Effects of Vinblastine and Vincristine on the function of chronic myeloid leukemic cells through expression of A20 and CYLD

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ABSTRACT

Chronic myelogenous leukemia (CML) is characterised by the translocation of regions of the BCR and ABL genes, leading to the fusion gene BCR-ABL forming the Philadelphia (Ph) chromosome. Vinblastine (Vinb) and Vincristine (Vinc) are Vinca alkaloids and frequently used in combination chemotherapy in leukemias and lymphomas. Deubiquitinating enzyme (DUB) genes such as A20, Otubain 1 and CYLD are known as inhibitors of functional activation of immune cells mediated through the NF-kB/STAT pathway. Little is known about the regulatory role of Vinb/Vinc on the function of CML cells and the contribution of the DUBs to those effects. In the end, the gene expression profile was determined by quantitative RT-PCR, physiological properties of CML cells by flow cytometry and cytokine production by ELISA. As a result, inactivated expression of the DUBs A20, CYLD, Otubain 1 and Cezanne and enhanced activation of CD11b+ and CD4T cells were observed in CML patients. Importantly, Vinc enhanced the expression of A20 and CYLD and inhibited the proliferation and survival of CML (K562) cells. The effects were abolished in the presence of A20 siRNA, while cell proliferation only depended on the presence of CYLD. In conclusion, the up-regulation of A20 by Vinc could involve inhibitory effects on the proliferation and survival of K562 cells. The events might contribute to the anticancer effect of Vinc on A20-sensitive CML cells.

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Introduction

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder characterized by the uncontrolled growth of myeloid lineage cells in both bone marrow and peripheral blood. The disease is mostly caused by the translocation of regions of BCR and ABL genes, leading to the fusion gene BCR-ABL, which forms the Philadelphia (Ph) chromosome (1). Overexpression of BCR-ABL fusion protein leads to aberrant activation of Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway, resulting in uncontrolled proliferation and resistance to apoptosis of CML cells (2). In most cases, CML dramatically improved survival rate after the advent of tyrosine kinase inhibitors (TKIs), which bind to the kinase domain of BCR-ABL fusion protein, leading to the elimination of leukemic cells (3, 4).

Several deubiquitinating enzyme (DUB) genes such as A20 (Trfaip3), CYLD and Otubain 1 are considered as inflammatory and tumor suppressors by negatively regulating cell proliferation/differentiation and survival through deubiquitinating several key signaling proteins such as NF-kB and STATs (5-7). A20 is known to inhibit proliferation and induce apoptosis of CML cell lines, CML, and CD34+CML cells via miR-17-92 (8). A20 expression is down-regulated in lymphoma (6) and chronic lymphocytic leukemia (CLL) (9), whereas other investigations on glioblastoma, hepatocellular carcinoma, and acute lymphoblastic leukemia (ALL) cells reveal its increased expression (10, 11). Similar to A20, CYLD is also indicated as a critical mediator in cell survival in ALL and CLL cells (7, 12). The presence of OTUB1 is linked to poor migration of colorectal cancer (13) and OTUB1-deficient mice exhibit late embryonic lethality (5). Up to now, investigations on the effects of the DUB genes such as A20, CYLD, Otubain 1, Otubain 2 and Cezanne on the function of myeloid leukemic cells have been poorly studied.

Vinblastine (Vinb) and vincristine (Vinc) are Vinca alkaloids derived from the leaves of Catharanthus roseus and extensively used as anti-mitotic drugs in combination with chemotherapy in a variety of neoplasms, including leukemias and lymphomas (14). The difference between Vinb and Vinc is that Vinb has a methyl group, while Vinc has a formyl group on the indole nitrogen of the vindoline skeleton (15). The small difference between Vinb and Vinc leads to distinct toxicity, although they both exert potential anticancer effects by inhibiting the assembly of tubulin and disrupting microtubule function, which causes...
multinucleated cells and cell death (15, 16). Side-effects of Vinb consist of toxicity to the nervous system. Unlike the toxic effect of Vinc, Vinb toxicity usually involves the hematopoietic system (17) and neurotoxicity is less frequent with Vinb as compared with Vinc (17). The anticarcinogenic effects of the two products have been shown to be partly mediated through the activation of NF-κB and STAT pathways (18).

Little is known regarding the involvement of the DUB genes in the regulation of proliferation and apoptosis of CML (K562) cells upon challenge with Vinb/Vinc. The present study has thus been performed to elucidate whether the presence of A20 and CYLD contribute to their regulatory effects on the function of CML cells in response to Vinb/Vinc. To this end, the gene expression profile, activation, proliferation and survival of CML cells were investigated.

Materials and Methods

Patients and control subjects
Total peripheral blood samples from untreated 96 BCR/ABL-positive CML patients with newly diagnosed chronic phase and 80 normal healthy volunteers without CML signs, used as controls, were collected at the 103 Hospital, Military Medical University and the National Institute of Hematology and Blood Transfusion, Hanoi, Vietnam. The patients were diagnosed as CML according to the criteria of the World Health Organization (WHO) (19). No individuals in the control population took any medication or suffered from any known acute or chronic disease. All patients and volunteers gave written consent to participate in the study. Person care and experimental procedures were performed according to Vietnamese law for the welfare of human and were approved by the Ethical Committee of the Institute of Genome Research, Vietnam Academy of Science and Technology.

Isolation of CML cells and PBMCs
Peripheral blood mononuclear cells (PBMCs) from CML patients and healthy donors, respectively, were collected by venipuncture and transferred to sterile tubes. CML patients and healthy donors, respectively, were isolated by density gradient centrifugation (Ficoll-Paque Plus, GE Healthcare Life Sciences). The cells were next counted in a Neubauer chamber, washed with PBS and used for further experiments.

Drugs and culture of K562 cells
BCR/ABL positive CML cell line K562 was purchased from American Type Culture Collection and cultured in RPMI 1640 (GIBCO) containing: 10 % FCS, 1 % penicillin/streptomycin, and 1 % glutamine. Cells were maintained in cell culture dishes at 37°C with a humidified atmosphere containing 5% CO₂. All experiments were performed on the cells at passages 3-8 with 80%-90% confluence. Vincristine and Vinblastine were purchased from Sigma Aldrich and dissolved in DMSO. The drugs were stored at-20°C and protected from light.

Immunostaining and flow cytometry
The surface expressions of CD11b, CD86, CD40, CD3, CD4, CD8, CD19, CD21, CD25, CD44 and CD45 were determined by using ELISA kits (Thermo Scientific). IL-1β, TNF-α, and IL-6 concentrations were determined by using ELISA kits (Thermo Scientific) according to the manufacturer’s protocol.

RNA extraction and real-time RT-PCR
Total mRNA was isolated using the Qiashredder and RNeasy Mini Kit from Qiagen according to the manufacturer’s instructions. For cDNA first-strand synthesis, 1 µg of total RNA in 12.5 µl DEPC-H₂O was mixed with 1 µl of oligo-dT primer (Invitrogen) and heated for 2 min at 70°C. To determine transcript levels of A20, CYLD, Otubain 1, Otubain 2, Cezanne, SHP-1, SHP-2, STAT-1, STAT-3, STAT-5, STAT-6 and GAPDH, the quantitative real-time PCR with the LightCycler System (Roche Diagnostics)...

Table 1. Primer sequences used for the quantitative PCR experiments.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5’→3’)</th>
<th>Reverse (5’→3’)</th>
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<tr>
<td>A20</td>
<td>TCTCAGGCTTTGTATTGGA</td>
<td>TGTGTACGTGTCAGTGTTTT</td>
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<tr>
<td>CYLD</td>
<td>TCCCTTTCAAACCTCGTTG</td>
<td>AAATCCTCTTCATCATCA</td>
</tr>
<tr>
<td>Otubain 1</td>
<td>ACAAGAGATCAAGACCTCCA</td>
<td>CAACTCTTCGTGTATCCA</td>
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<tr>
<td>Otubain 2</td>
<td>CTACAGTGCGCCCTTCATCA</td>
<td>GCCATGCGGTCATCTTCGT</td>
</tr>
<tr>
<td>Cezanne</td>
<td>AACATGTCGAGTTGCGCAGT</td>
<td>ACAGTGGGATCCACTTCACATTC</td>
</tr>
<tr>
<td>CYLD</td>
<td>TGCCCTCTAACTCGTCTTG</td>
<td>AATCCCGCTTCCAGTAGG</td>
</tr>
<tr>
<td>SHP-1</td>
<td>GCC CAG TTC ATT GAA ACC AC</td>
<td>GAG GGA ACC CTT GCC CTT CT</td>
</tr>
<tr>
<td>SHP-2</td>
<td>GAGAGCAATGACGCGGACATCTG</td>
<td>CCTCCACAAACGTGTAATTC</td>
</tr>
<tr>
<td>STAT1</td>
<td>CCTCTCTGGCTTTGGGATTTGAA</td>
<td>TCTCCGGGAGGCTACTCAGTA</td>
</tr>
<tr>
<td>STAT3</td>
<td>GGA GGA GTT GCA GCA AAG AA</td>
<td>TGG TGT TGG GCC CAG AAT GT</td>
</tr>
<tr>
<td>STAT5</td>
<td>CAGACCAAGTTTGGCACCA</td>
<td>CACACGACTTTACTGACCAT</td>
</tr>
<tr>
<td>STAT6</td>
<td>GCCCACTCACCACGAGGACCT</td>
<td>GGCTGTGTCAGGAGAAGTGACAT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GGAGCGAGATCCCTCCAAA</td>
<td>GGCTGTGTCATACTTCTCAT</td>
</tr>
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was applied. The qPCR primer information in this study is summarized in Table 1. PCR reactions were performed in a final volume of 20 µl containing 2 µl cDNA, 2.4 µl MgCl, (3 µM), 1 µl primer mix (0.5 µM of both primers), 2 µl cDNA Master SybrGreen 1 mix (Roche Molecular Biochemicals), and 12.6 µl DEPC-treated water. The target DNA was amplified during 40 cycles of 95°C for 10 s, 62°C for 10 s, and 72°C for 16 s, each with a temperature transition rate of 20°C/s, a secondary target temperature of 50°C, and a step size of 0.5°C. Melting curve analysis was performed at 95°C, 0 s; 60°C, 10 s; 95°C, 0 s to determine the melting temperature of primer dimers and the specific PCR products. The ratio between the respective gene and corresponding GAPDH was calculated per sample according to the ΔΔ cycle threshold method (20).

Transfection of cells with siRNAs
Control- or A20- or CYLD-targeted siRNA (Thermo Fisher Scientific) was transfected into cells (10⁵ cells/ml) with the help of Lipofectamine RNAiMAX Reagent (Invitrogen) according to the manufacturer’s recommendations. After 48h transfection, cells were treated with Vinc or Vinc for another 24 h at 37°C, 5% CO₂. After washing three times with PBS, cells were used for experiments.

Proliferation assay
The cells were washed twice with PBS and then labeled with a 10-μM solution of 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) (2x10⁵ cells/mL) and incubated at 37°C/5% CO₂ for 10 minutes. The cells were washed and centrifuged to remove excessive CFSE. CFSE-labeled cells were cultured in 24-well plates (2x10⁵ cells/mL) at 37°C/5% CO₂ for 5 days. The cells were harvested, washed twice and then resuspended in FACS buffer, and the fluorescence was analyzed with flow cytometry.

Phosphatidylserine translocation and propidium iodide incorporation
The presence of phosphatidylserine (PS) on the outer surface of apoptotic cells was detected with fluorescein isothiocyanate (FITC)-conjugated annexin V binding to PS at the cell surface and necrosis was assessed from the amount of propidium iodide (PI)-positive cells. In brief, 10⁵ cells were harvested and washed twice with Annexin washing buffer (AWB). The cell pellet was resuspended in 100 µl of Annexin V/PI labelling solution (eBioscience), and incubated for 15 min at room temperature. After washing with AWB, the cells were analyzed by flow cytometry.

Determination of ROS production
ROS production in CML cells was determined utilizing 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA, Sigma). After the treatment, the cells were collected and DCFDA was added to cell suspension at a final concentration of 10 µM. After 30 minutes of incubation in the dark at 37°C, cells were centrifuged and the pellet was washed twice with ice-cold PBS. The pellet was then resuspended in FACS buffer and the fluorescence was analyzed with flow cytometry.

Statistics
Data are provided as means ± SEM, n represents the number of independent experiments. Differences were tested for significance using Student’s unpaired two-tailed t-test or ANOVA, as appropriate. P<0.05 was considered statistically significant.

Results
Clinical correlations
Clinical features of the CML patient group are listed in Table 1. Results indicated that the percentage of female CML patients was 37%. The median age of CML patients at diagnosis was 46.71 years. Among clinical biomarkers assessed in Table 2, CML patients had all median total protein, Ferritin and LDH levels higher than normal values. Importantly, the percentages of CML patients with Ferritin and LDH levels higher than their clinical cutoff were

<table>
<thead>
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<th>Table 2. Clinical characteristics of CML patients.</th>
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<tr>
<td>Characteristics</td>
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<tr>
<td>Mean age at diagnosis (range)</td>
</tr>
<tr>
<td>Female (%)</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
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<tr>
<td>Glucose (mmol/l)</td>
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<tr>
<td>Creatinine (µmol/l)</td>
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<td>Total bilirubin (µmol/l)</td>
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<tr>
<td>Total protein (g/l)</td>
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<tr>
<td>Albumin (g/l)</td>
</tr>
<tr>
<td>Globulin (g/l)</td>
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<tr>
<td>Ferritin (µg/l)</td>
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<td>LDH (U/l)</td>
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AST: aspartate transaminase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.
48.96 and 51.04%, respectively.

**Analysis of gene expression profile in CML cells**

We firstly examined the expression of the DUBs genes and found that mRNA levels of A20, CYLD, Cezanne and Otubain1 were significantly down-regulated in CML cells. However, no difference in transcriptional expression of Otubain2 gene in the patient and control groups was detected (Figure 1A).

A20, CYLD and Otubain1 are known to inhibit inflammatory response through several signalling pathways, including SHPs, NF-κBs and STATs (5, 21, 22). Similar to the DUBs, the mRNA level of SHP-1 was significantly lower, while the expression of SHP-2 was significantly higher in the CML group compared to controls (Figure 1B). In agreement, several previous studies on patients with multiple myeloma and T-cell leukemia reported an inactivated expression of SHP-1 (23, 24), which is linked to the activation of the JAK/STAT pathway in these patients. As expected, mRNA levels of STAT1 and STAT6, but not STAT3 and STAT5, were significantly increased in CML cells (Figure 1C).

**Immunophenotypic change of CML cells**

Next, we investigated changes in activation of myeloid (CD11b+), CD4 T (CD3+CD4+), CD8 T (CD3+CD8+) and B (CD19+) cells present in CML by flow cytometry. The CD45+ cells were gated in all experiments. Flow cytometry analysis showed that numbers of CD11b+CD86+ and CD3+CD4+CD25+ expressing cells were significantly accumulated, whereas percentages of CD11b+CD40+CD3+CD4+CD44+, CD3+CD8+CD44+, CD19+CD25+ and CD3+CD4+CD25+ cells were lower in CML than that in healthy donors (Figure 2A-B). In addition, the percentage of CD3+CD8+CD25+ cells was higher in CML than that in controls but not reaching the statistical difference (Figure 2C).

**Effects of Vinb and Vinc on proliferation and survival of K562 cells**

Vinb and Vinc are known to induce apoptosis of lymphocytic leukemia and lymphoma cells (25, 26); therefore, we conducted experiments to ask whether the influence of Vinb (20-120nM) or Vinc (60-300nM) on the function of CML (K562) cells. As shown in Figure 3A, treatment of K562 cells with Vinb (120nM)/Vinc (300nM) affected the proliferation of K562 cells.

For cytokine analysis of sera, levels of IL-6, TNF-α, and IL-1β were measured by ELISA. Results showed that IL-6 and TNF-α concentrations in CML patients were found to be higher than in control individuals. However, CML patients showed no change in the serum level of IL-1β (Figure 2C). The evidences indicated that inactivated expression of A20, CYLD, Cezanne and Otubain1 in CML cells could be involved in the activation of CD11b+ and CD4 T cells and secretion of IL-6 and TNF-α in the circulatory system.
and survival of K562 cells rather than their activation and maturation.

**Effect of A20 and CYLD on the proliferation of Vinc-treated K562 cells**

Since expression of A20, CYLD, Cezanne and Otubain1 was downregulated in CML cells. Hence, we examined levels of the DUB genes when K562 cells were exposed to Vinc (20-120 nM) or Vinc (60-300 nM). Results indicated that mRNA levels of A20 and CYLD, but not Cezanne and Otubain1 were significantly higher when the cells were treated with Vinc at a concentration of 300nM (Figure 4A). In addition, the expression of the other DUBs was unaltered in the presence of Vinc (data not shown).

To ask whether the changes in the function of K562 cells are related to the expression of A20 and CYLD. The cells were transfected with control or A20 or CYLD siRNA and followed by Vinc (300nM) treatment. The efficiency of transcriptional expression was tested by real-time PCR, and results showed that A20/CYLD siRNA effectively decreased the expression of A20/CYLD in PBMCs (Figure 4B).

To examine whether the proliferation of K562 cells when exposed with Vinc (300nM) is related to expression of A20 and CYLD, the cells were stained with CFSE and cultured for 5 days. As shown in Figure 4C-D, the proliferation activity of K562 cells was markedly reduced when treated with Vinc. The effect was abolished in the presence of A20 or CYLD siRNA, pointing out that the inhibitory effect of Vinc on cell proliferation was sensitive to the presence of A20 and CYLD.

**Effect of A20 on Vinc-treated K562 cell death**

To examine whether the diminished proliferation rate of K562 cells is related to cell death, experiments using Annexin V/PI and DCFDA stainings were conducted in these cells. As seen in Figure 5A-D, treatment of Vinc (300nM) significantly enhanced the number of Annexin V/PI cells and accumulation of ROS. The effects were attenuated in the presence of A20 siRNA, but not CYLD siRNA.

**Discussion**

Inactivated expression of A20, CYLD, Cezanne and Otubain1 genes in CML patients was revealed for the first time. Recently, inactivation of A20 has been found in patients with acute lymphocytic leukemia (ALL) (27),

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**Figure 3. Effect of Vinc/Vinc on the function of K562 cells. A.** Arithmetic means ± SEM (n = 5 each) of CFSE−, Annexin V−/PI and ROS− cells, which were either untreated (white bars) or treated with Vinc (20, 60 and 120 nM, grey bars) or Vinc (60, 150 and 300 nM, black bars). * (p<0.05), ** (p<0.01) and *** (p<0.001) indicate significant differences from controls (ANOVA). B. Arithmetic means ± SEM (n = 5 each) of IL-6 and TNF-α concentrations are attained from supernatants of K562 cells, which were either untreated (white bars) or treated with Vinc (20, 60 and 120 nM, grey bars) or Vinc (60, 150 and 300 nM, black bars).

**Figure 4. Effect of A20 and CYLD on the proliferation of K562 cells. A.** Arithmetic means ± SEM (n = 5 each) of transcript levels of A20 and CYLD in K562 cells, which were either untreated (white bars) or Vinc (60, 150 and 300 nM, black bars). ** (p<0.01) indicates a significant difference from controls (ANOVA). B. Arithmetic means ± SEM (n = 5 each) of transcript levels of A20 and CYLD in PBMCs, which were transfected with control siRNA (white bars) or A20 siRNA/CYLD siRNA (40nM, black bars). GAPDH was used as a reference gene for relative quantification. * (p<0.05) indicates a significant difference from controls (ANOVA). C. Representative FACS histograms depicting CFSE fluorescence are attained from K562 cells, which were untreated or treated with Vinc (300 nM) in the presence or absence of A20 siRNA/CYLD siRNA (40nM). D. Arithmetic means ± SEM (n = 5 each) of CFSE positive cells, which were untreated (white bar) or treated with Vinc (black bars, 300 nM) in the presence or absence of A20 siRNA/CYLD siRNA (40nM). * (p<0.05) indicates significant difference from controls; † (p<0.05) and ‡ (p<0.01) indicate significant differences from Vinc-treated K562 cells (ANOVA).

**Figure 5. Effect of A20 and CYLD on survival of K562 cells. A, C.** Representative FACS histograms depicting Annexin V (A) and DCFDA (C) fluorescence were attained from K562 cells, which were untreated or treated with Vinc (300 nM) in the presence or absence of A20 siRNA (40nM). B, D. Arithmetic means ± SEM (n = 5 each) of Annexin V/PI (B) and ROS (D) cells, which were untreated (white bar) or treated with Vinc (black bars, 300 nM) in the presence or absence of A20 siRNA (40nM). ** (p<0.01) indicates significant difference from controls; † (p<0.05) and ‡ (p<0.01) indicate significant differences from Vinc-treated K562 cells (ANOVA).
Cezanne downregulation is linked to poor prognosis in hepatocellular carcinoma (28) and aberrant expression in CYLD gene is reported in leukemia and lymphoma (29, 30). A20, CYLD and Otubain1 are considered as negative regulators of immune reaction through NF-kB and/or STATs pathways (5, 21, 22), whose constitutive activation is related to the development of hematologic malignancy (2). As expected, we observed that the expression of STAT1 and STAT6 was significantly enhanced in CML patients as compared to controls. Activated STAT6 has been found in patients with solid cancers, lymphoma and leukemia and is involved in cellular proliferation and transformation (31). Moreover, we indicated that the level of SHP-1 was lower, while SHP-2 expression was higher in CML patients compared to controls. Consistently, several studies reported the inactivated expression of SHP-1 in hematological malignancies (23, 32) and SHP-2 phosphorylation is associated with imatinib-resistance phenotype in CML patients (33).

The immunophenotypic profile of CML cells indicated that CD11b+CD86+ and CD3’CD4’CD25’ expressing cells were recruited into the circulatory system. Recent studies show that level of CD86 is highly expressed in leukemia (34) and associated with T-cell exhaustion and relapse risk (35). The account of CD86-positive plasmacytoid dendritic cells is higher in CML cells than in normal PBMCs (35). Differently, the expression of CD40 has diverse effects on cell function. It is linked to inducing an immune response (36) and increasing malignant cell growth, survival, and immunogenicity (37). The level of CD25 is also indicated to express at a higher level on CML stem cells (38) and is associated with poor prognosis in CML (39). Gene expression is a process in which the information contained in the gene is used to obtain a functional product from it (40-42).

The promoting roles of Vinb and Vinc on cell death in lymphocytic leukemia and lymphoma are shown in several studies (25, 26). In this study, we observed the inhibitory effects of Vinb and Vinc on the proliferation and survival of K562 cells, as the reduced expression of CFSE-positive cells and the increased number of Annexin V+PI cells and ROS accumulation were detected in the presence of Vinb (120nM) or Vinc (300nM). Vinb and Vinc are known to exert inhibitory effects on cell proliferation by binding to the microtubules to prevent the development of the mitotic spindle (43). In addition, we showed that Vinb/Vinc did not affect the activation and maturation of K562 cells.

Importantly, the mRNA levels of A20 and CYLD were significantly enhanced in K562 cells and slightly increased in PBMCs (data not shown) when treated with Vinc. Therefore, the cells were silenced by using A20 or CYLD siRNA. As expected, the inhibitory effects on proliferation and survival of K562 cells were abolished by the presence of A20 siRNA and the cell proliferation only was sensitive to CYLD expression upon challenge with Vinc. This finding is supported by other reports indicating that the promoting effect of A20 on cell death has been investigated in several cell types, including lymphoma (6), CLL (9), and myeloid leukemia cells (8), whereas A20 is considered as a negative regulator of cell apoptosis in glioblastoma, hepatocellular carcinoma, and ALL (10, 11). Besides, A20 is also known to suppress cell proliferation and metastasis (8). In comparison with A20, the role of CYLD was a less potent effector in modulating the apoptosis of K562 cells. Up to now, there has been no report about the regulatory role of CYLD on the function of myeloid leukemic cells, although CYLD is reported as a positive regulator of cell apoptosis in ALL and CLL cells (7, 12). In addition, mRNA levels of A20 and CYLD were unaffected upon treatment of K562 cells with Vinb (data not shown). Clearly, Vinb and Vinc differ in their regulation of cellular biological activities. Vinc has the potential to induce neurotoxicity, while hematologic toxicity is rarely related to Vinc (17).

In conclusion, the present study discloses that Vinc inhibits cell proliferation and survival of K562 cells through upregulated A20 expression. The events might contribute to the antitumor effect of Vinc on A20-sensitive CML cells.

Competing interests
The authors of this paper declare that they have no financial/confidential conflicts of interest.

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References


