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Original Article

Imatinib mesylate induces apoptosis of K562 cell by down-regulating miR-139

Linhui Wang, Pengxiang Guo

Department of Hematology, The People's Hospital of Guizhou Province, Guiyang 550001, Guizhou, China

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Abstract: Leukemia has severely endangered people healthy. Imatinib mesylate is a newly-developed targeting drug for treating leukemia. This study aimed to investigate the molecular mechanism of imatinib mesylate on regulating K562 cell apoptosis via mediating micro RNA-139 (miR-139) expression. K562 cells were treated with imatinib mesylate and were examined for their growth, proliferation and apoptosis by flow cytometry. MiR-139 expression level was determined by RT-PCR. We also synthesized miR-139 expressing vectors and transfected them into K562 cells, followed by imatinib mesylate treatment and cell viability and apoptotic assays. The inhibition of K562 cell growth and proliferation occurred after imatinib mesylate treatment, accompanied with cell apoptosis. In leukemia patient sample, the expression level of miR-139 was significantly higher than normal people. K562 cell over-expressing miR-139 had depressed level of imatinib mesylate-induced cell apoptosis. Imatinib mesylate induces the apoptosis of K562 cells via suppressing miR-139 expression.

Keywords: Imatinib mesylate, microRNA-139, K562 cells, leukemia, cell apoptosis

Introduction

Leukemia is a family of hematological diseases manifested with malignant proliferation of hematopoietic stem cells (HSCs) [1] and constitutes a sever health concern for the population as a whole [2]. Current opinions accept that the abnormal differentiation in clonal leukemia cells and following inhibition of apoptosis and/or unregulated proliferation [3] can lead to accumulation of large amounts of abnormal leukemia cells within the bone marrow and further infiltration to other tissues/organs [4]. Meanwhile the inhibition of proliferation of normal HSCs eventually leads to organ failure such as spleen, liver, lymph node and bone marrow [5]. Therefore, the treatment of leukemia is mainly based on the pathogenesis mechanism, especially in the sense of inhibiting cell proliferation and apoptosis [6].

Imatinib mesylate is previously used in treatment gastrointestinal cancer and has obtained satisfactory effects [7]. Its application in chronic myeloid leukemia also received good clinical efficacy [8]. The detailed mechanism, however, remained unclear.

MicroRNA, also named as miRNA is a kind of small RNA molecules composed 18~25 nucleotide. Although no directly encoding proteins, various biological feature still exerted pluripotent biological functions [9]. So far more than 30,000 different kinds of miRNA have been identified in animals, plants and even in virus [10], and participate in various cellular events including signal transduction [11], cell growth [12] and proliferation [13], cell cycle modulation [14], cell apoptosis [15] and autophagy [16]. The exact role of miRNA in leukemia pathogenesis, however, requires further illustration.

This study thus utilized leukemia cell K562 as a model, on which the molecular regulatory mechanism of imatinib mesylate on K562 cell proliferation was investigated, in an attempt to provide further knowledge on the diagnosis and treatment of leukemia.

Materials and methods

Patient sample

All clinical samples were obtained from leukemia patients and healthy individuals from The

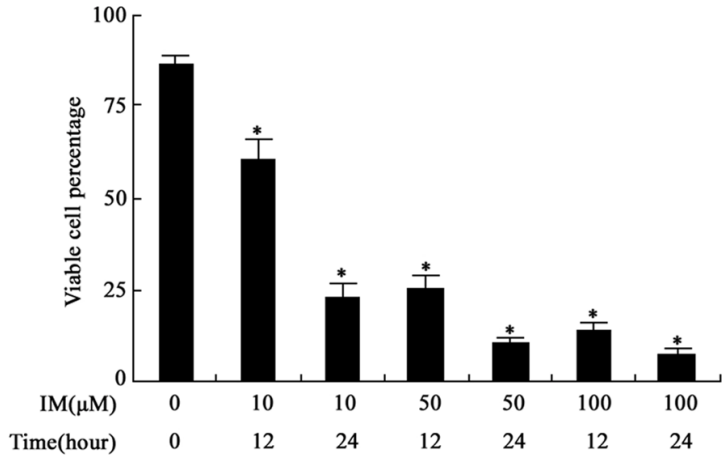

Figure 1. Imatinib mesylate inhibited K562 cell growth. IM, imatinib mesylate. *P<0.05 compared to cell with no IM treatment using one-way ANOVA analysis.

People's Hospital of Guizhou Province. This study has been approved by the ethical committee of The People's Hospital of Guizhou Province, and has obtained written consents from all participants involved.

Cell culture and transfection

K562 cells (American Type Culture Collection, ATCC, US) were resuscitated and thawed in 37°C water bath. After centrifugation to remove cell preserve buffer, cells were re-suspended in DMEM high-sugar culture medium (Gibco, US) containing 10% fetal bovine serum (FBS, Gibco, US) and were cultured in a humidified chamber perfused with 5% CO_2 gas maintaining at 37°C.

24 hours before transfection, cells were seeded into 6-well plate (10^5 cells per well) for reaching 90% density at the time of transfection. MiR-139 oligonucleotide (Sense, 5'-TGTAA AACGA CGGCC AGT-3'; Anti-sense, 5'-CAGGA AACAG CTATG ACC-3') and controlled RNA molecules were diluted in DMEM (1:200) with the addition of 1 μL Lipo2000 reagent (Invitrogen, US). After the addition of transfection mixture into cultured cells, they were incubated in a humidified chamber perfused with 5% CO_2 gas maintaining at 37°C.

MTT assay

Based on the effective concentration of imatinib mesylate in other tumor cell lines [1] and pilot study by us, gradient concentrations of imatinib mesylate (10 μM, 50 μM and 100 μM) were added into cultured K562 cells for 12 hours or 24 hours treatment in triplicates. (4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was then added into each well at 10 mg/mL, followed by 3 hour incubation at 37°C. Phosphate buffered saline (PBS) was used to stop the reaction, followed by the replenishment of 0.1 mL DMSO. Absorbance values at 490 nm in each well were quantified from a microplate reader.

Flow cytometry

K562 cells transfected with miR-139 and/or treated with imatinib mesylate were tested for cell apoptosis using phosphatidyl serine (PS) assay. In brief, cells were adjusted to 10^6 per mL concentration, and were mixed with Annexin V and Annexin V-EGFP (Dingguo Bio, China) at 250:50:1 ratio. After 30-min dark incubation at room temperature, cells were loaded for flow cytometry assay to detect the percentage of PS-positive cells.

Caspase activity was also examined using test kit (Beyotime, China) as described in the manual instruction. In brief, a standard curve was plotted based on standard samples. K562 cells from all groups were then lysed. Cell lysates were mixed with reaction reagents, followed by reading the absorbance value and deduction of caspase activity.

RT-PCR

Culture K562 cell and leukemia patient leukocytes were collected for total RNA extraction using Trizol kit (Invitrogen, US). RT-PCR was then performed using *in vitro* reverse transcription kit (Dingguo Bio, China) following the manual instruction. PCR was performed using specific primers for miR-139 to quantify the expressional profile of miR-139.

Statistical analysis

SPSS 16.0 software was used to process all collected data. All data were presented as mean ± standard deviation (SD). For comparison of no IM treatment with IM treatment at dif-

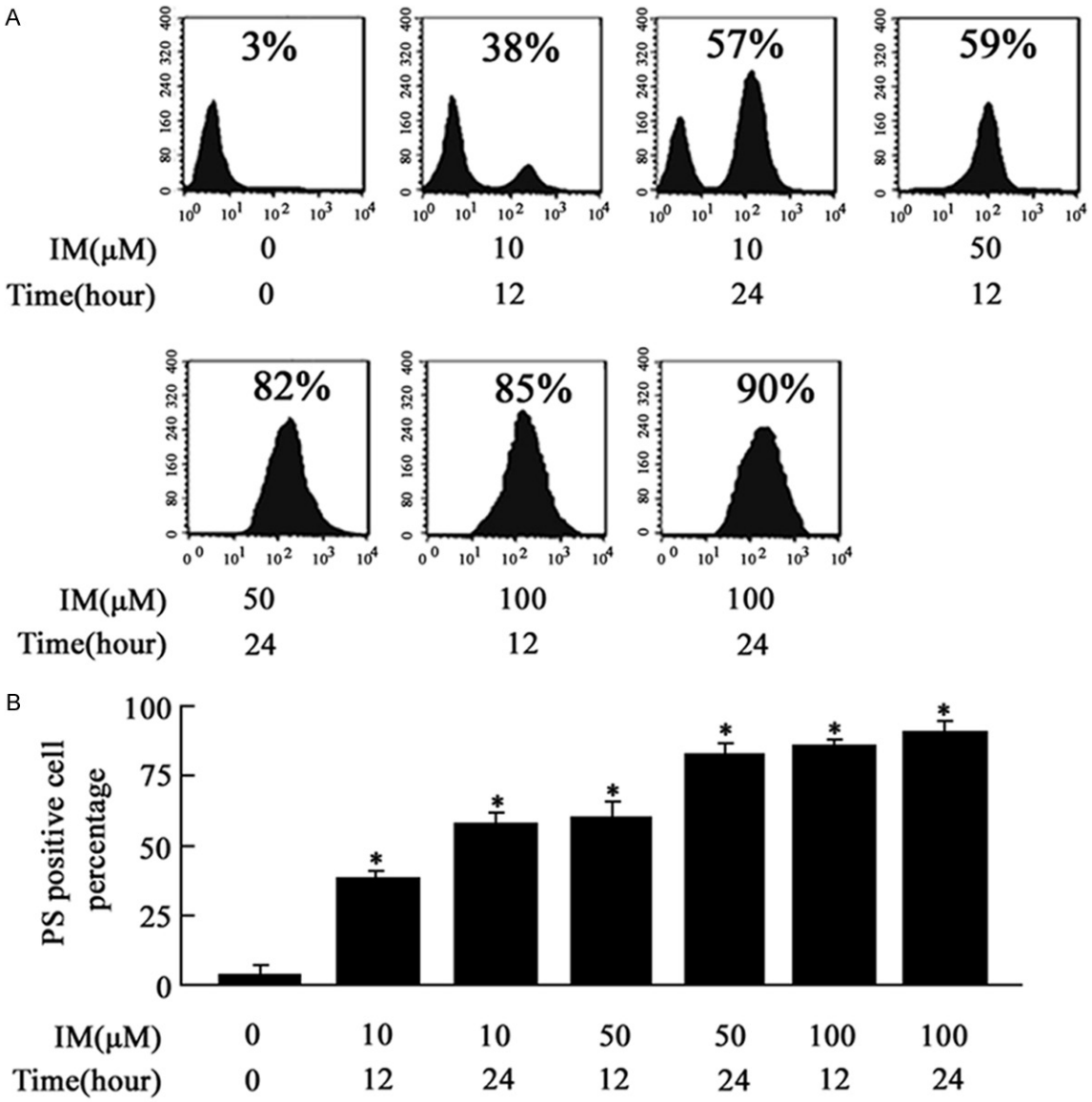

Figure 2. Induction of PS expression by imatinib mesylate. IM, imatinib mesylate. *P<0.05 compared to cell with no IM treatment using one-way ANOVA analysis.

ferent time points, data were assessed by one-way ANOVA. For comparison between control group and experiment group data were analyzed by unpaired Student *t*-test with 2-tailed *P* values. A statistical significance was defined when P<0.05.

Results

K562 cell growth was inhibited by imatinib mesylate

MTT assay showed a dose-dependent inhibition of K562 cell proliferation with imatinib mesylate treatment. As shown in **Figure 1**, at each time point, those cells receiving imatinib mesylate at different concentrations all had significantly inhibited cell viability when compared to control cells (P<0.05).

Induction of cell apoptosis by imatinib mesylate

The application of imatinib mesylate can induce the expression of PS (**Figure 2**) and elevated caspase-3 activity (**Figure 3**) in K562 cells in a dose- and time-dependent manner, suggesting the induction of cell apoptosis by imatinib

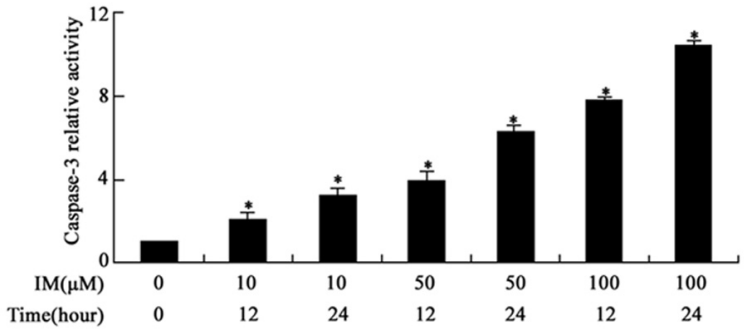

Figure 3. Caspase-3 activity of K562 cells. IM, imatinib mesylate. *P<0.05 compared to cell with no IM treatment using one-way ANOVA analysis.

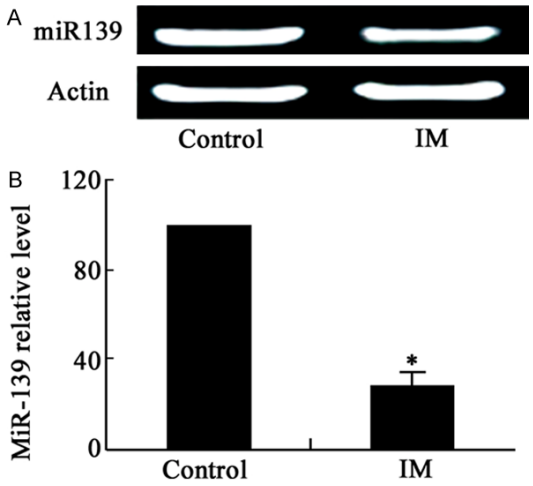

Figure 4. MiR-139 expression level in imatinib mesylate-treated K562 cells. IM, imatinib mesylate. *P<0.05 compared to controlled cells using 2-tailed unpaired Student *t*-test analysis.

mesylate. Compared to those cell without drug treatment, cells receiving imatinib mesylate at all concentrations had elevated PS positive rate and caspase-3 activity (P<0.05).

MiR-139 level in K562 cells

As shown in **Figure 4**, imatinib mesylate treatment significantly decreased the expression of miR-139 in K562 cells as compared to control group (P<0.05). These result suggested the possible involvement of miR-139 down-regulation in the anti-tumor mechanism of imatinib mesylate.

MiR-139 level in serum of leukemia patients

Using RT-PCR, we found significantly elevated blood-borne miR-139 expression levels in leukemia patients compared to healthy individuals (P<0.05, **Figure 5**). Such results indicated the possible relationship between up-regulation of miR-139 and leukemia pathogenesis.

Over-expression of miR-139 inhibited imatinib mesylate-induced cell apoptosis

The transfection of miR-139 or controlled miRNA did not cause significant apoptosis of K562 cells. When one treated miR-139 transfected K562 cells with imatinib mesylate, however, the cell apoptosis was significantly inhibited as compared to those cells transfected with controlled miRNAs (P<0.05, **Figure 6**). These results further supported the idea that imatinib mesylate-induced apoptosis of K562 cells were at least partially with miR-139 expression. The over-expression of miR-139 can inhibit imatinib mesylate-induced cell apoptosis.

Discussion

Due to its severe effects on patient's life, leukemia has brought heavy burdens for the whole family and society [17]. Therefore the elucidation of molecular mechanisms underlying the pathogenesis of leukemia has significant implications in both theoretical and practical senses [18]. Imatinib mesylate is one important anti-tumor drug in clinical practice [19]. Its molecular mechanisms in treating leukemia, however, require further elaborations. This study thus investigated the mechanism of imatinib mesylate on leukemia on K562 cell model. Our results showed that imatinib mesylate can inhibit the cell growth and induced apoptosis in a time- and dose-dependent manner, which is consistent with other chemotherapy drugs in tumor treatment [20].

Some novel findings have been demonstrated in this study: (1) Imatinib mesylate can inhibit the proliferation of K562 cells in both time- and dose-dependent manner. The drug also induced cell apoptosis in a similar manner. (2) MiR-139 expression was decreased by imatinib mesylate. Meanwhile miR-139 was up-regulated in leukemia patients' blood samples. (3) The over-

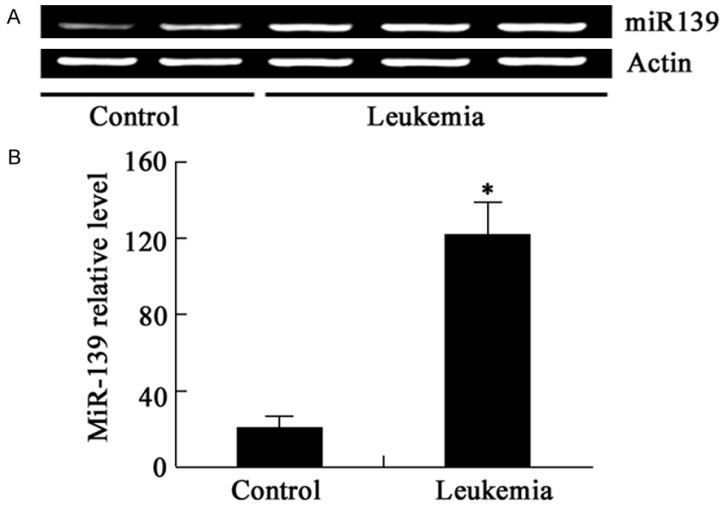

Figure 5. MiR-139 expression level in leukemia patients. *P<0.05 compared to control individuals using 2-tailed unpaired Student *t*-test analysis.

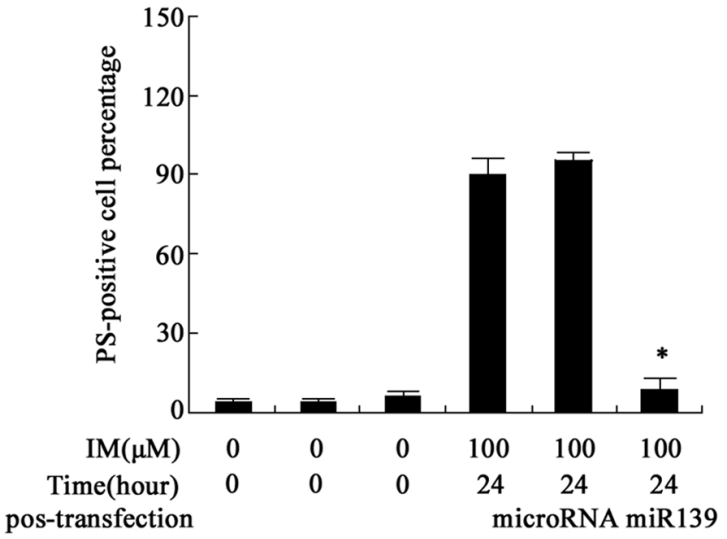

Figure 6. K562 cell apoptosis after miR-139 over-expression. IM, imatinib mesylate. *P<0.05 compared to those cells transfected with control microRNA using one-way ANOVA analysis.

expression of miR-139 can inhibit imatinib mesylate-induced apoptosis of K562 cells. Our results suggested the potency of miR-139 as a novel biomarker and potential drug target of leukemia. We also showed that the interference of miR-139 expression may potentiate the sensitivity of leukemia tumor cells against imatinib mesylate.

Our results had certain differences when compared to previous studies about microRNA, as previous scholars reported the facilitated cell apoptosis after over-expressing microRNA [21]. Such inconsistency may be attributed to the differential types of microRNA and host cells [22], as certain microRNA had pro-apoptotic function [23] while other were of anti-apoptotic nature [24]. The different experimental protocols or conditions may also explain such difference as unique microRNA expressional profiles across different types of cells [25].

Certain weakness and limitations existed in the current study as: (1) Only *in vitro* cell study regarding the molecular mechanism of imatinib mesylate-induced apoptosis had been elucidated. No clinical samples from leukemia patients undergone imatinib mesylate treatment or animal model has been tested for *in vivo* cell apoptosis or miR-139 level. (2) Although leukemia patients had elevated miR-139 levels, which can be suppressed by imatinib mesylate in K562 cells, no further investigation of miR-139 levels across leukemia patients with different disease severities have been performed. (3) No interference of miR-139 expression has been performed in this study to test the sensitivity of K562 cells against imatinib mesylate.

In summary, this study demonstrated the induced cell apoptosis of K562 cells by imatinib mesylate via down-regulating miR-139 expression, although further *in vivo* studies are required for further substantiation.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Pengxiang Guo, Department of Hematology, The People's Hospital of Guizhou Province, 83# Zhongshan East Road, Guiyang 550001, Guizhou, China. Tel: +86-0851-5922979; Fax: +86-0851-5922979; E-mail: guopengxiangbsa@163.com

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