CUEDC2 sensitizes chronic myeloid leukemic cells to imatinib treatment

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**A R T I C L E   I N F O**

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**A B S T R A C T**

CUEDC2, a newly reported protein, has been found to be ubiquitously expressed in human tissues and repress NF-κB activity. To study the role of CUEDC2 in chronic myeloid leukemia (CML), we explored the function of CUEDC2 in CML cells through using the CML cell line K562 and its imatinib resistant cells K562/G01. K562 cells expressed a relatively higher level of CUEDC2 compared to K562/G01 cells. Knockdown of CUEDC2 in K562 cells resulted in decreased cell apoptosis after imatinib treatment; when CUEDC2 was overexpressed in K562/G01 cells, imatinib induced more cell apoptosis. By analyzing the activity of NF-κB, the results indicated a negative association between the expression of CUEDC2 and NF-κB signaling pathway in these CML cells. Our data suggested that the expression level of CUEDC2 has an inverse correlation with imatinib resistance and activity of NF-κB signaling pathway in CML cells. CUEDC2 could regulate imatinib sensitivity in CML cells at least partially through NF-κB signaling pathway.

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1. Introduction

Chronic myeloid leukemia (CML) is a common hematologic malignancy, characterized by the formation of the Philadelphia (Ph) chromosome and Bcr-Abl fusion gene [1]. Bcr-Abl protein is a constitutively active tyrosine kinase and gives rise to uncontrolled growth of myeloid cells in the bone marrow through a series of downstream pathways [2]. According to the clinical stages, CML is divided into chronic phase (CP), accelerated phase (AP) and blast crisis (BC). The tyrosine kinase inhibitor (TKI) imatinib is a specific molecularly targeted molecular drug for the treatment of Ph chromosome-positive CML, particularly in CP [3–6]. Although it is considered as one of the most effective drugs and the first-line treatment for CML, resistance to imatinib seems unavoidable and occurs frequently during its clinical application. Due to the Bcr-Abl mutation or duplication, some other novel TKIs were developed, such as dasatinib, nilotinib and bosutinib [7]. However, abnormal expression of some other genes or alternative signaling pathways activation may also contribute to the resistance to imatinib [8,9].

The CUE domain-containing protein 2 (CUEDC2) is a newly reported protein, and its functions are still largely undefined. There is a CUE domain in the protein structure of CUEDC2, which is a moderately conserved ubiquitin-binding domain of ~40 amino acids found in many eukaryotic proteins. At present, an increasing amount of evidences suggest that CUEDC2 plays an important role in tumorigenesis [10]. It is ubiquitously expressed in human tissues and organs, not only highly expressed in the normal brain, heart and testis, but also some kinds of cancers, such as breast, ovarian and kidney cancers [11]. In these solid tumors, CUEDC2 expression was much higher than that in non-neoplastic tissues. It was initially reported that CUEDC2 could interact with estrogen receptor (ER) and progesterone receptor (PR) in breast cancer, promoted the degradation of the two receptors through the ubiquitin–proteasome pathway, and reduced the responsiveness of breast cancer cells to endocrine therapies, which suggested CUEDC2 might be involved in the progression of breast cancer [12–14]. Gao et al. demonstrated that high levels of CUEDC2 might result in earlier activation of the anaphase-promoting complex or cyclosome (APC/C), eventually resulting in chromosome missegregation and aneuploidy, and then promote tumor formation [11]. Another research indicated that CUEDC2 could act as an adaptor protein to target IkB kinase (IKK) for dephosphorylation and inactivation by recruiting protein phosphatase (PP1), and thus repressed activation of the transcription factor NF-κB [15]. Moreover, CUEDC2 has also been found to be a novel regulator of JAK1/STAT3 signaling, through binding to SOCS3 to increase its stability and thus inhibiting JAK1/STAT3 signaling [16].

However, the role of CUEDC2 in CML remains unclear, and thus need to be clarified. Our previous study found the expression level of CUEDC2 was associated with clinical stages in CML patients, the expression of CUEDC2 was significant higher in patient in CP than that in patients in AP and BC. Previous researches
demonstrated that response to imatinib in patients with advanced CML was less prominent than in CP [17,18]. Thus, we speculated that CUEDC2 might be implicated in the response of imatinib treatment.

In the present study, we attempted to explore that possible role of CUEDC2 in CML using the CML cell line K562 and its imatinib resistant cells K562/G01 as cellular model. In this report, it is demonstrated for the first time that CUEDC2 is associated with imatinib resistance in CML cells, and NF-κB signaling pathway might be involved in this process.

2. Materials and methods

2.1. Materials

We obtained RPMI1640 medium from Gibco-BRL Life Technologies, Inc. (Burlington, ON, Canada), fetal bovine serum (FBS) from HyClone (Logan, UT), imatinib from Novartis (Basel, Switzerland), Bay-117082 and G418 staining kit from Beyotime (Shanghai, China), mouse anti-GAPDH monoclonal antibody from Santa Cruz Biotechnology (Santa Cruz, CA), rabbit anti-CUEDC2 polyclonal antibody from ABGENT, and NF-κB Pathway Sampler Kit from Cell Signaling Technology (Beverly, MA, USA). Enhanced Chemiluminescence Reagent Plus (ECL) reagents were purchased from BD Transduction Laboratories. Annexin V-APC/PI Apoptosis Analysis Kit was purchased from Sungene Biotech (Tianjin, China).

2.2. Cell lines and culture

The CML cell line K562 (Bcr-Abl positive cell line) was preserved by our laboratory. K562/G01 cells are imatinib-resistant K562 cells, which was provided by pharmacology laboratory at Institute of Hematology, Chinese Academy of Medical Sciences and established by their group through incubation of K562 cells under increased concentrations of imatinib for several months [19]. The K562 and K562/G01 cells were cultured in RPMI1640 supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin mix (100IU/ml and 100 μg/ml, respectively) at 37 °C in a humidified atmosphere with 5% CO2. Imatinib at concentration of 1 μM was added to the culture system of K562/G01 cells to maintain resistance activity.

2.3. RNA isolation and real-time quantitative PCR

Total RNA was extracted using Trizol Reagent (Invitrogen, Grand Island, NY), treated with DNase I (Invitrogen, Grand Island, NY), and 2 μg RNA were reverse-transcribed using SuperScript II RT (Invitrogen, Grand Island, NY) following the manufacturer’s instructions in a total volume of 20 μl.

Primers for real-time quantitative PCR were designed using Primer premier software 5.0. Human GAPDH primers used as an internal control were 5′-GAAGGTGAAGGTCGGAGTC-3′ (forward) and 5′-GGGATTCCGATCACTCTTGG-3′ (reverse; 226 bp). Human CUEDC2 primers were 5′-GAATGCCAGGAA CAAAAGCAA-3′ (forward) and 5′-CTCATCTGGT GGTTCTGC-3′ (reverse; 147 bp). Real-time quantitative PCR was performed with SYBR Green PCR kit (Takara, Japan) on the ABI PRISM 7500 Sequence Detection System. Thermal cycling conditions were 95 °C for 30 s, followed by 40 cycles of 5 s at 95 °C, and 34 s at 60 °C. PCR reactions were performed in a total volume of 20 μl containing 2 μl of sample cDNA, 0.2 μM of each primer, and the SYBR Green PCR kit following the manufacturer’s instructions. The expression level of CUEDC2 was analyzed by the RQ value calculated through the 2−ΔΔCt method (∆ΔCt = (∆CtCUEDC2 − ∆Ct IgG)sample − (∆CtCUEDC2 − ∆Ct IgG)reference). Data are represented as mean ± SD of at least three independent experiments performed in triplicate.

2.4. Western blot

Total protein was extracted using RIPA lysis buffer with 1 mM PMSF (Sigma, USA) and other protease inhibitors. Protein concentration was determined by the BCA assay (Solarbio, Beijing, China) according to the manufacturer’s instructions. The whole-cell lysates were heat-denatured at 100 °C for 10 min before being run on 8–12% gradient SDS-PAGE. After SDS-PAGE, the proteins were electrotransferred onto nitrocellulose membranes, blotted with each primary antibody, incubated in secondary antibody and then detected with enhanced chemiluminescence detection reagent.

2.5. Short hairpin RNA-mediated RNA interference studies

The cDNA sequence of CUEDC2 was obtained from Genebank (NM_024040). Two different CUEDC2-specific targeting sequences were designed with the software from Ambion. And shRNA expressing plasmids specifically targeting CUEDC2 (termed as CUEDC2-shRNA1 and 2) were constructed by GenePharma Corporation (Shanghai, China) using pGPU6/GFP/Neo vector. An unrelated shRNA sequence with no homology to any human genes was used as a negative control (shRNA NC). These plasmids were then transfected into K562 cells (termed as K562-NC, K562-sh1 and K562-sh2). Transfected cells were selected by G418 48 h after transfection for at least 2 weeks and stable clones were obtained. Inhibition of CUEDC2 expression was measured by real-time quantitative PCR as well as by Western blot using a rabbit anti-CUEDC2 polyclonal antibody.

2.6. Plasmid construction for CUEDC2 overexpression and transfection

Human CUEDC2 was generated by PCR from a human normal cDNA library with the forward primer 5′-ACGCGATATTC GAGGCACCACTCG-3′ and the reverse primer 5′-GACGTCACGC/CAATGAGGCGCAGATCTC-3′ and were cloned into pIRE2-ScGFP (GenePharma Corporation, Shanghai, China). The recombinant plasmid was termed pIRE2-ScGFP-CUEDC2. K562-sh2 cells were then transfected into K562/G01 cells (termed as K562/G01-CUEDC2 and K562/G01-ScGFP) by lipofection. Transfected cells were selected by G418 48 h after transfection for at least 2 weeks for stable clones. Detection of CUEDC2 expression was measured by real-time quantitative PCR as well as by Western blot using a rabbit anti-CUEDC2 polyclonal antibody.

2.7. Measurement of cell viability

The viability of K562, K562/G01 cells and its transfectants were examined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. 3 × 104 cells per well were seeded into 96-well plates in 100 μl volume and grown in RPMI1640 medium supplemented with 10% fetal bovine serum at 37 °C. Cells were cultured for 24, 48, 72, 96 h, respectively. After indicated time of incubation, 20 μl of MTT reagent was added. Then the cells were incubated at 37 °C for an additional 4 h, and 100 μl triple lysate (sodium dodecyl sulfate/soybutanol/H2O) was added to each well. The absorbance of formazan product was measured by Versa Max tunable microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 570 nm. Each experiment was repeated at least thrice in quadruplicate.

2.8. Analysis of apoptosis by flow cytometric assay and nuclear morphology

Phosphatidylinositol externalization was analyzed with Annexin V-APC/PI Apoptosis Analysis Kit by a FACScalibur flow cytometer (BD) for cell apoptosis following the manufacturer’s instructions. Apoptosis was quantified as the percentage of Annexin V positive cells. Apoptosis was also judged by nuclear condensation stained by Hoechst 33258 following the manufacturer’s instructions.

2.9. Immunofluorescence assay

For immunofluorescence localization of NF-κB by laser confocal scanning microscopy, cells (106) were fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton x100 and blocked with goat serum, then cells were incubated with a rabbit monoclonal antibody against the p65, followed by TRITC-conjugated goat anti-rabbit IgG antibody. The nuclei were stained with Hoechst 33258. Cells were imaged on Lesa TCS F2 confocal laser microscope.

2.10. Statistical analysis

All experiments were conducted at least three times and data are presented as mean ± SD. Statistical analysis was performed with the SPSS software package (version 13.0; SPSS). Two-tailed P-values <0.05 were considered statistically significant.

3. Results

3.1. Detection of expression of CUEDC2 in CML cell lines

Real-time quantitative PCR assays were performed to selectively amplify CUEDC2 in K562 and imatinib resistant K562/G01 cells to detect the expression level of CUEDC2. The results showed that the expression level of CUEDC2 in K562 was significantly higher than that in K562/G01 cells (P <0.05) (Fig. 1A and B). The results of Western blot also revealed that K562 cells expressed a relatively higher level of CUEDC2 compared with K562/G01 (Fig. 1C and D). These results suggested a possible association between CUEDC2 expression level and imatinib sensitivity.

3.2. CUEDC2 knock-down impaired sensitivity to imatinib in K562 cells

Two different CUEDC2 specific shRNA plasmids were used to downregulate CUEDC2 expression in K562 cells, which had a relatively higher expression level of CUEDC2. As expected, stable
transfection experiments showed both shRNA plasmids could significantly inhibit CUEDC2 expression in K562 cells in mRNA and protein level (Fig. 2A–C). MTT assay showed CUEDC2 knockdown did not affect the viability of K562 cells (Fig. 2D).

In order to detect and quantify the apoptosis, we used Annexin V-APC/PI double staining to determine the effect of CUEDC2 knockdown on apoptosis in K562 cells with or without imatinib treatment. The results showed that CUEDC2 knockdown reduced
the percentage of apoptotic cells after incubation with 1 μM imatinib for 24 h compared with K562-NC, which was demonstrated by significantly decreased Annexin-V positive rate (18.81 ± 2.32%, 17.77 ± 1.69% VS 30.89 ± 2.39%, P<0.05) (Fig. 3A and B). Hoechst staining also showed a reduced proportion of condensed nuclei in K562-sh1 and K562-sh2 (Fig. 3C). But no difference was found in the cells without imatinib treatment.

Caspase-3 has been demonstrated as a key mediator of apoptosis, and its activation is taken as a hallmark of apoptosis [21,22]. More cleaved caspase-3 protein expression was observed in K562-NC cells compared with that in K562-sh1 and K562-sh2 after imatinib treatment (Fig. 3D). Our data indicated that knockdown of CUEDC2 impaired the sensitivity to imatinib in K562 cells.

3.3. CUEDC2 overexpression attenuated imatinib resistance in K562/G01 cells

In order to further study the effect of CUEDC2 expression on imatinib resistance, we transfected K562/G01 cells with CUEDC2 plasmid and observed an obviously increased expression of CUEDC2 in mRNA and protein level (Fig. 4A–C). MTT assay showed that the viability of K562/G01 cells was not influenced with CUEDC2 overexpression (Fig. 4D). Apoptosis assays were performed to investigate the correlation between CUEDC2 overexpression and imatinib sensitivity. As expected, significantly higher of Annexin V positive rate (28.7 ± 3.68% VS 14.10 ± 2.601%) (Fig. 5A and B) and an increased proportion of condensed nuclei (Fig. 5C), more cleaved caspase-3 protein expression (Fig. 5D) were observed in K562/G01-CUEDC2 cells compared with control. Similarly to the situation of CUEDC2 knockdown, no difference was observed in cells without imatinib treatment. These results indicated that CUEDC2 overexpression enhanced sensitivity to imatinib in K562/G01 cells, which had a relatively lower CUEDC2 expression level compared to sensitive K562 cells.

3.4. The role of NF-κB in the correlation between CUEDC2 and imatinib resistance

3.4.1. Constitutive NF-κB signaling pathway activity in K562 and K562/G01 cells

NF-κB has been suggested to play a role in determining resistance to treatment in numerous tumors [23,24]. Constitutive NF-κB activity has been demonstrated in several hematologic malignancies, such as acute myeloid leukemia (AML) and CML [25,26]. Classical activation of NF-κB involves phosphorylation and degradation of inhibitor of NF-κB (IκB), followed by nuclear translocation of NF-κB and subsequent activation of NF-κB target genes. And phosphorylation of IκBα depends on a multisubunit IKK complex, which is composed of 2 catalytic subunits, Iκκα and Iκκβ, and a noncatalytic subunit, IKKγ/NEMO [27]. To compare the constitutive NF-κB signaling pathway activity between K562 and K562/G01 cells, we detected the constitutive expression of Iκκα, Iκκβ, phospho-Iκκα/β (p-Iκκα/β), total IκBα, phospho-IκBα (p-IκBα) by Western blot and NF-κB nuclear translocation by immunofluorescence staining. The results showed that K562/G01 cells expressed a relatively higher p-Iκκα/β and p-IκBα compared with K562, while the total Iκκα and Iκκβ expression remained the same (Fig. 6A). NF-κB translocation from the cytosol to the nucleus was used as an index of its activation and a change in color of the nucleus was indicative of NF-κB translocation [27]. We localized NF-κB p65 with the immunostaining method described above and observed a relatively more NF-κB localized in nucleus in K562/G01...
cells compared with K562 cells (Fig. 6B). Our results indicated that imatinib resistant K562/G01 cells showed an elevated NF-κB activity than parental K562 cells, and there was an inverse correlation with the expression of CUEDC2.

3.4.2. The effects of CUEDC2 knockdown on NF-κB signaling pathway activity in K562 cells

To further rule out the correlation between CUEDC2 expression and NF-κB activity, we investigated the NF-κB signaling pathway activity when CUEDC2 was knocked down in K562 cells. The results showed that phosphorylation of IKKα/β and IκBα were obviously increased in K562-sh1 and K562-sh2 (K562-sh1/2) cells compared with K562-NC. Expression of total IκKα and IκKβ protein was similarly unchanged (Fig. 6C). In the K562-NC cells, NF-κB p65 protein was mainly located in the cytoplasm, while in CUEDC2 knockdown cells, an increase of NF-κB p65 translocated to the nucleus was observed (Fig. 6D). It was suggested that knockdown of CUEDC2 expression caused an increased activation of NF-κB, indicating CUEDC2 could inhibit the activity of NF-κB signaling pathway. After preincubation with NF-κB specific inhibitor Bay11-7082, phosphorylation of IκBα and the NF-κB nuclear translocation decreased in K562-sh1/2 cells, indicating that Bay11-7082 inhibited NF-κB activity induced by CUEDC2 knockdown in K562-sh1/2. Expression of cleaved caspase-3 after imatinib treatment confirmed that Bay11-7082 restored sensitivity to imatinib decreased by knockdown of CUEDC2 expression in K562 cells (Fig. 6E) and NF-κB signaling pathway played a role in the response to imatinib.

3.4.3. The effects of CUEDC2 overexpression on NF-κB activation in K562/G01 cells

As shown in Fig. 7, K562/G01 cells overexpressing CUEDC2 expressed a relatively lower level of phosphorylated IκKα/β, phosphorylated IκBα (Fig. 7A) and displayed less nuclear translocation of NF-κB compared to control cells (Fig. 7B). It was indicated that overexpression of CUEDC2 in K562/G01 resulted in inhibition of NF-κB signaling pathway activation.

4. Discussion

Bcr-Abl oncogenic tyrosine kinase plays a critical role in the pathogenesis of CML [1]. Imatinib, which was developed as a small molecule inhibitor of the Abl tyrosine targeting the oncogenic fusion protein Bcr-Abl, has been considered as the first-line therapy for all newly diagnosed CML at present [4,5]. However, imatinib resistance is becoming increasingly prominent due to various mechanisms, especially in more advanced CML. Recent researches indicate that other relevant targets in the Bcr-Abl pathways and strategies beyond targeting Bcr-Abl need to be explored for CML treatments [28,29].

CUEDC2 is a newly reported protein, and its functions are still under investigation at present. Recent studies have demonstrated that CUEDC2 was highly expressed in breast cancer, and modulated the expression of PR and ER through ubiquitin–proteasome pathway, indicating its role in the breast cancer [12,14]. Our previous study showed that CML patients in CP had significant higher CUEDC2 expression than that in CML patients with AP or BP (Data not shown). Clinical studies suggested that rates of imatinib resistance correlated with the stage of disease and CML patients in advanced stages were more prone to be refractory to imatinib treatment [17,18], which gave us an implication that CUEDC2 might be involved in CML treatment. In the present study, we examined the expression of CUEDC2 in CML cell line K562 and imatinib resistant K562/G01 cells. The results showed that the expression of CUEDC2 in K562 cell was significantly higher than that in imatinib resistance K562/G01 cells, which demonstrated that CUEDC2 was involved in the pathogenesis of CML, especially in imatinib resistance.

Pan et al. [14] found that CUEDC2 was inversely correlated with ER-α expression in breast cancer and elevated CUEDC2 expression...
impaired the responsiveness to tamoxifen treatment. Our results showed a relatively higher expression of CUEDC2 in imatinib sensitive K562 cells, indicating a different role of CUEDC2 in CML cells. K562 cells are considered a classical CML cell model because of its Bcr-Abl positive phenotype and sensitivity to imatinib treatment. In present study, knockdown of CUEDC2 in K562 cells by shRNA showed a decreased apoptosis rate induced by imatinib, indicating CUEDC2 conferred K562 the sensitivity to imatinib. Additionally, while overexpression of CUEDC2 in K562/G01 cells which expressed a relatively lower CUEDC2, imatinib induced increased apoptosis with the same treatment compared to the control cells. To combine the result of CUEDC2 expression level and apoptosis assay in CML cell lines, we found that higher expression of CUEDC2 was associated with higher sensitivity to imatinib, which may account for why CML patients in CP were more sensitive to imatinib treatment than patients in more advanced phases of the disease [5,17,30]. However, under the simple condition of knockdown of CUEDC2 expression in K562 cell or CUEDC2 overexpression in K562/G01 cells, cell viability was not affected and no obvious cell apoptosis was observed without imatinib treatment, herein, suggesting CUEDC2 played a role in imatinib induced apoptosis in CML cells. Our result further helped us to believe that CUEDC2 contributed to the sensitivity to imatinib in CML cells. However, the mechanism of CUEDC2 involved in this process still needs to be studied.

NF-κB is demonstrated downstream of Bcr-Abl and plays an important role in CML transformation [31,32]. Abnormal constitutive NF-κB/Rel activity has already been identified in CML [26,29]. Accumulating evidence indicates that targeting NF-κB is a potential therapeutic approach to overcome chemoresistance and radioreistance for cancer treatment [23,33,34]. Different kinds of NF-κB inhibitors have been developed to block the NF-κB signaling pathway and been demonstrated to inhibit cell growth, induce apoptosis in different cancers [35]. Li et al. reported that CUEDC2 could interact with IKK and promote dephosphorylation of IKK, and thus repressed activation of the transcription factor NF-κB [15]. Our results showed that K562/G01 cells displayed a relatively higher activity of NF-κB signaling pathway compared to K562 cells. Knockdown of CUEDC2 in K562 cells increased the activity of NF-κB signaling pathway. Bay11-7082, an IκB phosphorylation inhibitor, is an effective and specific NF-κB inhibitor [36]. In our study, Bay11-7082 blocked NF-κB signaling pathway activation induced by CUEDC2 knockdown through inhibiting the phosphorylation of IκBα and sensitized the K562 cells to imatinib, which further indicated NF-κB activity was associated with imatinib sensitivity in CML cells. The overexpression of CUEDC2...
Fig. 6. Detection of activity of NF-κB signaling pathway. (A, C) Western blot was performed to examine the expression of NF-κB signaling pathway relative proteins in indicated cells (NC, sh1, sh2 are short for K562-NC, K562-sh1 and K562-sh2 respectively); (B, D) immunofluorescence assay was performed to observe NF-κB p65 nuclear translocation. Red indicated NF-κB p65 staining, blue was Hoechst staining for nuclei; (E) Western blot was performed to detect the expression of cleaved caspase-3 induced by imatinib with or without Bay11-7082 pretreatment in CUEDC2 silenced K562 cells. Red indicated NF-κB p65 staining, blue was Hoechst staining for nuclei. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 7. Effect of CUEDC2 overexpression on activity of NF-κB signaling pathway in K562/G01 cells. (A) Western blot was performed to analyze the expression of NF-κB signaling pathway relative proteins; (B) immunofluorescence assay was performed to observe NF-κB p65 nuclear translocation. Red indicated NF-κB p65 staining, blue was Hoechst staining for nuclei. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
rendered K562/G01 cells decreased NF-κB signaling pathway activity and increased imatinib sensitivity. These data confirmed a negative correlation between expression of CUECD2 and activity of NF-κB signaling pathway in CML cells, consistent with the study of Li et al. [15], which demonstrated a negative regulatory role of CUECD2 on NF-κB signaling pathway activity. Our study strongly suggested that CUECD2 was implicated in the resistance to imatinib through NF-κB signaling pathway in CML cells.

Previous researches have demonstrated that mechanisms of imatinib resistance in CML were mostly attributed to Bcr-Abl gene amplification or mutations. Nowadays, additional relevant targets in the Bcr-Abl signaling pathway, for example, NF-κB signaling pathway is gradually being concerned. Cilloni et al. [29] demonstrated that the IKK inhibitor PS1145 could inhibit the proliferation of CML cell lines and primary BM cell, especially caused an increase of apoptosis with imatinib in resistant cell lines and BM cells from resistant patients. The data of Lounnas et al. [26] also strongly suggested the targeting of NF-κB as a promising new therapeutic opportunity for the treatment of imatinib resistant CML patients in particular in the case of T315I patients. These researches indicate blocking NF-κB signaling pathway activity is an effective therapy target for CML. Consistently, in our study, more cell apoptosis induced by imatinib was associated with decreased activity of NF-κB signaling pathway, and we found CUECD2 as a new inhibitor of NF-κB activity was involved in response of imatinib treatment. Our data provide a new insight of reversing imatinib resistance in CML.

In conclusion, higher expression of CUECD2 confers sensitivity to imatinib in CML cells. CUECD2 affects the imatinib sensitivity at least partially through NF-κB signaling pathway in CML cells. While it appears that CUECD2 might represent a novel therapy strategy and serve as an indicator for disease progression and therapy response in CML, a more detailed investigation of the association of CUECD2 and imatinib resistance, as well as the mechanism of adjusting the CUECD2 expression in CML should be performed.

Conflict of interest

We declare here none of our authors has financial or other conflicts of interest that might be construed as influencing the results interpretation or our study.

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