**Research Article**

**Overexpression of miR-202 resensitizes imatinib resistant chronic myeloid leukemia cells through targeting Hexokinase 2**

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**Introduction**

Chronic myeloid leukemia (CML), originating from a constitutively active tyrosine kinase, BCR/ABL1 which occurs spontaneously, is a myeloproliferative disease affecting older adults typically [1,2]. CML accounts for 15–20% of the newly diagnosed cases of adult leukemias [3]. It is well studied that the BCR/ABL fusion protein is essential and sufficient for the malignant transformation of CML [4,5]. Moreover, the mutant BCR/ABL protein confers malignant differentiation and proliferation of hematopoietic cells, resulting in leukemogenesis [6].

Recent studies have evaluated the molecular and cellular mechanisms contributing to CML. Imatinib is tyrosine kinase inhibitor (TKI), which is first used as a CML treatment strategy through targeting the tyrosine kinase activity of Bcr-Abl [7]. It is a TKI of the 2-phenylamino pyrimidine class through blocking the inactive conformation of BCR-ABL protein [7]. Initially, imatinib treatments...
displayed high efficacy and low toxicity in CML patients [7]. However, the development of resistance to imatinib treatment which occurs in CML patients greatly impaired the clinical application of imatinib [8,9]. Therefore, investigating the molecular mechanisms of the imatinib resistance is an important task to develop novel therapeutic strategy for improvement of the chemotherapeutic outcomes in CML.

miRNAs are a family non-coding RNAs consisting of 19–24 nts, which affect gene expression through binding to 3’-UTR within target mRNAs [10]. MiRNAs play essential roles in multiple cellular processes such as stem cell division [11], development [12], cellular metabolism [13] and carcinogenesis [14,15]. It has been reported that miRNAs act as either tumor suppressors or oncogenes [14]. Moreover, aberrant miRNAs exhibit a global down-regulation, which is observed in cancers [16], suggesting miRNAs are tumor suppressors at overall level. A growing evidence has revealed specific miRNAs in the pathogenesis of hematological malignancies such as chronic lymphocytic leukemia (CLL), B-cell lymphomas, acute promyelocytic leukemias, acute lymphocytic leukemia (ALL), and CML [17]. Recently, a miRNA microarray study comparing the miRNAs expression in K562 cell line with human healthy blood samples implicated a number of miRNAs were down-regulated in CML cell lines [18], indicating miRNAs might contribute to therapeutics in CML patients.

Previous studies reported that miR-202 was down-regulated in multiple cancers, such as breast cancer [19], liver cancer [20], gastric cancer [21], pancreatic cancer [22], non-small-cell lung cancer [23], and cervical cancer [24]. Moreover, overexpression of miR-202 inhibited tumor growth, suggesting that miR-202 plays suppressive roles in multiple cancer types and might contribute to enhancement of chemotherapy. However, the functions and molecular mechanisms of miR-202 in human leukemia as well as imatinib sensitivity have not been documented. In the present study, the roles of miR-202 in mediating imatinib sensitivity will be studied. By comparing the cellular metabolic profiles between K562 imatinib sensitive and resistant cells, the mechanisms of imatinib resistance in CML will be explored. Our study will provide new insights into miR-202 as a potential molecular target for development of anticancer agents against CML.

Materials and methods
Patient samples and ethics
Fifteen patients with newly diagnosed CML (eight males and seven females, aged 19–62 years) were recruited in the present study. None was treated with chemotherapeutic agents before. The control samples were from ten healthy donors (five males and five females, aged 19–60 years). Blood samples from healthy volunteers and CML patients were collected after obtaining informed consents according to procedures approved by the Ethics Committee at Affiliated Cancer Hospital and Institute of Guangzhou Medical University, Guangzhou, China.

CML cell lines
The human CML cell lines K562 and KU812 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, U.S.A.). EM2, EM3, LAMA 84, KCL-22, and HL-60 were obtained from the German Resource Center for Biological Material (DSMZ) (Germany). Cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 1% penicillin and streptomycin, and cultured at 37°C in a humidified incubator with 5% CO2.

Antibodies and reagents
Antibodies used in the present study were purchased from Cell Signaling Technology (Danvers, MA, U.S.A.); rabbit anti-Glut1 monoclonal antibody: (#12939); rabbit anti-Hexokinase 2 (HK2) monoclonal antibody: (#2867); rabbit anti-LDHA monoclonal antibody: (#3582); total PARP and cleaved PARP: (#9532) mouse anti-β-actin monoclonal antibody: (#3700). Imatinib mesylate, DAPI, 2-deoxyglucose (2-DG), and Oxamate were purchased from Sigma–Aldrich (Shanghai, China).

Leukocytes isolation
The leukocytes were isolated according to the previous reports [18]. Briefly, peripheral blood samples were drawn from newly diagnosed CML patients and from healthy volunteers. Samples were treated with red blood cell lysis buffer for 30 min. Blood samples were then mixed with erythrocyte lysis buffer (Qiagen, Shanghai, China) and centrifuged at 400 × g for 10 min at 4°C. The leukocyte pellet was washed and centrifuged again. The remaining leukocytes were collected and frozen for experiments in the present study.
Real-time PCR for detection of miRNAs and mRNAs

MiRNA real-time RT-PCR was performed using the TaqMan Small RNA primer and probe sets (Applied Biosystems, U.S.A.) according to the manufacturer’s instructions. Total RNA was isolated from cell lines and leukocytes purified from blood of CML patients and of healthy volunteers using TRIzol method according to the previous reports [18]. RNA was reverse-transcribed with miRNA specific stem-looped primers (Applied Biosystems, U.S.A.). Mixture was incubated at 16°C for 30 min; 42°C for 30 min; and 85°C for 5 min. Real-time PCR was performed in duplicates using the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min. U6-snRNA was used as an internal control. For detection of mRNAs of glycolytic enzymes, the total RNA was isolated from cell lines by TRIzol method. Total RNA (1 μg) of each cell line was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The cDNA reaction was diluted to 1:10 for use as a template for real-time RT-PCR. The 18S ribosomal primers and probes (Applied Biosystems, Foster City, CA) were used as internal controls. PCR amplifications were performed in a final reaction volume of 20 μl containing, 10 μl of TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 1 μl of the primers and probes mix and 9 μl of the cDNA diluted solution. The cycling conditions were as follows: one cycle of 2 min at 50°C, one cycle of 10 min at 95°C, 38 cycles of denaturation (30 s at 95°C) and annealing/extension (1 min at 55°C and 15 s at 72°C). All quantitative PCR reactions were carried out in triplicate and repeated at least twice. Relative mRNA or miRNA expression was calculated using the formula 2^(-ΔΔCt). The primers used for real-time PCR were: Glut1 (forward: 5’-AATCTTCCAGCCAGGTCCAC-3’; reverse: 5’-CACAGTGAAGTATGAGGAC-3’); HK2 (forward: 5’-AAAGTGACAGTGGGTGTGG-3’; reverse: 5’-GCCAGGTCTCTCACGTCTC-3’); and LDHA (forward: 5’-TTGGTCCAGCGTAACGTGAAC-3’; reverse: 5’-CCAGGATGTGACCTTTGGAG-3’).

DAPI staining

Untreated and treated K562 or KU812 imatinib sensitive and resistant cells were harvested by centrifugation and dripped on to a glass slide. Cells were fixed with 4% paraformaldehyde, and membrane permeabilized by exposure for 30 min to 0.1% Triton X-100 in PBS at room temperature. Cells were stained with DNA-binding fluorochrome DAPI for 10 min. The cells were then observed through a fluorescence microscope (Leica Microsystems AG, Wetzlar, Germany).

miRNA and plasmid DNA transfection

Cells were transfected using the Lipofectamine® LTX Transfection reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. miRNA transfection was performed with 100 nM and plasmid DNA was transfected with 2 μg. Forty-eight hours after transfection, cells were collected and prepared for further analysis.

Glycolysis assay

The glucose uptake and lactate production assays were performed using Glucose Uptake Colorimetric Assay Kit (# K676, Biovision, Milpitas, CA, U.S.A.) and Lactate Colorimetric Assay Kit (#K627, Biovision, Milpitas, CA, U.S.A.) according to the manufacturer’s protocols. The relative glucose uptake/lactate production results were normalized to the amount of total protein compared with the control cells.

MTT assay

Cells were seeded into a 96-well plate at a density of 5 × 10^3 cells per well in 200 μl medium and treated under specified glycolysis inhibitors or imatinib for 24 h. After treatments, MTT (5 mg/ml in PBS) was added to each well and incubated for 3-4 h. Suspension cells in the 96-well plate were centrifuged and the MTT solution was removed from the wells by aspiration and the formazan crystals were dissolved in DMSO. Absorbance was measured at 570 nm using a model 450 microplate reader (Bio-Rad Laboratories). Each experiment was performed in triplicate.

BrdU assay

The BrdU assay was performed using the BrdU Cell Proliferation ELISA Kit (colorimetric) (ab126556, Abcam) according to the manufacturer’s instructions. Absorbance was measured at 450 nm using a model 450 microplate reader (Bio-Rad Laboratories). Each experiment was performed in triplicate.
Figure 1. miR-202 is down-regulated in CML cell lines and suppresses CML cell proliferation

(A) The expressions of miR-202 seven CML cell lines and isolated leukocytes from a pool of seven healthy blood samples were measured using qRT-PCR. U6 snRNA was used as a loading control. (B) Control mimic or miR-202 mimic was transfected into K562 and KU812 cells for 48 h, followed by the measurements of miR-202 by qRT-PCR. U6 snRNA was used as a loading control. (C) Control mimic or miR-202 mimic was transfected into K562 and (D) KU812 cells for 48 h, followed by the measurements of cell proliferation at 0, 12, 24, 48 and 72 h by MTT assay and (E,F) BrdU assay. The results are presented as mean ± S.D. of triplicates from each of three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001.

Caspase-3 assay

The activity of Caspase-3 was measured using the Caspase 3 Assay Kit (Colorimetric) (ab39401, Abcam) according to the manufacturer’s instructions. Absorbance was measured at 405 nm using a model 450 microplate reader (Bio-Rad Laboratories). Each experiment was performed in triplicate.

Western blots

Cells were collected and pelleted by centrifugation at 13000 × g for 10 min at 4°C, washed with ice-cold PBS, and lysed with radioimmunoprecipitation assay (RIPA) buffer (50 mmol/l TrisHCl (pH 7.4), 150 mmol/l NaCl, 2 mmol/l EDTA, 1% NP40, and 0.1% SDS), plus protease inhibitors (complete cocktail tablets; Roche Applied Science). Protein concentration was assessed by the Bradford assay according to the previous description to ensure equal protein loading of each sample. Proteins were separated by SDS/PAGE and transferred to nitrocellulose membrane (Bio-Rad Laboratories) for immunoblotting. Membranes were blocked with 5% BSA solution for 1 h at room temperature. Blots were incubated with primary antibodies at a dilution of 1:1000 at 4°C for overnight. After washing by PBS, membranes were incubated with secondary antibodies at a dilution of 1:3000 at room temperature for 1 h. Proteins were visualized using the SuperSignal detection substrate (Pierce Biotechnology).

Statistics analysis

Differences were statistically evaluated by Student’s t test using Prism 5.0 software. P < 0.05 was considered to be statistically significant. Experiments were done in triplicates.

Results

miR-202 is down-regulated in CML cells

It has been known that miRNAs play important roles in multiple hematological malignancies [17]. With the objective of deciphering the potential roles of miR-202 in CML, we analyzed the miR-202 expression levels in multiple CML cell lines, including KU812, K562, KCL-22, EM2, HL-60, LAMA84, and EM3 and isolated leukocytes from a pool of healthy blood samples. MiR-202 expressions were then evaluated by quantitative reverse-transcription PCR (qRT-PCR) and our results demonstrated that miR-202 was significantly down-regulated in seven CML cell lines compared with leukocytes from normal blood sample (Figure 1A), suggesting that miR-202 might act as a tumor suppressor in CML.
Overexpression of miR-202 suppresses proliferation of CML cell lines

The above results showed a strong down-regulation of miR-202 in CML cell lines. We, therefore, investigated the functional significance of miR-202 in the regulation of cell proliferation of CML cell lines. We transfected the CML cell line K562 and KU812 with hsa-miR-202 mimic or control mimic. Compared with the transfection of control mimic, miR-202 was significantly overexpressed in the K562 and KU812 cells after transfection with miR-202 mimic (Figure 1B). Compared with control mimic, cells with forced expression of miR-202 exhibited significantly decreased proliferation from MTT and BrdU assays (Figure 1C–F), supporting that miR-202 displays tumor suppressive functions in CML cell lines.

Imatinib resistant cells display down-regulated miR-202

We continued to explore the functions of miR-202 in the chemosensitivity of CML cell lines. In the present study, we focused on imatinib which is a commonly used chemotherapeutic agent for CML. Interestingly, in both cell lines, K562-s and KU812, treatment with 1 μM of imatinib for 24 and 48 h significantly suppressed miR-202 expressions (Figure 2A,B). To further investigate the potential roles of miR-202 in imatinib sensitivity of CML cell lines, we established imatinib resistant cell line originating from K562 parental CML cell lines according to the previously reported methods [25]. K562 parental cells were treated with gradually increased concentrations of imatinib for three consecutive months. The survival cell clones were saved and pooled for the following experiments in the present study. Results in Figure 2C,D showed the K562 resistant cells could tolerate higher concentrations of imatinib treatments than parental cells. The IC₅₀ of K562 resistant cells was 2.16 μM, which is approximately six-fold higher than that of parental cells (0.37 μM). Moreover, the K562 parental cells showed a higher percentage of cleaved PARP than the resistant cells under imatinib treatments (Figure 2E). Taken together, the above results validated the selection of imatinib resistant K562 cell line. In order to observe the direct evidence for supporting the roles of miR-202 in imatinib resistance, we compared the expressions of miR-202 in imatinib sensitive and resistant cells. As we expected, miR-202 is significantly down-regulated in imatinib resistant cells (Figure 2F), indicating miR-202 might contribute to the sensitization of the CML imatinib.
Elevated glycolysis in imatinib resistant CML cell lines

It has been known that the altered glucose metabolism is a biochemical fingerprint of cancer cells and contributes to chemosensitivity [26]. To investigate the mechanisms for the miR-202-associated imatinib resistance in CML cell lines, we compared the glycolysis of imatinib sensitive and resistant CML cell lines. The imatinib resistant K562 and KU812 cells showed obviously different glucose metabolism profiles compared with imatinib sensitive cells. The imatinib resistant K562 and KU812 cells exhibited significantly higher levels of glucose uptake and lactate production than sensitive cells (Figure 3A,B). Consistently, we observed the glycolysis key enzymes, Glut1, HK2, and LDHA were up-regulated in imatinib resistant cells at both protein and mRNA levels (Figure 3C,D). Furthermore, to assess whether miR-202 could regulate glycolysis enzymes expressions, we compared protein and mRNA expression of Glut1, HK2, and LDHA in CML cells with or without miR-202 overexpression. K562 and KU812 cells with miR-202 overexpression demonstrated significantly down-regulated glycolysis enzymes expressions (Figure 3E,F). These results demonstrated that the imatinib resistant cells exhibit a highly glycolytic phenotype, suggesting that targeting the dysregulated glycolysis in CML cell lines might contribute to overcome imatinib resistance.

Imatinib resistant CML cell lines are sensitive to glycolysis inhibitors

Since the imatinib resistant CML cell lines have higher glucose metabolic rate, we hypothesized inhibition of glycolysis might sensitize CML cell lines to imatinib. To test it, we first treated both cells with low glucose medium to assess the cell survival rates. As we expected, the imatinib resistant K562 or KU812 cells were more susceptible to low glucose supply. Under low glucose conditions, more than 80% imatinib resistant cells died, lower than that of sensitive cells (25%) (Figure 4A,B). We then treated both imatinib sensitive and resistant K562 or KU812 cells with glycolysis inhibitors, 2-DG or Oxamate. 2-DG is a glucose analog, inhibits glycolysis then glucose cannot be fully oxidized [27] and Oxamate is a cytosolic inhibitor of LDHA [28]. Consistent with the above results in Figure 4A,B, MTT and Caspase-3 activity assays demonstrated imatinib resistant CML cell lines were more sensitive to glycolysis inhibitors compared with imatinib sensitive cells (Figure 4C–F), indicating the combination of glycolysis inhibitor with imatinib might display a synergistically inhibitory effect on CML cell lines.
**miR-202** inhibits glycolysis through targeting HK2

Our results revealed a negative correlation between miR-202 and imatinib resistance and a positive correlation between glycolysis and imatinib resistance in CML cell lines. Following that, we investigated whether miR-202 could directly regulate glycolysis in CML cell lines. By analyzing the 3′-UTR of glycolytic enzymes from public miRNA database microRNA.org, we observed that the 3′-UTR of HK2 contains miR-202 binding sites (Figure 5A), suggesting that miR-202 might directly target HK2. To assess the effect of miR-202 on HK expression, we performed Western blot analysis. The K562 and KU812 cells were transfected with control mimic or miR-202 mimic. Results in Figure 5B illustrated that overexpression of miR-202 inhibited HK2 protein expression. To further explore whether HK2 is a direct target of miR-202, we cloned the full-length HK2 3′-UTR or mutant HK2 3′-UTR into a luciferase reporter vector. 293 T cells were co-transfected with control mimic or miR-202 mimic with vector containing the full-length HK2 3′-UTR or mutant HK2 3′-UTR. As we expected, luciferase assay revealed that miR-202 directly bound to HK2 3′-UTR, and by which it remarkably reduced luciferase activities (Figure 5C). However, mutation of the putative miR-202 binding sites in the 3′-UTR of HK2 abrogated luciferase responsiveness to miR-202 (Figure 5C).

**Overexpression of miR-202 sensitizes imatinib resistant CML cell lines by inhibiting glycolysis**

To understand whether miR-202 suppresses glycolysis of CML cell lines through directly targeting HK2, we transfected CML cell lines with control mimic, miR-202 mimic, or miR-202 mimic with HK2 overexpression vector. Results illustrated co-transfection of miR-202 mimic with HK2 could restore the original level of HK2 in K562 cells (Figure 6A). Moreover, the glucose uptake and lactate production were recovered by the rescue of HK2 (Figure 6B,C).
Figure 5. miR-202 targets HK2 in K562 cells
(A) The sequence alignment of miR-202 and 3'-UTR regent of HK2 analyzed from microRNA.org. (B) K562 and KU812 cells were transfected with control mimic or miR-202 mimic for 48 h, followed by Western blot analysis. α-Tubulin is the loading control. (C) Luciferase assay showed that miR-202 could bind 3'-UTR of wild-type HK2, but could not bind to 3'-UTR mutant HK2. The results are presented as mean ± S.D. of triplicates from each of three independent experiments. ***P < 0.001.

Figure 6. Restoration of HK2 renders K562 cells resistant to imatinib
(A) K562 cells were transfected with control mimic, miR-202 mimic, or miR-202 mimic plus HK2 overexpression vector for 48 h, followed by Western blot analysis. α-Tubulin is the loading control. (B) K562 cells were transfected with control mimic, miR-202 mimic, or miR-202 mimic plus HK2 overexpression vector for 48 h, followed by measurements of glucose uptake or (C) lactate production. (D) K562 imatinib resistant cells were transfected with control mimic, miR-202 mimic, or miR-202 mimic plus HK2 overexpression vector for 48 h, followed by the treatments of imatinib at 0, 0.4, 0.8, 1, 2 or 4 μM for 24 h. Cell viabilities were measured by MTT assay. The results are presented as mean ± S.D. of triplicates from each of three independent experiments. *P < 0.05; **P < 0.01.
suggesting miR-202-mediated glycolysis inhibition was through targetting HK2. Importantly, our results demonstrated suppression of HK2 by miR-202 sensitized imatinib resistant K562 cells. Conversely, restoration of HK2 renders miR-202 overexpressing cells resistant to imatinib (Figure 6D), indicating miR-202 sensitizes imatinib resistant CML cell lines through targetting HK2.

Invert correlation of miR-202 and HK2 expressions in CML patients

We next compared the expressions of miR-202 in leukocytes from CML patients and normal healthy blood samples. Consistent with in vitro results, leukocytes isolated from ten CML blood samples exhibited significantly down-regulation of miR-202 (Figure 7A). The above in vitro results showed miR-202 could directly target HK2 in CML cell lines (Figure 5). To assess whether these in vitro results are also clinically applicable, we analyzed the correlation of miR-202 and HK2 expressions in 15 CML patient samples by qRT-PCR. We observed a significantly negative correlation between miR-202 and HK2 expressions (P < 0.001, R² = 0.5753) in CML patients (Figure 7B). In summary, this clinical analysis indicated that miR-202 could target HK2 in CML patients.

Discussion

In the present study, we report a tumor suppressive role of miR-202 in CML cell lines and patients. miRNAs and their roles in both normal physiological and disease contexts have been widely studied recently [10-18]. A bulk of evidence indicated that miRNAs play crucial roles in myeloid development and leukemogenesis. For example, miR-155 and miR-31 were found to be down-regulated, and some tumor suppressor miRs (miR-16-1, miR-15a, miR-101, miR-568) were up-regulated in CML [18]. Another publication reported that miR-203 was shown to be epigenetically suppressed in CML [29], suggesting a tumor inhibitory function of miR-203 in CML. MiR-202 has been reported as a tumor suppressor miRNA and was obviously down-regulated in breast carcinoma [19], gastric cancer [21], and lung cancer [23]. Moreover, aberrant expression of miR-202 is associated with tumorigenesis, tumor progression, and metastasis in multiple cancer types [19-24], suggesting overexpressing miR-202 contributes to development of novel anticancer agent. Up to date, however, little study described the role of miR-202 in CML as well as imatinib chemosensitivity. In the present study, we observed that miR-202 was significantly down-regulated in CML cell lines and patients compared with normal blood cells. Moreover, overexpression of miR-202 could suppress proliferation, migration and increase chemosensitivity of CML cell lines. Taken together, our study reveals a novel role of miR-202 in CML.

In 1926, Otto Warburg proposed that cancer cells exhibit high glycolysis even in the presence of oxygen, which was one of the hallmark of cancer development [30]. Metabolic changes in cancer have been recognized with crucial roles in tumorigenesis. Cancer cells up-regulate the rate-limiting enzymes of glycolysis such as GLUT1, HK2, PKM2, and LDHA as a result of the expression of oncogenes including RAS, SRC, or EGFR [31]. In addition, it is widely studied that the dysregulated glucose metabolism contributes to chemo-resistance [31]. Consistently, our data demonstrated
Imatinib resistant CML cell lines displayed up-regulated glycolytic rates and were sensitive to glycolysis inhibitors, suggesting inhibition of glycolysis in CML chemoresistant cells might be a novel therapeutic strategy.

Imatinib is a commonly used chemotherapeutic agent that targets the tyrosine kinase activity of Bcr-Abl because of its high efficacy, low toxicity, and ability to maintain durable hematological responses [7]. However, the development of resistance to imatinib became problems over time. Currently, several mechanisms for the imatinib resistance observed in patients with CML have been studied such as mutations on the BCR-ABL kinase domain, increased BCR-ABL expression, and overexpression of drug-efflux proteins (ABCB1 and ABCG2) [32]. A recent publication reported that glycolysis was highly correlated with imatinib resistance in CML [33], consistent with our observations in this study. In addition, we demonstrated miR-202 could directly target HK2, which is an essential glycolysis enzyme in the glucose metabolism pathway. We further reported overexpression of miR-202 sensitized imatinib resistant CML cell lines, suggesting miR-202 might be a novel therapeutic target against imatinib resistance. Although the detailed mechanisms for the imatinib regulated miR-202 are still not fully understood, our continuing project will use in vivo model to further illustrate the axis of miR-202-HK2-glycolysis-imatinib sensitivity in CML.

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**Author contribution**
Y.D. and X.L. designed the research. Y.D., X.L., and J.F. performed the research and analyzed the data. X.Z. wrote and edited the manuscript.

**Competing interests**
The authors declare that there are no competing interests associated with the manuscript.

**Abbreviations**
CML, chronic myeloid leukemia; HK2, hexokinase 2; qRT-PCR, quantitative reverse-transcription PCR; TKI, tyrosine kinase inhibitor; 2-DG, 2-deoxyglucose.

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