European Review for Medical and Pharmacological Sciences 2019; 23: 7008-7015

MicroRNA-577 promotes the sensitivity of chronic myeloid leukemia cells to imatinib by targeting NUP160

X.-T. ZHANG[1], S.-H. DONG[2], J.-Y. ZHANG[3], B. SHAN[4]

[1]Department of Pediatric Clinic, Qilu Hospital, Shandong University, Jinan, China
[2]Laboratory Medicine, Binzhou Yangxin Traditional Chinese Medicine Hospital, Binzhou, China
[3]Shandong University, Jinan, China
[4]Department of Blood Collection Center, Qilu Hospital (Qingdao), Shandong University, Qingdao, China

Abstract. – OBJECTIVE: To explore the effect of microRNA-577 on the drug sensitivity of chronic myeloid leukemia (CML) and the underlying mechanism.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to detect the expression of microRNA-577 in peripheral blood of patients with chronic myeloid leukemia. Meanwhile, the expression of microRNA-577 was detected in CML cell line after imatinib treatment. Cell counting kit-8 (CCK-8) and flow cytometry assay were applied to verify the effect of microRNA-577 on cell proliferation and cycle. NUP160 was identified as a target gene of microRNA-577 by dual luciferase reporter gene assay. Cell reverse test was performed to figure out whether microRNA-577 can enhance the sensitivity of CML to imatinib.

RESULTS: QRT-PCR results revealed that microRNA-577 level was notably decreased in peripheral blood of patients with CML, and microRNA-577 could inhibit the proliferation and cycle of CML cells. In addition, the result of dual luciferase reporting assay indicated that microRNA-577 had a binding relationship with NUP160, and up-regulation of microRNA-577 in CML cell lines reduced the expression of NUP160, and vice versa. Lastly, cell reverse experiments confirmed that microRNA-577 can alleviate the resistance of CML to imatinib.

CONCLUSIONS: We found that microRNA-577 promotes the sensitivity of chronic myeloid leukemia cells to imatinib by down-regulating the expression of NUP160.

Key Words:
Leukemia, MicroRNA-577, Imatinib, NUP160.

Introduction

Chronic myeloid leukemia (CML) is a malignant hematological tumor derived from hematopoietic stem cells. It can be clinically divided into Chronic Phase (CP), Accelerated Phase (AP), and Blastic Phase (BP)[1]. CML patients are often characterized by chromosomal ectopic, of which the C-ABL oncogene on chromosome 9 is translocated to the breakpoint cluster (BCR) of the long arm of chromosome 22 to form the BCR-ABL fusion gene. Abnormally expressed BCR-ABL protein, which regulates cytokine expression, leads to a massive proliferation of bone marrow stem progenitor cells, decreased apoptosis and decreased adhesion of bone marrow stromal cells, resulting in the release of a large number of immature myeloid cells into peripheral blood and then the occurrence of CML[2-4].

Chemotherapy (including direct chemotherapy drugs and indirect topoisomerase inhibitors) can cause DNA damage to tumor cells, which can eventually lead to tumor cell death or aging[5]. A specific Tyrosine Kinase Inhibitor (TKI) targeting BCR-ABL kinase, such as Imatinib Mesylate (IM), is a first-line chemotherapy for chronic myeloid leukemia treated by this disease. The use of imatinib can greatly improve the prognosis of CML. However, the use of imatinib alone does not cure the disease. Two independent studies[6,7] have indicated that stem/progenitor cells of CML do not entirely depend on BCR-ABL activity for survival. Besides, the early application of imatinib is critical. In the early stages of treatment of chronic myeloid leukemia, imatinib has a very good effect, but for patients with accelerated or acute changes, the treatment is not effective. At the same time, more and more patients develop drug resistance in drug therapy (discontinuous treatment), and there are many factors that cause drug resistance

Corresponding Author: Bo Shan, MM; e-mail: qdbaihexuan @163.com

in CML patients. Therefore, an in-depth study of the survival/growth mechanisms of these cells and exploring the principles of drug resistance may provide new ideas for continuing to improve the treatment of this disease.

MicroRNAs (miRNAs) are a class of non-coding small RNAs of about 18-25 nucleotides in length, which can target the 3'-untranslated region (3'-UTR) of the target genes to block translation and accelerate degradation of target genes at post-transcription level[8]. Xu et al[10] have shown that miRNAs are involved in a variety of biological processes including tumorigenesis, development, differentiation, invasion, and other processes including leukemia, as well as chemotherapy drug resistance[9]. Studies have shown that overexpression of miR-138 inhibits BCR-ABL1 and Cyclin D3 expression by binding to the coding region and the 3'-UTR region, respectively. Increased expression of miR-150 and miR-146a and decreased expression of miR-142-3p and miR-199b-5p were observed after 2 weeks of IM treatment in CML patients, suggesting that IM may affect tumor cells by modulating tumor cell miRNA profiles growth and apoptosis. In addition, overexpression of miR-122 promotes the sensitivity of cells to sorafenib by down-regulating IGF-1R and thereby inhibiting the activation of RAS/RAF/ERK signaling[10].

Despite this, the role of miRNA has not been extensively studied in the mechanisms of resistance to chronic myeloid leukemia. In this study, we found that microRNA-577 was notably expressed in peripheral blood of CML patients by previous experiments, which was further increased in CML cells cultured with imatinib, so we would like to further explore the role of microRNA-577 in CML and its relationship to drug resistance.

Patients and Method

Clinical Samples

The study included 18 CML patients and 28 healthy individuals from the Qilu Hospital. All patients signed the informed consent. The investigation received the approval of the Ethics Committee of Qilu Hospital. Peripheral blood mononuclear cells (PBMC) were isolated from the patients using Ficoll-Hypaque separation fluid according to the manufacturer's instructions (StemCell Technologies, Vancouver, Canada) and then stored in a refrigerator at -80°C.

Cell Line and Cell Culture

Peripheral blood leukocytes, human CML cell lines (K562 and KG-1a) (all kept by our laboratory) were cultured in Roswell Park Memorial Institute (RPMI) medium (Gibco, Rockville, MD, USA) (containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 0.1 mg/mL streptomycin) in a 5% CO_2, 37°C incubator.

Cell Transfection

The cells were inoculated one day before the transfection, and the next day, the relevant reagents were transfected according to the state of the cells. The final concentration of miRNA mimics, inhibitors and their respective negative control sequences was 50-100 nM. Diethyl pyrocarbonate (DEPC) tips and Eppendorf (EP) tubes were required for transfection. The transfection tool reagent used was Lipofectamine 3000 from Invitrogen (Carlsbad, CA, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from cell specimens and mononuclear cells of patient specimens using the TRIzol method (Invitrogen, Carlsbad, CA, USA). The RNA content and purity were determined by ultraviolet spectrophotometer and stored in a refrigerator at -80°C.

The expression levels of the target gene were detected with IRD SYBR real time qPCR kit using an ABI StepOnePlus real time quantitative fluorescent PCR instrument. The reaction system in each well contained 10 µL SYBR GreenMaster Mix, 1 µL upstream primer, 1 µL downstream primer, 0.4 µL 50x Rox Dye, 2 µL cDNA, and 5.6 µL enzyme-free water. The data were analyzed by StepOne Software version v2.1 (Applied BioSystems, Foster City, CA, USA) software and the primer sequences were as follows: microRNA-577: (F: 5'-ACACTCCAGCTGGGTAGATAAAATATTGG-3' R: 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCAGGTACC-3') U6 (F: CTCGCTTCGGCAGCAGCACATATA, R: AAATATGGAACGCTTCACGA);

Immunoblotting

According to the molecular weight of the target protein, 50 µg of proteins were applied for Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) gel electrophoresis and then transferred to the polyvinylidene difluoride (PDVF) membrane (Millipore, Billerica,

MA, USA). Immunoblots were blocked in 5% skim milk at room temperature for 1 h, added with the diluted primary antibody (concentration according to the antibody instructions) and shaken overnight in a 4°C cold room. In the next day, immunoblots were incubated with the diluted secondary antibody for 1 h at room temperature. After washed with Tris-Buffered Saline and Tween (TBST), protein bands were detected by enhanced chemiluminescence (ECL).

Luciferase Reporting Assay

The transcript 3'-UTR sequence of the NUP160 gene was cloned into the vector pGL3 containing the luciferase reporter gene, which was regarded as the WT3' UTR group. The MUT3' UTR group was constructed by mutating the core region of the miRNA binding on the 3'-UTR to a null binding sequence using a site-directed mutagenesis kit. *Renilla* luciferase internal reference plasmid and microRNA-577 mimics were transfected into each group. After 24 hours of transfection, the cell culture medium was drained, and the appropriate amount of lysate was added to fully lyse the cells according to the requirements of the kit. After centrifugation at 10,000 g for 5 min, 100 μL of the supernatant of the lysate was taken for measurement. The dual luciferase was used as an internal reference, and the RLU value obtained by the firefly luciferase assay was divided by the RLU value obtained by the *Renilla* luciferase assay. The degree of activation of the reporter gene for different sample purposes was compared based on the ratio obtained.

Cell Cycle Detection

The cells were plated in 6-well plates, grouped for corresponding transfection and other treatments. On the 2nd day, the cells were collected by trypsinization, washed twice with ice phosphate-buffered saline (PBS), then added with 1 mL of DNA Staining Solution, vortexed and mixed, and incubated at room temperature in the dark. After 30 min, the samples were tested by MACS flow cytometry (FACSCalibur; BD Biosciences, Detroit, MI, USA).

Cell Counting Kit-8 (CCK-8) Assay

The transfected cells were seeded into 96-well plates at a density of 3-5×10^3 per well, with 10 replicate wells per day. 10 μL of CCK-8 reagent (Dojindo, Kumamoto, Japan) was added to each well and cells were incubated in a 37-degree incubator for 1 hour. The optical density (OD) value of each replicate well at 450 nm was detected by the spectrometer.

Statistical Analysis

The measurement data were expressed as mean ± standard deviation. For the two sets of data, the *t*-test was used to examine the mean difference between the groups. Multiple sets of data were analyzed by variance analysis. $p<0.05$ was considered to be statistically significant.

Results

Induction of MicroRNA-577 Increased After Imatinib Treatment

First, we examined the expression of microRNA-577 in peripheral blood of 18 patients with CML and 28 healthy peripheral blood (HPB). The results showed that microRNA-577 was notably decreased in the peripheral blood of CML patients (Figure 1A). We then examined the expression of microRNA-577 in CML cell lines (K562 and KG-1a) and found that microRNA-577 expression was notably reduced in human CML cell lines compared to peripheral blood cells (Figure 1 B). To investigate the relationship between microRNA-577 and CML resistance to imatinib, we examined the expression of microRNA-577 after 3 days of imatinib (10 nmol/L) addition to K562 and KG-1a cells. The results showed that the expression of microRNA-577 was notably elevated after imatinib treatment (Figure 1C, 1D) (*$p\leq0.05$), suggesting that imatinib could upregulate the microRNA-577 in CML.

MicroRNA-577 Inhibited Proliferation of CML Cells

To explore the role of microRNA-577 in CML, we up-regulated or down-regulated the expression of microRNA-577 in CML cell lines and then examined the effects on cell proliferation. After testing the transfection efficiency of microRNA-577 mimic and inhibitor (Figure 2A, 2B), we investigated the effect of microRNA-577 on the proliferation of K562 and KG-1a cells by CCK-8 assay. The results showed that the overexpression of microRNA-577 decreased the cell proliferation ability and inhibited the proliferation of cells (Figure 2C, 2D). Then, we investigated the effect of microRNA-577 on cell cycle by flow cytometry. The results showed that up-regulating the expression of microRNA-577 notably blocked the cell cycle. In contrast, inhibition of

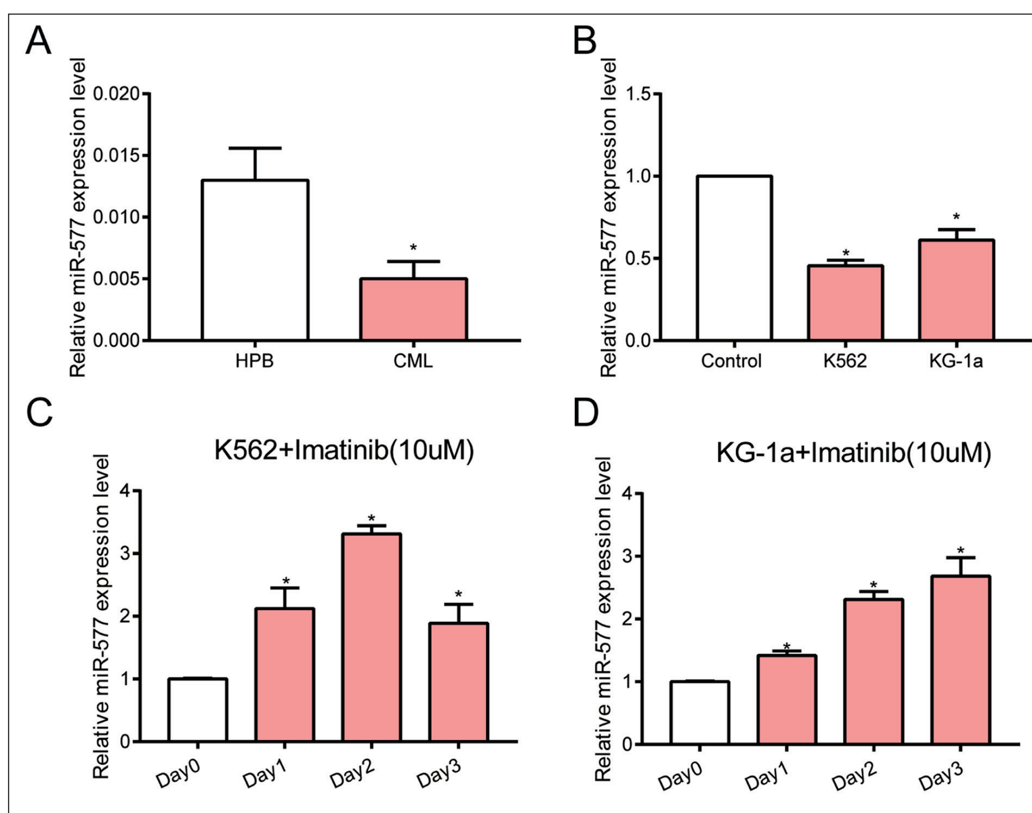

Figure 1. MiR-577 level was increased after imatinib treatment. ***A,*** The expression of miR-577 in peripheral blood of 18 patients with CML was significantly lower than that in 28 healthy peripheral blood (HPB). ***B,*** The expression of miR-577 was decreased in human CML cell lines (K562 and KG-1a) compared to peripheral blood cells. ***C-D,*** In K562 and KG-1a cells, the expression of miR-577 was increased after imatinib (10 nmol/L) treatment for 3 days (*$p \leq 0.05$).

miR-577 notably promoted the cell cycle (Figure 2E, 2F) (*$p \leq 0.05$). These results demonstrated that miR-577 could regulate the cell proliferation as well as the cell cycle.

NUP160 Was the Target Gene of MicroRNA-577

Through bioinformatics site prediction, we found that NUP160 and microRNA-577 have potential binding sites (Figure 3A). Further confirmed by dual luciferase reporter gene, experiments revealed that microRNA-577 had a binding relationship with NUP160 (Figure 3B, 3C). To further verify that NUP160 was a target gene of microRNA-577, we up-regulated and inhibited microRNA-577 expression in K562 and KG-1a cells and detected protein expression levels of NUP160. The results showed that after up-regulating the expression of microRNA-577, the protein expression of NUP160 was notably reduced, whereas the expression of NUP160 protein was notably increased after inhibition of microRNA-577 (Figure 3D, 3E), which further confirmed that NUP160 served as a target gene of microRNA-577. We then examined NUP160 expression in peripheral blood of patients with CML and found that it was notably higher than in healthy peripheral blood (HPB) (Figure 3F). At the same time, we also detected NUP160 expression in CML cell lines (K562 and KG-1a) and found that NUP160 expression in human CML cell line was also notably higher than normal cell line (Figure 3G) (*$p \leq 0.05$). Thus, we suggested that NUP160 was the target gene of microRNA- 577 in CML.

MicroRNA-577 Can Alleviate Imatinib Resistance in CML

To investigate the effects of microRNA-577 on imatinib resistance in CML, we up-regulated the expression of microRNA-577 while treating cells with imatinib. Cell proliferation assays showed that up-regulation of microRNA-577 can further enhance the inhibition effect of imatinib on CML cells proliferation (Figure 4A, 4B). Similarly, cell

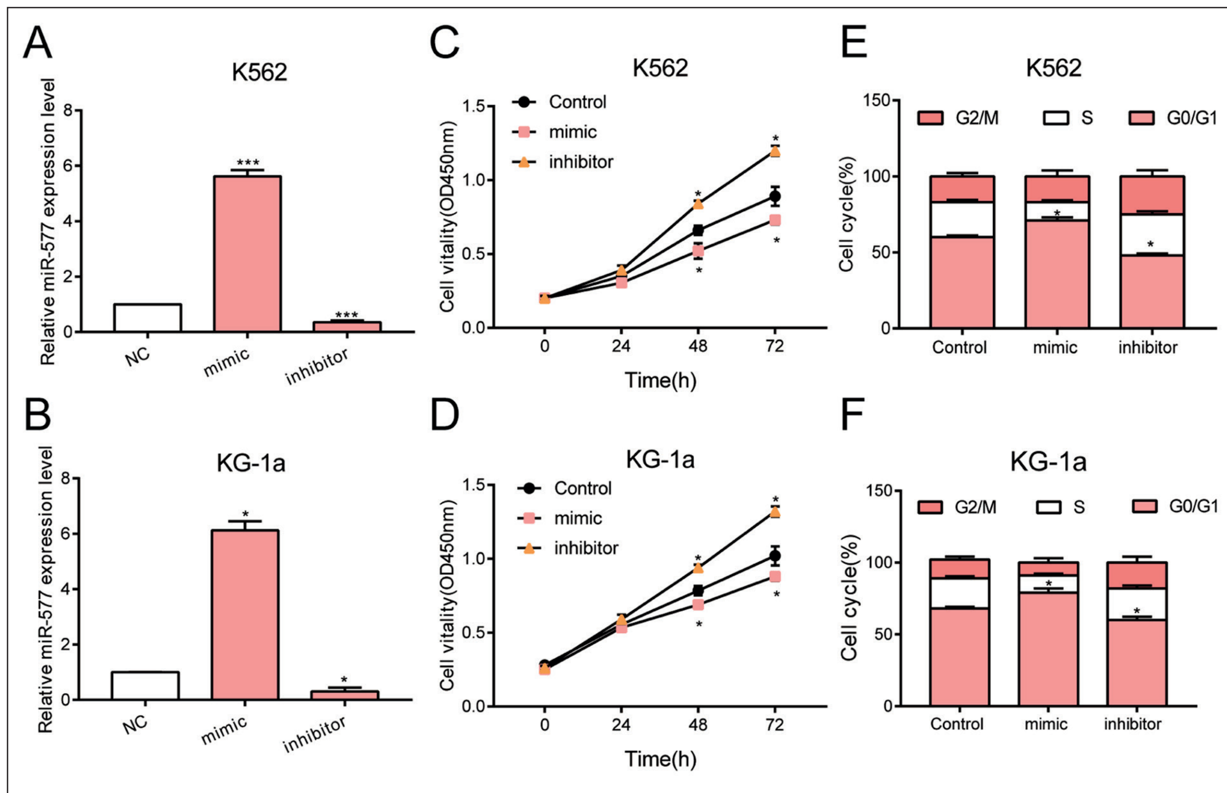

Figure 2. MiR-577 inhibits proliferation of CML cells. ***A, B,*** Transfection efficiency of miR-577 mimic and inhibitor IN K562 and KG-1a cells was shown. ***C, D,*** In K562 and KG-1a cells, overexpression of miR-577 decreased cell proliferation, while inhibiting miR-577 increased cell proliferation. ***E, F,*** In K562 and KG-1a cells, overexpression of miR-577 arrested cell cycle, while inhibition of miR-577 expression accelerated it (*$p \leq 0.05$).

cycle experiments showed that imatinib further enhanced CML cell cycle arrest after up-regulation of microRNA-577 (Figure 4C, 4D). The above results indicated that microRNA-577 can enhance the effect of imatinib, thereby alleviating the resistance of CML to imatinib (*$p \leq 0.05$ compared with control, #$p \leq 0.05$ compared with imatinib group).

Discussion

MicroRNA-577 acts as a tumor suppressor gene in a variety of tumors. For example, in glioblastoma, microRNA-577 inhibits tumor growth by regulating Wnt signaling pathway[11]. In breast cancer, microRNA-577 can block the progression of the tumor by down-regulating the expression of Rab25[12]. Besides, in colon cancer, in addition to inhibiting tumor growth, microRNA-577 can also enhance the sensitivity of colon cancer to chemotherapy drugs[13]. In this work, we examined the expression of microRNA-577 in peripheral blood of patients with CML and found that microRNA-577 was notably less expressed in CML patients than the control group. At the same time, we also found that microRNA-577 was also notably expressed in CML cell lines compared with human normal white blood cells, which indicated that microRNA-577 may play a certain role in the process of CML. To explore its mechanism, we up-regulated or down-regulated the expression of microRNA-577 in CML cell lines and verified its effect on cell proliferation and cycle. The results showed that up-regulation of microRNA-577 could inhibit cell proliferation and cycle.

The NUPl60 gene (NC 000011.10) is located on human chromosome 11 and contains 36 exons. The transcript mRNA (NM 015231) contains 5383 nucleotides, of which nucleotides 86 to 4396 encode nucleosides. The number of acid-encoding nucleotides is 4,311. The NUPl60 gene is highly expressed in HEK293 cells, HeLa cells, and B lymphoblastoid cells, and is also expressed in different degrees in tissues such as kidney, heart, liver, hypothalamus, bone marrow, and

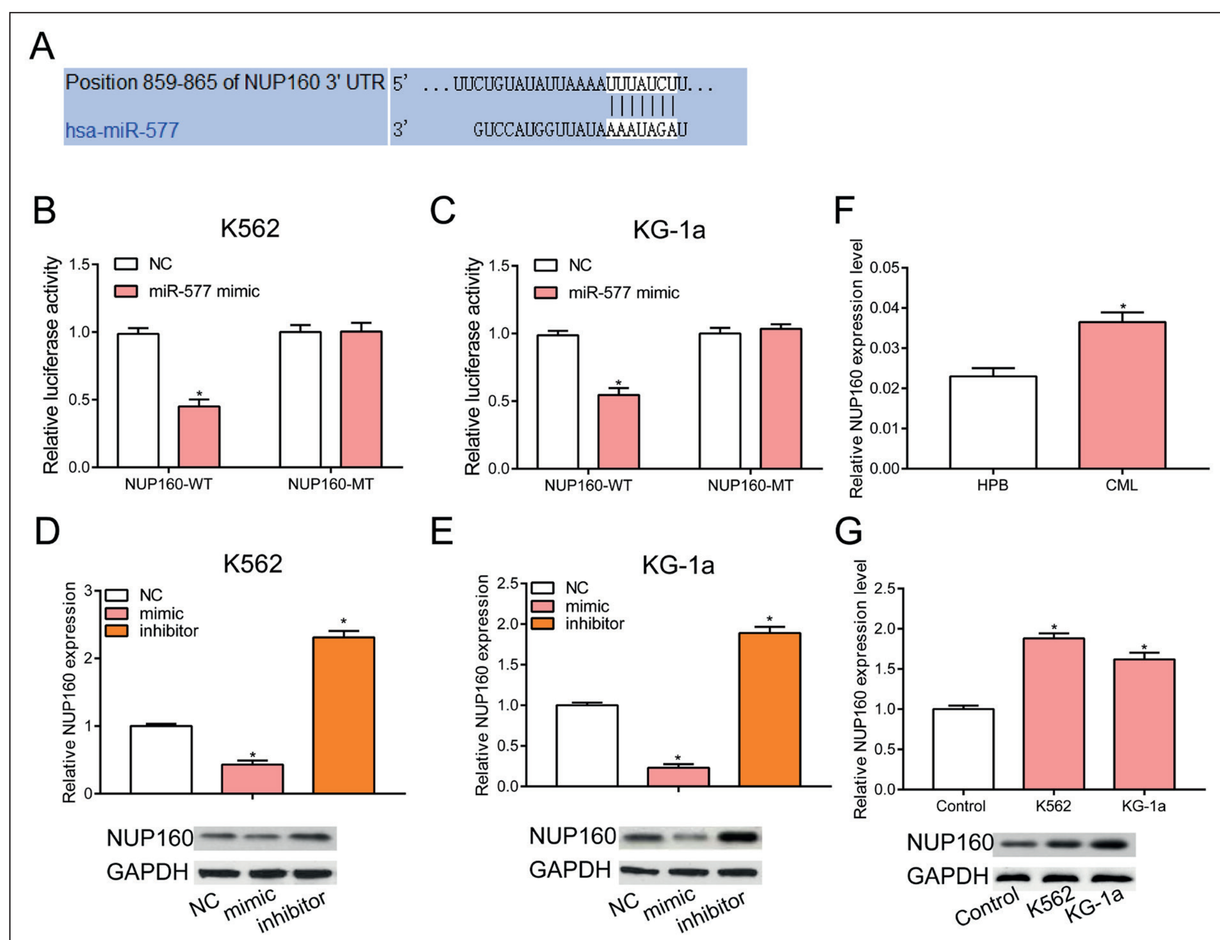

Figure 3. NUP160 is the target gene for miR-577. ***A,*** The website was used to predict the binding sites between NUP160 and miR-577. ***B, C,*** Luciferase reporter gene experiments showed that miR-577 could bind to NUP160. ***D, E,*** In K562 and KG-1a cells, the expression of NUP160 protein was decreased after overexpression of miR-577, while the opposite result was observed after inhibition of miR-577. ***F,*** The expression of NUP160 in peripheral blood of 18 patients with CML was significantly higher than that in 28 healthy peripheral blood (HPB). ***G,*** NUP160 expression was higher in human CML cell lines (K562 and KG-1a) than in peripheral blood cells (*$p \leq 0.05$).

lymph nodes[14]. The nuclear pore protein Nupl60 (NP 056046.1) encoded by NUPl60 gene contains 1436 amino acids with a molecular weight of 160 kDa and is an important component of the nuclear pore complex (NPC) central scaffold[15]. In this study, we found through site prediction that NUP160 may be a potential target gene of microRNA-577 with two potential binding sites, which was confirmed by the dual luciferase reporter gene assay. Further experiments showed that microRNA-577 could regulate NUP160 level. In addition, NUP160 was found highly expressed in peripheral blood or cell lines of CML patients.

Because miRNAs also play an important role in chemoresistance[16], we further explored whether microRNA-577 also played a role in CML resistance. First, after one to three days of cell culture with imatinib, we extracted cellular RNA and detected the expression of microRNA-577. The results showed that the expression of microRNA-577 was notably increased after treatment with imatinib, which suggested that the chemotherapy drug imatinib can increase the expression of microRNA-577, and microRNA-577 may enhance the sensitivity of chemotherapy drugs.

Current research indicates that miRNA can enhance the sensitivity of tumors to chemotherapy drugs by inducing apoptosis and inhibiting proliferation. It has been reported that miR-370 increases the sensitivity of K562 cells to homoharringtonine through targeting FoxM1 to induce cell apoptosis[17]. In addition, overexpression of microRNA-21 can notably increase IM-induced CML cell apoptosis[18], and make K562 cells more

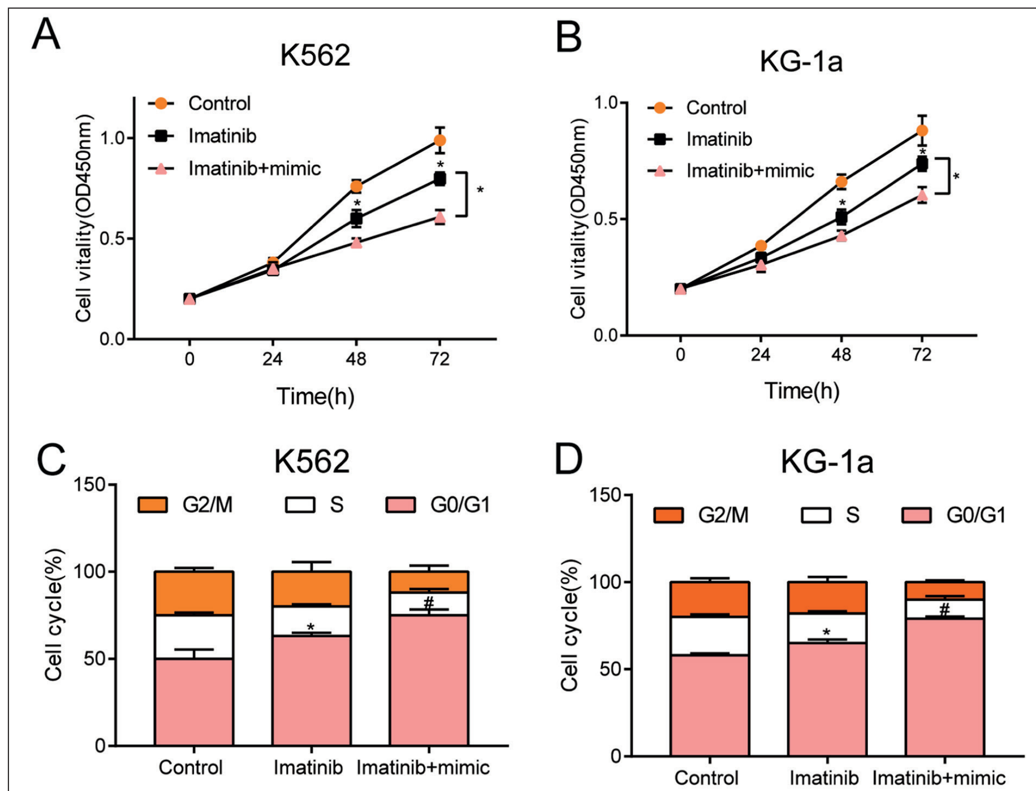

Figure 4. MiR-577 can alleviate imatinib resistance in CML treatment. ***A, B,*** CCK8 results showed that miR-577mimic could promote the inhibitory effect of imatinib on the proliferation of CML cells. ***C, D,*** miR-577 mimic could promote the inhibitory effect of imatinib on cell cycle of CML cells (*$p \leq 0.05$ compared with control, $^{\#}p \leq 0.05$ compared with imatinib group).

sensitive to dasatinib[19]. Additionally, microRNA-17 and microRNA-20a have been verified to be related to the BIM-S-mediated leukemia cell resistance to VP-16[20]. In this study, in order to investigate whether microRNA-577 can also potentiate the effects of imatinib by inhibiting cell proliferation and cycle, we up-regulated the expression of microRNA-577 while treating cells with imatinib. CCK-8 and flow cytometry were performed to examine its effects on cell proliferation and cycle. The results showed that imatinib can inhibit the proliferation and cycle of CML cells, and up-regulation of microRNA-577 expression can further enhance this effect. This also indicated that microRNA-577 can enhance the sensitivity of CML to imatinib, thereby promoting the effect of imatinib.

Conclusions

Based on the above results, we concluded that microRNA-577 could further enhance the inhibitory effect of imatinib on cell proliferation and cycle through down-regulating the expression of NUP160, thereby increasing the sensitivity of CML to imatinib. This may provide a new therapeutic target for the prevention and treatment of CML and the alleviation of resistance to imatinib.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Sherbenou DW, Druker BJ. Applying the discovery of the Philadelphia chromosome. J Clin Invest 2007; 117: 2067-2074.
2) Notari M, Neviani P, Santhanam R, Blaser BW, Chang JS, Galietta A, Willis AE, Roy DC, Caligiuri MA, Marcucci G, Perrotti D. A MAPK/HNRPK pathway controls BCR/ABL oncogenic potential by regulating MYC mRNA translation. Blood 2006; 107: 2507-2516.
3) Mohi MG, Boulton C, Gu TL, Sternberg DW, Neuberg D, Griffin JD, Gilliland DG, Neel BG. Combination of rapamycin and protein tyrosine kinase

(PTK) inhibitors for the treatment of leukemias caused by oncogenic PTKs. Proc Natl Acad Sci U S A 2004; 101: 3130-3135.

4) Marjanovic G. The use of inexpensive broad spectrum lower toxicity therapeutics in chronic lymphocytic leukemia. J BUON 2017; 22: 288-294.

5) Bouwman P, Jonkers J. The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. Nat Rev Cancer 2012; 12: 587-598.

6) Corbin AS, Agarwal A, Loriaux M, Cortes J, Deininger MW, Druker BJ. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. J Clin Invest 2011; 121: 396-409.

7) Hamilton A, Helgason GV, Schemionek M, Zhang B, Myssina S, Allan EK, Nicolini FE, Muller-Tidow C, Bhatia R, Brunton VG, Koschmieder S, Holyoake TL. Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival. Blood 2012; 119: 1501-1510.

8) Wang L, Wang Y, Lin J. MiR-152-3p promotes the development of chronic myeloid leukemia by inhibiting p27. Eur Rev Med Pharmacol Sci 2018; 22: 8789-8796.

9) Liu D, Wang B, Zhu Y, Yan F, Dong W. Carnosic acid regulates cell proliferation and invasion in chronic myeloid leukemia cancer cells via suppressing microRNA-708. J BUON 2018; 23: 741-746.

10) Xu Y, Huang J, Ma L, Shan J, Shen J, Yang Z, Liu L, Luo Y, Yao C, Qian C. MicroRNA-122 confers sorafenib resistance to hepatocellular carcinoma cells by targeting IGF-1R to regulate RAS/RAF/ERK signaling pathways. Cancer Lett 2016; 371: 171-181.

11) Zhang W, Shen C, Li C, Yang G, Liu H, Chen X, Zhu D, Zou H, Zhen Y, Zhang D, Zhao S. MiR-577 inhibits glioblastoma tumor growth via the Wnt signaling pathway. Mol Carcinog 2016; 55: 575-585.

12) Yin C, Mou Q, Pan X, Zhang G, Li H, Sun Y. MiR-577 suppresses epithelial-mesenchymal transition and metastasis of breast cancer by targeting Rab25. Thorac Cancer 2018; 9: 472-479.

13) Jiang H, Ju H, Zhang L, Lu H, Jie K. MicroRNA-577 suppresses tumor growth and enhances chemosensitivity in colorectal cancer. J Biochem Mol Toxicol 2017; 31:

14) Yoshida T, Shimada K, Oma Y, Kalck V, Akimura K, Taddei A, Iwahashi H, Kugou K, Ohta K, Gasser SM, Harata M. Actin-related protein Arp6 influences H2A.Z-dependent and -independent gene expression and links ribosomal protein genes to nuclear pores. Plos Genet 2010; 6: e1000910.

15) Schmid M, Arib G, Laemmli C, Nishikawa J, Durussel T, Laemmli UK. Nup-PI: the nucleopore-promoter interaction of genes in yeast. Mol Cell 2006; 21: 379-391.

16) Yang N, Kaur S, Volinia S, Greshock J, Lassus H, Hasegawa K, Liang S, Leminen A, Deng S, Smith L, Johnstone CN, Chen XM, Liu CG, Huang Q, Katsaros D, Calin GA, Weber BL, Butzow R, Croce CM, Coukos G, Zhang L. MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer. Cancer Res 2008; 68: 10307-10314.

17) Zhou M, Zeng J, Wang X, Guo Q, Huang T, Shen H, Fu Y, Wang L, Jia J, Chen C. MiR-370 sensitizes chronic myeloid leukemia K562 cells to homoharringtonine by targeting Forkhead box M1. J Transl Med 2013; 11: 265.

18) Wang WZ, Pu QH, Lin XH, Liu MY, Wu LR, Wu QQ, Chen YH, Liao FF, Zhu JY, Jin XB. Silencing of miR-21 sensitizes CML CD34+ stem/progenitor cells to imatinib-induced apoptosis by blocking PI3K/AKT pathway. Leuk Res 2015; 39: 1117-1124.

19) Nishioka C, Ikezoe T, Yang J, Nobumoto A, Tsuda M, Yokoyama A. Downregulation of miR-217 correlates with resistance of Ph(+) leukemia cells to ABL tyrosine kinase inhibitors. Cancer Sci 2014; 105: 297-307.

20) Yu Y, Yang L, Zhao M, Zhu S, Kang R, Vernon P, Tang D, Cao L. Targeting microRNA-30a-mediated autophagy enhances imatinib activity against human chronic myeloid leukemia cells. Leukemia 2012; 26: 1752-1760.