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Original Article

Hypoxia-initiated Cysteine-rich protein 61 secretion promotes chemoresistance of acute B lymphoblastic leukemia cells

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Abstract: The drug resistance is a major obstacle in acute B-lymphoblastic leukemia (B-ALL) treatment. Our previous study has indicated that increased levels of Cysteine-rich protein 61 (Cyr61) in the bone marrow can mitigate the chemosensitivity of B-ALL cells, though the specific source of Cyr61 in the bone marrow remains unknown. In this study, we aimed to investigate whether hypoxia can induce Cyr61 production in B-ALL cells, delineates the underlying mechanisms, and evaluates the effect of Cyr61 on the chemosensitivity of B-ALL cells under hypoxia conditions. The results indicate that hypoxia promotes Cyr61 production in B-ALL cells by activating the NF-κB pathway. Increased Cyr61 expression appears to reduce the chemosensitivity of B-ALL cell to vincristine (VCR) and daunorubicin (DNR) through autophagy under hypoxia. Notably, inhibition of Cyr61 restores the chemosensitivity of B-ALL cells to both chemotherapeutic agents. This study is the first time to report that hypoxia decreases the chemosensitivity of B-ALL cells by inducing Cyr61 production, suggesting that targeting Cyr61 or its associated pathways could potentially improve the clinical response of B-ALL patients.

Keywords: Cysteine-rich protein 61, acute B lymphoblastic leukemia, daunorubicin, vincristine, hypoxia

Introduction

Acute B-lymphoblastic leukemia (B-ALL) is an aggressive B lymphoproliferative disorder. Currently, combination chemotherapy with vincristine (VCR) and daunorubicin (DNR) serves as the primary treatment method, and showing promising clinical results [1]. However, the development of chemotherapeutic resistance in B-ALL cells often leads to treatment failures, with a significant number of patients relapsing [2, 3]. The challenge of chemotherapy resistance has become a major issue in the clinical treatment of B-ALL.

Previous research on leukemia resistance has primarily focused on the leukemia cells themselves. However, there is now growing evidence that interactions between the bone marrow (BM) microenvironment and leukemia cells plays a crucial role in the development of chemotherapeutic resistance in B-ALL [4-6].

Cysteine-rich 61 (Cyr61, also known as CCN1), an important extracellular matrix protein belonging to the CCN family [7, 8], is implicated in the growth and progression of various tumors, including breast cancer, gastric cancer, ovarian cancer, glioma, and leukemia [9-13]. Interestingly, studies have demonstrated that Cyr61 is also connected to chemotherapy resistance, shown to reduce the chemotherapy sensitivity in breast cancer, pancreatic adenocarcinoma, and acute myeloid leukemia (AML) [14-19]. In our previous research, we observed the elevated levels of Cyr61 in both serum and bone marrow of B-ALL patients, where it enhances cell survival and contributes to chemotherapy resistance [13].

Leukemia cells are known to proliferate rapidly, consume a large amount of oxygen, and thus create a hypoxic environment within the bone marrow of B-ALL [20, 21]. It is well established that hypoxia can impact cellular physiological processes including metabolism, survival, and proliferation. Importantly, hypoxia not only contributes to the development of leukemia, but also enhances the chemotherapy resistance in leukemic cells [5, 22]. Li et al. found that hypoxia can promote glucocorticoid resistance in B-ALL cells [23], while Zhu et al. indicated that hypoxia can increase the resistance of B-ALL cells to Adriamycin [24]. Frolova et al. further observed that hypoxia reduces the chemosensitivity of ALL cells to vincristine, methotrexate and etoposide [22]. These studies collectively demonstrate the pivotal role of hypoxia in leukemia drug resistance, although its underlying mechanism is still unclear. Previous studies have shown that hypoxia can induce the production of Cyr61 production in various cells, such as nasal polyp fibroblasts, retinal vascular endothelial cells, and breast cancer cells [25-27]. Considering that Cyr61 plays an important role in the in B-ALL cell resistance, the potential of hypoxia mediating drug resistance through the induction of Cyr61 in B-ALL cells has not been reported.

In this study, we investigated the effects of hypoxia on Cyr61 production its role in regulating the sensitivity of B-ALL cells to VCR and DNR under the hypoxic environment. Our findings demonstrated that hypoxia promotes Cyr61 production in B-ALL cells through the activation of NF-κB pathway and reduces the chemosensitivity of B-ALL cells to VCR and DNR via autophagy. Collectively, these results indicate that hypoxia-induced Cyr61 production contributes to drug resistance in B-ALL cells, and that targeting Cyr61or its effector pathways could potentially improve the clinical outcomes of B-ALL patients.

Materials and methods

Cells culture and hypoxia conditions

The human acute B-lymphoblastic leukemia cell line Nalm-6 and Reh was obtained from Guangzhou Gineo Biotechnology Co., and the Shanghai Cell Bank of the Chinese Academy of Sciences, respectively. Cells were cultured in RPMI-1640 medium (HyClone, Logan, UT) supplemented with 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA), 100 U/ml penicillin (HyClone, Logan, UT) and 100 ug/ml streptomycin (HyClone, Logan, UT), in a humidified 5% CO_2 atmosphere at 37°C.

Hypoxic conditions were established using a sealed hypoxic workstation maintained at 1% O_2, 94% N_2 and 5% CO_2, with a constant temperature of temperature 37°C and humidity (90%).

Cyr61 overexpression and knockdown

Lentivirus vectors for overexpressing (pGLV5-Cyr61) and silencing (pGLV3-shCyr61) Cyr61, alongside control vectors (pGLV5-NC, pGLV3-shNC), were purchased from Shanghai GeneChem Co., Ltd. The specific shRNA target sequence for Cyr61 was 5'-CAACGAGGACTGCAGCAAA-3'. The viral particles were prepared with a standard method following the manufacturer's instructions (Shanghai GeneChem Co., Ltd.). Viruses were collected at 72 h post transfection and used to infect Nalm-6 and Reh cells. The successful transduction efficiency of Nalm-6 and Reh cells was confirmed to be > 97% before selection with 0.5 μg/ml puromycin (Sigma-Aldrich, St Louis, MO, USA) for 5 days. The overexpression and knockdown efficiency of Cyr61 were verified by western blotting.

Cell viability assay

To evaluate the effect of DNR and VCR (Selleck Biotechnology, Shanghai, China) on B-ALL cell viability under hypoxia, cells were treated with increasing concentrations of DNR and VCR in RPMI-1640 medium for 72 hours. Cell viability was quantified using the Cell Counting Kit-8 (CCK-8; Beyotime Biotechnology, Jiangsu, China) according to the manufacturer's instructions. In brief, 5×10^4 B-ALL cells in 100 μl of culture medium were seeded into 96-well plates under hypoxia for 72 hours. Different concentrations of DNR and VCR were added and cells were incubated for an additional 24 hours. Then cell viability was measured by adding 10 μl of CCK8 reagent and incubated for 2 hours. The optical density (OD) of the plates was measured at 450 nm using a microplate reader (Tecan, Männedorf, Switzerland), and the 50% inhibitory concentration (IC50) was calculated using GraphPad Prism version 8 (GraphPad Software, La Jolla, CA).

Apoptosis assay

Apoptotic B-ALL cells were measured using the Annexin V-APC Kit (BD Biosciences, Franklin Lakes, NJ, USA) following the manufacturer's instructions. Post 72-hour hypoxia exposure, B-ALL cells (5×10^5/ml, 1 ml) with up/down-regulated Cyr61 expression and control cells were treated with DNR (100 ng/ml) and VCR (100 ng/ml) for 24 hours in 24-well plates. The percentages of apoptotic cells were quantified by flow cytometric analysis. Flow cytometry was performed using a FACS Calibur cytometer (BD Biosciences, San Jose, CA, USA) and analyzed with Cellquest software (BD Biosciences, San Jose, CA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from specimens using a TriPure Isolation Reagent (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. One microgram of RNA was reverse-transcribed into first-strand cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). qRT-PCR was performed with SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA). The primers sequences used in this study were as follows: Cyr61, Forward, TCCAGCCCAACTGTAAACATCA; Cyr61, Reverse, GGACACAGAGGAATGCAGCC; β-actin, Forward, CCTGGCACCCAGCACAAT; β-actin, Reverse, GGGCCGGACTCGTCATAC. β-actin served as an internal control, and relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

To analyze the levels of the target proteins under hypoxia, western blot was performed. In briefly, cells were lysed using lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's instructions, and protein concentration was determined with a BCA Protein Assay Kit (Beyotime Biotechnology, Haimen, China). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% nonfat milk in PBS, incubated with primary antibodies overnight at 4°C, and subsequently with horseradish peroxidase conjugated secondary antibodies at room temperature for 1 hour. After washing, the target proteins were examined with the ECL system (Millipore Corporation, Bedford, MA, USA) and visualized with autoradiography film. Antibodies against Cyr61, NF-κB, phosphorylated NF-κB, JNK, phosphorylated JNK, Beclin 1, LC3B, and β-actin (Cell Signaling Technology Inc., Beverly, MA, USA) were employed.

Probing of signaling pathways involved in hypoxia-induced Cyr61 in B-ALL

To investigate the activation of signaling pathways in B-ALL cells under hypoxia, B-ALL cells were cultured in hypoxic and normoxic incubator for 8 hours, followed by protein extraction. Western blot was conducted to detect the phosphorylation of NF-κB/p65 and JNK. Meanwhile, B-ALL cells pretreated with or without SP600125 (an inhibitor of JNK; Abcam Biotechnology, USA) and PDTC (an inhibitor of NF-κB; Abcam Biotechnology, USA) for 2 hours, and were cultured for 72 hours under hypoxia. The expression level of Cyr61 was detected by western blotting as previously described.

Luciferase reporter gene assays

Cyr61 promoter activity was assessed using luciferase reporter constructs. Luciferase activity was determined according to the manufacturer's instructions (Dual Luciferase Kit, Promega, Madison, WI). Briefly, Nalm-6 cells (4×10^5 cells/well in a 24-well plate) were transfected with Cyr61 WT promoter and Cyr61 mNF-κB promoter constructs (GeneChem Co., Ltd., Shanghai, China) together with a Renilla luciferase expression vector using Lipofectamine 2000 Reagent (Thermo Scientific, Maryland, USA). After 8 hours, cells were washed and then cultured under hypoxia for 48 hours. The luciferase assay was performed with the Dual Luciferase Assay by Promega using Renilla luciferase as an internal control.

Chromatin immunoprecipitation (ChIP) assays

The ChIP assay was performed to examine NF-κB binding to the Cyr61 promoter using the ChIP kit (Beyotime Biotechnology, Jiangsu, China) following the manufacturer's instructions. Nalm-6 cells (5×10^6 cells) cultured under hypoxic or normoxic conditions for 48 hours were fixed with formaldehyde, lysed, and soni-

cated to shear chromatin. Immunoprecipitation was performed using antibodies against NF-κB and normal rabbit IgG (Cell Signaling Technology Inc., Beverly, MA, USA). The immunoprecipitated DNA was quantified by qPCR with primers targeting the NF-κB binding site on the Cyr61 promoter: Forward 5'-CAAAAGAGGGAAGGGCTGGA-3'; Reverse 5'-AAACTTGCCCATCGTCTGGC-3'.

Statistics

Data are presented as mean ± standard error of the mean (SEM). The significant differences between groups were assessed by Student's t test for single comparisons or by analysis of variance for multi-group comparisons, with a significance threshold of $P < 0.05$. Statistical analyses were carried out using SPSS software version 20.0.

Results

Cyr61 decreases the chemosensitivity of B-ALL cells to DNR and VCR under hypoxia

Previous studies have shown that Cyr61 is able to reduce the chemosensitivity of B-ALL cells under normoxia, but it is not clear whether Cyr61 can mediate chemoresistance in B-ALL cells under hypoxia. In this study, we upregulated the expression of Cyr61 in B-ALL cells (**Figure 1A** and **1D**), and used the CCK8 assay to detect the effect of Cyr61 on the chemosensitivity of B-ALL cells to DNR and VCR under hypoxia. The results showed that up-regulation of Cyr61 significantly increased the IC50 of DNR and VCR on B-ALL cells (**Figure 1B**, **1C**, **1E** and **1F**). To further verify the above results, we down-regulated the expression of Cyr61 in B-ALL cells (**Figure 1G** and **1J**), and found that down-regulation of Cyr61 significantly decreased the IC50 of DNR and VCR in B-ALL cells (**Figure 1H**, **1I**, **1K** and **1L**). Together, these results indicate that, under hypoxia, Cyr61 also decreases the chemosensitivity of B-ALL cells to DNR and VCR.

Cyr61 effectively inhibits DNR and VCR-induced apoptosis of B-ALL cells under hypoxia

It is well established that chemotherapeutic drugs such as DNR and VCR induce apoptosis in tumor cells [28-30], while Cyr61 is known to mediate chemotherapeutic resistance by reducing drug-induced apoptosis [31, 32]. To determine whether Cyr61 similarly protect B-ALL cells from apoptosis under hypoxia, flow cytometry was employed. The results, as presented in **Figure 2A** and **2B**, showed that under hypoxia, up-regulation of Cyr61 decrease DNR or VCR-induced apoptosis in B-ALL cells, while down-regulated Cyr61 could increase DNR or VCR-induced apoptosis in B-ALL cells (**Figure 2C** and **2D**). Taken together, these data indicate that Cyr61 can inhibit DNR and VCR-induced apoptosis of B-ALL cells under hypoxia.

Cyr61 promotes autophagy of B-ALL cells under hypoxia

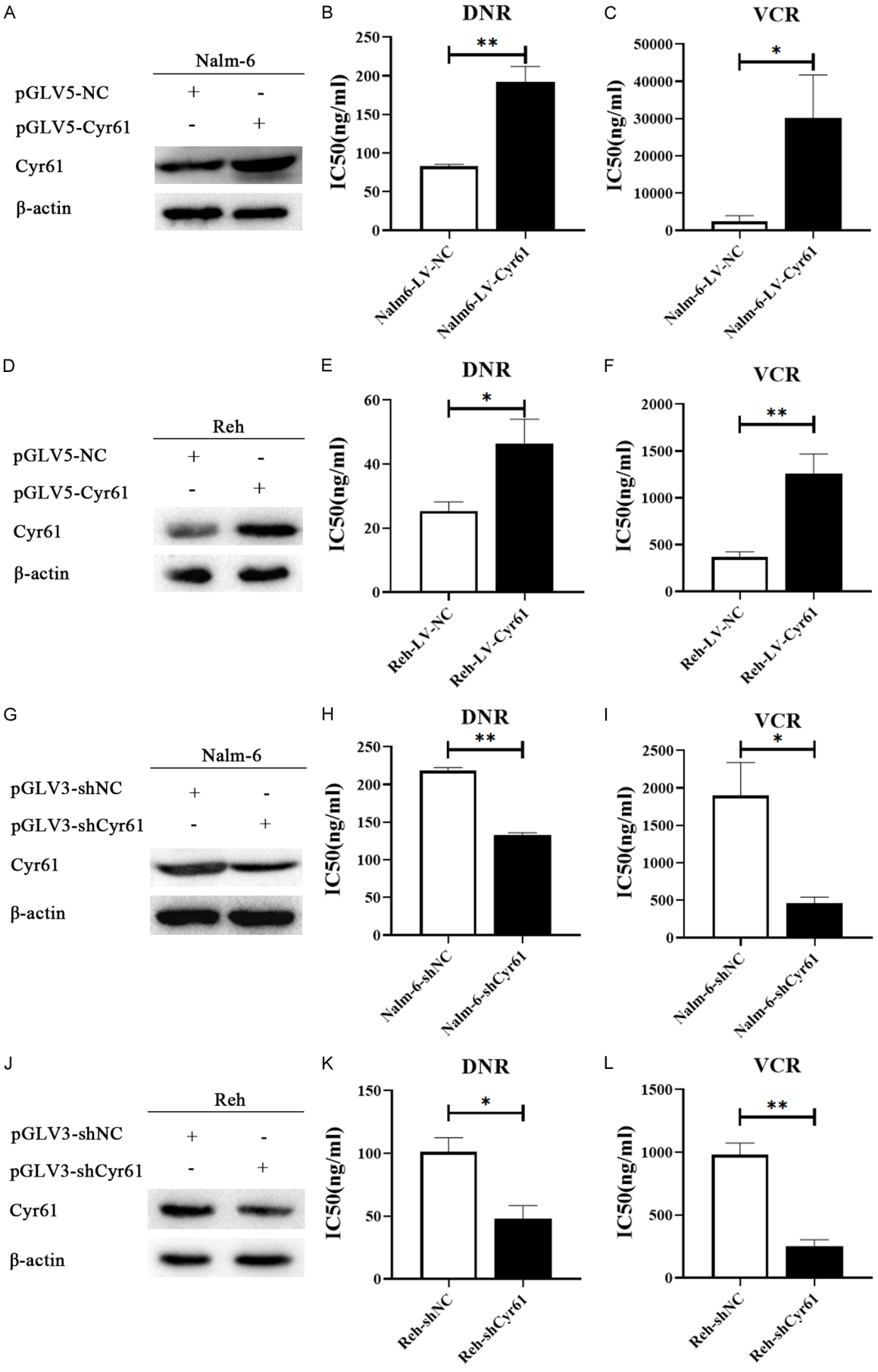
Previous studies have shown that autophagy plays a crucial role in the development, progression and treatment of hematological malignancies, often reducing the sensitivity of B-ALL cells to chemotherapeutic drugs [33, 34]. In order to further explore whether Cyr61-induced drug resistance in B-ALL cells is also related to autophagy, we specifically up-regulated and down-regulated the expression of Cyr61 in B-ALL cells, and found that upregulation of Cyr61 expression in B-ALL cells could increase Beclin 1 levels and the LC3BII/I ratio (**Figure 3A** and **3B**), while its downregulation had the opposite effect (**Figure 3C** and **3D**). Together, these results suggested that Cyr61 promotes the autophagy of B-ALL cells under hypoxia.

Hypoxia promotes Cyr61 production in B-ALL cells

We further investigated whether hypoxia induces Cyr61 production in B-ALL cells. Nalm-6 and Reh cells were cultured under hypoxia for varying durations, showing a significant induction of Cyr61 mRNA expression in a time-dependent manner (**Figure 4A** and **4B**). Consistent with these observations, western blotting showed that the protein levels of Cyr61 in Nalm-6 and Reh cells were significantly increased under hypoxia, supporting the observation (**Figure 4C** and **4D**) that hypoxia can promote Cyr61 production, potentially contributing to the higher concentration of Cyr61 in the bone marrow of B-ALL patients.

Hypoxia-induced Cyr61 production in B-ALL cells depends on the NF-κB activation

Our findings demonstrate that hypoxia stimulates Cyr61 production in B-ALL cells. Previous


A
Nalm-6
pGLV5-NC + -
pGLV5-Cyr61 - +
Cyr61
β-actin
B
DNR
IC50(ng/ml)
Nalm6-LV-NC
Nalm6-LV-Cyr61
C
VCR
IC50(ng/ml)
Nalm-6-LV-NC
Nalm-6-LV-Cyr61
D
Reh
pGLV5-NC + -
pGLV5-Cyr61 - +
Cyr61
β-actin
E
DNR
IC50(ng/ml)
Reh-LV-NC
Reh-LV-Cyr61
F
VCR
IC50(ng/ml)
Reh-LV-NC
Reh-LV-Cyr61
G
Nalm-6
pGLV3-shNC + -
pGLV3-shCyr61 - +
Cyr61
β-actin
H
DNR
IC50(ng/ml)
Nalm-6-shNC
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VCR
IC50(ng/ml)
Nalm-6-shNC
Nalm-6-shCyr61
J
Reh
pGLV3-shNC + -
pGLV3-shCyr61 - +
Cyr61
β-actin
K
DNR
IC50(ng/ml)
Reh-shNC
Reh-shCyr61
L
VCR
IC50(ng/ml)
Reh-shNC
Reh-shCyr61

Figure 1. Cyr61 decreases the chemosensitivity of B-ALL cells to DNR and VCR under hypoxia. A. Western blot was used to detect the levels of Cyr61 protein in Nalm-6 cells infected with lentivirus pGLV5-Cyr61 and pGLV5-NC. B, C. Nalm-6-LV-NC and Nalm-6-LV-Cyr61 cells were treated with different concentrations of DNR and VCR for 24 h under hypoxia. The inhibition rate of DNR and VCR on cells was detected by CCK-8 assay. D. Western blot was used to detect the levels of Cyr61 protein in Reh cells infected with lentivirus pGLV5-Cyr61 and pGLV5-NC. E, F. Reh-LV-NC and Reh-LV-Cyr61 cells were treated with different concentrations of DNR and VCR for 24 h under hypoxia. The inhibition rate of DNR and VCR on cells was detected by CCK-8 assay. G. Western blot was used to detect the levels of Cyr61 protein in Nalm-6 cells transfected with lentivirus pGLV3-shCyr61 and pGLV3-shNC. H, I. Under hypoxia, Nalm-6-shNC and Nalm-6-shCyr61 were treated with different concentrations of DNR and VCR for 24 h, and the inhibition rate of DNR and VCR on cells was detected by CCK-8 assay. J. Western blot was used to detect the levels of Cyr61 protein in Reh cells transfected with lentivirus pGLV3-shCyr61 and pGLV3-shNC. K, L. Under hypoxia, Reh-shNC and Reh-shCyr61 were treated with different concentrations of DNR and VCR for 24 h, and the inhibition rate of DNR on cells was detected by CCK-8 assay. All the IC50 was calculated using GraphPad Prism 8.0. Data represent the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

studies have shown that both NF-κB and JNK signaling pathways are involved in the expression of Cyr61 [35-38]. To investigate the role of the NF-κB and JNK signaling pathways in hypoxia-induced Cyr61 production in B-ALL cells, we analyzed the phosphorylation of JNK and the NF-κB under hypoxia, and the results showed that hypoxia enhances the phosphorylation levels of both JNK and NF-κB p65 (**Figure 5A-D**). Using pharmacological inhibitors, PDTC (an inhibitor of NF-κB) and SP600125 (an inhibitor of JNK), we observed that inhibition of NF-κB significantly reduced hypoxia-induced Cyr61 production, whereas JNK inhibition did not affect Cyr61 levels (**Figure 5E** and **5F**). These data indicate that the NF-κB pathway, rather than JNK, is involved in hypoxia-induced Cyr61 production in B-ALL cells.

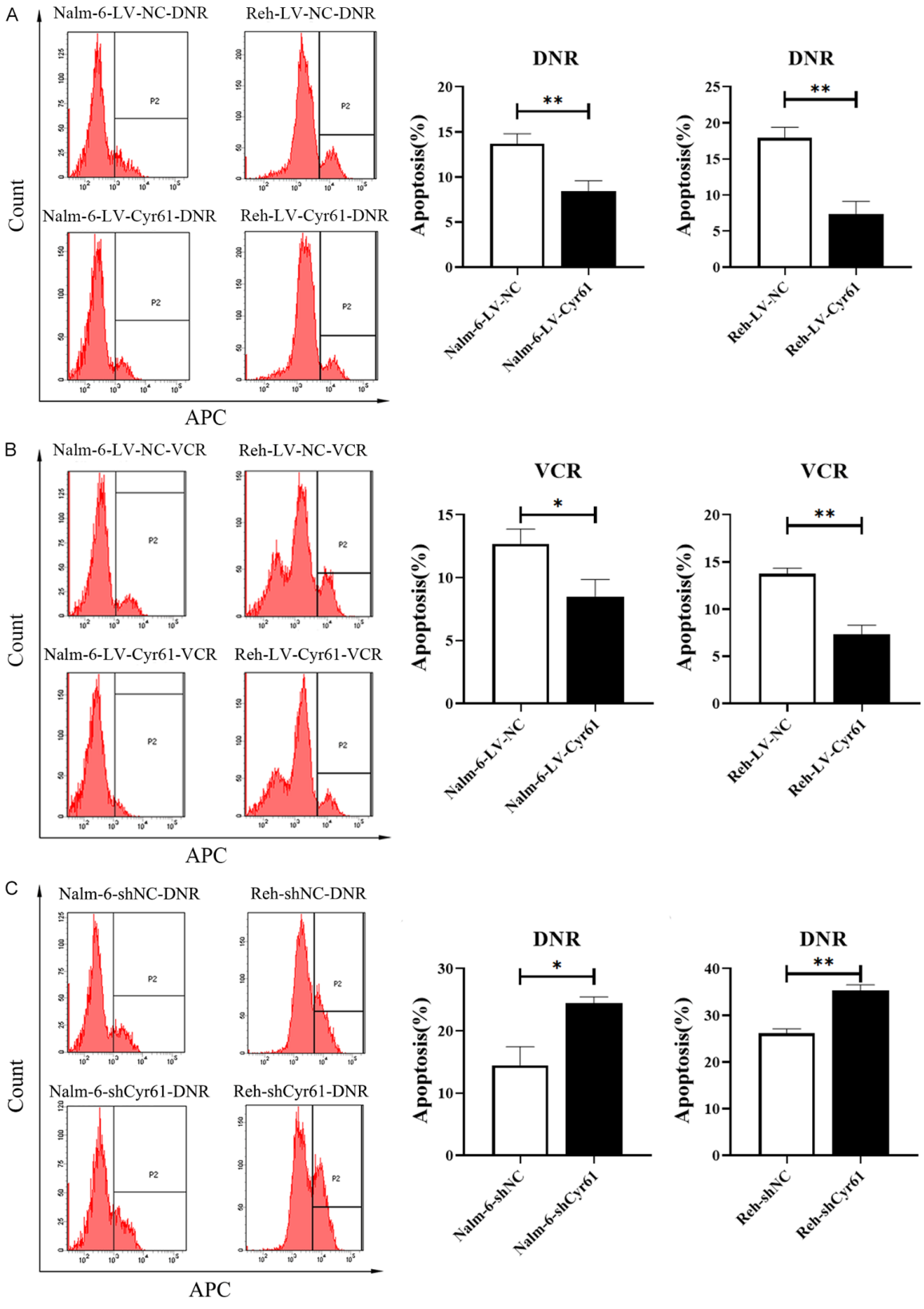
To clarify the molecular mechanisms of hypoxia-induced Cyr61 production, we constructed luciferase reporter genes under the control of a Cyr61 wild type promoter (Cyr61 WT) and a Cyr61 promoter with mutations in the NF-κB binding sites (Cyr61 mNF-κB). These constructs were transfected into Nalm-6 cells, which were then subjected to hypoxia. As shown in **Figure 5G**, luciferase assays revealed that that activity from the Cyr61 WT promoter increased significantly under hypoxia compared to normoxic conditions, while activity from the Cyr61 mNF-κB promoter showed no significant change, underscoring the essential role of NF-κB binding sites in hypoxia-driven Cyr61 transcription. Additionally, chromatin immunoprecipitation (ChIP) assays were performed to assess the binding of the NF-κB p65 subunit to the Cyr61 promoter in vivo under hypoxic conditions. The ChIP results indicated an increased binding of p65 to the Cyr61 promoter, thereby increasing the transcriptional activity and suggesting a pivotal role for NF-κB in hypoxia-induced production of Cyr61 in B-ALL cells (**Figure 5H**).

Cyr61 monoclonal antibody (093G9) enhances the sensitivity of B-ALL cells to DNR and VCR under hypoxia

Our results have demonstrated that hypoxia promotes Cyr61 production by activating the NF-κB pathway in B-ALL cells. To verify the effect of Cyr61 in B-ALL cells under hypoxia, Nalm-6 and Reh cells were cultured under hypoxia with the anti-Cyr61 monoclonal antibody (093G9), generously provided by Dr. Ningli Li Shanghai Jiao Tong University School of Medicine, Shanghai, China. The results showed that the application of 093G9 under hypoxic conditions significantly decreased the IC50 values of DNR and VCR in B-ALL cells (**Figure 6A-D**), and concurrently increased the apoptosis of B-ALL cells induced by these chemotherapeutic agents (**Figure 6E** and **6F**). These data substantiated that 093G9 effectively restores the chemosensitivity of B-ALL cells to DNR and VCR, supporting the hypothesis that hypoxia-induced Cyr61 production contributes to drug resistance B-ALL cells.

Discussion

Chemotherapy remains the primary treatment for B-ALL patients, however, chemotherapy resistance significantly hinders clinical outcomes. Thus, it is necessary to thoroughly investigate the mechanism of chemotherapy resistance in the treatment of B-ALL [39]. In this study, we discovered that hypoxia stimulates the production of Cyr61 in B-ALL cells via the NF-κB pathway, which in turn reduces the sensitivity of B-ALL cells to VCR and DNR under hypoxia.


A
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Reh-LV-Cyr61-DNR
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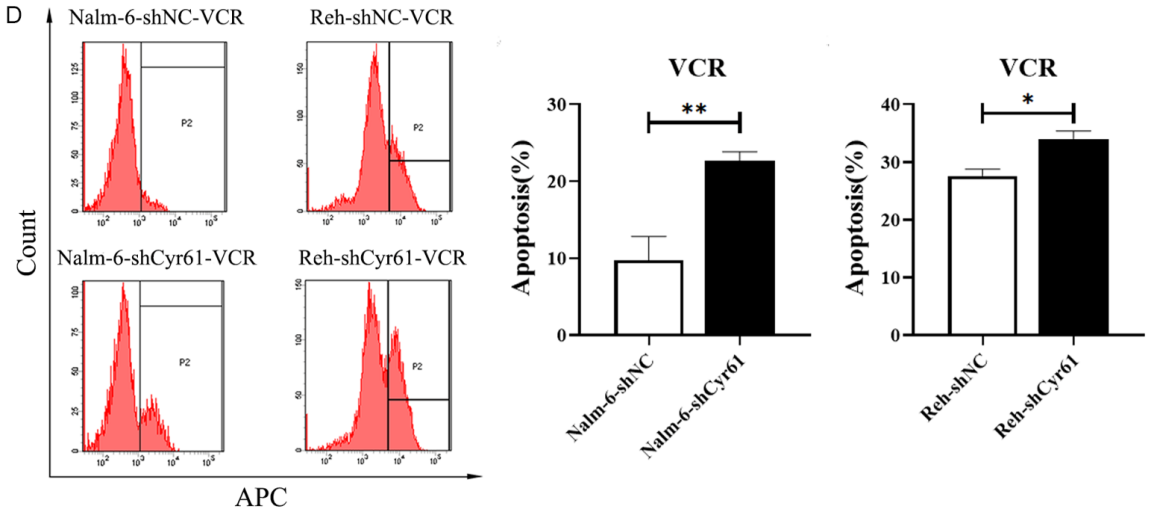
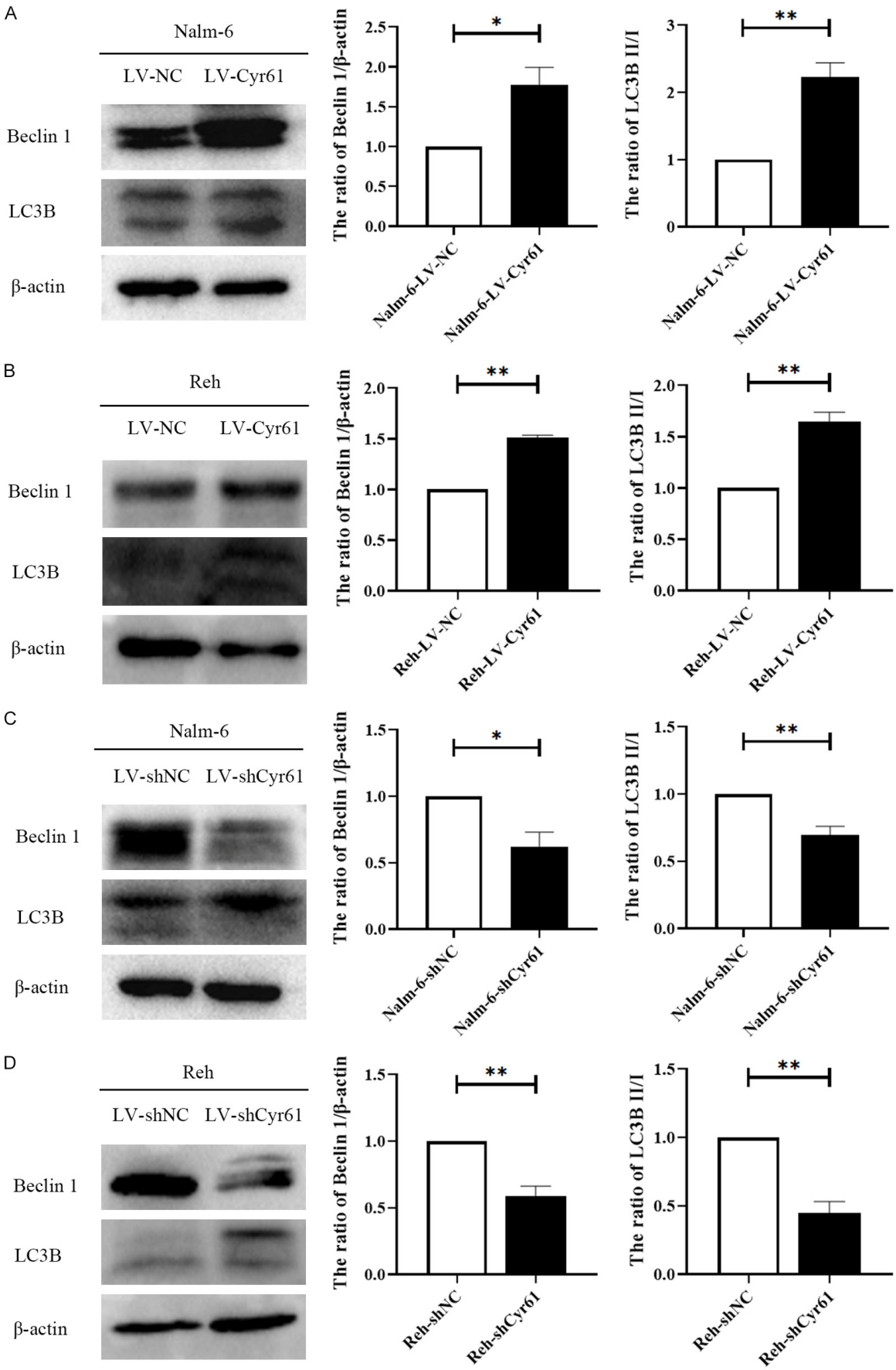


Figure 2. Cyr61 effectively inhibits DNR and VCR-induced apoptosis of B-ALL cells under hypoxia. A, B. Under hypoxia, Nalm-6-LV-NC, Nalm-6-LV-Cyr61, Reh-LV-NC and Reh-LV-Cyr61 cells were treated with DNR and VCR for 24 h, and the apoptosis of cells was detected by FCM. C, D. Under hypoxia, Nalm-6-shNC, Nalm-6-shCyr61, Reh-shNC and Reh-shCyr61 cells were treated with DNR and VCR for 24 h, the apoptosis of cells was detected by FCM. Data represent the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

Cyr61, a crucial extracellular matrix protein, has been extensively documented to facilitate cell proliferation, migration, adhesion and differentiation. Recent studies highlight its significant role in various tumors by modulating angiogenesis, cell proliferation, metastasis, and resistance to drugs [31, 40-43]. Our previous research demonstrated that Cyr61 was able to reduce the chemosensitivity of B-ALL cells under normoxia. Given the hypoxic nature of the bone marrow microenvironment in B-ALL, the impact of Cyr61 on drug resistance under such conditions remains unclear. In this study, we firstly found that Cyr61 also decreases the chemosensitivity of B-ALL cells to DNR and VCR under hypoxia. Furthermore, the administration of Cyr61-blocking antibody (093G9) restored chemosensitivity, reinforcing the pivotal role of Cyr61 in the resistance of B-ALL to these drugs and suggesting that targeting Cyr61 could be an effective strategy to overcome drug resistance and relapse in B-ALL.

Autophagy is crucial for cellular homeostasis through phagocytosis, degradation of damaged proteins and cellular components, and has been linked to preventing cellular damage and promoting cell survival. Several studies have reported the association between Cyr61 and autophagy. For instance, Pan et al. demonstrated that Cyr61 mediates the development of lupus nephritis by inducing the autophagy in renal podocytes [44], and Guo et al. found similar effects in renal tubular epithelial cells in an inflammatory environment [45]. Recent studies have highlighted autophagy's crucial role in the pathogenesis of hematologic malignancies, particularly its function in promoting the survival of B-ALL cells and reducing their chemosensitivity [46-48]. Our research indicates that Cyr61 increased the production of autophagy-related molecules, such as Beclin 1, and increases the LC3B II/I ratio, indicating that Cyr61 facilitates autophagy under hypoxic conditions. Moreover, we observed that Cyr61 reduced the apoptosis of B-ALL cells induced by DNR and VCR under similar conditions. Based on our results and other reports, Cyr61 might exert an anti-apoptotic role through autophagy in a hypoxic environment, thus protecting leukemia cells from chemotherapeutic drugs and reducing their drug sensitivity. Supporting this, previous studies have shown that autophagy protects cells from apoptosis [49-52]. Importantly, Su et al. reported that Cyr61 triggers autophagy in cardiomyocytes to limit its apoptotic activities, which is similar to the results of our study [53]. Similarly, Kim H et al. identified that Cyr61 serves as an anti-apoptotic protein and promotes chemoresistance in breast cancer cells by upregulating *survivin* expression [54]. These studies indicate that,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Nalm-6
LV-NC
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The ratio of Beclin 1/β-actin
The ratio of LC3B II/I
Nalm-6-LV-NC
Nalm-6-LV-Cyr61
B
Reh
LV-NC
LV-Cyr61
Beclin 1
LC3B
β-actin
The ratio of Beclin 1/β-actin
The ratio of LC3B II/I
Reh-LV-NC
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C
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LV-shCyr61
Beclin 1
LC3B
β-actin
The ratio of Beclin 1/β-actin
The ratio of LC3B II/I
Nalm-6-shNC
Nalm-6-shCyr61
D
Reh
LV-shNC
LV-shCyr61
Beclin 1
LC3B
β-actin
The ratio of Beclin 1/β-actin
The ratio of LC3B II/I
Reh-shNC
Reh-shCyr61

Figure 3. Cyr61 promotes the autophagy of B-ALL cells under hypoxia. A, B. The levels of Beclin 1 and LC3B protein in Nalm-6-LV-NC, Nalm-6-LV-Cyr61, Reh-LV-NC and Reh-LV-Cyr61 cells were detected by Western blot after 72 h of hypoxia. C, D. The levels of Beclin 1 and LC3B protein in Nalm-6-shNC, Nalm-6-shCyr61, Reh-shNC, and Reh-shCyr61 cells were detected by Western blot after 72 h of hypoxia. Data represent the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

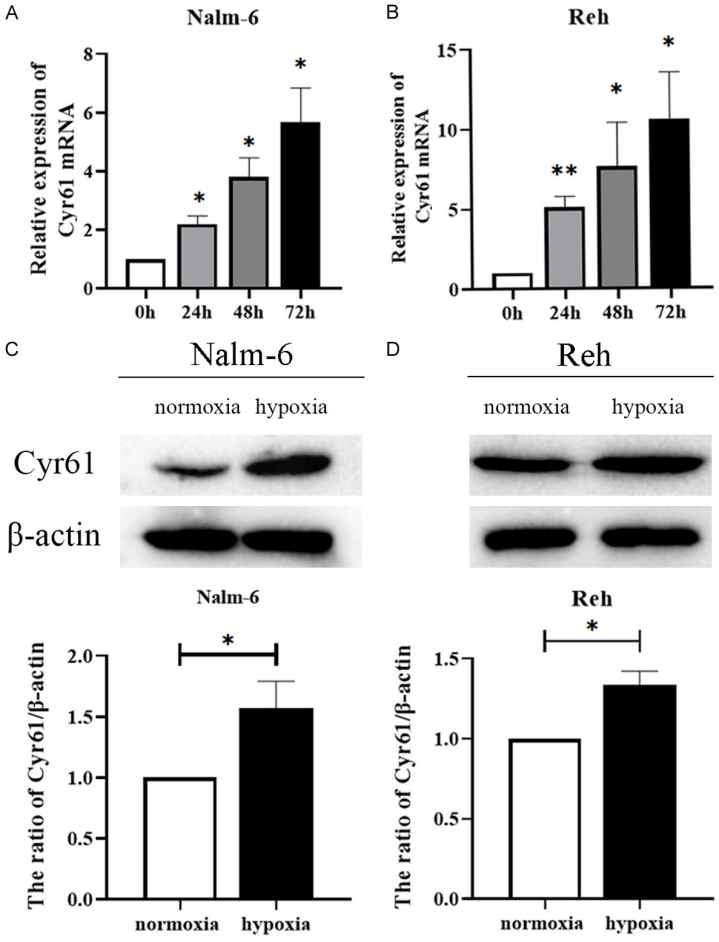

Figure 4. Hypoxia promotes Cyr61 production in B-ALL cells. Hypoxia promotes Cyr61 production in B-ALL cells. A, B. Nalm-6 and Reh cells were treated with hypoxia for different time, and the expression of Cyr61 mRNA was detected by Real-time PCR. C, D. Nalm-6 and Reh cells were treated with hypoxia for 72 h, the levels of Cyr61 protein were detected by Western blot. Data represent the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

Cyr61 can exhibit anti apoptotic activity through various mechanisms depending on the cellular context. In conclusion, our study reinforces that Cyr61 significantly mitigates the sensitivity of B-ALL cells to DNR and VCR in hypoxic environments, potentially through autophagy. However, the specific signaling pathways involved require further exploration.

Our previous research has identified that Cyr61 levels are elevated in both the serum and bone marrow of B-ALL patients, promoting the survival of B-ALL cells. Building upon these findings, our current study further demonstrates that elevated Cyr61 also reduced the chemosensitivity of B-ALL cells under hypoxia, reinforcing its role in drug resistance. It is well-documented that hypoxia can upregulate Cyr61 production across various cell types, including nasal polyp fibroblasts, retinal vascular endothelial cells, and breast cancer cells [25-27].

Consistent with this broader understanding, our study reveals that hypoxia similarly promotes Cyr61 production in B-ALL cells. This observation suggests that hypoxia-induced Cyr61 expression could be a significant contributor to the elevated levels of Cyr61 observed in the bone marrow of B-ALL patients.

A large number of studies have found that the NF-κB and JNK pathways are implicated in the regulation of Cyr61 expression, with hypoxia known to activate these pathways [18, 55-59]. Our research demonstrated that that hypoxia specifically activated both the NF-κB and JNK pathways in B-ALL cells. However, only the NF-κB signaling pathway was directly involved in the production of Cyr61. These findings indicate that hypoxia promotes Cyr61 production predominantly through the NF-κB pathway, rather than the JNK pathway. Interestingly, this observation contradicts the findings of [60], Shun CT [25], and Pinru Wu [37], who proposed that hypoxia promotes Cyr61 production via both pathways. This discrepancy could potentially be explained by the presence of molecules that inhibit the JNK pathway in B-ALL, warranting further validation. Additionally, in melanoma cells, the

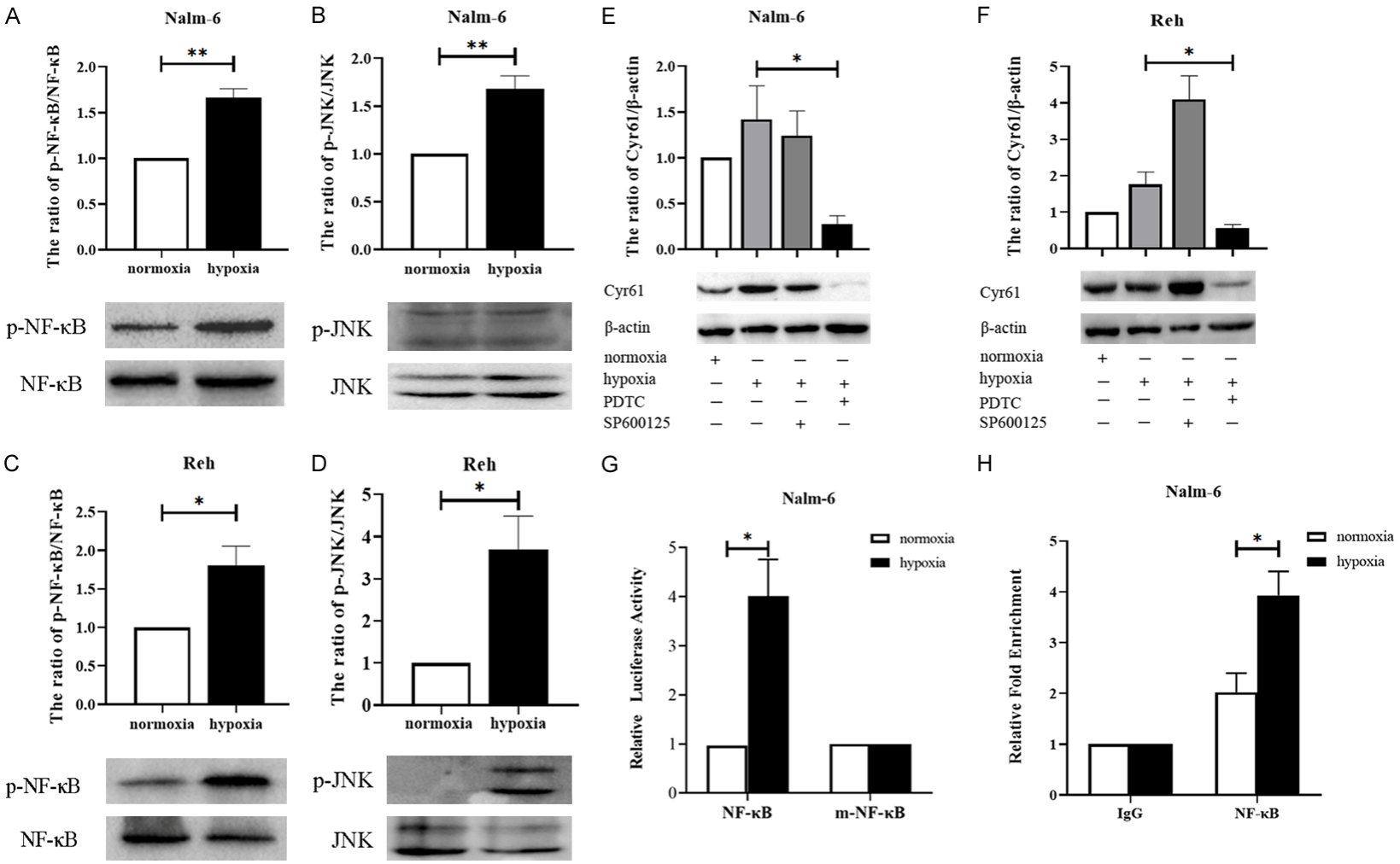

Figure 5. Hypoxia-induced Cyr61 production in B-ALL cells depends on NF-κB activation. Hypoxia-induced Cyr61 production in B-ALL cells depends on NF-κB activation. A-D. Nalm-6 and Reh cells were cultured under hypoxia for 8 h, the activation of NF-κB and JNK pathway was detected by Western blot. E, F. Nalm-6 and Reh cells were treated with NF-κB pathway inhibitor (PDTC) or JNK pathway inhibitor (SP600125) under hypoxia for 72 h, the levels of Cyr61 protein in Nalm-6 cells was detected by Western blot. G. Nalm-6 cells were transfected Wild-type (Cyr61 WT) or mutant vectors (mNF-κB) with the control vector pRL-TK for 4 hours, and cultured under hypoxia for 48 h. The luciferase activity relative to control was indicated after normalization with Renilla luciferase activity by dual-luciferase reporter assay. H. Nalm-6 cells were cultured under hypoxia for 48 h. The binding of NF-κB to Cyr61 promoter was investigated by CHIP assay, and the relative amount of Cyr61 promoter DNA bound to p65 was detected by RT-PCR. Data represent the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

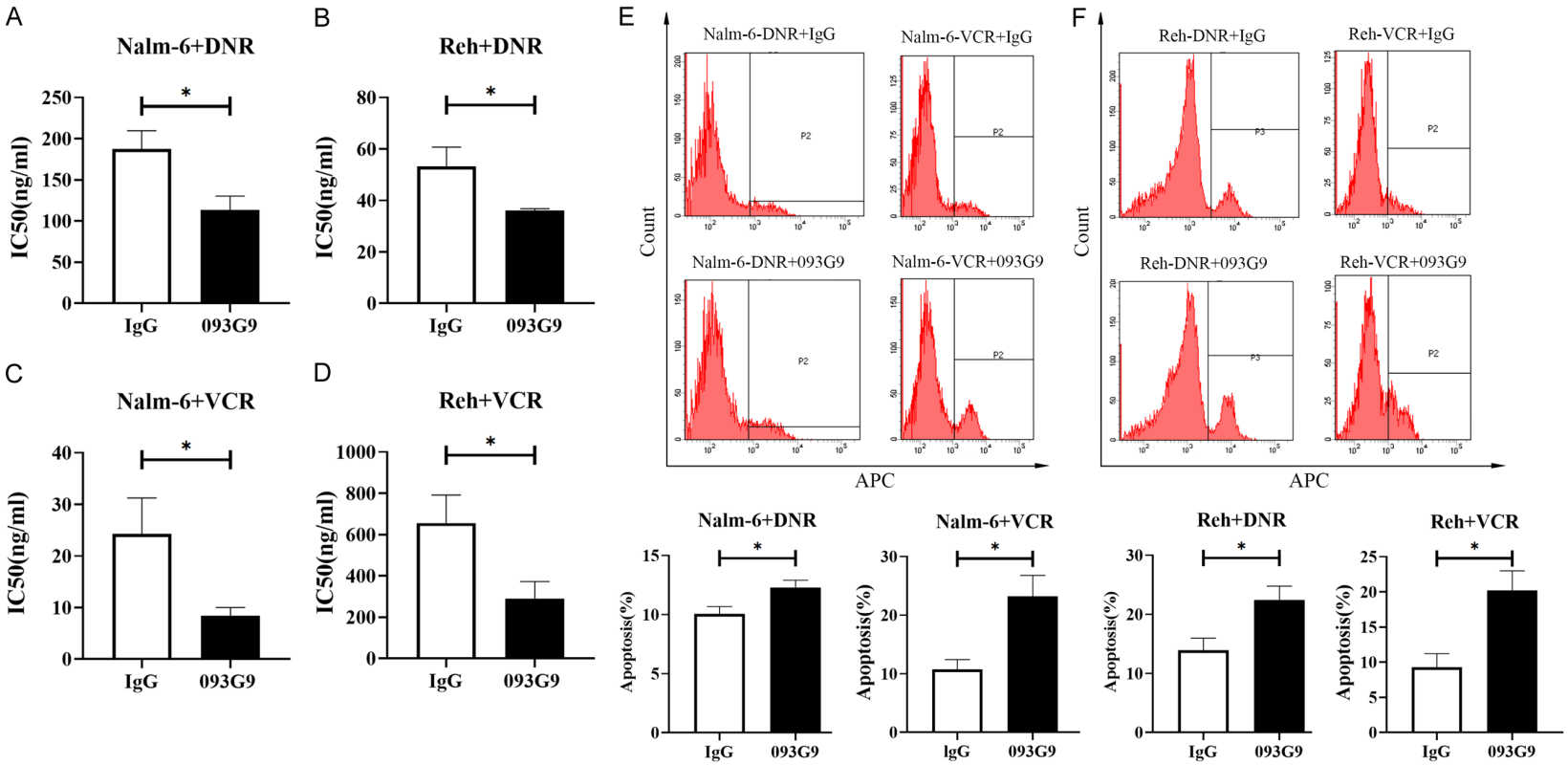

Figure 6. Cyr61 monoclonal antibody (093G9) improves the sensitivity of B-ALL cells to DNR and VCR under hypoxia. A-D. Under hypoxia, Nalm-6 and Reh cells were pretreated with 093G9 or IgG for 6 h, and then were treated with different concentrations of DNR and VCR for 24 h, the inhibition rate of DNR and VCR on cells was detected by CCK-8 assay. All the IC50 was calculated using GraphPad Prism 8.0. E, F. Under hypoxia, Nalm-6 and Reh cells were pretreated with 093G9 or IgG for 6 h, and then were treated with DNR and VCR for 24 h, the apoptosis rate of DNR and VCR on cells was detected by flow cytometry. Data represent the mean ± SEM of at least 3 independent experiments. *P < 0.05, **P < 0.01.

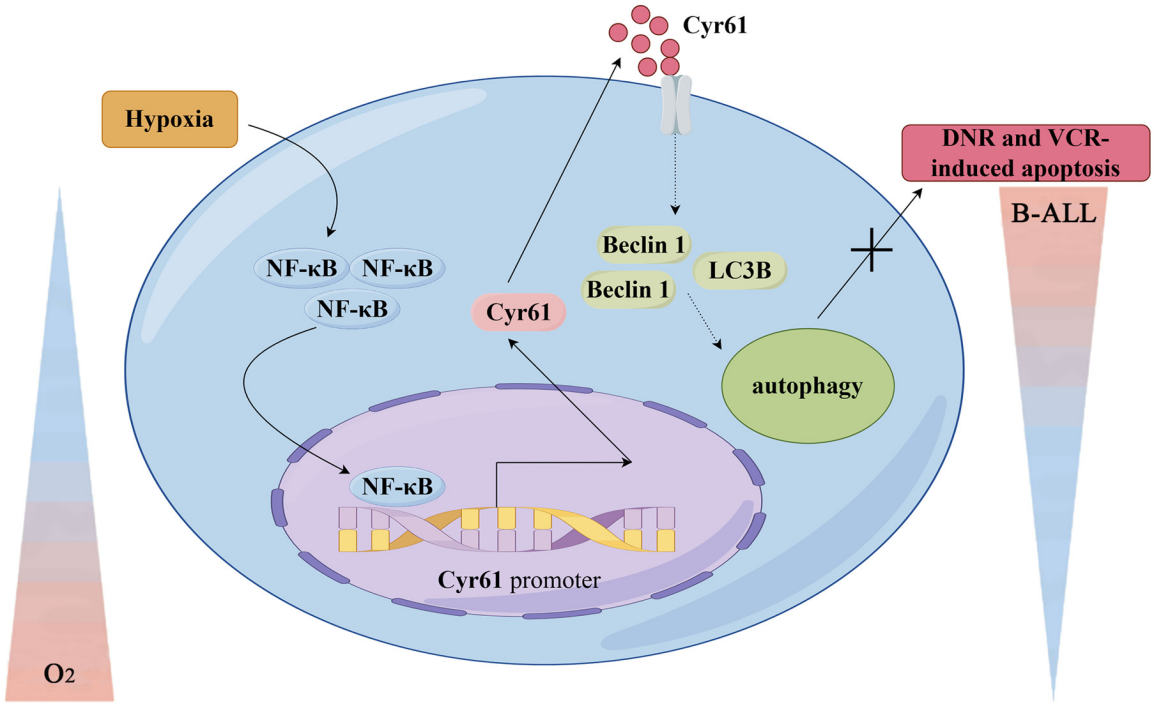

Figure 7. The graphic representation illustrates hypoxia promotes Cyr61 production by activating the NF-κB pathway in B-ALL cells, thereby reducing the chemosensitivity of B-ALL cells to VCR and DNR (By Figdraw).

interaction between hypoxia-inducible factor-1alpha and c-Jun/AP-1 may play a role in regulating Cyr61 transcription under hypoxia, pointing to tumor-specific heterogeneity in the mechanisms regulating Cyr61 [61]. This suggests that different tumor cells may exhibit unique regulatory mechanisms influenced by hypoxia.

Both contemporary and historical research indicates that hypoxia stimulates the production of Cyr61 in B-ALL cells. The increased Cyr61 is helpful in enhancing the survival of B-ALL cells while simultaneously decreasing their sensitivity to chemotherapy. Furthermore, the abundant presence of B-ALL cells consumes substantial amounts of oxygen, perpetuating a hypoxic state within the bone marrow microenvironment. This interaction establishes a deleterious positive feedback loop that accelerates the progression of B-ALL with Cyr61 performing crucial functions within this cycle, thereby highlighting its critical function in the pathogenesis of B-ALL.

Taken together, our findings reveal that that hypoxia-induced Cyr61 production in B-ALL cells, facilitated by the activation of the NF-κB pathway, significantly reduces the chemosensitivity of these cells to VCR and DNR, frequently culminating in chemotherapy resistance (**Figure 7**). These insights confirm the integral role of the hypoxia-Cyr61 axis in modulating the chemoresistance of B-ALL cells. Consequently, targeting this pathway could offer a promising therapeutic strategy to circumvent drug resistance and prevent relapse in B-ALL treatment.

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Disclosure of conflict of interest

None.

Abbreviations

B-ALL, Acute B lymphoblastic leukemia; AML, Acute myeloid leukemia; Cyr61, Cysteine-rich 61; DNR, Daunorubicin; VCR, Vincristine; IC50, 50% concentration of inhibition; FBS, Fetal

bovine serum; PBS, Phosphate buffered solution; BM, Bone marrow microenvironment; ChIP, Chromosomal immunoprecipitation; RT-PCR, Reverse transcription polymerase chain-reaction.

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References

[1] Kato M and Manabe A. Treatment and biology of pediatric acute lymphoblastic leukemia. Pediatr Int 2018; 60: 4-12.

[2] Friend BD and Schiller GJ. Closing the gap: novel therapies in treating acute lymphoblastic leukemia in adolescents and young adults. Blood Rev 2018; 32: 122-129.

[3] Inaba H and Mullighan CG. Pediatric acute lymphoblastic leukemia. Haematologica 2020; 105: 2524-2539.

[4] Zhang LP, Zhang MY and Liu WJ. Mechanism of resistance and therapeutic prospect of leukemia mediated by signaling pathway in bone marrow microenvironment. Eur Rev Med Pharmacol Sci 2019; 23: 6419-6428.

[5] Benito J, Shi Y, Szymanska B, Carol H, Boehm I, Lu H, Konoplev S, Fang W, Zweidler-McKay PA, Campana D, Borthakur G, Bueso-Ramos C, Shpall E, Thomas DA, Jordan CT, Kantarjian H, Wilson WR, Lock R, Andreeff M and Konopleva M. Pronounced hypoxia in models of murine and human leukemia: high efficacy of hypoxia-activated prodrug PR-104. PLoS One 2011; 6: e23108.

[6] Szymczak D, Dybko J and Kuliczkowski K. The role of hypoxia-inducible factors in leukemias. Adv Clin Exp Med 2018; 27: 271-275.

[7] Zhao G, Huang BL, Rigueur D, Wang W, Bhoot C, Charles KR, Baek J, Mohan S, Jiang J and Lyons KM. CYR61/CCN1 regulates sclerostin levels and bone maintenance. J Bone Miner Res 2018; 33: 1076-1089.

[8] Emre Y and Imhof BA. Matricellular protein CCN1/CYR61: a new player in inflammation and leukocyte trafficking. Semin Immunopathol 2014; 36: 253-259.

[9] Lin Z, Song Y, Qiu Y, Shi P, Zeng M, Cao Y and Zhu X. Serum CYR61 as a potential biomarker to improve breast cancer diagnostics. Biomark Med 2022; 16: 1121-1128.

[10] Quan X, Zhang Z, Qin Y, Gai X, Tian Q, Guo Y, Qian J and Yao J. Expression of Shh, Gli1, and Cyr61 in gastric cancer predicts overall survival of patients: a retrospective study. Cancer Control 2022; 29: 10732748221134398.

[11] Shi J, Huo R, Li N, Li H, Zhai T, Li H, Shen B, Ye J, Fu R and Di W. CYR61, a potential biomarker of tumor inflammatory response in epithelial ovarian cancer microenvironment of tumor progress. BMC Cancer 2019; 19: 1140.

[12] Xie D, Yin D, Tong X, O'Kelly J, Mori A, Miller C, Black K, Gui D, Said JW and Koeffler HP. Cyr61 is overexpressed in gliomas and involved in integrin-linked kinase-mediated Akt and beta-catenin-TCF/Lef signaling pathways. Cancer Res 2004; 64: 1987-1996.

[13] Zhu X, Song Y, Wu C, Pan C, Lu P, Wang M, Zheng P, Huo R, Zhang C, Li W, Lin Y, Cao Y and Li N. Cyr61 participates in the pathogenesis of acute lymphoblastic leukemia by enhancing cellular survival via the AKT/NF-κB signaling pathway. Sci Rep 2016; 6: 34018.

[14] Maity G, Ghosh A, Gupta V, Haque I, Sarkar S, Das A, Dhar K, Bhavanasi S, Gunewardena SS, Von Hoff DD, Mallik S, Kambhampati S, Banerjee SK and Banerjee S. CYR61/CCN1 Regulates dCK and CTGF and causes gemcitabine-resistant phenotype in pancreatic ductal adenocarcinoma. Mol Cancer Ther 2019; 18: 788-800.

[15] Menendez JA, Vellon L, Mehmi I, Teng PK, Griggs DW and Lupu R. A novel CYR61-triggered 'CYR61-alphavbeta3 integrin loop' regulates breast cancer cell survival and chemosensitivity through activation of ERK1/ERK2 MAPK signaling pathway. Oncogene 2005; 24: 761-779.

[16] Lai D, Ho KC, Hao Y and Yang X. Taxol resistance in breast cancer cells is mediated by the hippo pathway component TAZ and its downstream transcriptional targets Cyr61 and CTGF. Cancer Res 2011; 71: 2728-2738.

[17] Lee KB, Byun HJ, Park SH, Park CY, Lee SH and Rho SB. CYR61 controls p53 and NF-κB expression through PI3K/Akt/mTOR pathways in carboplatin-induced ovarian cancer cells. Cancer Lett 2012; 315: 86-95.

[18] Lin MT, Chang CC, Chen ST, Chang HL, Su JL, Chau YP and Kuo ML. Cyr61 expression confers resistance to apoptosis in breast cancer MCF-7 cells by a mechanism of NF-kappaB-dependent XIAP up-regulation. J Biol Chem 2004; 279: 24015-24023.

[19] Long X, Yu Y, Perlaky L, Man TK and Redell MS. Stromal CYR61 confers resistance to mitoxantrone via spleen tyrosine kinase activation in human acute myeloid leukaemia. Br J Haematol 2015; 170: 704-718.

[20] Jing X, Yang F, Shao C, Wei K, Xie M, Shen H and Shu Y. Role of hypoxia in cancer therapy by

regulating the tumor microenvironment. Mol Cancer 2019; 18: 157.
[21] Park MH, Kim AK, Manandhar S, Oh SY, Jang GH, Kang L, Lee DW, Hyeon DY, Lee SH, Lee HE, Huh TL, Suh SH, Hwang D, Byun K, Park HC and Lee YM. CCN1 interlinks integrin and hippo pathway to autoregulate tip cell activity. Elife 2019; 8: e46012.
[22] Frolova O, Samudio I, Benito JM, Jacamo R, Kornblau SM, Markovic A, Schober W, Lu H, Qiu YH, Buglio D, McQueen T, Pierce S, Shpall E, Konoplev S, Thomas D, Kantarjian H, Lock R, Andreeff M and Konopleva M. Regulation of HIF-1α signaling and chemoresistance in acute lymphocytic leukemia under hypoxic conditions of the bone marrow microenvironment. Cancer Biol Ther 2012; 13: 858-870.
[23] Li Y, Wei Y and Gu L. Effect of hypoxia on proliferation and glucocorticoid resistance of T-cell acute lymphoblastic leukaemia. Hematology 2021; 26: 775-784.
[24] Zhu B, Pan S, Liu J, Wang S, Ni Y, Xiao L, Wei Q, Peng Y, Ding Z and Zhao W. HIF-1α forms regulatory loop with YAP to coordinate hypoxia-induced adriamycin resistance in acute myeloid leukemia cells. Cell Biol Int 2020; 44: 456-466.
[25] Shun CT, Lin SK, Hong CY, Huang HM and Liu CM. Hypoxia induces cysteine-rich 61, vascular endothelial growth factor, and interleukin-8 expressions in human nasal polyp fibroblasts: an implication of neutrophils in the pathogenesis of nasal polyposis. Am J Rhinol Allergy 2011; 25: 15-18.
[26] You JJ, Yang CM, Chen MS and Yang CH. Regulation of Cyr61/CCN1 expression by hypoxia through cooperation of c-Jun/AP-1 and HIF-1α in retinal vascular endothelial cells. Exp Eye Res 2010; 91: 825-836.
[27] Hirschfeld M, zur Hausen A, Bettendorf H, Jäger M and Stickeler E. Alternative splicing of Cyr61 is regulated by hypoxia and significantly changed in breast cancer. Cancer Res 2009; 69: 2082-2090.
[28] Qu P, Zhou F, Tan LF, Wang ZJ, Wang ML, Jin RM and Han J. Effect of huaier aqueous extract combined with routine chemo-therapeutic drugs on human acute lymphoblastic leukemia cells Nalm-6 and Sup-B15. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2020; 28: 1451-1458.
[29] Duan YJ, Liu C, Chen XY, Wu WQ, Zheng JR, Zhang YC and Zhu XF. Navitoclax combined with daunorubicin promotes apoptosis of erythroleukemia cell lines K562, HEL and TF-1. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2020; 28: 724-730.
[30] Chu M, Yin K, Dong Y, Wang P, Xue Y, Zhou P, Wang Y and Wang Y. TFDP3 confers chemoresistance in minimal residual disease within childhood T-cell acute lymphoblastic leukemia. Oncotarget 2017; 8: 1405-1415.
[31] Song Y, Kang Y, Lin Z, Zeng M, Shi P, Lin J, Lu P, Luo L, Cao Y and Zhu X. Cyr61 mediates oxaliplatin resistance in colorectal cancer cells by regulating Bcl-xL expression. J Cancer 2021; 12: 1952-1959.
[32] Rho SB, Woo JS, Chun T and Park SY. Cysteine-rich 61 (CYR61) inhibits cisplatin-induced apoptosis in ovarian carcinoma cells. Biotechnol Lett 2009; 31: 23-28.
[33] García Ruiz O, Sánchez-Maldonado JM, López-Nevot MÁ, García P, Macauda A, Hernández-Mohedo F, González-Sierra PA, Martínez-Bueno M, Pérez E, Reyes-Zurita FJ, Campa D, Canzian F, Jurado M, Rodríguez-Sevilla JJ and Sainz J. Autophagy in hematological malignancies. Cancers (Basel) 2022; 14: 5072.
[34] Boustani H, Khodadi E and Shahidi M. Autophagy in hematological malignancies: molecular aspects in leukemia and lymphoma. Lab Med 2021; 52: 16-23.
[35] Zhang Q, Wu J, Cao Q, Xiao L, Wang L, He D, Ouyang G, Lin J, Shen B, Shi Y, Zhang Y, Li D and Li N. A critical role of Cyr61 in interleukin-17-dependent proliferation of fibroblast-like synoviocytes in rheumatoid arthritis. Arthritis Rheum 2009; 60: 3602-3612.
[36] Kim SM, Park JH, Chung SK, Kim JY, Hwang HY, Chung KC, Jo I, Park SI and Nam JH. Coxsackievirus B3 infection induces cyr61 activation via JNK to mediate cell death. J Virol 2004; 78: 13479-13488.
[37] Wu P, Xu H, Li N, Huo R, Shen B, Lin X and Ma G. Hypoxia-induced Cyr61/CCN1 production in infantile hemangioma. Plast Reconstr Surg 2021; 147: 412e-423e.
[38] Su RL, Qiao Y, Guo RF and Lv YY. Cyr61 overexpression induced by interleukin 8 via NF-kB signaling pathway and its role in tumorigenesis of gastric carcinoma in vitro. Int J Clin Exp Pathol 2019; 12: 3197-3207.
[39] Wang J, Xue HM, Chen YR, Xu HG, Lin SF, Tang XK and Chen C. Evaluation of insulin-mediated regulation of AKT signaling in childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2019; 41: 96-104.
[40] Gery S, Xie D, Yin D, Gabra H, Miller C, Wang H, Scott D, Yi WS, Popoviciu ML, Said JW and Koeffler HP. Ovarian carcinomas: CCN genes are aberrantly expressed and CCN1 promotes proliferation of these cells. Clin Cancer Res 2005; 11: 7243-7254.
[41] Hesler RA, Huang JJ, Starr MD, Treboschi VM, Bernanke AG, Nixon AB, McCall SJ, White RR and Blobe GC. TGF-β-induced stromal CYR61 promotes resistance to gemcitabine in pancreatic ductal adenocarcinoma through downregulation of the nucleoside transporters hENT1

and hCNT3. Carcinogenesis 2016; 37: 1041-1051.
[42] Kok SH, Chang HH, Tsai JY, Hung HC, Lin CY, Chiang CP, Liu CM and Kuo MY. Expression of Cyr61 (CCN1) in human oral squamous cell carcinoma: an independent marker for poor prognosis. Head Neck 2010; 32: 1665-1673.
[43] Hyun HB, Moon JY and Cho SK. Quercetin suppresses CYR61-mediated multidrug resistance in human gastric adenocarcinoma AGS cells. Molecules 2018; 23: 209.
[44] Pan X, Chen S, Shen R, Liu S and You Y. HOXA11-OS participates in lupus nephritis by targeting miR-124-3p mediating Cyr61 to regulate podocyte autophagy. Mol Med 2022; 28: 138.
[45] Guo P, Ma Y, Deng G, Li L, Gong Y, Yang F and You Y. CYR61, regulated by miR-22-3p and MALAT1, promotes autophagy in HK-2 cell inflammatory model. Transl Androl Urol 2021; 10: 3486-3500.
[46] Colturato-Kido C, Lopes RM, Medeiros HCD, Costa CA, Prado-Souza LFL, Ferraz LS and Rodrigues T. Inhibition of autophagy enhances the antitumor effect of thioridazine in acute lymphoblastic leukemia cells. Life (Basel) 2021; 11: 365.
[47] Gong Y, Wu J, Yang R, Zhang L and Ma Z. Rapamycin-induced autophagy plays a pro-survival role by enhancing up-regulation of intracellular ferritin expression in acute lymphoblastic leukemia. Exp Oncol 2020; 42: 11-15.
[48] Huang FL, Yu SJ and Li CL. Role of autophagy and apoptosis in acute lymphoblastic leukemia. Cancer Control 2021; 28: 10732748211019138.
[49] Wang B, Huang X, Liang H, Yang H, Guo Z, Ai M, Zhang J, Khan M, Tian Y, Sun Q, Mao Z, Zheng R and Yuan Y. PLK1 inhibition sensitizes breast cancer cells to radiation via suppressing autophagy. Int J Radiat Oncol Biol Phys 2021; 110: 1234-1247.
[50] Xiong X, Lu B, Tian Q, Zhang H, Wu M, Guo H, Zhang Q, Li X, Zhou T and Wang Y. Inhibition of autophagy enhances cinobufagin-induced apoptosis in gastric cancer. Oncol Rep 2019; 41: 492-500.
[51] Fan J, Yang X, Li J, Shu Z, Dai J, Liu X, Li B, Jia S, Kou X, Yang Y and Chen N. Spermidine coupled with exercise rescues skeletal muscle atrophy from D-gal-induced aging rats through enhanced autophagy and reduced apoptosis via AMPK-FOXO3a signal pathway. Oncotarget 2017; 8: 17475-17490.
[52] Zong DD, Liu XM, Li JH, Ouyang RY, Long YJ, Chen P and Chen Y. Resveratrol attenuates cigarette smoke induced endothelial apoptosis by activating Notch1 signaling mediated autophagy. Respir Res 2021; 22: 22.
[53] Su BC, Hsu PL and Mo FE. CCN1 triggers adaptive autophagy in cardiomyocytes to curb its apoptotic activities. J Cell Commun Signal 2020; 14: 93-100.
[54] Kim H, Son S, Ko Y, Lim H, Lee J, Lee KM and Shin I. CYR61 confers chemoresistance by up-regulating survivin expression in triple-negative breast cancer. Carcinogenesis 2024; [Epub ahead of print].
[55] Kwon EJ, Park EJ, Choi S, Kim SR, Cho M and Kim J. PPARγ agonist rosiglitazone inhibits migration and invasion by downregulating Cyr61 in rheumatoid arthritis fibroblast-like synoviocytes. Int J Rheum Dis 2017; 20: 1499-1509.
[56] Kim KH, Min YK, Baik JH, Lau LF, Chaqour B and Chung KC. Expression of angiogenic factor Cyr61 during neuronal cell death via the activation of c-Jun N-terminal kinase and serum response factor. J Biol Chem 2003; 278: 13847-13854.
[57] Malekan M, Ebrahimzadeh MA and Sheida F. The role of hypoxia-inducible factor-1alpha and its signaling in melanoma. Biomed Pharmacother 2021; 141: 111873.
[58] Wu F, Gao F, He S, Jiang Y, Luo G and Xiao Y. c-Jun promotes the survival of H9c2 cells under hypoxia via PTEN/Akt signaling pathway. J Physiol Biochem 2019; 75: 433-441.
[59] Yamamoto T, Kanda A, Kase S and Ishida S. Hypoxia induces galectin-1 expression via autoinduction of placental growth factor in retinal pigment epithelium cells. Invest Ophthalmol Vis Sci 2021; 62: 22.
[60] Glaus Garzon JF, Pastrello C, Jurisica I, Hottiger MO, Wenger RH and Borsig L. Tumor cell endogenous HIF-1α activity induces aberrant angiogenesis and interacts with TRAF6 pathway required for colorectal cancer development. Neoplasia 2020; 22: 745-758.
[61] Kunz M, Moeller S, Koczan D, Lorenz P, Wenger RH, Glocker MO, Thiesen HJ, Gross G and Ibrahim SM. Mechanisms of hypoxic gene regulation of angiogenesis factor Cyr61 in melanoma cells. J Biol Chem 2003; 278: 45651-45660.