Daunorubicin induces Cysteine-rich protein 61 to decreases the Chemosensitivity through the ATM/NF-κB pathway in B-acute lymphoblastic leukemia

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Abstract

Background: Acute lymphoblastic leukemia（ALL）is the second most common hematologic malignancy worldwide with B-acute lymphoblastic leukemia（B-ALL）accounting for 70%-80%. Cysteine-rich protein 61 (Cyr61), a potential tumor-promoting factor, is increased in both serum and bone marrow of B-ALL patients in our previous study. We aimed to elucidate the role of Cyr61 in B-ALL as well as to explore the source of Cyr61 in the bone marrow of B-ALL patients.

Methods: The human B-ALL cell line Nalm-6 was used. Cyr61 expression levels were measured by using quantitative real-time PCR (qRT-PCR), enzyme-linked immunosorbent assay (ELISA) and western blot analysis. The biological functions of Cyr61 in regulating B-ALL cell chemosensitivity to DNR and VCR was detected by cell viability assay and flow cytometry analysis. The production mechanisms of Cyr61 in the bone marrow were measured by qRT-PCR and western blot analysis.

Results: Knockdown of Cyr61 increased the chemosensitivity of Nalm-6 cells to DNR and VCR, and overexpression of Cyr61 decreased the chemosensitivity of Nalm-6 cells to DNR and VCR. Mechanistically, we found that Cyr61 attenuated chemotherapeutic drug-induced apoptosis by the upregulation of Bcl-2. Importantly, Cyr61 can be up-regulated by the chemotherapeutic drug DNR through the ATM-dependent NF-κ B pathway, however, VCR has no effect on the expression of Cyr61 in B-All cells.

Conclusions: In conclusion, our study revealed that DNR can induce the production of Cyr61 in B-ALL cells; further, increasing Cyr61 decreased the chemosensitivity of B-ALL cells to chemotherapeutic drugs. Thus, targeting Cyr61 may be a promising therapeutic strategy to increase the chemotherapy sensitivity in patients with B-ALL.

Background

Acute lymphoblastic leukemia(ALL), the second most common hematologic malignancy worldwide, originates from an uncontrolled clonal proliferations of immature lymphocytes in bone marrow with B-acute lymphoblastic leukemia（B-ALL）accounting for 70%-80%[1]. Despite, combination chemotherapy has achieved good clinical outcomes on B-ALL, there still remain some patients with chemotherapy insensitivity; moreover, currently available drugs have limited benefits for patients who relapse[2]. Therefore, there is an urgent need to clear the recurrence mechanism of the patients with B-ALL to improve the prognosis and long-term disease-free survival (DFS).

A large number of studies indicate that the bone marrow microenvironment plays an indispensable role in leukemia [3]. Abnormal bone marrow microenvironment favors the production of cytokines, chemokines and matrix proteins to maintain the malignant cloning of leukemia cells and decrease the sensitivity of chemotherapeutic drugs [4–8].

Cysteine-rich protein 61 (Cyr61/CCN1), one of the CCN (Cyr61/CTGF/NOV) family members, is highly expressed in a variety of tumors [9–13], moreover, patients with Cyr61 high expression are indicative of

poor prognosis in the clinic [14–19]. Our previous study showed that the expression of Cyr61 is elevated in patients with ALL and its concentrations are positively correlated with the proportions of leukemia cells in bone marrow [20]. Interestingly, in a follow-up study, it was found that Cyr61 can decrease the chemosensitivity of ALL cells to cytarabine [21]. It is well known that DNR and VCR are first-line chemotherapy agents for ALL. However, the effects of Cyr61 on the chemosensitivity of DNR and VCR are unclear; moreover, the source of Cyr61 in bone marrow remains largely unknown.

Studies suggest that chemotherapy drugs can induce cells to produce soluble molecules (such as cytokines, growth factors, matrix proteins, etc.) to protect tumor cells from the cytotoxicity of chemotherapy drugs [22–24]. Gilbert *et.* find that doxorubicin can cause DNA damage, which activates the thymic epithelial cells to induce the production of IL-6 [23]. Zhong *et.* reveal that rituximab induce diffuse large B-cell lymphoma (DLBCL) cells to release IL-6, which protects DLBCL cells from drug-induced apoptosis [25]. However, whether chemotherapy can induce B-ALL cells to release Cyr61 remains largely unknown.

In this study, we found that Cyr61 can decrease the chemotherapeutic sensitivity of VCR and DNR through up-regulating the expression of Bcl-2. Furthermore, Cyr61 had been found to be elevated after DNR stimulation in B-ALL cells through the ATM-dependent NF-κB pathway, but VCR has no such effect. Collectively, our study further cleared the biological function of Cyr61 on B-ALL and elucidated the molecular mechanism of releasing Cyr61 by B-ALL cells in bone marrow, and targeting Cyr61 might be a promising therapeutic strategy to improve the chemosensitivity in patients with B-ALL.

Materials And Methods

Patients and specimens

The peripheral blood of B-ALL patients at initial diagnosis was collected from Fujian Medical University Union Hospital, Fuzhou, China. These studies were performed in accordance with the ethical guidelines under the protocols approved by the Institutional Medical Ethics Review Board of Fujian Medical University Union Hospital, Fuzhou, China. The human acute B-lymphocytic leukemia cell line Nalm-6 was cultured with RPMI 1640 medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) and 1% penicillin/streptomycin (HyClone, Logan, UT, USA) in a 37°C incubator supplied with 5% CO_2. The cell line was routinely checked by mycoplasma contamination test, and was recently evaluated using short Tandem Repeat (STR) DNA profiling.

Drugs and antibodies

DNR and VCR (Selleckchem, Houston, TX, USA) were dissolved in dimethyl sulfoxide (DMSO) according to the supplier's guidelines. Drugs were stored in aliquots at − 20°C. Cyr61 monoclonal antibody (093G9) was donated by Dr. Li Ningli (Medical College of Shanghai Jiaotong University, Shanghai, China). Other antibodies were all purchased from Cell signaling Technology.

Enzyme-linked immunosorbent assay (ELISA)

The concentration of Cyr61 in the culture supernatant of Nalm-6 cells was determined by using human Cyr61 ELISA Systems (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

Cell viability assay

To explore the effect of VCR and DNR on the growth of Nalm-6-shCyr61, Nalm-6-shNC, Nalm-6-LV-Cyr61 and Nalm-6-LV-NC cells, these cells were cultured in RPMI 1640 medium containing different concentration of VCR and DNR for 24h. Cell viability was measured by using Cell Counting Kit-8 (CCK8, Beyotime Biotechnology, Jiangsu, China) according to kit instructions. In brief, 5.0×10^3 cells were seeded into 96-well plate for 24h, and 10 μL CCK8 reagents were added into each well for another 2h. Then, optical density (OD) of plates was determined at 450 nm using a microplate reader (BIO-TEK), and the 50% inhibitory concentration (IC50) values for VCR and DNR were obtained. Each sample was assayed in triplicate and the experiments were repeated three times.

Western blot analysis

Nalm-6 cells were collected and washed with ice-cold PBS. The cells were added to RIPA lysis buffer for 20 minutes. Western blotting (Protein immunoblotting) is described early[13, 26, 27]. The following antibodies were used in this study: anti-human cyr61 monoclonal antibody (093G9) was donated by Dr. Li Ningli (Medical College of Shanghai Jiao tong University, Shanghai, China). Anti-NF-κBp65 (4764; Cell Signaling Technology, Danvers, MA, USA), anti-P- NF-κBp65 (3033; Cell Signaling Technology, Danvers, MA, USA), anti-Bcl-2 (4233; Cell Signaling Technology, Danvers, MA, USA), anti-PI3K/AKT (9272; Cell Signaling Technology, Danvers, MA, USA), anti-p- PI3K/AKT (9271; Cell Signaling Technology, Danvers, MA, USA), anti-p38 MAPK (9212; Cell Signaling Technology, Danvers, MA, USA), anti-p-p38 MAPK (9211; Cell Signaling Technology, Danvers, MA, USA), anti-ERK(p44/42) (9202; Cell Signaling Technology, Danvers, MA, USA), anti-p-ERK(p44/42) (9201; Cell Signaling Technology, Danvers, MA, USA).

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from specimens using RNAeasy™ Animal RNA Isolation Kit with Spin Column (Beyotime, Shanghai, China) according to the manufacturer's instructions. Total RNA (1 μg) was reverse-transcribed into first-strand cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). Real-time PCR was carried out using SYBR Green Master Mix (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. The primers used in this study were as follows: Cyr61 forward: TCCAGCCCAACTGTAAACATCA reverse: GGACACAGAGGAATGCAGCC; GAPDH forward: CACATGGCCTCCAAGGAGTA revers: TGAGGGTCTCTCTCTTCCTCTTGT; Bcl-2 forward: CTGGTGGGAGCTTGCATCAC revers: ACAGCCTGCAGCTTTGTTTC; Bcl-xl forward: TCAGGCTGCTTGGGATAAAGAT revers: AGAGGCTTCTGGAGGACATTTG; Survivin forward: GGAAGAAGTAGCGTCACTC revers: TGACGACCCCATAGAGGAACA; XIAP forward: TTGAGGAGTGTCTGGTAAG revers: CCATTCGTATAGCTTCTTGT.

Apoptosis assay

Apoptotic Nalm-6 cells were detected by APC Annexin-V Apoptosis Detection Kit according to the manufacturers' instructions (BD Biosciences, Franklin Lakes, NJ, USA). Briefly, 5.0×10^5 cells were washed with ice-cold PBS, resuspended in 195 μL binding buffer, and stained for 10 minutes at room temperature with 5 μL APC conjugated anti-Annexin-V antibody. Unbound Annexin-V antibody was removed by washing with binding buffer. Percentage of apoptotic Nalm-6 cells (Annexin-V positive) was determined by flow cytometry analysis. The cell apoptosis rate (Annexin-V positive) was determined by FACSCanto II cytometer (BD Biosciences, San Jose, CA).

Cyr61 knockdown

Cyr61 knockdown was done as described previously [26]. Briefly, Nalm-6 cells (5×10^4 cells/mL) were infected by lentivirus carrying shNC or shCyr61 (Shanghai GeneChem Co., Ltd, Shanghai, China). The infection efficiency of cells was ensured to be > 95% before selection with 4 μg/mL puromycin (Sigma-Aldrich, St. Louis, MO) for 5 days. The knockdown efficiency of Cyr61 was measured by western blot analysis and the frequency of GFP^+ cells was measured by flow cytometry.

Cyr61 overexpression

The lentivirus particles containing the Cyr61 sequence were purchased from Shanghai GeneChem Co., Ltd. (Shanghai, China). First, Nalm-6 cells (5×10^4 cells/mL) were infected with the lentivirus particles containing the Cyr61 sequence for 18-24h according to the manufacturer's protocol. The infection efficiency of Nalm-6 cells was confirmed to be > 95% before selection with 4 μg/mL puromycin (Sigma-Aldrich, St. Louis, MO) for 5 days. The knockdown efficiency of Cyr61 was measured by western blot analysis and the frequency of GFP^+ cells was measured by flow cytometry.

Statistical analysis

SPSS 22.0 statistical software (Version 22.0 SPSS, Chicago, IL; USA) was used for statistical analysis. All data was expressed as mean ± standard errors of the mean (SEM). Student's test was used to compare the two groups, A value of $P < 0.05$ was statistically significant (*$P < 0.05$; **$P < 0.01$).

Results

Cyr61 decreased the chemosensitivity of B-ALL cells to DNR and VCR

Since Cyr61 can decrease the chemosensitivity of ALL cells to cytarabine [21], moreover, DNR and VCR have limited benefits for patients with B-ALL who relapse [28–31]; therefore, there is a need to determine whether Cyr61 can decrease the chemosensitivity of DNR and VCR in B-ALL cells. The plasmids with Cyr61 overexpression and low expression were respectively constructed in Nalm-6 (acute B-lymphocytic leukemia cell line) cells via lentiviral infection and a CCK8 assay was used to measure the cell viability. Compared with the Nalm-6-shNC cell group (51.63 ± 4.438 ng/mL), the Nalm-6-shCyr61 cell group (33.50 ± 1.432 ng/mL) showed a lower IC50 value to DNR, which indicated that knockdown of Cyr61 increased

the chemosensitivity of Nalm-6 cells to DNR (Fig. 1A). Furthermore, compared with the Nalm-6-shNC cell group (37.51 ± 3.780 ng/mL), the Nalm-6-LV-Cyr61 cell group (52.87 ± 4.280 ng/mL) showed a higher IC50 value to DNR, which indicated that overexpression of Cyr61 decreased the chemosensitivity of Nalm-6 cells to DNR (Fig. 1B). Additionally, we also observed that the IC50 of VCR in Nalm-6-shCyr61 cells group (1.12 ± 0.39 ng/mL) was significantly lower than the control group (7.14 ± 3.77 ng/mL) (Fig. 1C); Moreover, the IC50 of VCR in Nalm-6-LV-Cyr61 cells group (34.10 ± 13.29 ng/mL) was significantly higher than the control group (2.30 ± 1.25 ng/mL) (Fig. 1D). These results indicated that Cyr61 could reduce the chemosensitivity of B-ALL cells to DNR and VCR.

Cyr61 decreased the apoptosis of B-ALL cells induced by DNR and VCR

Accumulating evidence suggests that Cyr61, also known as potential tumor-promoting factor, has been involved in chemo-resistance and relapse in multiple cancers [14–19]. However, the mechanism of Cyr61 decreasing the chemosensitivity of B-ALL cells remain unclear. Since multiple factors are contributed to drug resistance, including decreasing drug-induced cell apoptosis, up-regulating drug resistance genes and activating drug efflux systems [32, 33]; therefore, we explored the apoptosis condition of B-ALL cells with stimulation of DNR and VCR, as well as examined the effect of Cyr61-antibody (093G9). The result showed that knockdown of Cyr61 in Nalm-6 cells increased DNR and VCR-induced apoptosis (Fig. 2A, 2D); conversely, overexpression of Cyr61 in Nalm-6 cells decreased DNR and VCR-induced apoptosis (Fig. 2B, 2E). Additionally, we also observed that anti-Cyr61 antibody (093G9) could increase the DNR and VCR-induced apoptosis (Fig. 2C, 2F). Together, these results indicated that Cyr61 was involved in decreasing the chemosensitivity of B-ALL cells through decreasing the apoptosis induced by DNR and VCR.

Cyr61 can regulate the expression of Bcl-2

As suggested by the above experiments, Cyr61 could decrease the apoptosis of B-ALL cells induced by DNR and VCR. However, its mechanism remains poorly characterized. As Bcl-2 family molecules are important regulators of cellular apoptosis [34–36]; therefore, we detected the mRNA expressions of Bcl-2, Bcl-xl, Survivin and XIPA in Cyr61-knockdown Nalm-6 cells and DNR-treated Nalm-6 cells. Fortunately, we observed that the mRNA expression of Bcl-2 was decreased in Cyr61-knockdown Nalm-6 cells and was increased in Cyr61-overexpression Nalm-6 cells with or without DNR-treated, however, the expressions of Bcl-xl, Survivin and XIPA mRNA were not significantly changed (Fig. 3A, 3C). In addition, we also observed that the expression of Bcl-2 protein was significantly decreased in Cyr61-knockdown Nalm-6 cells and increased in Cyr61-overexpression Nalm-6 cells with or without DNR-treated (Fig. 3B, 3D). Together, considering that Bcl-2 is an anti-apoptotic protein, these results suggested that Cyr61 impaired the apoptosis induced by DNR and VCR possibly through the upregulation of Bcl-2.

DNR up-regulated the production of Cyr61 in B-ALL cells

Studies reveal that chemotherapeutic drugs promote tumor cells or stromal cells to generate a stronger barrier against cytotoxic effects of these chemotherapeutic drugs via producing large amounts of growth factors, cytokines and antiapoptotic proteins [23, 37–40]. However, whether DNR and VCR can induce tumor cells to release Cyr61 remain unclear, thus, we treated Nalm-6 cells with different concentrations of DNR and VCR. As shown in Fig. 4A, the mRNA expression of Cyr61 was significantly increased with the stimulation of DNR, and peaked at 2.5μg/mL; however, VCR had no significant effect on it (Fig. 4B). Additionally, we also observed that DNR induced Nalm-6 cells to produce Cyr61 mRNA in a time-dependent manner (Fig. 4C). Moreover, the protein levels of Cyr61 were significantly increased in DNR-treated Nalm-6 cells (Fig. 4D, 4E). Importantly, we were glad to found that the Cyr61 mRNA expression was increased in DNR-treated peripheral blood lymphocytes from newly diagnosed B-ALL patients (Fig. 4F). Taken together, these results clearly demonstrated that DNR could up-regulate the production of Cyr61 in B-ALL cells.

DNR induced Cyr61 through the ATM/NF-κB signaling pathway

Unfortunately, there is a paucity of research on the mechanism of Cyr61 expression. However, since several studies show that PI3K/AKT, p38 MAPK, ERK(p44/p42), and NF-κB signaling pathways are associated with the production of anti-apoptotic proteins [41–44], therefore, we decided to test these several pathways treated with DNR. Delightfully, we found that the phosphorylation of NF-κB/p65 was markedly increased in DNR-treated Nalm-6 cells; however, the phosphorylation of PI3K/AKT, p38 MAPK and ERK (p44/p42) had no significant change (Fig. 5A). Additionally, we observed that the expression of Cyr61 in Nalm-6 cells pretreated with NF-κB/p65 inhibitor (PDTC) was decreased in respond to the stimulation of DNR (Fig. 5B). Together, these results indicated that the activation of NF-κB pathways is involved in DNR-induced Cyr61 production in B-ALL cells.

As an anthracycline antibiotic, DNR is known as non-specific cell cycle drug causing strong DNA damage response (DDR) [45]. Therefore, combining with studies about drug-resistance induced by chemotherapy drugs [22–24], we inferred that DNA damage response may be involved in DNR-induced Cyr61 production in B-ALL cells. As expected, the phosphorylation of ATM and H2A·X, the markers of DNA damage response, was significantly increased in DNR-treated Nalm-6 cells (Fig. 6A). Some studies indicate that DDR can activate NF-κB pathway [46]. Fortunately, we also observed that the phosphorylation of NF-κB was down-regulated accompanied with a marked reduction of Cyr61 in Nalm-6 cells were pretreated with KU55933 (an inhibitor of ATM phosphorylation) (Fig. 6B). Together, these results suggested that DNR-induced Cyr61 production depend on the ATM/NF-κB signaling pathway.

Discussion

ALL is the second most common hematologic malignancy of which patients with B-ALL account for 80% [1]. Despite combination chemotherapy has achieved remarkable success on B-ALL; however, about 20 percent of patients with B-ALL still show insensitive to chemotherapy drugs [2]. As is well-know, the

ultimate goal of clinical therapy is to overcome the drug-resistance and reduce the recurrence. In this study, we demonstrated for the first time that Cyr61 can decrease the chemosensitivity of B-ALL cells to both DNR and VCR; Simultaneously, we also found that DNR can induce the production of Cyr61 in B-ALL cells through the ATM-dependent NF-κB pathway.

Cyr61 is a matrix protein and a member of CCN (Cyr61/CTGF/NOV) family. Cy61 plays an important role in maintaining the normal physiological function of the body [47–54]. Recent studies have found that Cyr61 contributes to the occurrence and development of tumors [9–13]. Our previous study has shown that the level of Cyr61 in both serum and bone marrow of ALL patients is increased, and the increasing Cyr61 can promote ALL cell survival [21]. However, the role of Cyr61 in the chemosensitivity of B-ALL cells and the origin of Cyr61 in bone marrow remain unclear.

Although, DNR and VCR, common chemotherapy drugs for hematologic tumors, have good clinical response to B-ALL patients, however, there still remain some patients to develop drug resistance to therapy [29]. In this study, we were surprised to find that Cyr61 could decrease chemosensitivity of B-ALL cells to DNR and VCR through regulating the cell apoptosis. Combining with our previous study [20], we confirmed that Cyr61 would play an important role in inducing multidrug resistance by affecting chemotherapy-induced apoptosis in B-ALL cells as well as blocking the function of Cyr61 maybe an interesting target for B-ALL.

It is well known that the regulation of cellular apoptosis is determined by the balance of anti-apoptotic and pro-apoptotic factors of Bcl-2 family proteins [55, 56]; therefore, we evaluated the effect of Cyr61 on the expression of Bcl-2, Bcl-xL, XIAP, and Survivin; Interestingly, our data indicated that Cyr61 can increase the expression of Bcl-2 without affecting Bcl-xL, XIAP, and Survivin levels. Our findings reported here were consistent with our previous results in which Cyr61 can promote the survival of chronic myeloid leukemia (CML) cells by upregulating Bcl-2 expression [57]. Considering that Bcl-2 is the classical antiapoptotic protein of the Bcl-2 family [58], we propose that Cyr61 decrease the apoptosis of B-ALL cells through the Bcl-2 pathway.

There is no doubt that Cyr61 plays an important role in decreasing the chemosensitivity of DNR and VCR in B-ALL; however, how this protein is produced in bone marrow remains enigmatic. Many studies have indicated that chemotherapy can induce drug resistance via producing a variety of cytokines, which can shelter the tumor cells from chemotherapy drugs [29, 37, 59]; Delightfully, as expected, our study showed that DNR, known as common first-line chemotherapy drug for B-ALL, could up-regulate the expression of Cyr61 in Nalm-6 cells through ATM/ NF-κB pathway. We speculated that inducing B-ALL cells to release Cyr61 by DNR might be a self-protective mechanism by tumor cells for survival; moreover, the increased Cyr61 could protect B-ALL cells from the drugs killing effects, which explained the high recurrence rate in later clinical treatment as well as the mechanism of minimal residual leukemia (MRL). In summary, our study has indicated that up-regulation of Cyr61 expression in Nalm-6 cells by DNR may proceed via ATM/NF-κB pathway and this may provide a target for us to clear the chemo-resistant remnant B-ALL cells.

DNR is one of anthracycline antibiotics and also belongs to cell cycle non-specific drug which can cause strong DNA damage respond (DDR). ATM, also known as a central molecule for DDR, can lead to a cell-cycle delay, which facilitates DNA repair prior to replication [55–57]. ATM downstream molecule NF-κB mediates the abnormal expression of anti-apoptotic genes in leukemic cells [58]. Based on the studies presented above, we speculated that DNR may up-regulate the expression of Cyr61 in Nalm-6 cells through ATM/ NF-κB pathway. In this study, our data confirmed this assumption, and it was found that Nalm-6 cells had obvious DDR under the stimulation of DNR, the phosphorylation of ATM had been significantly increased and the expression of Cyr61 had been up-regulated after NF-κB activating

Taken together, our data showed that the Cyr61 can reduce the chemosensitivity of DNR and VCR in B-ALL cells via activating Bcl-2; moreover, DNR can induce the production of Cyr61 in B-ALL cells via an ATM-dependent NF-κB pathway. Blocking the Cyr61 expression in B-ALL cells may overcome the protection effect of therapy-induced BM niche and potentially prevent the occurrence of B-ALL relapse, which thereby significantly improve the efficacy of chemotherapeutics.

Abbreviations

ALL
Acute lymphoblastic leukemia
B-ALL
B-acute lymphoblastic leukemia
Cyr61
Cysteine-rich protein 61
DNR
daunorubicin
VCR
vincristine
DFS
disease-free survival
qRT-PCR
Quantitative real-time PCR
ELISA
Enzyme-linked immunosorbent assay
IL-6
interleukin-6
DLBCL
diffuse large B-cell lymphoma
DMSO
Dimethyl sulfoxide
XIAP
X-linked inhibitor of apoptosis

NF-κB
Nuclear factor kappa B
PI3K/AKT
Phosphatidylinositol-3-kinase
ERK
extracellular signal-regulated kinase
SEM
standard errors of the mean
CCK-8
Cell Counting Kit-8
OD
Optical density
IC50
50% inhibitory concentration
ATM
Axia-telangiectasia mutated
DDR
DNA damage respond
CML
chronic myeloid leukemia
MRL
minimal residual leukemia.

Declarations

Ethics approval and consent to participate

These studies were performed in accordance with the ethical guidelines under the protocols approved by the Institutional Medical Ethics Review Board of Fujian Medical University Union Hospital, Fuzhou, China. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZX and CY had a supervision and designed this study; SP and LZ performed the experiments, analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript.

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Figures

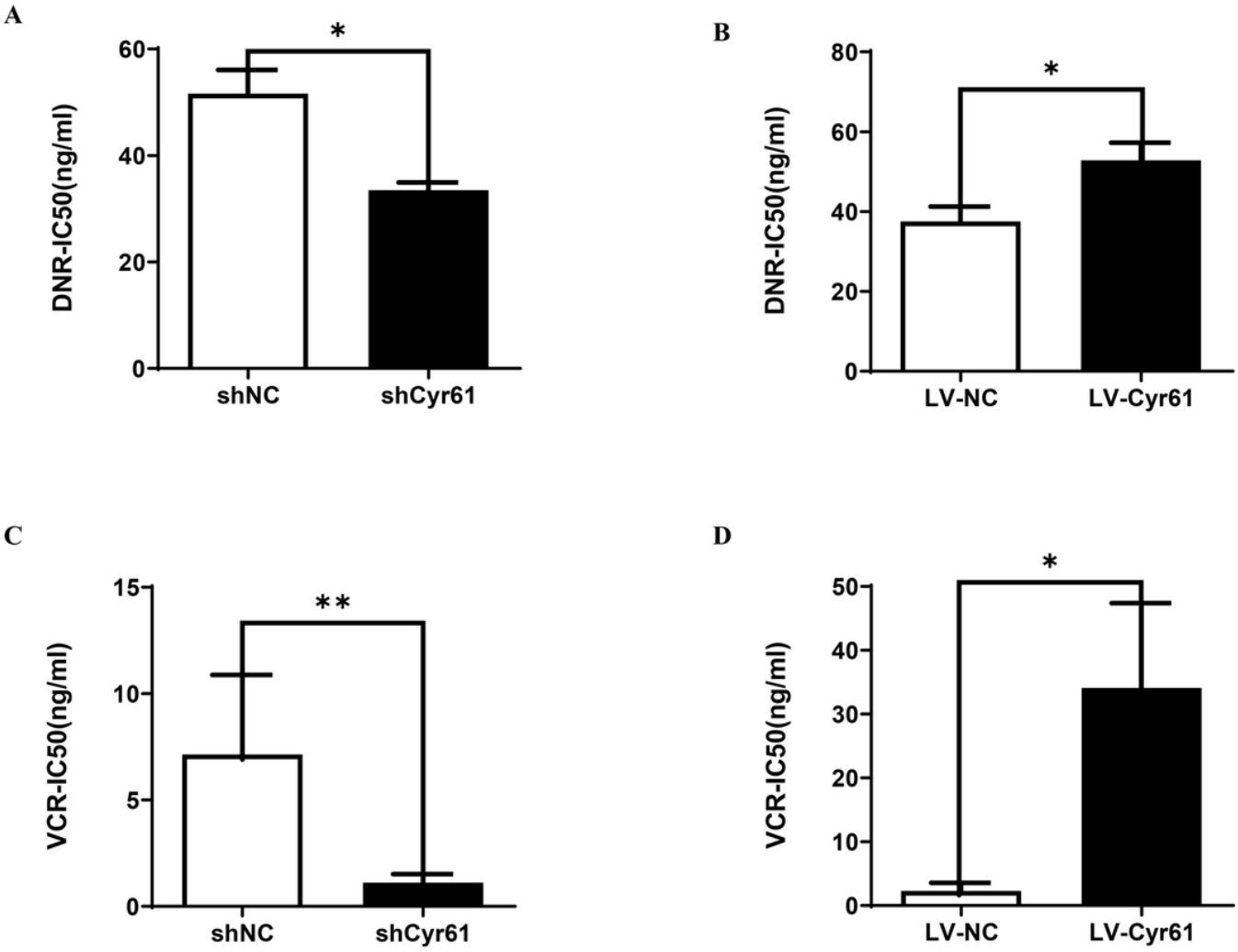

Figure 1

Cyr61 decreased the chemosensitivity of B-ALL cells to DNR and VCR. **A B** Nalm-6-shNC, Nalm-6-shCyr61, Nalm-6-LV-Cyr61 and Nalm-6-LV-NC cells were treated with various concentrations of DNR and the cell viability was analyzed by CCK8 assays. **C D** Nalm-6-shNC, Nalm-6-shCyr61, Nalm-6-LV-Cyr61 and Nalm-6-LV-NC cells were treated with various concentrations of VCR and the cell viability was analyzed by CCK8 assays. IC50 was calculated from the dose-response curves show. Data represented the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

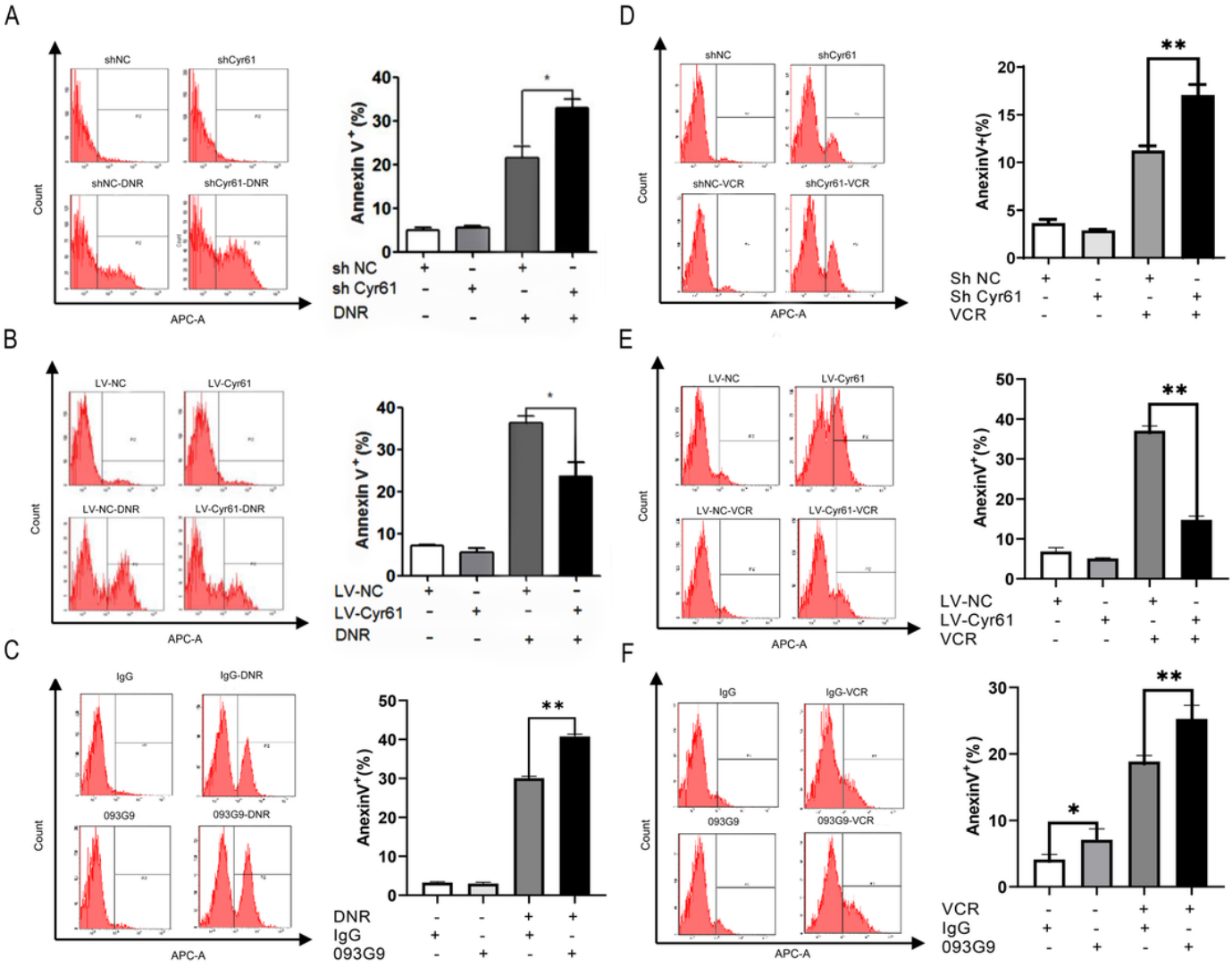

Figure 2

Cyr61 decreased the apoptosis of B-ALL cells induced by DNR and VCR. **A B** Nalm-6-shCyr61 cells, Nalm-6-shNC cells, Nalm-6-LV-Cyr61 cells and Nalm-6-LV-NC cells were treated with 50ng/mL DNR for 24h, then the apoptotic rates were determined by flow cytometric analysis. **C** Nalm-6 cells were treated with Cyr61 monoclonal antibody (1μ/mL) and DNR (50ng/mL) for 24h, then the apoptotic rates were determined by flow cytometric analysis. **D E** Nalm-6-shCyr61 cells, Nalm-6-shNC cells, Nalm-6-LV-Cyr61 cells and Nalm-6-LV-NC cells were treated with 5ng/mL VCR for 24h. then the apoptotic rates were determined by flow cytometric analysis. **F** Nalm-6 cells were treated with Cyr61 monoclonal antibody (1μ/mL) and VCR (5ng/mL) for 24h, and then the apoptotic rates were determined by flow cytometric analysis. Data represented the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

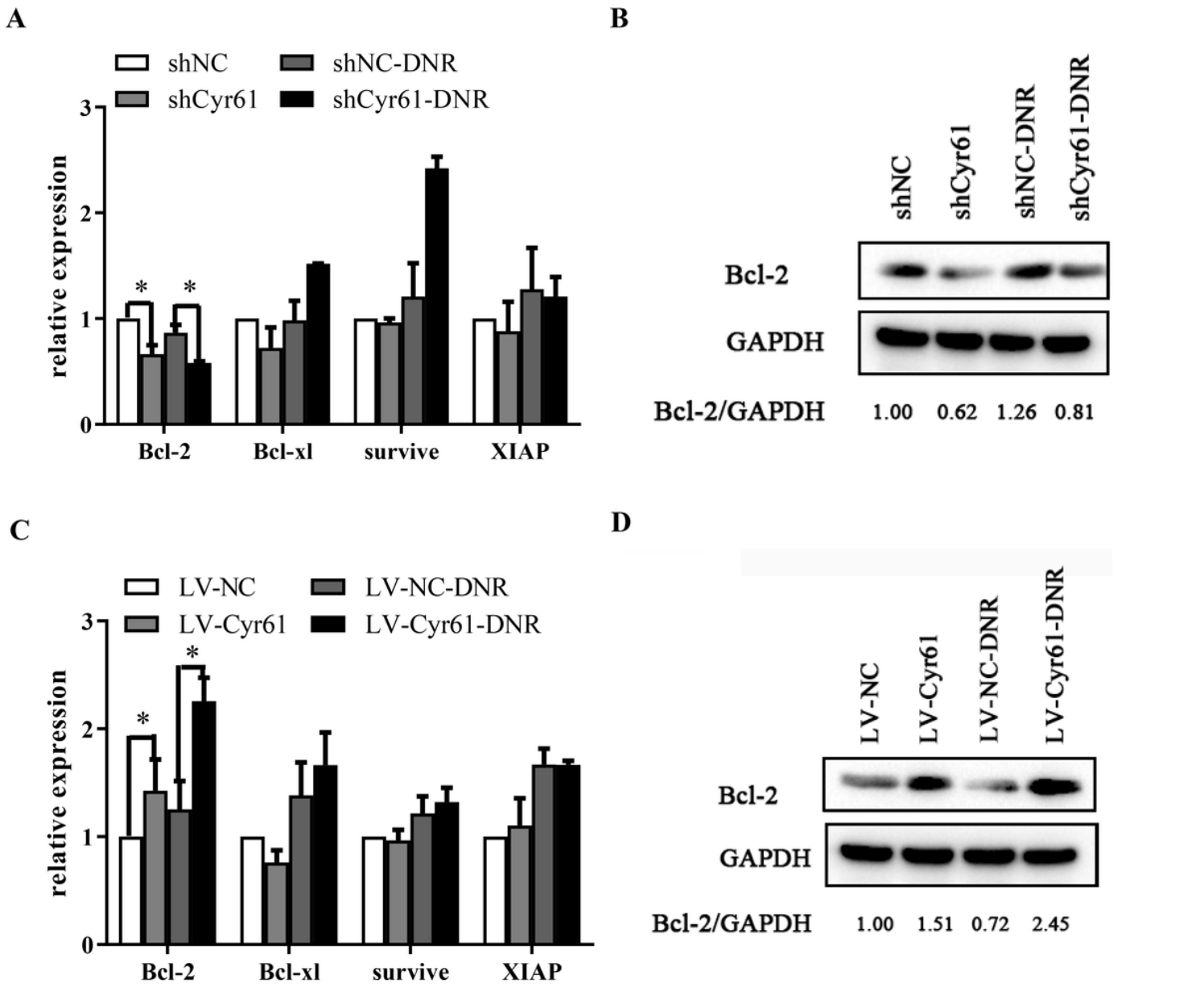

Figure 3

Cyr61 can regulate the expression of Bcl-2. **A C** Nalm-6-shCyr61 cells, Nalm-6-shNC cells, Nalm-6-LV-NC cells and Nalm-6-LV-Cyr61 cells were treated with DNR for 24h, then detected the mRNA expressions of Bcl-2, Bcl-xl, Survivin and XIAP by real-time PCR. **B D** Nalm-6-shNC cells, Nalm-6-shCyr61 cells, Nalm-6-LV-NC cells and Nalm-6-LV-Cyr61 cells were treated with DNR for 24h, then detected the protein production of Bcl-2 by western blot. Band intensity of Bcl-2 was quantified by densitometry and normalized to GAPDH. Data represented the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

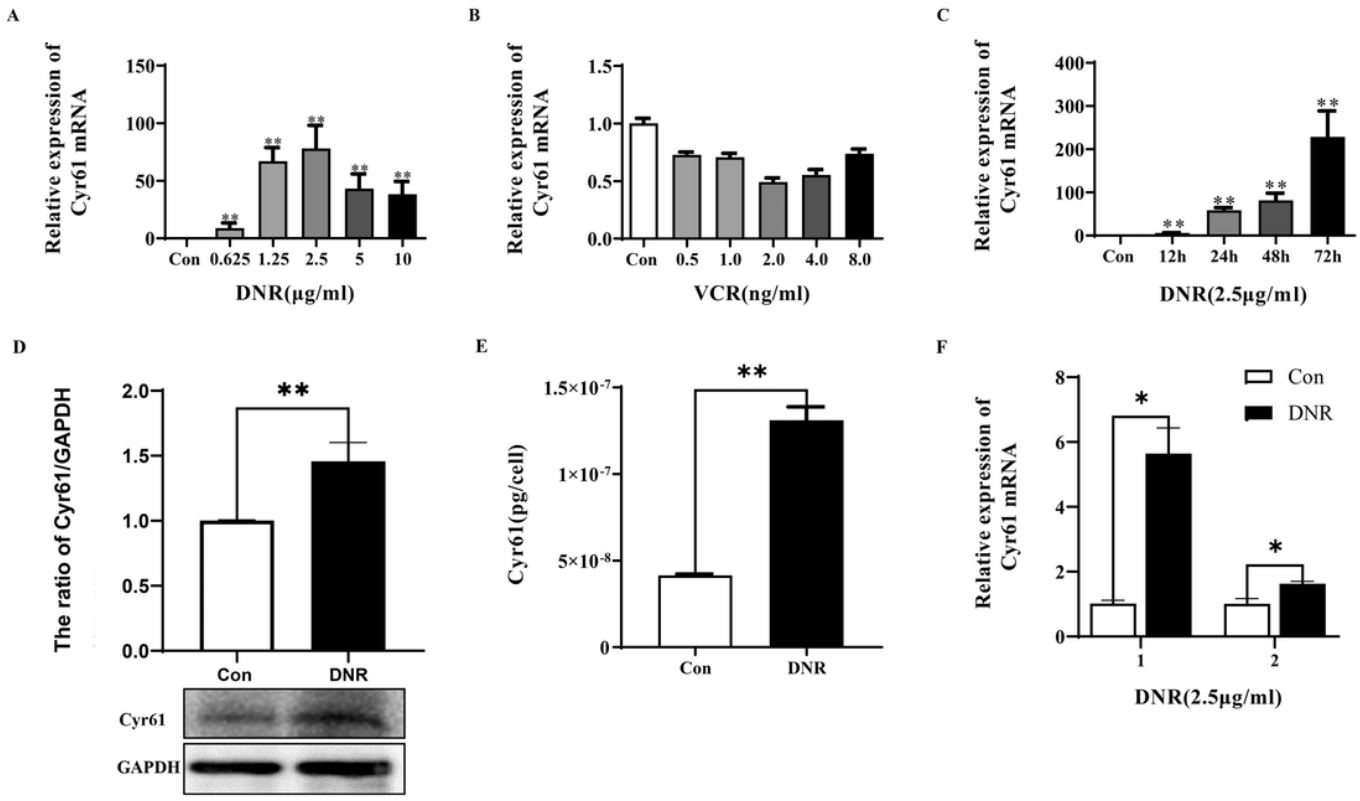

Figure 4

DNR up-regulated the expression of Cyr61 in B-ALL cells. **A B** Nalm-6 cells were treated with different concentrations of DNR and VCR for 24h, and then the mRNA expression of Cyr61 was detected by real-time PCR. **C** Nalm-6 cells were treated with 2.50μg/mL DNR for different time, and then the mRNA expression of Cyr61 was detected by real-time PCR. **D** Nalm-6 cells were treated with 2.50μg/mL DNR for 24h, and then the protein levels of Cyr61 were detected by western blotting. The band intensity of Cyr61 was quantified by densitometry and normalized to GAPDH. **E** Nalm-6 cells were treated with 2.50μg/mL DNR for 24h, and then the protein levels of Cyr61 in cell culture supernatants were measured by ELISA. **F** Peripheral blood lymphocytes from newly diagnosed patients with B-ALL were treated with 2.50μg/mL DNR for 24h, and then the mRNA expression of Cyr61 was detected by real-time PCR. Data represented the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

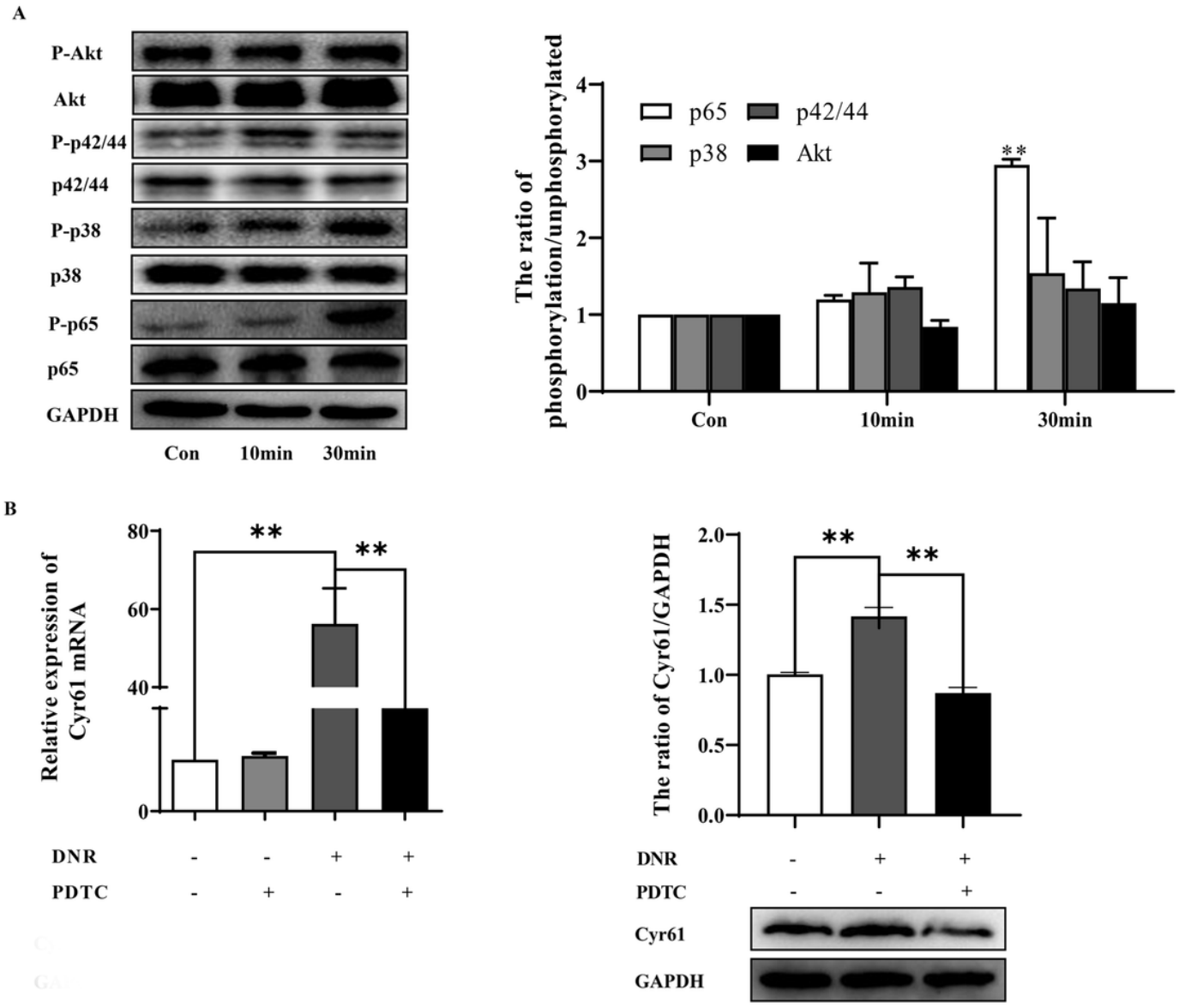

Figure 5

DNR induced Cyr61 through the ATM/NF-κB signaling pathway. **A** Nalm-6 cells were treated with 2.50μg/mL DNR for 10mins and 30mins, and the phosphorylation of PI3K/AKT, p38 MAPK, ERK (p44/p42) and NF-κB pathway was detected by western blot. **B** Nalm-6 cells were treated with 2.50μg/mL DNR in combination with or without 40μM PDTC for 24h. The mRNA and protein levels of Cyr61 were detected by real-time PCR (left panel) and by western blot (right panel), respectively. The band intensity of Cyr61 was quantified by densitometry and normalized to GAPDH. Data represented the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

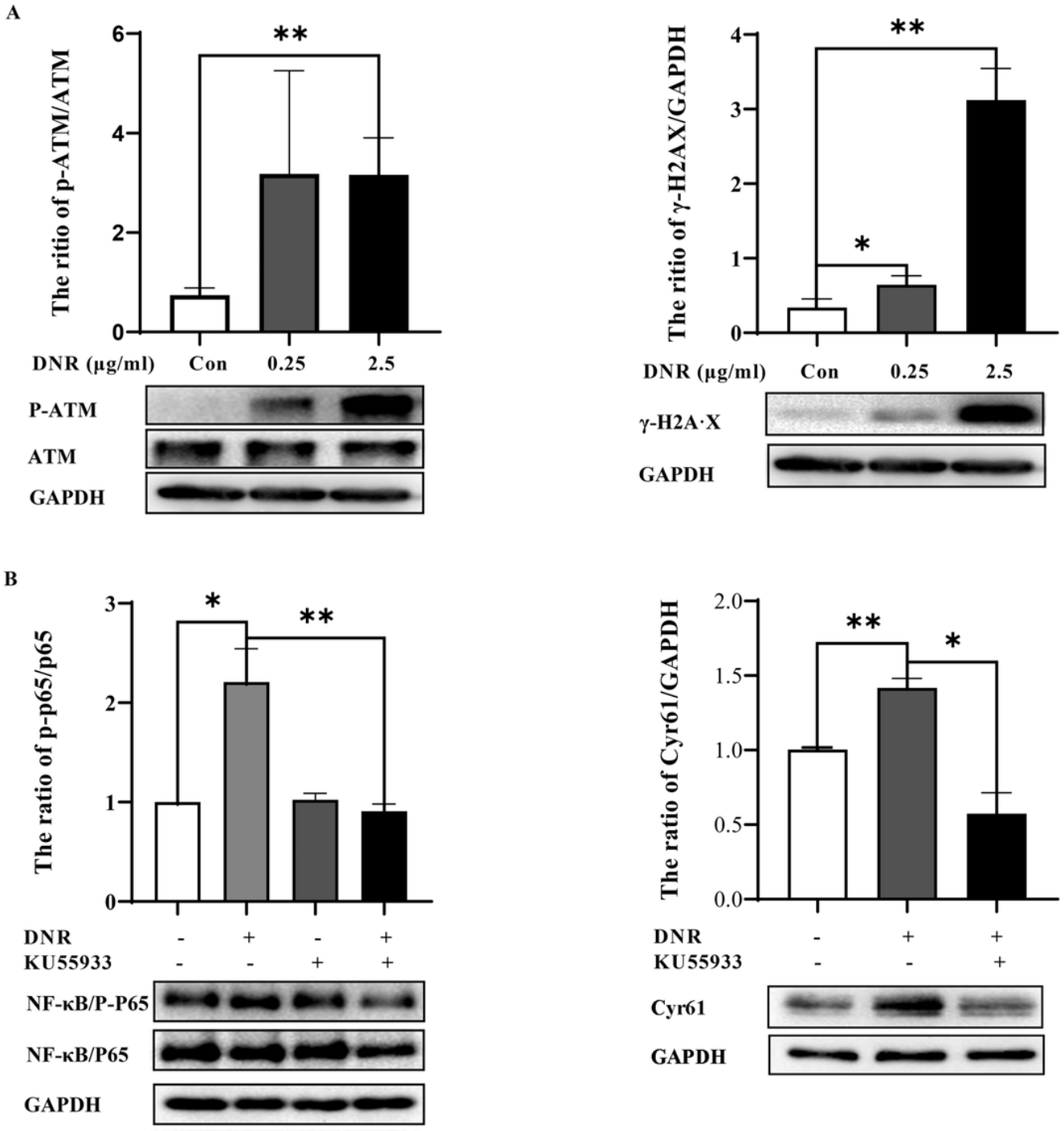

Figure 6

DNR induced Cyr61 through the ATM/NF-κB signaling pathway. **A** Nalm-6 cells were treated with 2.50μg/mL DNR for 30mins, and the phosphorylation of ATM (left panel) H2A·X (right panel) was detected by western blot. **B** Nalm-6 cells were treated with 2.50μg/mL DNR in combination with or without 10μM KU55933 for 30mins, and the phosphorylation of NF-κB (left panel) and Cyr61 protein levels (right panel) were detected by western blot. The band intensity of Cyr61 was quantified by densitometry and normalized to GAPDH. Data represented the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

Supplementary Files

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- Supplementarymeterial.pdf