib and gilteritinib have been shown to reduce FLT3-ITD protein levels [47, 48]. Inhibiting deubiquitinating enzymes degrades FLT3-ITD in crenolanib-sensitive cells [49]. Our study suggests that increased E2 ubiquitin-conjugase activity can induce FLT3-ITD degradation via the proteasome. Specifically, we demonstrate that decursin preferentially suppresses the survival of FLT3-ITD AML cells and significantly increases the level of UBE2L6, leading to FLT3-ITD degradation. Compared to MOLM-13, decursin seems to be more efficacious against MV4-11 in the same concentration, which may be due to the fact that MOLM-13 carries a FLT3-ITD heterozygote, whereas MV4-11 carries a FLT3-ITD homozygote. Previous studies have shown that gilteritinib inhibits MV4-11 more than MOLM-13 and is ineffective against FLT3-WT cells [35], which is similar to our experimental results.

UBE2L6 is a potential new target for FLT3-ITD-positive AML therapy. However, its biological role depends on the type of cancer. For example, esophageal squamous cell carcinoma cells overexpress UBE2L6 [50], which appears to be an oncogene. UBE2L6 is significantly downregulated in nasopharyngeal carcinoma, which is related to poor prognosis [51], and can promote apoptosis in cervical cancer cells [52]; thus, UBE2L6 seems to be a tumor suppressor gene. Nevertheless, the effect of UBE2L6 in leukemia has not been determined. Our results showed that shRNA-mediated knockdown of UBE2L6 inhibited decursin-induced apoptosis. Although the overexpression of SIAH1 results in the turnover of FLT3-ITD [21], decursin only affects SIAH1 expression. These findings suggest that UBE2L6, rather than SIAH1, plays an essential role in decursin-induced FLT3-ITD degradation. The TCGA database also revealed that the overall expression of UBE2L6 in AML patients carrying FLT3-ITD mutations is low (Fig. 6C), which may indicate that the limited expression of UBE2L6 is a prerequisite for the stabilization of the levels of FLT3-ITD and other oncoproteins [53, 54]. Dysregulation of UBE2L6 may be a step in the process of cellular transformation.

Degradation of cancer proteins by enhancing ubiquitin–proteasome activity has been shown to be a very effective treatment [48, 55]. For example, docosahexaenoic acid can induce the estrogen receptor (αERα) degradation [56]. Lenalidomide can bind to the E3 ubiquitin ligase cereblon and subsequently ubiquitinate the degradation of IKZF1 and IKZF3 [57]. In contrast, our current work shows that decursin increases the UBE2L6 level, which amplifies its E2-conjugase activity. Nevertheless, whether there is a direct interaction between decursin and UBE2L6 that influences the ability of UBE2L6 to bind to its substrate requires further investigation.

In addition, decursin alleviated the leukemia burden and prolonged survival in mice AML model, confirming the antileukemic effect of decursin in vivo. However, xenograft mouse models are difficult to use to mimic the pathogenesis of FLT3-ITD-positive leukemias; therefore, FLT3-ITD knock-in transgenic mice could be used to better assess the role of decursin in the development of FLT3-ITD-positive AML.

The use of FLT3 inhibitors often leads to compensatory upregulation of FLT3, which is associated with the development of drug resistance [58]. Therefore, inducing FLT3 degradation may be more promising than using kinase inhibitors. Here, we confirmed that decursin, a FLT3 degrader, not only effectively inhibits the FLT3-ITD pathway, but also shows anti-leukemic activity in CDX and PDX models. Previous studies have shown that the combination of a FLT3 inhibitor and venetoclax can still maintain compensatory upregulation of FLT3 [48], which may cause drug resistance and AML progression. We found that inducing FLT3-ITD degradation also synergizes with venetoclax, and this combination does not lead to compensatory upregulation of FLT3-ITD. These encouraging results suggest that inducing FLT3-ITD degradation may be used as an alternative to FLT3 inhibitors as a new therapeutic strategy for targeting FLT3-ITD AML. However, to date, there are no drugs in clinical treatment that specifically target FLT3-ITD degradation. Considering that decursin has no obvious side effects and can effectively degrade the FLT3-ITD protein, combination chemotherapy or other drugs may enhance clinical efficacy in AML patients carrying the FLT3-ITD mutations.

Conclusion

In summary, our study identified decursin as a natural product that degrades FLT3-ITD by increasing UBE2L6 expression, preferentially induces apoptosis in FLT3-ITD-positive AML cells, and showed anti-leukemic activity in preclinical AML mice models. Therefore, decursin might become a natural product to develop FLT3-ITD-targeted drug. Our research lays a solid foundation for degrading FLT3 as a new therapeutic strategy.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12964-025-02157-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Authors' contributions
Tianxin Zhang: investigation, data curation, writing the original manuscript. Yuchen Li: methodology, technical support, visualization. Wenhao Liao: supervision, writing-review, editing. Yu Mou: validation, data curation. Xue Zhan: formal analysis, software. Qiongying Hu: conceptualization, writing the original manuscript. Ziyi Zhao: methodology, project management. Daqian Xiong: supervision, writing the original manuscript, funding acquisition.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (2023KZL-005), in line with the Declaration of Helsinki, and informed consent was obtained from all participants.

Competing interests
The authors declare no competing interests.

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