Research Article

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Knockdown of G1P3 inhibits cell proliferation and enhances the cytotoxicity of dexamethasone in acute lymphoblastic leukemia

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Abstract: Drug resistance contributes to treatment failure and relapse in acute lymphoblastic leukemia (ALL). G1P3 (also known as IFI6, interferon, alpha-inducible protein 6) has been regarded as an antiapoptotic protein in myeloma cells and contributes to chemoresistance in breast cancer. However, the role of G1P3 in the proliferation and chemosensitivity of ALL is largely unknown. Data from colony formation and bromo-deoxyuridine (BrdU) incorporation assays showed that siRNA-mediated downregulation of G1P3 repressed cell proliferation of glucocorticoids-resistant human leukemic cells (CEM-C1), while overexpression of G1P3 promoted the cell proliferation. Cell apoptosis of CEM-C1 was suppressed by G1P3 overexpression accompanied by a decrease in cleaved caspase-3 and caspase-9. Knockdown of G1P3 increased protein expression of cleaved caspase-3 and caspase-9 to promote the cell apoptosis of CEM-C1. Moreover, silencing of G1P3 reduced cell viability and promoted cell apoptosis of CEM-C1 exposed to dexamethasone. The proapoptotic protein B-cell lymphoma 2 interacting mediator of cell death (Bim) was enhanced by the interference of G1P3 in CEM-C1. Silencing of Bim attenuated G1P3 interference-induced decrease in cell viability and increase in cell apoptosis in

CEM-C1 exposed to dexamethasone. Conclusively, knockdown of G1P3 inhibited cell proliferation of ALL and sensitized glucocorticoid-resistant ALL cells to dexamethasone through upregulation of Bim-mediated cell apoptosis.

Keywords: G1P3, ALL, proliferation, glucocorticoids-resistant, dexamethasone, Bim, apoptosis

1 Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by the replacement of normal hematopoietic cells in the bone marrow with undifferentiated and highly proliferating lymphoid precursors of T or B origin (called T-ALL or B-ALL, respectively) [1]. Genetic alterations in the development of ALL leads to leukemogenesis through cell apoptosis suppression, promoting cell proliferation and drug resistance [2]. Glucocorticoids are widely used to treat lymphoma and leukemia due to their ability to induce apoptosis and proliferation stagnation of lymphoid cells [3]. Dexamethasone is a synthetic glucocorticoid analogue and a major component in the treatment of hematopoietic malignancies, including ALL [4]. However, the resistance of ALL to glucocorticoids has become an important factor in the poor prognosis of patients with ALL [5].

G1P3 (also known as IFI6, interferon, alpha-inducible protein 6) is a member of the interferon stimulating genes (ISG) family and functions as mitochondrial protein to stabilize the mitochondrial function through antagonism of apoptosis [6]. Therefore, G1P3 was widely investigated in innate immunity and cancers [6]. For example, non-coding RNA psoriasis susceptibility-related RNA gene induced by stress (PRINS) promoted expression of G1P3 in keratinocytes, and knockdown of G1P3 increased cell apoptosis of keratinocytes during the progression of psoriasis [7]. G1P3 was found to be overexpressed in gastric cancer cells and suppressed mitochondrial-mediated apoptosis [8]. G1P3

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also contributed to the metastatic capacity of breast cancer cells through antiapoptotic function [9]. Moreover, depletion of G1P3 inhibited esophageal squamous cell carcinoma cell proliferation and promoted apoptosis [10]. In myeloma cells, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced cell apoptosis was antagonized by G1P3 [11]. A previous study showed that G1P3, as an estrogen- and interferon-induced survival protein, is implicated in the poor prognosis, chemoresistance, and hyperplasia of breast cancer [12]. However, the biological effects of G1P3 on cell proliferation of ALL and chemosensitivity of ALL to glucocorticoids have not been reported yet.

In this study, the effects of G1P3 on cell proliferation and apoptosis of glucocorticoid-resistant human leukemic cells (CEM-C1) were first investigated, and the role of G1P3 in chemosensitivity of CEM-C1 to dexamethasone was then evaluated. The results might provide a combinational strategy with glucocorticoids for the treatment of ALL.

2 Materials and methods

2.1 Cell culture and transfection

Glucocorticoid-resistant human leukemic cells (CEM-C1) were purchased from ATCC (Manassas, VA, USA) and cultured in RPMI 1640 medium (Gibco, Carlsbad, CA, USA) containing antibiotics (penicillin–streptomycin) and 10% fetal bovine serum (Sigma-Aldrich, St Louis, MO, USA). siRNA targeting G1P3 (si-G1P3) and the negative control (si-NC), si-Bim, pcDNA-G1P3, and the negative control (pcDNA) were provided by Genepharma (Suzhou, China). CEM-C1 cells were transfected with siRNAs or pcDNAs via Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

2.2 qRT-PCR

TRIzol reagent (Invitrogen) was used to extract RNAs from CEM-C1, and the RNAs were then transcribed into cDNAs under Reverse Transcription System (Promega, Madison, WI, USA). qRT-PCR analysis of G1P3 was performed using SYBR Green Master (Roche, Mannheim, Germany) under Applied Biosystems 7500 System. Specific primers: G1P3 (forward: 5'-TGAGCTGGTCTGCGATCC-3' and reverse: 5'-CCATCAGGGCACCAATATTACC-3') and GAPDH (forward: 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse: 5'-GAAGATGGTGATGGGATTTC-3') were used in this study.

2.3 BrdU incorporation and colony formation assays

CEM-C1 cells were seeded into a 96-well plate and transfected with siRNAs or pcDNAs for 48 h. The cells were incubated with 10 mM BrdU according to the BrdU Cell Proliferation Kit (Sigma-Aldrich) for 4 h and then fixed in methanol. Following incubation with a peroxidase-conjugated specific antibody against BrdU (Abcam, Cambridge, MA, USA), cells were treated with peroxidase substrate (tetramethylbenzidine), and then the absorbance was measured with a microplate reader (Abcam) at 450 nm. For colony formation assay, CEM-C1 cells posttransfections were seeded in a 6-well plate and cultured in the RPMI-1640 medium for 14 days. Cells were fixed in methanol and stained with crystal violet before measurement under a light microscope (Olympus, Tokyo, Japan).

2.4 Cell viability and flow cytometry

CEM-C1 with or without transfection of siRNAs or pcDNAs were seeded in 96-well plates and incubated with or without 75 µg/mL dexamethasone (Sigma-Aldrich) for 24 h. CCK8 solution (Beyotime, Beijing, China) was added to each well and incubated for 4 h. The absorbance at 450 nm was measured under the microplate reader. CEM-C1 were transfected with siRNAs or pcDNAs for 48 h. Cells with siRNAs or pcDNAs transfection were incubated with or without 75 µg/mL dexamethasone (Sigma-Aldrich) for 24 h. Cells were harvested following trypsin treatment and then resuspended in the binding buffer according to Annexin V-FITC Apoptosis Staining/Detection Kit (Abcam). PI and annexin V-FITC were then added into the cell suspensions before analysis under a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

2.5 Western blot

CEM-C1 were lysed in RIPA lysis buffer (Beyotime, Beijing, China), and the protein concentration was detected with a Micro BCA protein kit (Pierce, Rockford, IL, USA). Protein samples were separated with 10% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked with 5% defatted milk and probed with specific antibodies: anti-G1P3 and anti-β-actin (1:2,000, Abcam), anti-cleaved caspase-3, and anti-cleaved caspase-9 (1:2,500, Abcam). Following incubation with horseradish

Figure 1: Knockdown of G1P3 suppressed cell proliferation of CEM-C1. (a) G1P3 was up-regulated in primary acute myeloid leukemia tissues ($n = 173$) compared to the normal tissues ($n = 70$) based on TCGA database. (b) Transfection with si-G1P3 reduced mRNA expression of G1P3 in CEM-C1, while transfection with pcDNA-G1P3 enhanced the expression. (c) Transfection with si-G1P3 reduced the protein expression of G1P3 in CEM-C1, while transfection with pcDNA-G1P3 enhanced the expression. (d) Silencing of G1P3 reduced the number of BrdU-positive cells in CEM-C1, while overexpression of G1P3 enhanced the number of BrdU-positive cells. (e) Silencing of G1P3 reduced the number of cell colonies in CEM-C1, while overexpression of G1P3 enhanced the numbers of cell colonies. $n = 3$. ** vs pcDNA, $p < 0.01$. ##, vs si-NC, $p < 0.01$.

Figure 2: Knockdown of G1P3 promoted cell apoptosis of CEM-C1. (a) Silencing of G1P3 promoted cell apoptosis in CEM-C1, while overexpression of G1P3 suppressed apoptosis. (b) Silencing of G1P3 increased the expression of cleaved caspase-3 and caspase-9 in CEM-C1, while overexpression of G1P3 decreased the expression. $n = 3$. *, ** vs pcDNA, $p < 0.05$, $p < 0.01$. ##, vs si-NC, $p < 0.01$.

Figure 3: Knockdown of G1P3 promoted glucocorticoid sensitivity in CEM-C1. (a) Incubation with dexamethasone decreased the cell viability of CEM-C1, and transfection with si-G1P3 further reduced the cell viability of CEM-C1 exposed to dexamethasone. (b) Incubation with dexamethasone promoted cell apoptosis of CEM-C1, and transfection with si-G1P3 further promoted cell apoptosis of CEM-C1 exposed to dexamethasone. (c) Incubation with dexamethasone promoted the apoptosis ratio of CEM-C1, and transfection with si-G1P3 further promoted the cell ratio of CEM-C1 exposed to dexamethasone. $n = 3$. ^{**} vs Dex + si-NC, $p < 0.01$. ^{##}, vs control, $p < 0.01$.

peroxidase-conjugated secondary antibody (1:3,000, Abcam), the protein bands were visualized using chemiluminescence (Sigma-Aldrich) and normalized to β -actin by Image J.

2.6 Statistical analysis

All the data with at least triple replicates were expressed as mean $\pm$ SEM and analyzed by Student's t -test or one-

way analysis of variance with SPSS software. A p value of <0.05 was considered statistically significant.

3 Results

3.1 Knockdown of G1P3 suppressed cell proliferation of CEM-C1

G1P3 was up-regulated in primary acute myeloid leukemia tissues ($n = 173$) compared to the normal tissues ($n = 70$) based on TCGA database (Figure 1a). Glucocorticoid-resistant human leukemic cells (CEM-C1) were transfected with si-G1P3 or pcDNA-G1P3 to investigate the role of G1P3 in ALL. Transfection with si-G1P3 reduced mRNA (Figure 1b) and protein (Figure 1c) expression of G1P3 compared with the cells transfected with siNC. However, the expression of G1P3 was enhanced by pcDNA-G1P3 transfection (Figure 1b and c). Silencing of G1P3 reduced the number of BrdU-positive cells in CEM-C1, while overexpression of G1P3 enhanced the number of BrdU-positive cells (Figure 1d). The colony numbers of CEM-C1 were upregulated by overexpression of G1P3, while they were downregulated by silencing of G1P3 (Figure 1e), suggesting the antiproliferative effect of G1P3 silence on glucocorticoids-resistant ALL.

3.2 Knockdown of G1P3 promoted cell apoptosis of CEM-C1

Cell apoptosis of CEM-C1 was repressed by transfection with pcDNA-G1P3, while transfection with si-G1P3 promoted cell apoptosis (Figure 2a). Moreover, overexpression of G1P3 decreased the protein expression of cleaved caspase-3 and caspase-9 in CEM-C1, whereas silencing of G1P3 increased the expression (Figure 2b), demonstrating the proapoptotic effect of G1P3 silencing on glucocorticoids-resistant ALL.

3.3 Knockdown of G1P3 promoted glucocorticoid sensitivity in CEM-C1

Incubation with dexamethasone decreased the cell viability of CEM-C1 (Figure 3a). Transfection with si-G1P3 further reduced the cell viability of CEM-C1 exposed to dexamethasone (Figure 3a). Dexamethasone treatment also promoted cell apoptosis of CEM-C1 (Figures 3b and c), and knockdown of G1P3 exaggerated the cell apoptosis of

CEM-C1 exposed to dexamethasone (Figures 3b and c), revealing that knockdown of G1P3 promoted glucocorticoid sensitivity of glucocorticoid-resistant ALL.

3.4 Knockdown of G1P3 promoted glucocorticoid sensitivity in CEM-C1 through upregulation of Bim

Overexpression of G1P3 decreased the protein expression of Bim while silencing of G1P3 increased the expression of Bim (Figure 4a). In addition, the knockdown of Bim attenuated G1P3 interference-induced decrease in cell viability (Figure 4b) and increased apoptosis (Figure 4c) in CEM-C1 exposed to dexamethasone, indicating that knockdown of G1P3 sensitized glucocorticoids-resistant ALL to dexamethasone through upregulation of Bim.

4 Discussion

Interferon-stimulated genes (ISGs) are widely known as inhibitors of virus infection by regulating the virus life

cycle [13]. ISGs are also implicated in distinct biological functions, such as antiviral immune response, innate immunity, cancer progression, and metastasis [14]. For example, interferon-induced protein with tetratricopeptide repeat 2, the prominent ISGs, promoted the apoptotic death of leukemic cells [15]. Moreover, a previous study has shown that ISGs were involved in the radioresistance and chemoresistance of cancer cells through interaction with tumor-infiltrating lymphocytes [16]. Since G1P3 was reported to be an antiapoptotic factor, the roles of G1P3 in cell proliferation of ALL and chemoresistance were investigated in this study.

Knockdown of G1P3 suppressed cell proliferation of glucocorticoid-resistant ALL cells. Interferons have been widely used in cancer therapy by modulating tumor cell proliferation, differentiation, anti-angiogenic, and immunomodulatory responses [17]. Interferons treatment-induced ISGs and ISGs exerted apoptotic functions by interaction with TRAIL, XIAP associated factor-1, caspases, and other signaling pathways [17]. G1P3 has been shown to interact with calcium and integrin-binding protein to inhibit mitochondrial-mediated apoptosis of gastric cancer [8] and antagonize TRAIL-induced myeloma cell apoptosis [11]. This

Figure 4: Knockdown of G1P3 promoted glucocorticoid sensitivity in CEM-C1 through upregulation of Bim. (a) Overexpression of G1P3 decreased the protein expression of Bim in CEM-C1, while silencing of G1P3 increased the expression. (b) Silence of Bim attenuated G1P3 interference-induced decrease in cell viability in CEM-C1 exposed to dexamethasone. (c) Silence of Bim attenuated G1P3 interference-induced increase in apoptosis in CEM-C1 exposed to dexamethasone. $n = 3$. ** vs pcDNA or Dex + si-G1P3, $p < 0.01$. ##, vs si-NC or control, $p < 0.01$. @, vs Dex + si-NC, $p < 0.01$.

study showed that the silencing of G1P3 increased the protein expression of cleaved caspase-3 and caspase-9 to promote the cell apoptosis of glucocorticoid-resistant ALL cells. Anticancer agents also exerted therapeutic properties through regulation of cancer-associated inflammation [18], mismatch repair deficiency and microsatellite instability [19], oxidative stress [20,21], and cell cycle arrest [22]. Therefore, other molecular pathways involved in G1P3 silence-mediated glucocorticoid-resistant ALL cells should be investigated in further research.

miR-145 promoted cell apoptosis and autophagy to reverse the chemoresistance in ALL [23], and rapamycin sensitized glucocorticoids-resistant ALL to dexamethasone through induction of cell apoptosis [24]. Therefore, promoting cell apoptosis might be a potential strategy to sensitize the glucocorticoid-resistant ALL to dexamethasone. G1P3 has been reported to antagonize tamoxifen in breast cancer cells by regulating Bcl-2 family proteins, thus leading to chemoresistance in breast cancer cells [12]. This study showed that silencing of G1P3 reduced cell viability of dexamethasone-induced CEM-C1 and promoted cell apoptosis, indicating that silencing of G1P3 promoted glucocorticoid sensitivity of glucocorticoid-resistant ALL cells.

Bim is widely known as a proapoptotic member in the Bcl-2 family and participates in chemotherapeutic agents and apoptotic stimulation-mediated processes [25]. Suppression of Bim promoted metastasis and chemoresistance of tumors, and therefore, Bim is regarded as targeted therapy for treating tumor-associated phenomena, including metastasis and chemoresistance [25]. Dexamethasone treatment has been shown to enhance the expression of Bim in ALL [26], while ALL cells with Bim knockout showed resistance to glucocorticoids [27]. Moreover, glucocorticoid resistance in xenografted mice with primary childhood ALL biopsies was related to the reduction of Bim levels [5]. G1P3 has been shown to reduce Bim protein during the anoikis of breast cancer cells [12]. In this study, overexpression of G1P3 reduced the protein expression of Bim, while interference of G1P3 enhanced the Bim expression. Moreover, silencing of Bim attenuated G1P3 interference-induced decrease in cell viability and increased apoptosis in dexamethasone-induced CEM-C1. These results suggested that G1P3 contributed to chemoresistance of ALL through the regulation of Bim. However, in addition to intrinsic apoptosis (Bcl-2), extrinsic apoptosis was also implicated in the cell apoptosis of dexamethasone-induced ALL [28]. Therefore, the role of G1P3 on the extrinsic apoptosis of dexamethasone-induced CEM-C1 should be investigated in further research.

In summary, knockdown of G1P3 suppressed cell proliferation of CEM-C1 and promoted cell apoptosis. Moreover, knockdown of G1P3 promoted glucocorticoid sensitivity of glucocorticoid-resistant ALL cells through upregulation of Bim. Therefore, G1P3 might be explored as a potential target in the treatment of drug-resistant ALL. However, additional analysis in animal studies and clinical trials is required.

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