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DUXAP10 regulates proliferation and apoptosis of chronic myeloid leukemia via PTEN pathway

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Abstract. – OBJECTIVE: To investigate the role of DUXAP10 in chronic myelogenous leukemia (CML) and its underlying mechanism.

PATIENTS AND METHODS: We detected DUXAP10 expression in 82 CML patients, 12 normal controls, and CML cell line by qRT-PCR (quantitative real-time polymerase chain reaction). After transfection of si-DUXAP10 or si-PTEN in CML cell lines (K652, KG-1), we detected proliferation, cell cycle, and apoptosis by CCK-8 (cell counting kit-8), colony formation assay, and flow cytometry, respectively. Finally, protein expressions of p21, CDK2, Bcl-2, Bax, and PTEN were detected by Western blot.

RESULTS: DUXAP10 was upregulated in CML tissues and cells, which was gradually increased in the chronic phase (CP), acceleration phase (AP), and blast phase (BP) of CML. Knockdown of DUXAP10 in K652 and KG-1 cells can remarkably inhibit cell proliferation, promote cycle arrest and apoptosis. Western blot and flow cytometry results demonstrated that DUXAP10 can reduce apoptosis by inhibiting PTEN expression.

CONCLUSIONS: Overexpressed DUXAP10 accelerates the development and progression of CML by promoting cell proliferation, reducing cell cycle arrest and apoptosis via inhibiting PTEN expression.

Key Words:
DUXAP10, Chronic myeloid leukemia, Cell proliferation, Apoptosis, PTEN.

Introduction

Chronic myelogenous leukemia (CML) is a clonal bone marrow stem cell disorder in which a proliferation of mature granulocytes (neutrophils, eosinophils, and basophils) and their precursors is found[1,2]. Globally, the prevalence of CML is 1-2/100,000 and the median age of CML patients is about 50-55 years[3]. Under normal circumstance, CML patients are in chronic phase (CP) at first with the normal function of mature blood cells[4,5]. The disease condition progresses to an acceleration phase (AP) after 3 to 5 years if not treated. This phase is characterized by an increased burden of disease and production of progenitor/progenitor cells rather than terminally differentiated cells. The last phase of CML is the blast phase (BP), which is characterized by the rapid proliferation of myeloid-differentiated BP cells[4-6]. Previous studies have shown that adhesion dysfunction, activation of mitotic genes, and inhibition of apoptosis are the underlying causes of CML.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs with over 200 nt in length, which are widely expressed in the nucleus and cytoplasm. LncRNA lacks an open reading frame and does not participate in protein coding[7]. Functionally, lncRNAs are involved in various biological processes, such as cell proliferation, cell cycle, differentiation, and apoptosis[8-10]. For example, lncRNA-PVT1 can promote cell proliferation, cell cycle, and stem cell performance[11]. Downregulated HOTAIR leads to the arrested cell cycle in G0/G1 and inhibition of *in situ* tumor growth[12]. MALAT1 is capable of inhibiting cell apoptosis in pancreatic cancer and prostate cancer[13].

RNA derived from transcriptional pseudogenes was previously considered to be a kind of molecule without any biological function. However, recent studies have found that some pseudogenes are greatly involved in tumorigenesis[14]. It is reported that DUXAP10 promotes the development of non-small cell carcinoma[15]. DUXAP10 is located on chromosome 14q 11.1, which regulates cell cycle and reproductive development of tumor cells[8]. Previous researches have shown that DUXAP10 can inhibit PTEN expression in colorectal cancer cells[16], but whether DUXAP can exert the same biological role in CML remains to be studied.

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The purpose of this study was to investigate the role of DUXAP10 in chronic myelogenous leukemia (CML) and its underlying mechanism.

Patients and Methods

Patients

All subjects were outpatients and inpatients from The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University who were pathologically diagnosed as CML through bone marrow cell morphology, cytogenetics, and chromosome examinations. Enrolled subjects were newly diagnosed as blast phase of CML, and acceleration phase of CML without treatment or without remission after treatment. In this experiment, there were 27 cases with chronic phase, 25 cases with acceleration phase, and 30 cases with blast phase of CML. Meanwhile, 12 cases in the control group were healthy donors of hematopoietic stem cell transplantation. This study was approved by The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University Medical Ethics Committee. All the subjects signed the informed consent.

RNA Extraction and qRT-PCR (Quantitative Real-Time Polymerase Chain Reaction)

The total RNA was extracted from tissue samples by TRIzol (Invitrogen, Carlsbad, CA, USA) method and then transcribed into complementary Deoxyribose Nucleic Acid (cDNA) according to the instructions of SYBR® Green Master Mix (TaKaRa, Otsu, Shiga, Japan). The amplification condition for PCR was as follows: 94°C for 5 min, 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, for a total of 40 cycles. Primers used in this study were: DUXAP10 (F: 5'-CTGTAGGAGGCCAAGACAGG-3', R: 5'-CATTGTCTCAAGGTCTGCTGAA-3'), PTEN (F: 5'-TGGATTCGACTTAGACTTGACCT-3', R: 5'-GGTGGGTTATGGTCTTCAAAAGG-3'), GAPDH (F: 5'-ACCCACTCCTCCACCTTTGA-3', R: 5'-CTGTTGCTGTAGCCAAATTCGT-3').

Cell Culture

THP-1, KG-1 and K562 cells were purchased from ATCC (Manassas, VA, USA). Cells were cultured in RPMI-1640 (Roswell Park Memorial Institute-1640) containing 10% FBS (fetal bovine serum), and incubated in a 5% CO_2 incubator at 37°C (Gibco, Rockville, MD, USA). Culture medium was replaced every day.

Cell Transfection

Cells in logarithmic growth phase were seeded in the 6-well plates. DUXAP10 NC, si-DUXAP101#, si-DUXAP10 2# or si-PTEN were transfected according to the instruments of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Culture medium was replaced after transfection for 6 h. Primer sequences were as follows: si-DUXAP10 1# (5'-GGAACUUCCCAAACCUCCAUGAUUU-3'), si-DUXAP10 2# (5'-CAGCAUACUUCAAAUUCACAGCAAA-3'), si-PTEN (5'-GACGGGAAGACAAGUUCAUTT-3'), si-NC (5'-UUCUCCGAACGUGUCACGUTT-3').

CCK-8 (Cell Counting Kit-8) Assay

Transfected cells were seeded into 96-well plates with 1×10^4 per well. 10 μL of the CCK-8 solution (Dojindo, Kumamoto, Japan) was added in each well. The absorbance at 450 nm of each sample was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

Colony Formation Assay

Cells at logarithmic growth were routinely digested, centrifuged, and adjusted to a density of 1×10^4/mL. A single cell suspension was added to 6-well plates. After incubation for 3-4 days, 500 μL of 1% crystal violet was utilized to stain cells at room temperature for 1h. Under an inverted microscope (Nikon, Tokyo, Japan), 5 randomly selected fields in each well were captured.

Cell Apoptosis Detection

The cell suspension was collected into marked flow tubes, and digested with EDTA-free trypsin. After centrifugation, 1×10^5 cells were washed with PBS (phosphate buffer saline) twice. We then added 5 μL of Annexin V-FITC fluorescent probe and 1 μL of propidium iodide (PI) to the cell precipitation, followed by incubation without light for 5 min. Subsequently, cell apoptosis was analyzed by flow cytometry (Partec AG, Arlesheim, Switzerland) through FL1 and FL3 dual channels after adding 400 μL of 1 × loading buffer.

Cell Cycle Detection

For cell cycle detection, cell density was adjusted to 1×10^5/mL and fixed with pre-cooled 75% ethanol overnight. 100 μL of RNaseA was then added for 30-min incubation, followed by the addition of 10 mL of PI (Propidium Iodide) agent for mixing. The mixture

was incubated at room temperature for 15 min without exposure to light. Subsequently, cell cycle was analyzed by flow cytometry at the wavelength of 488 nm.

Western Blot

The protein sample was extracted by PMSF (phenylmethylsulfonyl fluoride) and the concentration of each sample was detected by a BCA (bicinchoninic acid) kit (Beyotime, Shanghai, China). Briefly, 50 μg of total protein was separated by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) under denaturing conditions and transferred to PVDF (polyvinylidene difluoride) membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% skimmed milk, followed by the incubation of specific primary antibodies overnight. After washed with PBS (phosphate-buffered saline) for three times, membranes were incubated with the corresponding secondary antibody (1:5000) at room temperature for 1 h. Immunoreactive bands were exposed by enhanced chemiluminescence (ECL) method.

Statistical Analysis

We used Statistical Product and Service Solutions (SPSS) 22.0 Software for all statistical analysis (IBM, Armonk, NY, USA). GraphPad Prism 5 (La Jolla, CA, USA) was introduced for image editing. The quantitative data were represented as mean ± standard deviation (`x ± s). The *t*-test was used for comparing differences between the two groups. $p < 0.05$ was considered statistically significant.

Results

DUXAP10 Was Upregulated in CML Samples and Cells

To investigate the association between DUXAP10 and CML, we used qRT-PCR to detect the expression level of DUXAP10 in CML samples, control tissues and CML cell lines, respectively. The results showed a higher expression of DUXAP10 in CML samples than that of the control group (Figure 1A), indicating that DUXAP10 may be involved in the occurrence of CML. Furthermore, we detected DUXAP10 expression in AP, CP, and BP of CML samples. The data illustrated that DUXAP10 expression was gradually increased as the disease phase developed (Figure 1B). *In vitro* experiments also demonstrated that DUXAP10 was overexpressed in CML cell lines, especially in K652 and KG-1 cells (Figure 1C). Hence, we selected K652 and KG-1 cells for the following experiments.

DUXAP10 Knockdown Inhibited Proliferation, Promoted Cell Cycle Arrest and Apoptosis of K652 and KG-1 Cells

Transfection efficacy was evaluated after si-DUXAP10 1# or si-DUXAP10 2# were transfected in K652 and KG-1 cells for 48 h, respectively (Figure 2A). We then carried out the CCK-8 assay for detecting cell proliferation. The data showed that DUXAP10 knockdown led to inhibited proliferative ability after transfection for 6, 24, 48, and 72 h, respectively (Figure 2B). Colony formation assay obtained the similar results (Figure 2C). Furthermore, cell cycle and

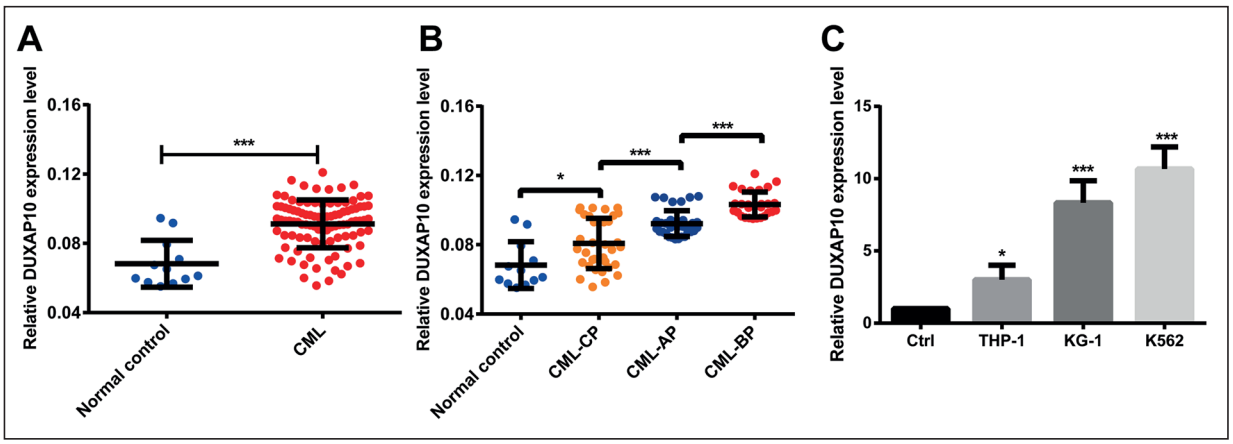

Figure 1. DUXAP10 was upregulated in CML samples and cells. ***A,*** Higher expression of DUXAP10 in CML samples than that of the control group. ***B,*** DUXAP10 expression was gradually increased as the disease phase developed. ***C,*** DUXAP10 was overexpressed in CML cell lines, especially in K652, and KG-1 cells.

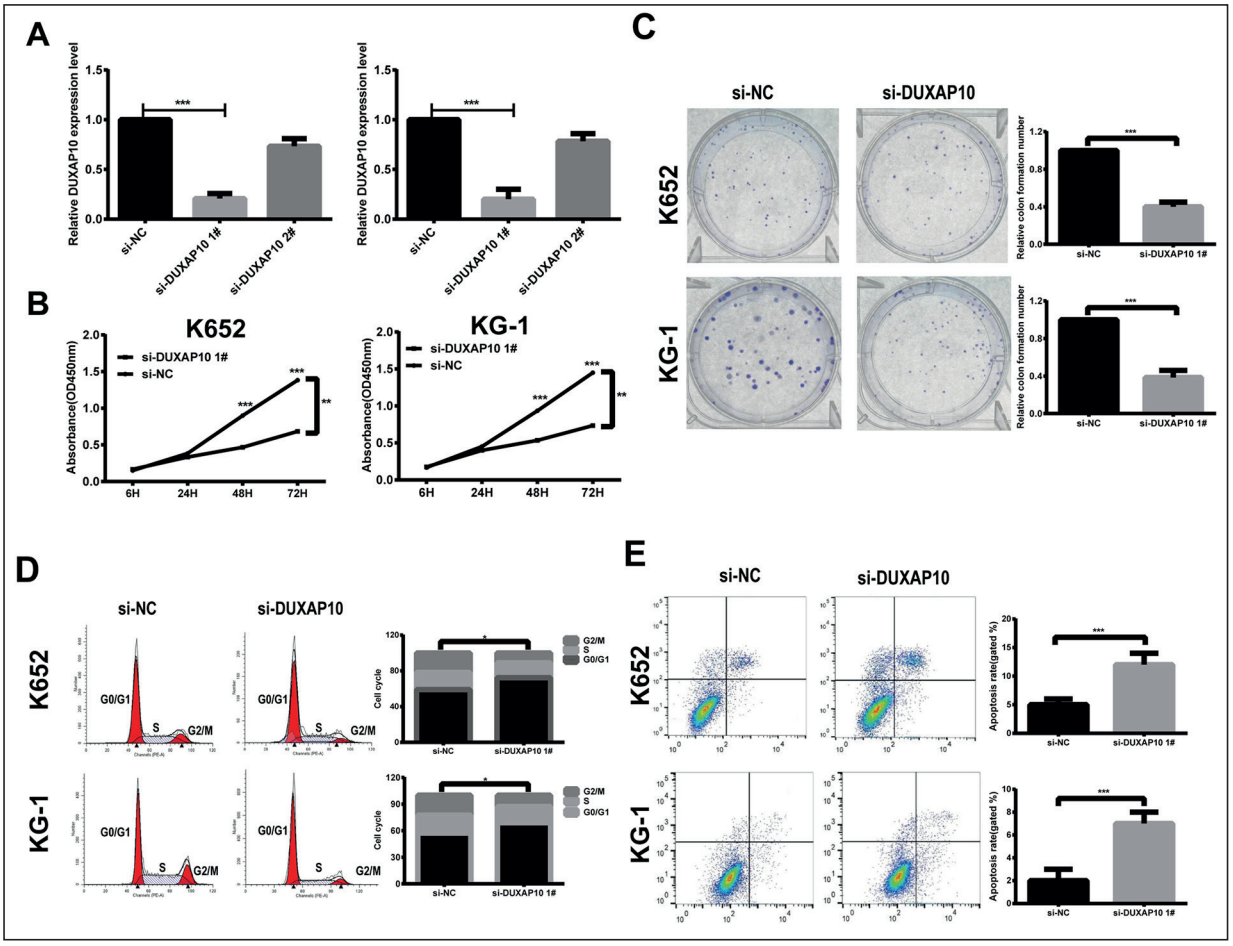

Figure 2. DUXAP10 knockdown inhibited proliferation, promoted cell cycle arrest and apoptosis of K652 and KG-1 cells. ***A,*** Transfection efficacy was evaluated after si-DUXAP10 1# or si-DUXAP10 2# were transfected in K652 and KG-1 cells for 48 h, respectively. ***B,*** DUXAP10 knockdown led to inhibited proliferative ability after transfection for 6, 24, 48, and 72 h, respectively. ***C,*** DUXAP10 knockdown led to inhibited colony formation ability. ***D, E,*** Arrested cell cycle (***D***) and increased apoptosis (***E***) were detected after inhibition of DUXAP10.

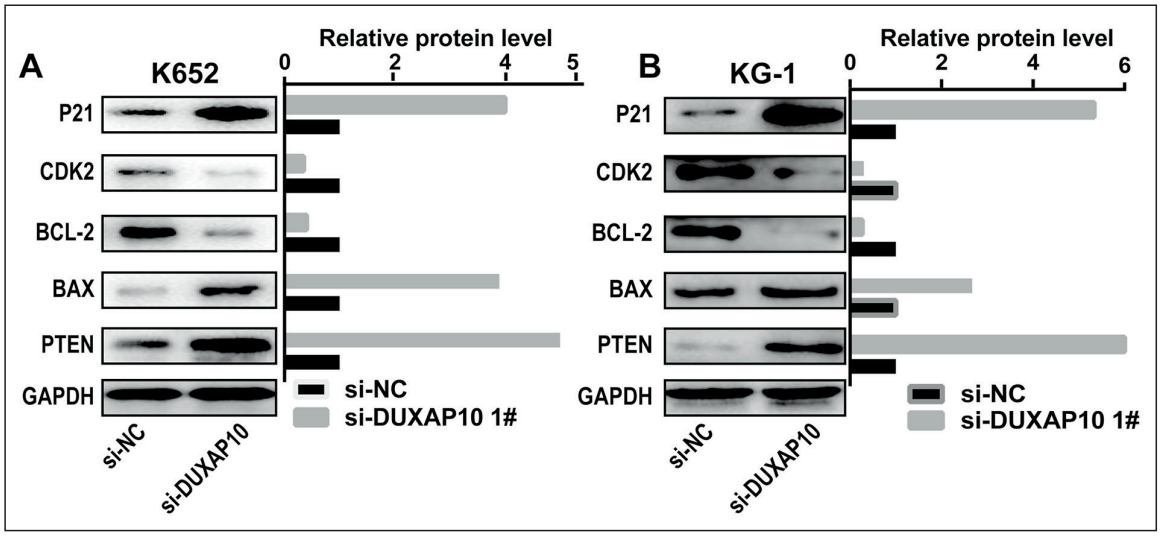

Figure 3. DUXAP10 knockdown increased PTEN expression. ***A, B,*** Downregulated CDK2 and Bcl-2, as well as upregulated p21 and PTEN were found after K652 and KG-1 cells were transfected with si-DUXAP10 1# for 48 h.

apoptosis were detected by flow cytometry. Inhibited DUXAP10 resulted in arrested cell cycle and increased apoptosis in K652 and KG-1 cells (Figure 2D and 2E). The above data suggested that DUXAP10 knockdown inhibited proliferation, promoted cell cycle arrest and apoptosis of K652 and KG-1 cells.

DUXAP10 Knockdown Increased PTEN Expression

Protein expressions of p21, CKD2, Bcl-2, BAX, and PTEN were detected after K652 and KG-1 cells were transfected with si-DUXAP10 1# for 48 h. Downregulated CDK2 and Bcl-2, as well as upregulated p21 and PTEN after DUXAP10 knockdown indicated that DUXAP10 participates in CML *via* regulating PTEN expression (Figure 3A and 3B).

DUXAP10 Effect Was Rescued by PTEN Inhibition

Protein and mRNA levels of PTEN were decreased after K652 and KG-1 cells were transfected with si-PTEN (Figure 4A and 4B). Moreover, cell apoptosis was promoted after the DUXAP10 knockdown, which was rescued by inhibiting PTEN expression (Figure 4C and 4D).

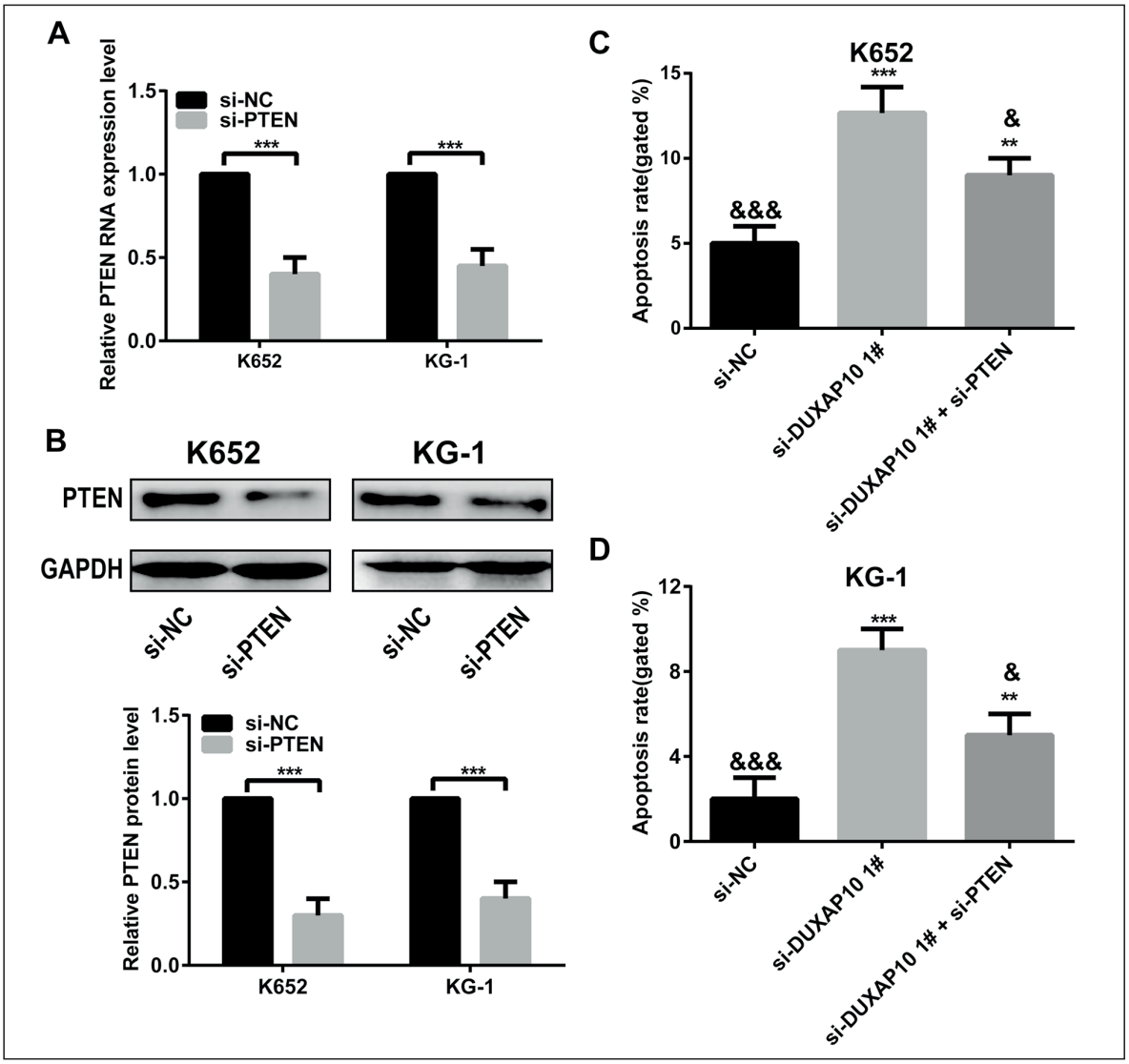

Figure 4. The DUXAP10 effect was rescued by PTEN. ***A, B,*** Protein (***A***) and mRNA (***B***) levels of PTEN were decreased after K652 and KG-1 cells were transfected with si-PTEN. ***C,*** Cell apoptosis was promoted after the DUXAP10 knockdown. ***D,*** Cell apoptosis was inhibited after co-transfection with si-DUXAP10 and si-PTEN (**$p < 0.01$, ***$p < 0.0001$ *vs.* si-NC; $^{\&}p < 0.05$, $^{\&\&\&}p < 0.0001$ *vs.* si-DUXAP10).

Discussion

CML is a kind of hematological malignancy with high heterogeneity. Its specific pathogenesis, however, remains unclear. In recent years, various etiological factors such as oncogenes, tumor suppressor genes, and apoptosis-suppressor genes are considered to be involved in the pathogenesis of leukemia. Among them, tumor suppressor genes have become an important cause of the malignant proliferation of blood cells.

In the hematopoietic system, several lncRNAs participate in the occurrence and development of hematological malignancies. For example, downregulated H19 has been found in many hematological malignancies, such as CML, chronic lymphocytic leukemia (CLL), and bone marrow proliferative disorders[17]. LncRNA HOTAIR and lncRNA asoct4-pg5 inhibit expressions of some certain transcription factors, including HOX and OCT4[18]. LncRNA p21 is downregulated in CLL patients, which is related to the disease stage of CLL[19]. Previous studies[20-22] have shown that DUXAP10 can promote cell proliferation and inhibit apoptosis in many diseases. In this study, we found that DUXAP10 expression was remarkably elevated in CML samples and cells. Knockout of DUXAP10 inhibited the proliferation, promoted cycle arrest and apoptosis of K652 and KG-1 cells.

PTEN is an essential tumor suppressor, the function of which is similar to that of p53. PTEN-encoded proteins exhibit dual-specific phosphatase activity in the cytoplasm, namely protein tyrosine phosphatase activity and lipid phosphatase activity. PTEN activates/inhibits multiple intracellular and nuclear signaling pathways and is therefore widely involved in the proliferation, apoptosis, and drug resistance of human tumor cells. As a consequence, PTEN has become one of the most crucial tumor suppressor genes[23-25]. Current studies have shown that PTEN exerts a role in suppressing cell proliferation by inducing apoptosis or inhibiting angiogenesis mainly through PKB/AKT pathway. PTEN deficiency results in the production of leukemia-derived stem cells (LSCs) and the loss of hematopoietic stem cells (HSCs), eventually threatening patients' health[26].

In the present study, transfection of si-DUXAP10 remarkably increased expressions of PTEN-related proteins and apoptosis of CML cells, which was partially reversed by inhibition of PTEN expression.

Conclusions

Overexpressed DUXAP10 accelerates the development and progression of CML by promoting cell proliferation, reducing cell cycle arrest and apoptosis via inhibiting PTEN expression.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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