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In vitro anti-leukemia activity of dual PI3K/mTOR inhibitor Voxtalisib on HL60 and K562 cells, as well as their multidrug resistance counterparts HL60/ADR and K562/A02 cells

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ABSTRACT

Current treatment strategies for leukemia still have some limitations such as severe side effects and drug resistance. Less toxic and more effective drugs for leukemia patients are therefore expected. In the present study, the efficacy of a dual PI3K/mTOR inhibitor, Voxtalisib, on acute myeloid leukemia (AML) cell line HL60 and chronic myeloid leukemia (CML) cell line K562, as well as their Adriamycin (ADR)-selected multi drug resistance (MDR) counterparts HL60/ADR and K562/A02, was investigated. Voxtalisib exhibited potent anti-proliferative activity on these four cell lines dose-dependently, with IC_{50} values as 2.23 μM for HL60, 4.79 μM for HL60/ADR, 4.20 μM for K562 and 3.90 μM for K562/A02 cells. Voxtalisib arrested cell cycle progression at G1 phase in all cell lines by upregulating p27, downregulating cyclin D1 and p-pRb. When combined with ADR, Voxtalisib reversed the ADR-resistance of HL60/ADR and K562/A02 cells, possibly by reducing MDR1 and MRP1 expression. In conclusion, Voxtalisib showed anti-leukemia activity on AML and CML cell lines as well as their multidrug resistant ones, suggesting Voxtalisib might become a promising drug candidate for therapy of AML and CML in the future.

1. Introduction

Leukemia, a hematological disorder, usually begins in the bone marrow, the site where hematopoietic stem cells are cloned abnormally [1]. According to the disease progression and the type of white blood cells affected, leukemia can be divided into four types as acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML) [2].

AML is heterogeneous and accounts for the majority in leukemia cases [3]. It has been well known that Fms-like tyrosine kinase 3 (FLT3) is expressed in malignant cells of most AML patients. FLT3 is a transmembrane receptor that regulates the growth, proliferation and differentiation of hematopoietic progenitor cells through its downstream RAS/RAF/MEK/ERK and PI3K/Akt/mTOR pathways [4,5]. Several FLT3 inhibitors such as sunitinib and midostaurin, have been approved as molecular targeted anticancer drugs [6]. Unfortunately, targeted therapeutics against AML did not evolve profoundly in the past decades because of frequent FLT3 mutations and development of drug resistance [7]. CML is characterized by the Philadelphia chromosome and its chimeric oncoprotein BCR-ABL [8]. BCR-ABL protein which bears constitutive tyrosine kinase activity, drives sustained activation of downstream signaling pathways such as PI3K/Akt/mTOR, finally leading to uncontrolled clonal proliferation of leukemia cells. Tyrosine kinase inhibitors (TKI) which target BCR-ABL, developed rapidly in the past years [9–11]. However, insufficient response of some CML patients to TKI therapy and the drug resistance development present major therapeutic challenges. As a result, there is still an urgent need to develop less toxic and more effective drugs for leukemia patients [12,13].

Voxtalisib, also named XL765, is a dual inhibitor of PI3K/mTOR with specific adenosine triphosphate (ATP)-competitive binding to the catalytic domains of PI3K and mTOR. Voxtalisib is now in clinical trial [14]. Preclinical studies have indicated that Voxtalisib exhibits potent antitumor activity against multiple tumors by inhibiting PI3K/Akt/mTOR signaling [15]. However, the effect of Voxtalisib on AML and CML cells has not yet been reported.

Therefore, we recently investigated the anti-leukemia activity of Voxtalisib on AML HL60 and CML K562 cells, as well as their multidrug resistant counterparts HL60/ADR and K562/A02.

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2. Materials and methods

2.1. Reagents

Voxtalisib and Adriamycin (ADR) were purchased from Selleck (London, ON, Canada). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) reagent was from Amresco (Solon, OH, USA). Propidium iodide (PI) was from Sigma-Aldrich (St. Louis, MO, USA). Annexin V-FITC, anti-p-pRb (S780) (1500:), anti-p27 (11,000:) and anti-cyclin D1 (11,000:) antibodies were obtained from BD Biosciences Pharmingen (San Jose, CA, USA). Anti-MDR1 (11,000:), anti-MRP1(1500:) and anti-Lamin B antibody (1500:) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-p-Akt (Ser473) (11,000:), anti-Akt (11,000:), anti-p-mTOR (Ser2448) (11,000:), β-actin (11,000:), anti-mouse and anti-rabbit HRP (horse radish peroxidase)-conjugated secondary antibodies (12,000:) were obtained from Cell Signaling Technology (Danvers, MA, USA).

2.2. Cell culture

The human CML K562 and AML HL60 cell lines were purchased from Cell Resource Center, Peking Union Medical College (Beijing, China). Their ADR-selected multidrug resistance (MDR) counterparts K562/A02 and HL60/ADR cell sub-lines were obtained from the Institute of Hematology, Chinese Academy of Medical Sciences (Tianjin, China). Human peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood sample of a healthy voluntary donor by using Human Lymphocyte Separation Medium (Dakewe, Beijing, China). The performance was approved by the Tianjin Medical University Institutional Review Board. The cells were cultured in 10% (v/v) fetal bovine serum (FBS) in RPMI 1640 (Biological Industries, Israel) medium supplemented with 100 μg/ml of kanamycin and 0.44 μg/ml of glutamine. All cell lines were maintained in a humidified atmosphere with 5% CO_2 at 37 ℃. For culture of the MDR cell lines, 1 μg/ml (final concentration) of ADR was added to the culture medium of K562/A02 and HL60/ADR cells to sustain the MDR phenotype. The cells were further cultured in drug-free medium for 10 days before experiments.

2.3. MTT assay

MTT assay was performed to assess cell viability of K562, K562/A02, HL60 and HL60/ADR as previously described [16,17]. Briefly, 200 μl of cell suspension (2×10^4 cells/ml) was seeded in each well of a 96-well plate and treated with Voxtalisib or ADR for 24 h, 48 h or 72 h at 37 °C. Concentrations of each drug were as follows: 1) Voxtalisib: 0, 0.3125, 0.625, 1.25, 2.5, 5, 10 μM; 2) ADR: 0, 0.1, 0.5, 1, 2, 5, 10 μM for K562 and HL60 cells; 3) ADR: 0, 0.2, 2, 10, 20, 40, 100 μM for K562/A02 and HL60/ADR cells. After treatment for 48 h, the cells were exposed to MTT (5 mg/ml) for 4 h. Then, the culture medium was removed, and the purple formazan crystals were dissolved in DMSO. The absorbance value optical density (*OD*) was measured at 490 nm by using a microplate reader iMark (BIO-RAD, Hercules, CA, USA) in triplicate. The number of viable cells was calculated as follows: Cell number (%) = $(OD_{Sample} - OD_{Blank})/(OD_{Control} - OD_{Blank})$. Blank represents the well containing medium only and Control represents the well containing cells treated with DMSO. The IC_{50} values were determined according to the logistic curve analyzed by GraphPad Prism 5 Software (GraphPad Software, San Diego, CA, USA).

2.4. Colony formation assay

The colony formation assay was carried out as previously described [18]. The cells were treated with Voxtalisib (0, 0.625, 2.5, 10 μM) for 48 h at 37 °C. Then, the treated cells (1.2×10^4 cells/dish) suspended in complete media were added in the upper agar containing 0.3% agarose. The lower agar is consisted of 0.6% agarose in culture medium. A total of 3 ml fresh medium was added to the cells every two days. After incubation for 14 days at 37 °C, the colonies were stained with 0.5% crystal violet. The colony numbers were counted under a microscope and the rate of colony formation was calculated as follows: the rate of colony formation (%) = number of colonies in experimental group/number of colonies in control group × 100%. Each experiment was carried out at least in triplicate.

2.5. Flow cytometric analysis of cell cycle distribution

The effect of Voxtalisib on cell cycle distribution analysis was carried out by PI staining using flow cytometer as previously described [19,20]. The cells (4×10^5 cells/ml) were treated with Voxtalisib (0, 0.625, 1.25, 2.5, 5, 10 μM) for 48 h at 37 °C, fixed overnight in 75% ethanol at 4 °C. After centrifugation, the fixed cells were stained for 30 min in 300 μl PBS containing 50 μg/ml PI, 100 μg/ml RNase A and 0.5% Triton X-100 at 4 °C in the dark. The stained cells were subjected to flow cytometer BD Accuri C6 (BD Biosciences, San Jose, CA, USA) for cell cycle distribution analysis.

2.6. Flow cytometric analysis of apoptosis

Analysis of apoptosis was carried out by using flow cytometer after Annexin V-FITC/PI double staining as previously reported [21]. The cells (4×10^5 cells/ml) were treated with Voxtalisib (0, 2.5, 10 μM) for 48 h at 37 °C. After being harvested, the treated cells (1×10^5 cells) were stained in 100 μl of binding buffer containing Annexin V-FITC solution and PI solution for 15 min at room temperature in the dark. Finally, apoptosis analysis was conducted by BD FACS Verse flow cytometer (BD Biosciences, San Jose, CA, USA) and quantified using Flow Jo Software (Tristar, CA, USA).

2.7. Determination of resistance reversal effect of Voxtalisib

The resistance reversal effect of Voxtalisib was investigated by comparing the anti-proliferative activity of ADR alone with that combined with Voxtalisib, which was determined with MTT assay, as previously described [18]. The K562/A02 and HL60/ADR cells (2×10^4 cells/ml) were treated with various concentrations of ADR (0, 0.2, 2, 10, 20, 40, 100 μM) in combination with Voxtalisib (0, 0.3125, 0.625, 1.25, 2.5 μM) in a 96-well plate for 48 h at 37 °C. The resulting absorbance at 490 nm was measured by using a microplate reader iMark (BIO-RAD, Hercules, CA, USA). The reversal effect of Voxtalisib was assessed based on the reversal fold (RF) value which was calculated as the ratio of the IC_{50} value for ADR alone to that for drug combination.

2.8. Western blot analysis

Western blot analysis was performed as we reported previously [22,23]. The cells (4×10^5 cells/ml) were treated with different concentrations of Voxtalisib in a 6-well plate for 48 h at 37 °C. To extract the total protein, the cells were collected, harvested and lysed using RIPA lysis buffer (Roche Diagnostics, Basel, Switzerland). Nuclear protein was prepared using NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo Fisher Scientific, Waltham, MA, USA). After bicinchoninic acid (BCA) assay, equal amount of protein samples were isolated by 10% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocking in 5% non-fat milk, the membranes were exposed to specified primary antibodies and then the respective secondary antibodies. The blots were visualized with enhanced chemiluminescence (ECL) system and quantified using Image J software. The signals were quantified by comparison to that of β-actin, which is widely used as the internal reference in Western blot.

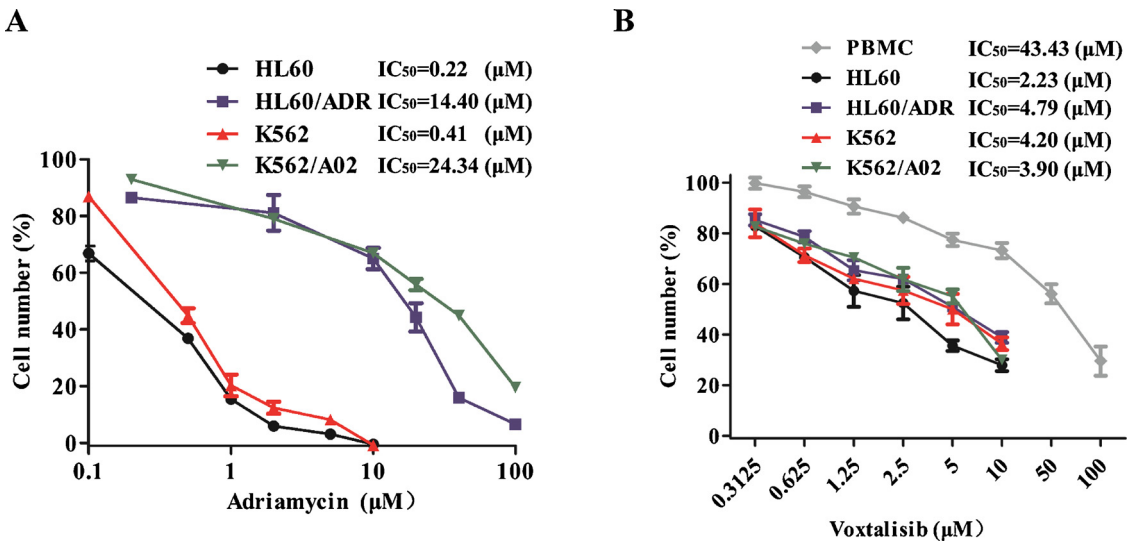

Fig. 1. Antiproliferative activity of Voxtalisib and ADR on HL60, HL60/ADR, K562 and K562/A02 cells. (A) HL60/ADR and K562/A02 cells showed resistance to ADR. The cells were treated with different concentrations of ADR: 0, 0.1, 0.5, 1, 2, 5 and 10 μM for HL60 and K562 cells; 0, 0.2, 2, 10, 20, 40 and 100 μM for HL60/ADR and K562/A02 cells. Cell viability was determined by MTT assay. (B) The effect of Voxtalisib on the growth of PBMCs, HL60, HL60/ADR, K562 and K562/A02 cells. The cells were treated with various concentrations of Voxtalisib for 48 h, and cell viability was determined by MTT assay. The results are mean ± *SD*, representative of three independent experiments ($n = 3$).

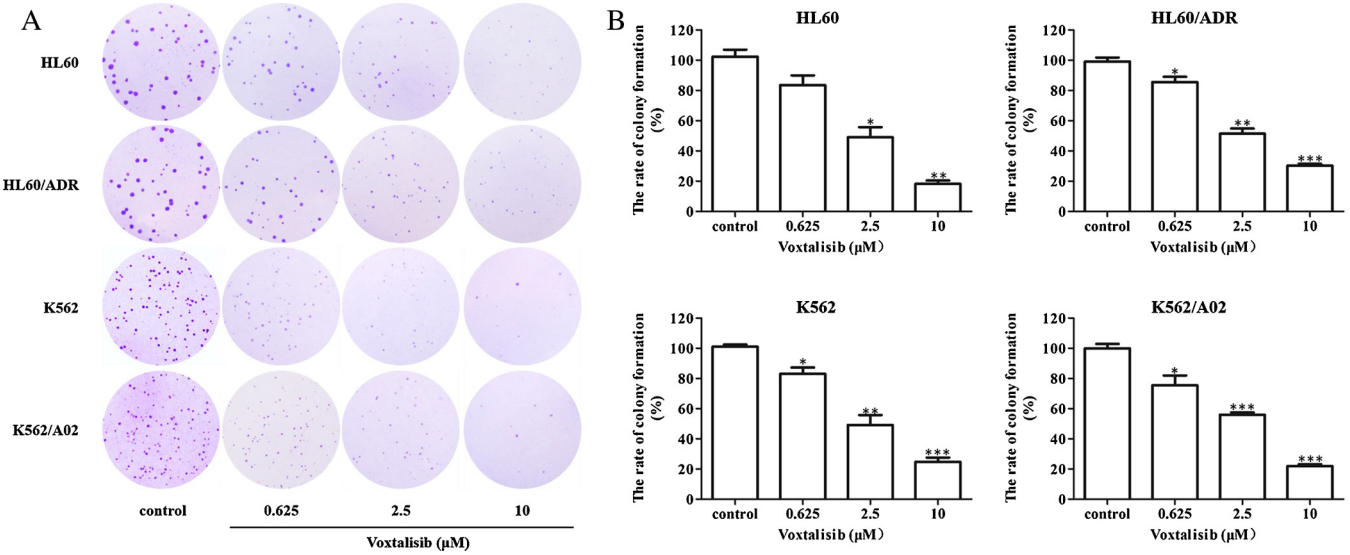

Fig. 2. Voxtalisib inhibited soft agar colony formation of HL60, HL60/ADR, K562 and K562/A02 cells. (A) HL60, HL60/ADR, K562 and K562/A02 cells pretreated with various concentrations of Voxtalisib (0, 0.625, 2.5 and 10 μM) for 48 h were further grown in soft agar for 14 days. The colonies were observed and counted under a microscope. (B) Quantification of the colonies formed by HL60, HL60/ADR, K562 and K562/ADR cells with or without Voxtalisib treatment was performed by counting the number of colonies. The results are mean ± *SD*, representative of three independent experiments ($n = 3$). $^{*}: p < 0.05$, $^{**}: p < 0.01$, $^{***}: p < 0.001$, compared with the respective control.

2.9. Statistical analysis

Data are presented as mean ± standard deviation (SD) from 3 independent experiments. Statistical comparisons between two groups were analyzed by student's *t*-test with GraphPad Prism 5 software (GraphPad, San Diego, CA, USA). Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Growth inhibitory effect of Voxtalisib on AML and CML cells

AML HL60 and CML K562 cells are known as chemosensitive cell lines, whereas HL60/ADR and K562/A02 cells are resistant to ADR as well as other chemotherapeutic drugs. To ascertain the MDR phenotype of HL60/ADR and K562/A02 cells, we examined the sensitivity of HL60/ADR and K562/A02 cells to ADR using MTT assay. As shown in Fig. 1A, ADR showed a far weaker growth inhibition against HL60/ADR (IC_{50} = 14.40 μM) and K562/A02 (IC_{50} = 24.34 μM) cells than that against their parental cells HL60 (IC_{50} = 0.22 μM) and K562 (IC_{50} = 0.41 μM), with the relative resistance (RR) as 65.50 and 59.40, respectively, suggesting that HL60/ADR and K562/A02 cell lines indeed showed significant resistance to ADR [24].

Then, we examined the antiproliferative effect of Voxtalisib on K562 cells after treatment for 24 h, 48 h and 72 h, respectively, by using MTT assay. Voxtalisib inhibited K562 cell proliferation with IC_{50} values as 9.20 μM (for 24 h), 4.20 μM (for 48 h) and 4.03 μM (for 72 h), respectively (Fig S1). K562/A02, HL60 and HL60/ADR cells were exposed to

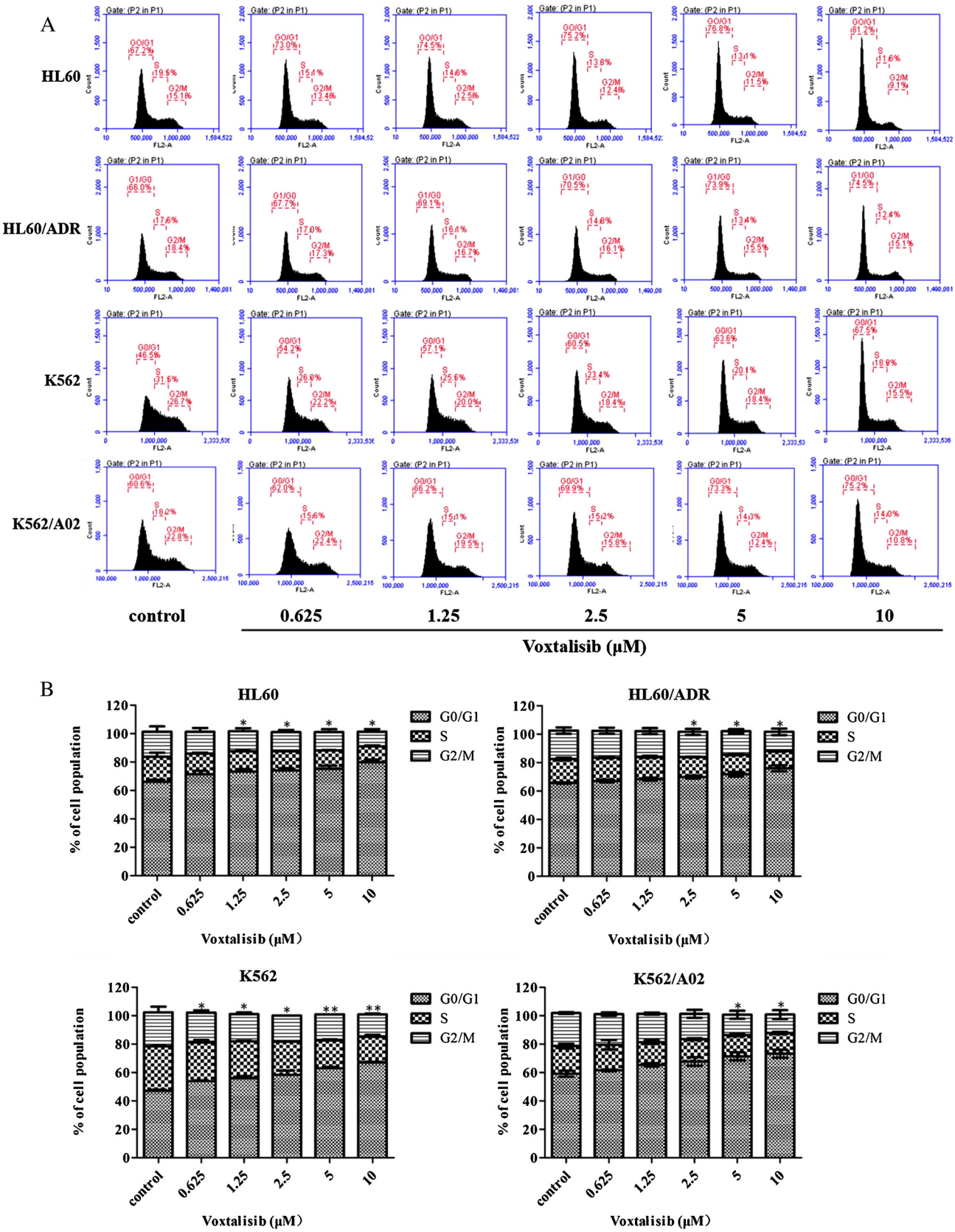

Fig. 3. Voxtalisib induced cell cycle arrest at G1 phase in HL60, HL60/ADR, K562 and K562/A02 cells. (A) Representative histograms of cell cycle distribution in HL60, HL60/ADR, K562 and K562/A02 cells. The cells were exposed to different concentrations of Voxtalisib (0, 0.625, 1.25, 2.5, 5 and 10 μM) for 48 h. The cell cycle was assessed by flow cytometry after PI staining. (B) The percentages of the cell population at G1, S, and G2/M phases were calculated. Data are mean ± *SD*, representative of three independent experiments ($n = 3$). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, compared with the respective control.

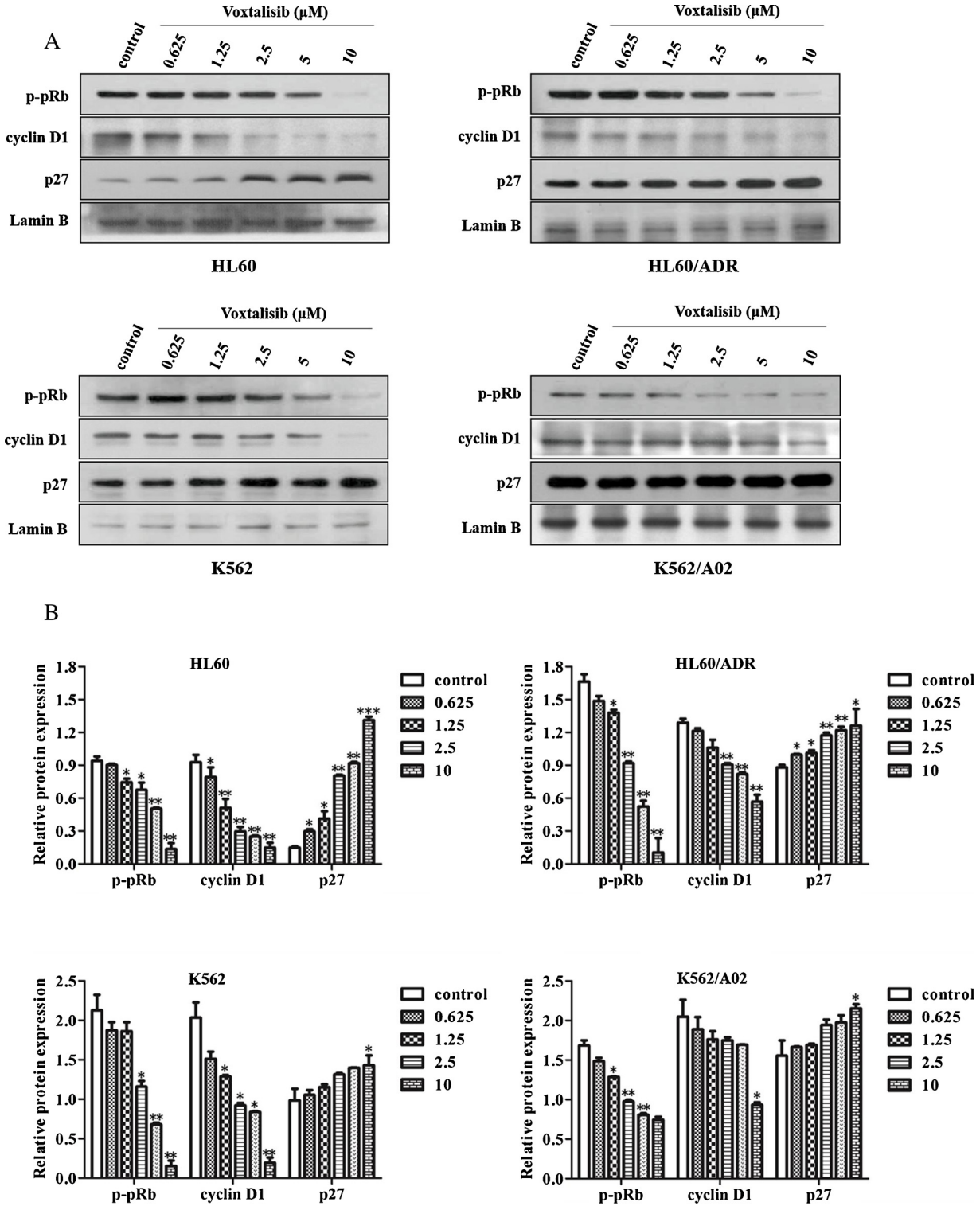

Fig. 4. Voxtalisib affected the cell cycle regulators. (A)HL60, HL60/ADR, K562 and K562/A02 cells pretreated with various concentrations of Voxtalisib (0, 0.625, 1.25, 2.5, 5 and 10 μM) for 48 h were harvested for western blot. The levels of cyclin D1, p27, and p-pRb in the nuclei were determined. (B) Quantified western blot results of cyclin D1, p27 and p-pRb. Data are mean ± *SD*, representative of three independent experiments ($n = 3$). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, compared with the respective control.

various concentrations of Voxtalisib for 48 h, and cell viability was determined by MTT assay. As shown in Fig. 1B, Voxtalisib reduced proliferation of K562/A02, HL60 and HL60/ADR cells in a dose-dependent manner, with the IC_{50} value to be 2.23, 4.79 and 3.90 μM, respectively. In contrast, Voxtalisib showed weak inhibition against normal cell PBMC with IC50 of 43.43 μM.

To further investigate the antitumor effect of Voxtalisib on HL60, HL60/ADR, K562 and K562/A02 cells, soft agar colony formation assay was used to assess tumorigenicity of cancer cells after treatment with Voxtalisib [25]. After being exposed to different concentrations of Voxtalisib for 48 h, cells were grown in soft agar for 14 days. As shown in Fig. 2A and B, the number of colonies was reduced by Voxtalisib

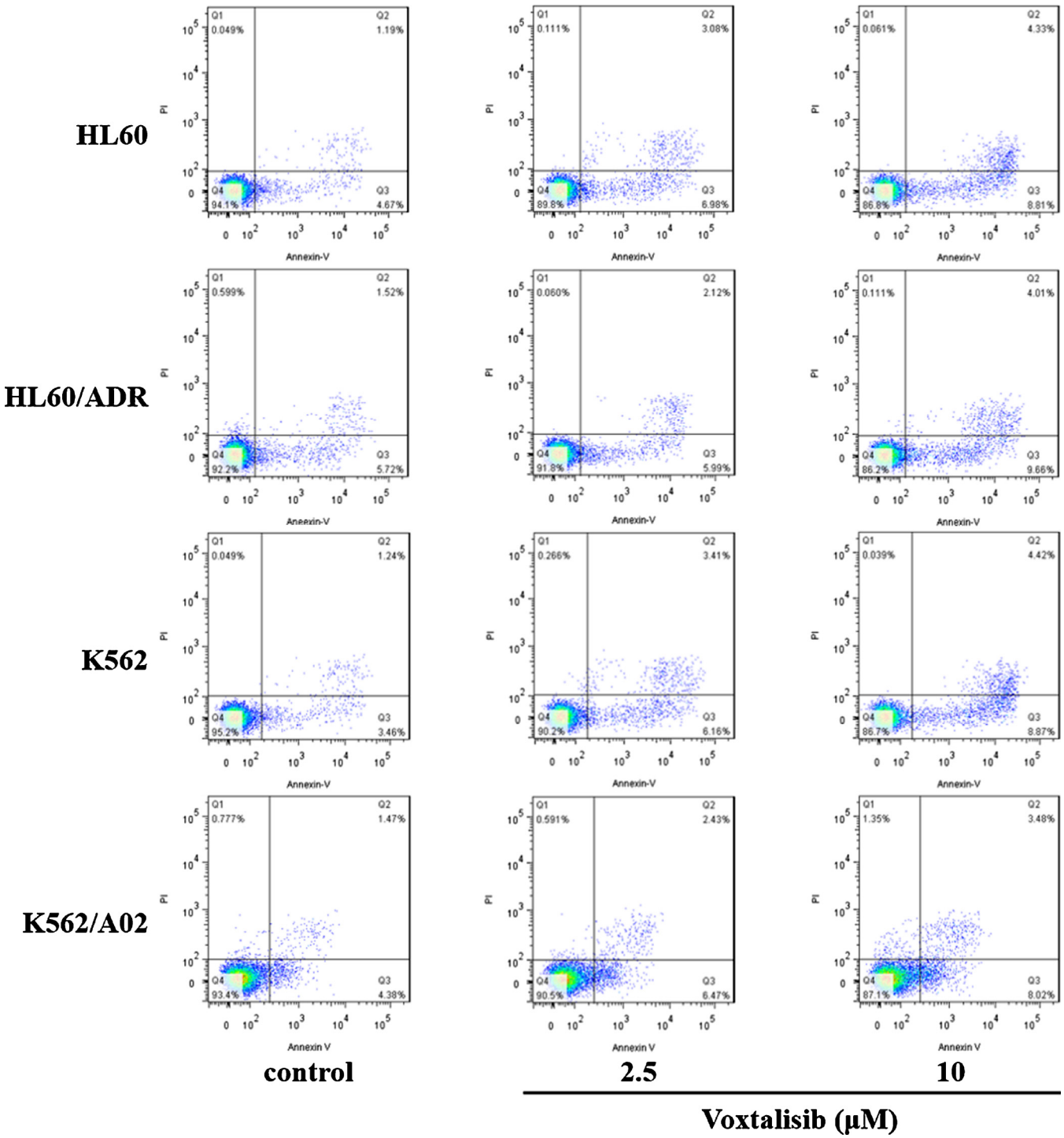

Fig. 5. Voxtalisib did not induce obvious apoptosis in HL60, HL60/ADR, K562 and K562/A02 cells. HL60, HL60/ADR, K562 and K562/A02 cells pretreated with indicated concentrations of Voxtalisib (0, 2.5 and 10 μM) for 48 h were double stained with Annexin V and PI to be available for flow cytometry.

treatment dose dependently, for either parental cell lines or the MDR sub-cell lines, suggesting Voxtalisib inhibited tumorigenicity of all the four cell lines.

3.2. Voxtalisib induced cell cycle arrest at G1 phase in AML and CML cells

To investigate whether the cell growth inhibition caused by Voxtalisib on AML and CML cells was due to its cell cycle arrest effect or not, we then detected cell cycle distribution after Voxtalisib exposure. As shown in Fig. 3A and B, no matter in the MDR cells HL60/ADR and K562/A02, or their parental cells HL60 and K562, Voxtalisib increased the proportion of cells in G1 phase dose-dependently. When exposed to 10 μM Voxtalisib, there was an increase of 14.00% (for HL60), 8.50% (for HL60/ADR), 21.00% (for K562) and 14.60% (for K562/A02), in G1 phase population compared to their respective control group, indicative of a G1 cell cycle arrest by Voxtalisib.

The cell cycle checkpoint proteins include cyclin-cyclin dependent kinases (CDK) complexes and CDK inhibitors. To investigate the underlying molecular mechanism of Voxtalisib-mediated G1 phase arrest, the key regulators of G1 to S phase transition such as cyclin D1, p27, as

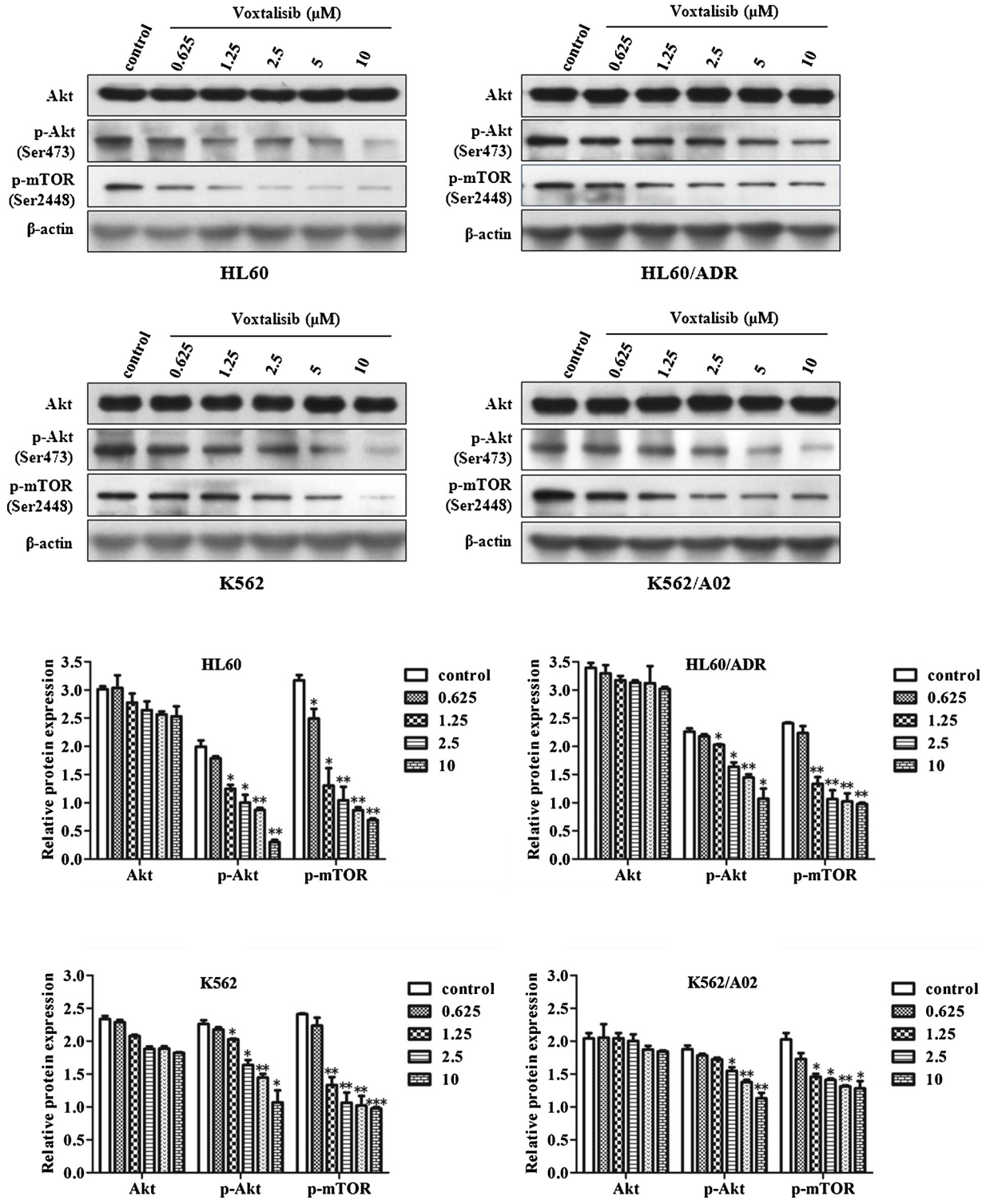

Fig. 6. Voxtalisib inhibited PI3K/Akt/mTOR pathway in leukemia cells. (A) HL60, HL60/ADR, K562 and K562/A02 cells were incubated with indicated concentrations of Voxtalisib (0, 0.625, 1.25, 2.5, 5 and 10 μM) for 48 h followed by total protein extraction. The levels of p-Akt (Ser473), Akt and p-mTOR (Ser2448) were determined by western blot. (B) Quantified western blot results of Akt, p-Akt (Ser473) and p-mTOR (Ser2448). Data are mean ± *SD*, representative of three independent experiments ($n = 3$). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, compared with the respective control.

well as the downstream pRb, was examined by western blot. Fig. 4A and B showed that, in each of the 4 cell lines, nuclear proteins cyclin D1 and p-pRb levels were significantly downregulated, while nuclear p27 expression was upregulated following Voxtalisib treatment.

3.3. Voxtalisib did not induce apoptosis in AML and CML cells

We further investigated whether Voxtalisib induced cell apoptosis in the tested leukemia cells. As shown in Fig. 5 and Fig S2, Voxtalisib did not show obvious cell apoptosis induction in HL60, HL60/ADR, K562 and K562/A02 cells, suggesting that the growth inhibitory effect of

Table 1
The resistance reversal effect of Voxtalisib on HL60/ADR cells.

Compounds		IC_{50}(μM)(reversal fold)
Adriamycin(μM)	Voxtalisib(μM)	
0.2–100	0	14.40(1.00)
	0.3125	7.00(2.06)
	0.625	3.94(3.65)
	1.25	2.84(5.07)
	2.5	2.64(5.45)

Table 2
The resistance reversal effect of Voxtalisib on K562/A02 cells.

Compounds		IC_{50}(μM)(reversal fold)
Adriamycin(μM)	Voxtalisib(μM)	
0.2–100	0	24.34(1.00)
	0.3125	22.19(1.10)
	0.625	19.78(1.23)
	1.25	14.34(1.69)
	2.5	8.50(2.86)

Voxtalisib on AML and CML cells might be unrelated to cell apoptosis induction.

3.4. Voxtalisib inhibited PI3K/Akt/mTOR pathway in AML and CML cells

It is well known that PI3K/Akt/mTOR signaling plays an important role in oncogenesis [26,27]. Since Voxtalisib is a dual PI3K/mTOR inhibitor, we speculate that Voxtalisib exerts anti-leukemia activity through PI3K/Akt/mTOR pathway. To test this hypothesis, HL60, HL60ADR, K562 and K562/A02 cells were treated with Voxtalisib, and the protein levels of specific signaling nodes in this pathway were analyzed. As shown in Fig. 6A and B, the phosphorylation levels of Akt (Ser473) and mTOR (Ser2448) were decreased in a dose-dependent manner after Voxtalisib treatment in all the cells. Collectively, these results indicate that Voxtalisib inhibits AML and CML cell growth and arrests cell cycle G1 phase probably by negatively regulating PI3K/Akt/mTOR pathway.

3.5. Voxtalisib reversed ADR resistance in HL60/ADR and K562/A02 cells

Since Voxtalisib exhibited similar anti-leukemia potency on parental cell lines and their MDR sub-cell lines, we speculated that Voxtalisib might not be a substrate of MDR or might have the ability to reverse MDR. To test this, we analyzed the sensitivity of HL60/ADR and K562/A02 cells to ADR in the presence of Voxtalisib with MTT assay. As shown in Tables 1 and 2, ADR alone (ranging from 0.2 to 100 μM) weakly inhibited proliferation of HL60/ADR and K562/A02 cells, while the combination with Voxtalisib significantly sensitized both cell lines. The IC_{50} of ADR toward HL60/ADR and K562/A02 cells were decreased dramatically, from 14.40 μM and 24.34 μM to 2.64 μM and 8.50 μM, in the presence of 2.5 μM Voxtalisib, and the reversal fold (RF) was 5.45 and 2.86, respectively. These results revealed that Voxtalisib reversed the ADR resistance of HL60/ADR and K562/A02 cells in a dose-dependent manner.

3.6. Voxtalisib diminished expression of MDR1 and MRP1 in HL60/ADR and K562/A02 cells

MDR1 and MRP1 are potential regulators rendering cancer cells

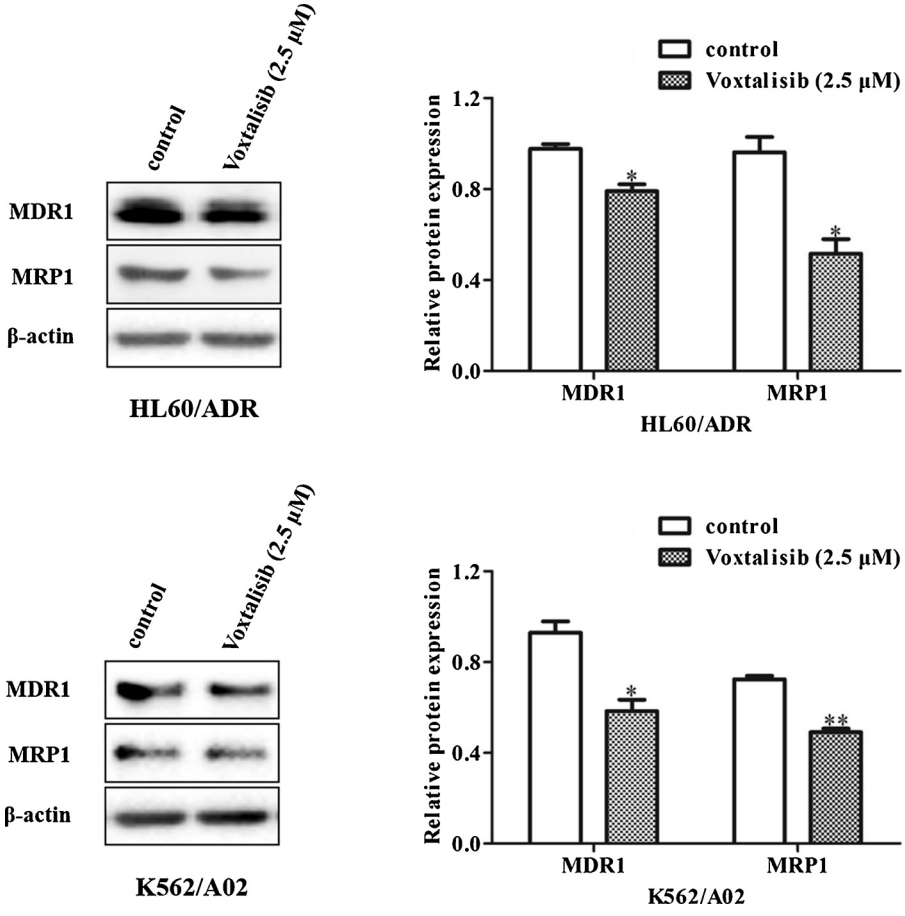

Fig. 7. Voxtalisib diminished expression of MDR1 and MRP1 in HL60/ADR and K562/A02 cells. HL60/ADR and K562/A02 cells were treated with Voxtalisib (0, 2.5 μM) for 48 h followed by total protein extraction. The expression levels of MDR1 and MRP1 were determined by western blot, and quantified by using Image J software (NIH, USA). Data are mean ± *SD*, representative of three independent experiments ($n = 3$). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, compared with the respective control.

resistant to anticancer drugs [28]. Since an obvious resistance reversal effect of Voxtalisib was observed, we then analyzed the effect of Voxtalisib on expression of MDR1 and MRP1 in HL60/ADR and K562/A02 cells. As shown in Fig. 7, western blot results clearly showed that MDR1 and MRP1 expressions were downregulated by Voxtalisib. The above results suggested that Voxtalisib might reverse MDR of HL60/ADR and K562/A02 cells by reducing the expression of MDR1 and MRP1.

4. Discussion

It is known that there are about 21,000 new diagnosis of AML cases annually, and over 10,000 deaths of this tumor each year in the USA [29]. Mutation of FLT3 gene is a crucial outcome factor in AML, with gloomier prognostic for patients. The encoded FLT3 promotes cell growth and survival by activating PI3K/Akt/mTOR [30]. On the other hand, the annual incidence of CML accounts for 15% of newly diagnosed adult leukemia patients [31]. It has been reported that CML is defined by the BCR-ABL fusion gene, but blocking BCR-ABL kinase activity is not sufficient to eradicate leukemogenic effects [32,33]. It's noteworthy that MDR, early relapse and persistence of leukemic stem cells are all obstacles to successful treatment of leukemia patients with chemotherapy [34,35]. Therefore, discovery of novel anti-leukemia agents that are effective on multidrug resistance is expected.

PI3K/Akt/mTOR signaling is known to play key roles in cellular processes involved in tumorigenesis. Dysregulation of the PI3K/Akt/mTOR signaling is widely observed in human cancers including AML and CML [36]. PI3Ks are lipid kinases that phosphorylate phosphatidylinositol 4, 5-bisphosphate (PIP2) at the 3-OH of the inositol ring to produce phosphatidylinositol 3, 4, 5-trisphosphate (PIP3), which in turn activates Akt and the downstream molecules to play key roles in tumorigenesis. Activation of Akt promotes cell cycle progression by regulating glycogen synthesis kinase 3β (GSK3β) and the downstream cyclin D1, maintain cell survival through inhibition of Bcl2-antagonist of cell death (BAD), and promotes cell growth by phosphorylation of the downstream mTOR [37]. Since both PI3K and mTOR are important oncology targets, we chose dual PI3K/mTOR inhibitor Voxtalisib [27,38], and investigated its anti-leukemia activity on HL60 and K562 cells, as well as the MDR cell sub-lines HL60/ADR and K562/A02.

Voxtalisib is currently in phase I/II clinical investigation, either as a single agent or in combination with other anticancer drugs, for therapy of human solid tumors [14,38]. Voxtalisib was reported to have a manageable safety profile in patients with advanced solid tumors, while side effects including fatigue, gastrointestinal and cutaneous adverse events were shown [38]. Our result demonstrated that Voxtalisib dose-dependently inhibited proliferation of all four leukemia cell lines with IC_{50} values lower than 5 μM. In contrast, Voxtalisib showed weak inhibition against normal cell PBMC with IC_{50} of 43.43 μM, suggesting the selectivity for leukemia cells over normal cells.

Moreover, Voxtalisib induced cell cycle G1 arrest, of which the mechanism might be related to upregulation of p27, downregulation of cyclin D1 and p-pRb. In addition, Voxtalisib did not induce obvious apoptosis effect in any of the four cell lines, suggesting Voxtalisib might exhibit anti-leukemia activity through growth inhibition and cell cycle G1 arrest, but not apoptosis induction.

Besides the role in promoting tumor cell growth and survival, aberrant PI3K/mTOR signaling is also known to be involved in resistance to chemotherapies [39]. Inhibition of PI3K pathway has been identified to sensitize leukemia cells to chemotherapies [40–42]. Thus, in this research, we examined whether Voxtalisib could reverse drug resistance of HL60/ADR and K562/A02 cells. Interestingly, we found that Voxtalisib showed similar growth inhibition efficacy on HL60/ADR and K562/A02 cells to that on HL60 and K562 cells. Moreover, Voxtalisib (2.5 μM) reversed ADR resistance of HL60/ADR and K562/A02 cells by 5.45-fold and 2.86-fold, respectively. Overexpression of the ATP-Binding Cassette (ABC) transporters plays a key role in the multidrug resistance of cancer cells to chemotherapeutic drugs, by increasing efflux of the anticancer drugs [43,44]. MDR1 and MRP1, 2 major ATP-binding cassette (ABC) transporters, play important roles in MDR [28]. Voxtalisib treatment diminished MDR1 and MRP1 expression in HL60/ADR and K562/A02 cells, suggesting Voxtalisib might reverse MDR of the two cell lines by reducing the expression of MDR1 and MRP1.

In conclusion, Voxtalisib, a dual PI3K/mTOR inhibitor, exhibits anti-leukemia activity on K562 and HL60 cells as well as their MDR cell sub-lines K562/A02 and HL60/ADR through blockade of PI3K/Akt pathway and arrest of cell cycle at G1 phase. Moreover, Voxtalisib could reverse the drug resistance of K562/A02 and HL60/ADR cells by decreasing expression of MDR1 and MRP1. These results suggest that Voxtalisib has the potential to be applied for therapy of AML and CML in the future.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2018.04.089.

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