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Decreased expression of microRNA-214 contributes to imatinib mesylate resistance of chronic myeloid leukemia patients by upregulating ABCB1 gene expression

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Abstract. The aim of the present study was to determine the expression of adenosine triphosphate binding cassette subfamily B member 1 (ABCB1) gene and its protein P-glycoprotein (PGP) in bone marrow mononuclear cells from chronic myeloid leukemia (CML) patients with imatinib mesylate (IM) resistance, or IM-resistant CML K562 cells. In addition, the molecular mechanism of action of microRNA (miR)-214 on ABCB1 in IM resistance was investigated. A total of 26 CML patients with IM resistance were included in the present study. In addition, 31 CML patients who did not have IM resistance were included as the control group. Bone marrow was collected from all subjects. The K562R cell line, which is a K562 cell line with IM resistance, was used for cellular studies. Reverse transcription-quantitative polymerase chain reaction was used to determine the expression of ABCB1 mRNA and miR-214 in cells. Western blotting was employed to determine the expression of PGP. Dual luciferase reporter assay was carried out to identify interactions between ABCB1 mRNA and miR-214. MTT assay was used to determine the survival rate of cells. ABCB1 mRNA and PGP expression was upregulated in bone marrow mononuclear cells from CML patients with IM resistance. K562R cells had higher ABCB1 and PGP expression than K562 cells, potentially due to their different sensitivity to IM. Expression miR-214 was decreased in bone marrow mononuclear cells from patients with IM resistance and K562R cells. Notably, miR-214 was able to bind with the 3'-untranslated region, seed region of ABCB1 mRNA to regulate its expression. In addition, elevated expression of miR-214 restored IM sensitivity to K562R cells potentially by affecting ABCB1 expression. The present study demonstrated that upregulated expression of ABCB1 mRNA and PGP in bone marrow mononuclear cells from CML patients with IM resistance may be associated with the downregulation of miR-214. In addition, miR-214 may participate in the IM resistance of CML patients by regulating ABCB1 expression.

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Introduction

Chronic myeloid leukemia (CML) is a malignant disease in which hematopoietic stem cells proliferate continuously, and it is the first tumor that is confirmed to be related to chromosome aberration (1). Epidemiology shows that the incidence of CML is about 1-2 cases/100,000 people, and most patients of CML are elderly people (median age on diagnosis, 65 years) (2). The prevalence of CML in males is higher than that in females, but the overall survival rate for females is higher than that for males (3). The incidence of CML in China is 0.36 case/100,000 people (4).

Imatinib mesylate (IM) is among the first generation of tyrosine kinase inhibitors (TKIs) that are authorized by the Food and Drug Administration of the USA in the treatment of chronic myelogenous leukemia in the chronic phase (CML-CP) (5). With the use of IM, CML-CP patients have achieved a survival rate longer than 5 years and it is predicted that their life span is close to or equivalent to that of non-leukemia patients (2,6-8). In the course of long-term follow-up of CML, some patients are found to be resistant to IM (9). The incidence of drug resistance in each year of the first 3 years of treatment is 2-4% for CML-CP patients, and the drug resistance rate is gradually increasing with the prolongation of drug use (10,11).

The mechanism of IM drug resistance is very complex, and the expression of multidrug transporters has attracted much attention (12,13). The most intensively studied mechanism is multidrug resistance (MDR) mediated by P-glycoprotein (PGP) and multidrug resistance-associated protein-1 (MRP1) (14-16). PGP is encoded by multidrug resistance gene (adenosine triphosphate binding cassette subfamily B member 1 (ABCB1), also known as MDR1), and involved in drug absorption, distribution, metabolism, and excretion (17).

Therefore, the regulation of ABCB1 has become an important research direction for IM resistance therapy.

microRNA (miRNA or miR) molecules are small-molecule non-encoding RNA molecules that widely exist in eukaryotic cells, and they regulate protein expression at mRNA level (18-20). Studies show that miRNA plays important biological roles in imatinib resistance (21,22). This process is also accompanied by alterations in the expression of multiple miRNA and proteins. The microRNA that is upstream of ABCB1 and regulates ABCB1 expression in CML has not been reported yet. In the present study, we determine the expression of ABCB1 and its upstream miRNA, and try to understand the mechanism of regulation between them.

Materials and methods

Patients. A total of 26 CML patients with IM resistance were included into the present study between December 2013 and June 2017. In addition, 31 CML patients who do not have IM resistance were included into control group (Table I). Bone marrow was collected from all subjects. Among the 26 patients with IM resistance, 15 were males and 11 were females (age range, 17-66 years; median age, 52.6 years). In addition, 20 patients with IM resistance were in chronic phase, while 6 patients were in accelerated phase. Among the 31 patients in control group, 19 were males and 12 were females (age range, 15-69 years; median age, 53.5 years). Moreover, 28 patients in control group were in chronic phase, while 3 patients in control group were in accelerated phase. All patients received treatments for IM. The standard for the diagnosis of IM resistance in CML patients includes: i) no hematologic remission was achieved within 3 months; ii) no complete hematologic remission or cytogenetic remission was achieved within 6 months; iii) failure to achieve major cytogenetic remission within 12 months or complete cytogenetic remission within 18 months and iv) loss of prior hematologic or cytogenetic remission, or the emergence of Abl kinase point mutations with high IM resistance (23). All procedures were approved by the Ethics Committee of Jining No. 1 People's Hospital (Shandong, China). Written informed consents were obtained from all patients or their families.

Cells. K562 cell line was a kind of CML cell line, while K562R cell line was K562 cell line with IM resistance. Both types of cell lines were originally purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and kept at our lab. One day before transfection, K562R cells in log-phase growth were seeded into 24-well plates ($3x10^5$ in each well) containing antibiotics-free F12/DMEM medium supplemented with 10% fetal bovine serum and cultured at 37˚C and 5% CO_2. When reaching 70% confluency, transfection began. In the first vial, 1 μl agomiR-214 (20 pmol/μl; miR-214 mimics group; Sangon Biotech Co., Ltd., Shanghai, China) was mixed with 50 μl Opti Mem medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Here, agomiR-214 was an artificially synthesized modified double-chain miR-214 with the same function with mature form of miR-214 (24). In the second vial, 1 μl Lipofectamine 2000 (Thermo Fisher Scientific, Inc.) was mixed with 50 μl Opti Memi medium. After standing still for 5 min, the two vials were combined before incubation at room temperature for 20 min. Then, the mixtures were added onto cells in respective groups. Six hour later, the medium was replaced with F12/DMEM medium containing 10% fetal bovine serum. After cultivation at 37˚C and 5% CO_2 for 48 h, the cells were collected for further assays.

To obtain mononuclear cells in bone marrow, bone marrow samples were first mixed with equal amount of phosphate-buffered saline (v/v, 1:1). Then, the mixture was added gently onto Ficoll-Paque Premium lymphocyte separation medium (GE Healthcare, Chicago, IL, USA) with a ratio of 2:1 before centrifugation at 2,000 rpm for 20 min. After centrifugation, the middle layer was aspirated and thoroughly mixed with 5 volumes of phosphate-buffered saline before centrifugation at 1,500 rpm for 10 min. After discarding supernatant, 5 volumes of phosphate-buffered saline were added again followed by thorough mixing. After centrifugation at 1,500 rpm for 10 min, the supernatant was discarded again. The cells that were attached on the bottom were mononuclear cells in bone marrow.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extract from cells using TRIzol reagent following the manufacturer's manual (Yeasen, Shanghai, China). Then, total RNA was extracted using phenol chloroform method. The concentration and quality of RNA was measured using ultraviolet spectrophotometry (Nanodrop ND2000; Thermo Fisher Scientific, Inc.). Then, cDNA was obtained by reverse transcription from 1 μg RNA and stored at -20˚C. Reverse transcription of mRNA was performed using TIANScript II cDNA First Strand Synthesis kit (Tiangen Biotech Co., Ltd., Beijing, China), and reverse transcription of miRNA was carried out using miRcute miRNA cDNA First Strand Synthesis kit (Tiangen Biotech Co., Ltd.).

SuperReal PreMix (SYBR-Green) RT-qPCR kit (Tiangen Biotech Co., Ltd.) was used to detect mRNA expression of Abcb1a, using GAPDH as internal reference. The sequences of Abcb1a were 5'-TGGGGCTGGACTTCCTCTCATGATGC-3' (sense) and 5'-GCAGCAACCAGCACCCCAGCACCAAT-3' (anti-sense). The sequences of GAPDH were 5'-AGAAGG CTGGGGCTCATTTG-3' (sense) and 5'-GGAACGCTTCAC GAATTTG-3' (anti-sense). The reaction system (25 μl) was composed of 12.5 μl SYBR Premix EXTaq, 0.5 μl upstream primer, 0.5 μl downstream primer, 1 μl cDNA and 10.5 μl ddH_2O. PCR condition was: Initial denaturation at 95˚C for 3 min; 40 cycles of denaturation at 95˚C for 10 sec, annealing at 60˚C for 30 sec, and elongation at 72˚C for 20 sec (iQ5; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The $2^{-\Delta\Delta Cq}$ method (25) was used to calculate the relative expression of Abcb1a mRNA against GAPDH. Each sample was tested in triplicate.

The expression of miR-214 was determined by miRcute miRNA RT-PCR kit (Tiangen Biotech Co., Ltd.), using U6 as internal reference. The sequences of miR-214 primers were 5'-AGCATAATACAGCAGGCACAGAC-3' (upstream) and 5'-AAAGGTTGTTCTCCACTCTCTCAC-3' (downstream). The sequences of U6 were 5'-ATTGGAACGATACAGAGA AGATT-3' (upstream) and 5'-GGAACGCTTCACGAA TTTG-3' (downstream). The reaction system (20 μl) contained 10 μl RT-qPCR-Mix, 0.5 μl upstream primer, 0.5 μl downstream universal primer, 2 μl cDNA and 7 μl ddH_2O. The

reaction protocol was: Initial denaturation at 95˚C for 3 min; 40 cycles of denaturation at 95˚C for 12 sec, annealing at 62˚C for 40 sec and 72˚C for 20 sec (iQ5; Bio-Rad Laboratories, Inc.). The $2^{-\Delta\Delta Cq}$ method (25) was used to calculate the relative expression of miR-214 against U6. Each sample was tested in triplicate.

Western blot analysis. Cells ($1x10^6$) in each group were collected and precooled Radio-Immunoprecipitation Assay (RIPA) lysis buffer (1,000 μl; 50 mM Tris-base, 1 mM EDTA, 150 mM NaCl, 0.1% sodium dodecyl sulfate, 1% TritonX-100, 1% sodium deoxycholate; Beyotime Institute of Biotechnology, Shanghai, China) was added to the cells. After lysis for 40 min on ice, the mixture was centrifuged at 12,000 rpm and 4˚C for 10 min. The supernatant was used to determine protein concentration by bicinchoninic acid (BCA) protein concentration determination kit (RTP7102, Real-Times Biotechnology Co., Ltd., Beijing, China). Protein samples were then mixed with 2x sodium dodecyl sulfate loading buffer before denaturation in boiling water bath for 10 min. Afterwards, the samples (20 μg) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (100 V). The resolved proteins were transferred to polyvinylidene difluoride membranes on ice (100 V, 2 h) and blocked with 5% skimmed milk at room temperature for 1 h. Then, the membranes were incubated with rabbit anti-human PGP (1:3,000; Abcam, Cambridge, UK) and rabbit anti-human β-actin (1:5,000; Abcam) polyclonal primary antibodies at 4˚C overnight. After extensive washing with phosphate-buffered saline with Tween 20 for 5 times of 5 min, the membranes were incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibodies (1:3,000; Abcam) for 1 h at room temperature before washing with phosphate-buffered saline with Tween 20 for 5 times of 5 min. Then, the membrane was developed with enhanced chemiluminescence detection kit (Abcam) for imaging. Image Lab v3.0 software (Bio-Rad Laboratories, Inc.) was used to acquire and analyze imaging signals. The relative content of target protein was expressed against β-actin.

Bioinformatics. Bioinformatics prediction is a powerful tool for the study of the functions of miRNAs. To understand the regulatory mechanism of ABCB1, we used miRanda (http://www.microrna.org/microrna/home.do), TargetScan (http://www.targetscan.org), PiTa (http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html), RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) and PICTA (http://pictar.mdc-berlin.de/) to predict miRNA molecules that might regulate ABCB1, and found that miR-214 was able to potentially regulate ABCB1 (Fig. 1).

Dual luciferase reporter assay. According to bioinformatics results, wild-type (WT) and mutant seed regions of miR-214 in the 3'-UTR (150 bp upstream and downstream of CCUGCUG) of ABCB1 gene were chemically synthesized *in vitro*, added with Spe-1 and HindIII restriction sites, and then cloned into pMIR-REPORT luciferase reporter plasmids. Plasmids (0.8 μg) with WT or mutant 3'-UTR DNA sequences were co-transfected with agomiR-214 (100 nM; Sangon Biotech Co., Ltd.) into 293T cells. After cultivation for 24 h, the cells were lysed using dual luciferase reporter assay kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's manual, and luminescence intensity was measured using GloMax 20/20 luminometer (Promega Corporation). Using Renilla luminescence activity as internal reference, the luminescence values of each group of cells were measured.

Table I. Clinical characteristics of CML and IM-resistant patients.

Characteristics	CML patients	IM-resistant patients
Sex (n)		
Male	19	15
Female	12	11
Age range (years)	15-69	17-66
Median age (years)	53.5	52.6
Treatment duration (months)	1-2	3-18
White blood cell count ($x10^9$/l)	78±33	108±56

CML, chronic myeloid leukemia; IM, imatinib mesylate.

MTT assay. After transfection, cells were seeded into 96-well plates at a density of $2x10^3$ cells per well and cultured in the presence of l μM IM. Each condition was tested in triplicate wells according to a previously published method (26). At 24, 48, and 72 h, 20 μl MTT (5 g/l, JRDC000003; JRDUN Biotechnology, Shanghai, China) was added into each well. On the last day, DMSO (150 μl per well) was added to dissolve purple crystals after incubation at 37˚C for 4 h. Then, absorbance of each well was measured at 490 nm with a microplate reader (Bio-Rad Laboratories, Inc.). Cell survival curves were plotted.

Statistical analysis. The results were analyzed using SPSS v.18.0 statistical software (SPSS, Inc., Chicago, IL, USA). The data were expressed as the mean ± standard deviation. Data were tested for normality. Multigroup measurement data were analyzed using one-way analysis of variance. In case of homogeneity of variance, the Least Significant Difference and Student-Newman-Keuls post hoc methods were used; in case of heterogeneity of variance, Tamhane's T2 or Dunnett's T3 post hoc methods were used. $P<0.05$ was considered to indicate a statistically significant difference.

Results

ABCB1 mRNA and PGP expression is up-regulated in bone marrow mononuclear cells from CML patients with IM resistance. To measure the expression of ABCB1 mRNA and its protein PGP in bone marrow mononuclear cells, RT-qPCR and western blotting were performed. The data showed that the levels of ABCB1 mRNA and PGP in mononuclear cells from patients with IM resistance were significantly higher than those from patients without IM resistance ($P<0.05$; Fig. 2A and B). The result suggests that ABCB1 mRNA and PGP expression is up-regulated in bone marrow mononuclear cells from CML patients with IM resistance.

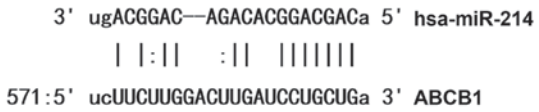

Figure 1. Direct interaction between miRNA-214 and ABCB1. Bioinformatics prediction was employed to understand the regulatory mechanism of ABCB1, using miRanda, TargetScan, PiTa, RNAhybrid and PICTA databases in order to predict the miRNA molecules that may regulate ABCB1. miR-214 was revealed to be able to potentially regulate ABCB1. miR/miRNA, microRNA; ABCB1, adenosine triphosphate binding cassette subfamily B member 1.

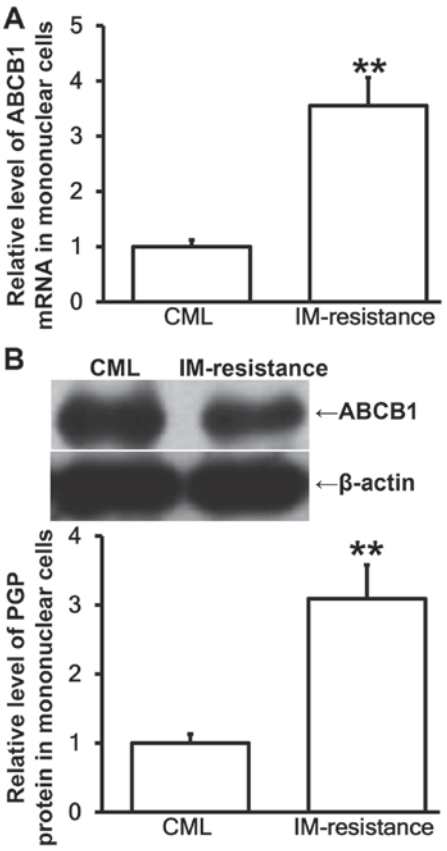

Figure 2. Expression of (A) ABCB1 mRNA and (B) PGP in mononuclear cells from CML patients without or with IM resistance. Reverse transcription-quantitative polymerase chain reaction was used to measure the expression of mRNA, while western blotting was used to determine protein expression. **P<0.01 vs. CML group. ABCB1, adenosine triphosphate binding cassette subfamily B member 1; PGP, P-glycoprotein; CML, chronic myeloid leukemia; IM, imatinib mesylate.

K562R cells have higher ABCB1 and PGP expression than K562 cells, probably due to their different sensitivity to IM. To determine the expression of ABCB1 mRNA and its protein PGP in K562 and K562R cells, RT-qPCR and western blotting were employed. The data showed that the levels of ABCB1 mRNA and PGP in K562R cells were significantly increased than those in K562 cells (P<0.05; Fig. 3A and B). The result indicates that K562R cells have higher ABCB1 and PGP expression than K562 cells, probably due to their different sensitivity to IM.

Expression miR-214 is decreased in bone marrow mononuclear cells from patients with IM resistance and K562R cells. To test the levels of miR-214 in bone marrow mononuclear cells and K562R cells, RT-qPCR was carried out. The data showed that miR-214 levels in bone marrow mononuclear cells from patients with IM resistance were significantly lower than that from patients without IM resistance (P<0.05; Fig. 4A). Similarly, miR-214 expression in K562R cells was significantly decreased than that in K562 cells (P<0.05; Fig. 4B). The results suggest that the expression miR-214 is decreased in bone marrow mononuclear cells from patients with IM resistance and K562R cells.

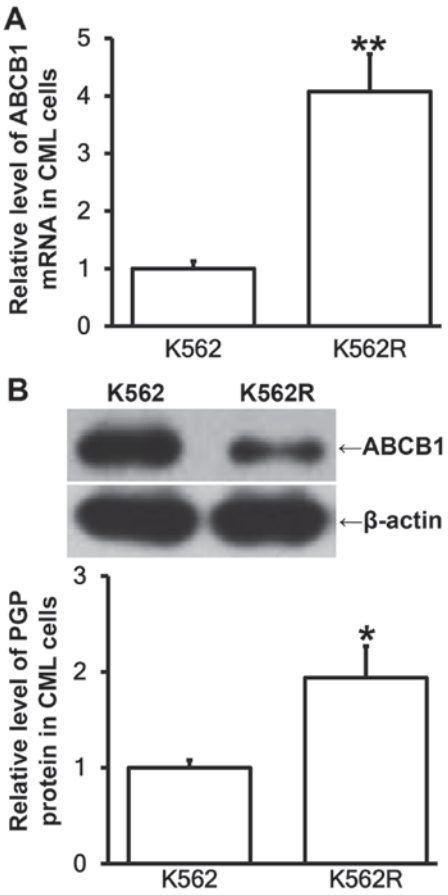

Figure 3. Expression of (A) ABCB1 mRNA and (B) PGP in CML cells. The K562 cell line is a CML cell line without IM resistance, while the K562R cell line is a K562 cell line with IM resistance. Reverse transcription-quantitative polymerase chain reaction was used to measure the expression of mRNA, while western blotting was used to determine protein expression. *P<0.05 and **P<0.01 vs. K562 group. ABCB1, adenosine triphosphate binding cassette subfamily B member 1; PGP, P-glycoprotein; CML, chronic myeloid leukemia; IM, imatinib mesylate.

miR-214 can bind with the 3'-UTR seed region of ABCB1 mRNA to regulate its expression. To identify the interaction between miR-214 and the 3'-UTR of ABCB1 mRNA, dual luciferase reporter assay was performed. The luminescence value of cells co-transfected with agomiR-214 and pMIR-REPORT-WT luciferase reporter plasmids was significantly lower than that in negative control group (P<0.05). By contrast, the luminescence value of cells co-transfected with agomiR-214 and pMIR-REPORT-mutant luciferase reporter plasmids was not significantly different from that in negative control group (P>0.05; Fig. 5). The result suggests that miR-214 can bind with the 3'-UTR seed region of ABCB1 mRNA to regulate its expression.

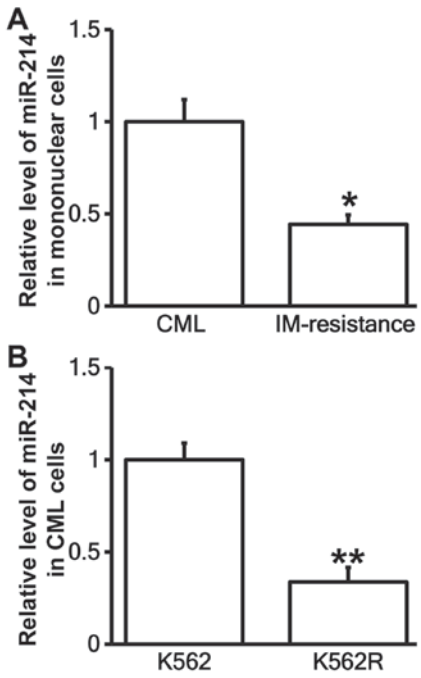

Figure 4. Expression of miR-214 in (A) mononuclear cells from CML patients with or without IM resistance and (B) CML K562 or K562R cells. Reverse transcription-quantitative polymerase chain reaction was used to measure the expression of miR-214. *P<0.05 and **P<0.01 vs. (A) CML group and (B) K562 group, respectively. miR, microRNA; CML, chronic myeloid leukemia; IM, imatinib mesylate.

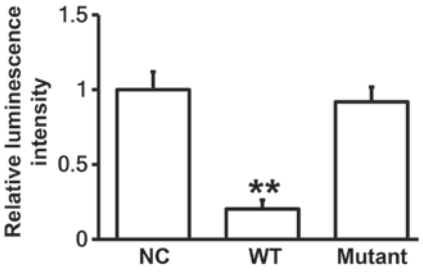

Figure 5. Identification of the interaction between miR-214 and ABCB1 using dual luciferase reporter assay. Plasmids (0.8 μg) with WT or mutant 3'-untranslated region DNA sequences were co-transfected with agomiR-214 into 293T cells. Following cultivation for 24 h, the cells were lysed using dual luciferase reporter assay kit, and luminescence intensity was measured using GloMax 20/20 luminometer. Using *Renilla* luminescence activity as the internal reference, the luminescence values of each group of cells were measured. **P<0.01 vs. NC group. miR, microRNA; ABCB1, adenosine triphosphate binding cassette subfamily B member 1; WT, wild-type; NC, negative control.

Elevated expression of miR-214 restores IM-sensitivity to K562R cells possibly by affecting ABCB1 expression. To test how miR-214 affects the expression of ABCB1 mRNA and PGP protein, we used RT-qPCR, western blotting and MTT assay. The data showed that expression of miR-214 in K562R cells after transfection with agomiR-214 was significantly enhanced (P<0.05; Fig. 6A). In addition, the expression of ABCB1 mRNA in K562R cells transfected with agomiR-214 was significantly lower than that in K562R cells transfected with negative control (P<0.05; Fig. 6B). Similarly, PGP expression in K562R cells transfected with agomiR-214 was significantly reduced than that in K562R cells transfected with negative control (P<0.05; Fig. 6C). Of note, MTT assay showed that the survival rate of K562R cells transfected with agomiR-214 was significantly reduced than those of K562R cells transfected with negative control or K562 cells at 24, 48 and 72 h in the presence of IM (P<0.05 for all points; Fig. 6D). The results indicate that elevated expression of miR-214 restores IM sensitivity to K562R cells possibly by affecting ABCB1 expression.

Discussion

CML is the first tumor that has targeted therapeutics drugs designed according to its disease-causing gene types (2). Discontinuation of IM treatment results in high frequency of disease recurrence, indicating that only a small proportion of patients may receive curative or prolonged remission after treatment (27,28). IM resistance has become an important issue in the treatment of CML. Studies show that overexpression of multidrug transporter and drug target enzyme may be involved in IM resistance of CML (29-31). Multidrug transporters, such as PGP, MRPS, LRP and BCRP, can increase intracellular drug efflux or vesicle isolation, resulting in decreased intracellular drug concentration or altered drug distribution. The best studied is MDR mediated by P-GP/MDRI and MRPI. It is first discovered in colchicine-resistant Chinese hamster ovary cells (32), and later found in all tissues of the organism (33). MDR prevents the body from absorbing harmful substances, mediates the output of substances, protects the brain and testis (34). It is reported that PGP expressed by ABCB1 gene in human body is closely related to drug distribution and translocation (34). PGP utilizes ATP in the organism to transport exogenous drugs and toxins outside the cells, reduces the concentration of intracellular drugs and toxins, decreases effect of drug treatment, and produces drug resistance (35). In the present study, we discover that expression of ABCB1 mRNA and PGP in mononuclear cells from CML patients with IM resistance is up-regulated, and similar trend is also observed for K562R that has IM resistance. This suggests that ABCB1 gene and its encoded protein play regulatory roles in IM resistance.

Our further study is then focused on the upstream miRNA that may regulate ABCB1 expression. It is reported that miRNA molecules cut mRNA and inhibits its translation to achieve a negative feedback regulation (36,37). miRNA molecules are important regulators in development, normal physiology, and diseases. Moreover, some miRNA molecules have become biomarkers for diseases (38,39). Using bioinformatics, we have discovered that miR-214 is an upstream regulator gene of ABCB1. It is reported that miR-214 can be used as a predictive factor for the diagnosis of gastric cancer, and it can also affect the proliferation and invasion of tumor cells (40). In addition, miR-214 can affect the proliferation and invasion of breast cancer through P53 (41). miRNA-214 can also inhibit bladder cancer growth by targeting PDRG1 gene (42). Moreover, Wan *et al* (43) report that miR-214 has a protective role in the post treatment for myocardial ischemia, and Izawa *et al* (44) discover that miR-214 effectively alleviates thioacetamide-induced cirrhosis and may have an anti-fibrotic effect. In the present study, we find that expression of miR-214 is reduced in both mononuclear cells from IM-resistant CML patients and K562R cells. Furthermore, dual luciferase

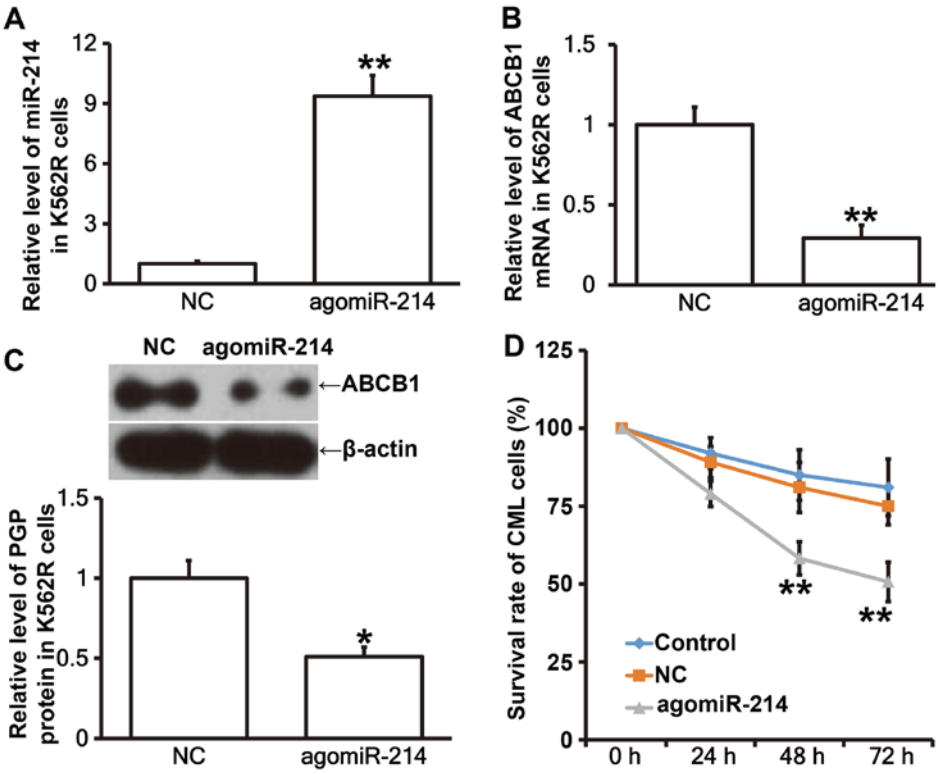

Figure 6. Effect of miR-214 expression on the expression of ABCB1 mRNA and PGP protein, as well as the survival of CML cells. (A) Expression of miR-214 in K562R cells transfected with NC or agomiR-214. Expression of (B) ABCB1 mRNA and (C) PGP in K562R cells transfected with NC or agomiR-214. (D) Survival rate of K562 cells (control), and K562R cells transfected with NC or agomiR-214 in the presence of IM (1 μM). Reverse transcription-quantitative polymerase chain reaction was employed to measure the expression of miR-214 and ABCB1 mRNA, western blotting was used to determine PGP expression, and MTT assay was performed to determine cell survival rate. *P<0.05 and **P<0.01 vs. NC group. miR, microRNA; ABCB1, adenosine triphosphate binding cassette subfamily B member 1; PGP, P-glycoprotein; CML, chronic myeloid leukemia; NC, negative control; IM, imatinib mesylate.

reporter assay demonstrates that miR-214 directly binds with the 3'-UTR seeding region of ABCB1 mRNA and regulates its expression. Then, we have transfected agomiR-214 into K562R cells to up-regulate the expression of miR-214 in these cells. After culturing the cells in IM medium, we find that the survival rate of K562R cells transfected with agomiR-214 is reduced in the presence of IM, suggesting that the sensitivity of K562R cells to IM is partially restored by overexpression of miR-214. The limitations of the study include low sequence homology between miRNA-214 and the ABCB1 gene and relatively small sample numbers in some experiments. We will improve this in future studies.

In conclusion, the present study demonstrates that miR-214 alters the expression of PGP by targeting ABCB1, and elevates IM resistance of CML cells. It plays an important biological role in IM resistance of CML patients.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JJ and JY collaborated on the design of the present study, performed the experiments, and analyzed the data. FY, ZJ and DL contributed to the literature review, and assisted with the experimental design and completion. SW was responsible for experimental design, data collection and analysis, and writing the manuscript. All authors collaborated to interpret the results and develop the manuscript. The final version of the manuscript has been read and approved by all authors.

Ethics approval and consent to participate

All procedures were approved by the Ethics Committee of Jining No. 1 People's Hospital (Shandong, China). Written informed consents were obtained from all patients or their families.

Consent for publication

Written informed consent for the publication of any associated data and accompanying images were obtained from all patients, or their parents, guardians or next of kin.

Competing interests

The authors declare that they have no competing interests.

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