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Knock-down of CIAPIN1 sensitizes K562 chronic myeloid leukemia cells to Imatinib by regulation of cell cycle and apoptosis-associated members *via* NF-κB and ERK5 signaling pathway

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ABSTRACT

CIAPIN1 (cytokine-induced apoptosis inhibitor 1) was recently identified as an essential downstream effector of the Ras signaling pathway. However, its potential role in regulating myeloid leukemia cells sensitivity to Imatinib remains unclear. In this study, we found depletion of CIAPIN1 inhibited proliferation and triggered more apoptosis of K562CML (chronic myeloid leukemia) cells with or without Imatinib treatment. Meanwhile, CIAPIN1 depletion decreased ERK5 phosphorylation and NF-κB activity. Importantly, treating CIAPIN1-depleted K562 cells with ERK5 signaling pathway specific inhibitor, XMD8-92, further inhibited proliferation and promoted apoptosis with or without Imatinib treatment. Treatment with the NF-κB specific inhibitor, Bay 11-7082, induced nearly the same inhibition of proliferation and promotion of apoptosis conferred by CIAPIN1 depletion as was observed with XMD8-92 treatment. Further, XMD8-92 and Bay 11-7082 synergistically inhibited proliferation and promoted apoptosis of CIAPIN1-depleted K562 cells with or without Imatinib treatment. The nude mice transplantation model was also performed to confirm the enhanced sensitivity of CIAPIN1-depleted K562 cells to Imatinib. Thus, our results provided a potential management by which CIAPIN1 knock-down might have a crucial impact on enhancing sensitivity of K562 cells to Imatinib in the therapeutic approaches, indicating that CIAPIN1 knock-down might serve as a combination with chemotherapeutical agents in leukemia diseases therapy.

1. Introduction

CML (chronic myeloid leukemia), is a clonal disorder of the pluripotent hematopoietic stem cell (resulting in a progressive granulocytosis), in which a reciprocal translocation t(9;22)(q34;q11) forms a Ph (Philadelphia) chromosome and creates a novel fusion gene, BCR–ABL [1]. Its corresponding protein has a constitutively activated tyrosine kinase that is central to the pathogenesis of CML [2]. The disease follows a triphasic course: an initial CP (chronic phase) lasting 3–5 years, an AP (accelerated phase) lasting 6–18 months and the final phase called BC (blast crisis) or acute leukemia, defined hematologically by the increase of leukemic blasts (myeloid or lymphoid) in peripheral blood and/or bone marrow (more than 20%). At this stage of the disease, many patients died between three and six months, because they are refractory to most treatments, including resistance to Imatinib [3].

Imatinib (STI571, Gleevec) has emerged as the leading compound to treat CML. It targets the ATP-binding site of different tyrosine kinases including BCR–ABL, the platelet-derived growth factor receptor [4], and C-KIT [5]. Imatinib selectively induces growth arrest and apoptosis of BCR–ABL positive leukemia cells with minimal effect on normal hematopoietic progenitors [6–8]. Of

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note, this agent has proven very effective in patients in chronic phase of CML [9] and to a lesser extent, in patients in accelerated phase and blast crisis [7]. Although treatment with Imatinib achieves complete hematologic remission in the great majority of patients with CML, total cytogenetic and molecular responses are relatively rare events [10].

CIAPIN1 (cytokine-induced apoptosis inhibitor 1) is a newly identified apoptosis inhibitor with no homology to apoptosis regulatory molecules of the Bcl-2 family, caspase family or signal transduction molecules, and is proven to be a mediator of the Ras signaling pathway and has an important role in fetal liver hematopoiesis [11]. The expression of CIAPIN1 is dependent on stimulation with growth factors such as interleukin 3, stem cell factor, and thrombopoietin in factor-dependent hematopoietic cell lines, and forced expression of CIAPIN1 confers resistance to apoptosis caused by growth factor deprivation [12]. In addition, CIAPIN1 protects Ba/F3 cells against etoposide, γ radiation and staurosporine, but the mechanisms by which CIAPIN1 confers resistance in Ba/F3 cells to these apoptosis inducing factors is still unclear [13]. Most importantly, CIAPIN1 expression is found to be related to the malignant phenotypes of some cancers and may play potential roles in mediating some functions [12,14]. Some previous studies reported that CIAPIN1 could affect the cell proliferation and cell cycle progression and it might be involved in regulating MDR in some cancers [14–20]. Owing to its prognostic value for human tumors and involvement in cancer progression and tumor cell resistant to anticancer agents, CIAPIN1 has been proposed as an attractive target for new anticancer interventions.

K562 is a hematopoietic progenitor cell line established from a human CML patient in blast crisis [21,22]. K562 cells possess the capability of unlimited proliferation, but are unable to proceed with differentiation. In the current study, we proved that CIAPIN1 was highly expressed in CML patients and K562CML cell line. Then, we evaluated the effect of CIAPIN1 knock-down on the sensitivity of K562 cells to Imatinib and further investigated the underlying mechanisms. This is the first time to examine the role of CIAPIN1 in the sensitivity of myeloid leukemia cells to Imatinib, which may provide a theoretical basis for development of novel therapeutic strategies against leukemia diseases.

2. Materials and methods

2.1. Reagents and chemicals

We obtained RPMI 1640 medium, penicillin and streptomycin from Gibco-BRL Life Technologies, Inc. (Burlington, ON, Canada), FBS (fetal bovine serum) from HyClone (Logan, UT, USA), Imatinib from Novartis (Basel, Switzerland), XMD8-92, Bay 11-7082 and Hoechst 33258 staining kit from Beyotime (Shanghai, China), Cell Cycle analysis kit and Annexin V-APC/PI apoptosis analysis kit from Sungene Biotech (Tianjin, China). For western blotting analysis, antibodies GAPDH, Cyclin D1, P21, anti-phosphospecific ERK5 ($pTpY^{218/220}$), p38 ($pTpY^{180/182}$), JNK1-2/SAPK ($pTpY^{183/185}$), and nonphosphorylated ERK5, p38, JNK, were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), antibody CIAPIN1 from Abcam (MA, USA), Bcl-2 apoptosis analysis kit, caspase apoptosis kit and NF-κB pathway sampler kit from Cell Signaling Technology (Beverly, MA, USA). ECL (enhanced chemiluminescence reagent plus) reagents were purchased from BD Transduction Laboratories (CA, USA).

2.2. Cell cultures and bone morrow samples

The K562 cell line was preserved by our laboratory. K562 cells were cultured in RPMI 1640 medium (Gibco-BRL Life Technologies, Canada) supplemented with 10% FBS (fetal bovine serum, HyClone, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco-BRL Life Technologies, Canada) at 37 °C in a humid atmosphere with 5% CO_2.

We collected bone marrow samples of patients from Hospital of Blood Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College. Inclusion criteria for our study were based on the European Leukemia Net criteria. Clinical evaluation of patients was performed with physical examination and laboratory monitoring. All the patient samples were treated in accordance with the Helsinki Declaration. Before the start of treatment, each patient gave written informed consent. Within 24 h after sampling, mononuclear cells from bone marrow samples from patients were isolated by density gradient centrifugation using Lymphoprep and then centrifuged at $480 \times g$ for 15 min at room temperature. Molecular analysis included quantitative measurement of CIAPIN1 transcript level. Results were expressed as a ratio of CIAPIN1 to GAPDH transcript's copy number on the basis of an intra-laboratory standard.

2.3. Plasmids and transfection

The independent shRNA (small hairpin RNA) targeting CIAPIN1 was achieved using software from Ambion. The hairpin was synthesized and cloned into the eukaryotic vector pGPU6/GFP/Neo (GenePharma, China). The plasmid pGPU6-shCIAPIN1 (CIAPIN1 interfering DNA plasmid) and pGPU6-scramble (CIAPIN1 unrelated sequence DNA plasmid) were prepared from transformed *Escherichia coli* DH5α cells and transfected into K562 cells according to Lipofectamine™ 2000 (Invitrogen, USA) protocol. K562 cells in exponential phase were collected and seeded into the 24-well plate with 2×10^5 cells per well, in 500 μl RPMI 1640 medium without antibiotics. Dilute 1 μg recombinant plasmid DNA with 2 μl Lipofectamine™ 2000 in 50 μl volume of Opti-MEM, respectively and then combined the diluted DNA with Lipofectamine™ 2000 slightly and incubated for 20 min at room temperature. Subsequently, the mixture was added to the 24-well culture plate containing cells and incubated for about 24 h at 37 °C. The transfectants were selected with G418 at the concentration of 450 μg/ml for at least 2 weeks.

2.4. Real-time quantitative PCR

Trizol kit (Invitrogen, Grand Island, NY) was utilized to extract total RNA from cultured cells as suggested by the manufacturer and the concentration of total RNA was measured by spectrophotometer after treatment with DNase I (Invitrogen, Grand Island, NY). 2 μg RNA was used for reverse transcription in a 20 μl reaction with EasyScript RT (Trans, CA). Real-time quantitative PCR was performed with ABI 7500 system Instrument with SYBR Green PCR kit (Takara, Japan). Thermal cycling program consisted of 95 °C for 10 s, followed by 40 cycles of 5 s at 95 °C and 40 s at 60 °C. Each test was amplified in a total volume of 20 μl reaction following the manufacturer's instructions in one experiment and each test was repeated three times at least. Primer premier software 5.0 was used to design the primers for real-time quantitative PCR. The expression level of indicated genes was analyzed by the RQ value calculated through $2^{-\Delta\Delta Ct}$.

2.5. Western blotting

Cells with indicated treatments were harvested and rinsed three times with ice-cold PBS. The harvested cells were then lysed on ice in RIPA lysis buffer (RIPA: 150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM Tris (pH 8.0)) with protease inhibitors, and 1 mM PMSF (Sigma, USA) for 30 min and centrifuged at 12,000 rpm for 5 min at 4 °C.