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LncRNA MEG3 Regulates Imatinib Resistance in Chronic Myeloid Leukemia via Suppressing MicroRNA-21

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Abstract

Imatinib resistance has become a major clinical problem for chronic myeloid leukemia. The aim of the present study was to investigate the involvement of MEG3, a lncRNA, in imatinib resistance and demonstrate its underlying mechanisms. RNAs were extracted from CML patients' peripheral blood cells and human leukemic K562 cells, and the expression of MEG3 was measured by RT-qPCR. Cell proliferation and cell apoptosis were evaluated. Western blotting was used to measure the protein expression of several multidrug resistant transporters. Luciferase reporter assay was performed to determine the binding between MEG3 and miR-21. Our results showed that MEG3 was significantly decreased in imatinib-resistant CML patients and imatinib-resistant K562 cells. Overexpression of MEG3 in imatinib-resistant K562 cells markedly decreased cell proliferation, increased cell apoptosis, reversed imatinib resistance, and reduced the expression of MRP1, MDR1, and ABCG2. Interestingly, MEG3 binds to miR-21. MEG3 and miR-21 were negatively correlated in CML patients. In addition, miR-21 mimics reversed the phenotype of MEG3-overexpression in imatinib-resistant K562 cells. Taken together, MEG3 is involved in imatinib resistance in CML and possibly contributes to imatinib resistance through regulating miR-21, and subsequent cell proliferation, apoptosis and expression of multidrug resistant transporters.

Key Words: Chronic myeloid leukemia, Imatinib, Drug resistance, MEG3, MiR-21

INTRODUCTION

Chronic myeloid leukemia (CML) is a major myeloproliferative neoplasm, which accounts for around 15% of newly diagnosed leukemia in adults (Jabbour and Kantarjian, 2014). One of the first line treatments for CML is imatinib mesylate (IM) (Hughes *et al*., 2015; Arora *et al*., 2016), a tyrosine kinase inhibitor (TKI) that competitively binds to the ATP-binding site of the breakpoint cluster region (*BCR*)-Abelson murine leukemia (*ABL*) protein and inhibits the subsequent phosphorylation and activation of downstream signal transduction (Schindler *et al*., 2000). As the result, the proliferation of leukemic cells is reduced and the apoptosis of leukemic cells is induced. The treatment with imatinib has dramatically improved the 8-year overall survival rate of CML to 80-90% (Wei *et al*., 2010). However, imatinib resistance has emerged as a major problem in the treatment of CML (Tauchi and Ohyashiki, 2004; Salizzato *et al*., 2016). Several mechanisms that lead to imatinib resistance have been reported, including amplification or mutation of the *BCR/ABL* gene (Barthe *et al*., 2001), aberrant expression of drug transporters (Takahashi and Miura, 2011), dysregulation of antiapoptotic members of the Bcl-2 protein family (Bellodi *et al*., 2009), and epigenetic alterations such as DNA hypermethylation (Boultwood and Wainscoat, 2007). However, the above mechanisms do not explain all cases of imatinib resistance. Therefore, novel mechanism to explain and new molecules to target are urgently needed to overcome imatinib resistance in CML.

Based on the transcript size, noncoding RNAs (ncRNAs) can be briefly classified into long noncoding RNAs (lncRNAs, >200 nt) and small noncoding RNAs (<200 nt) (Xia and Hui, 2014). The best-studied small noncoding RNAs are microRNAs (miRNAs), which are usually 20-25 nt and primarily bind to mRNAs through sequence complementarity to promote

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mRNA degradation or to prevent mRNA translation (Taft *et al*., 2010; Silveira *et al*., 2014). On the other hand, less is known regarding the biology and function of lncRNAs. Growing data have indicated that lncRNAs play important regulatory roles in the pathological process of tumorigenesis and the development of therapeutic resistance (Lalevee and Feil, 2015). One such lncRNA gene is maternally expressed gene 3 (*MEG3*), which is located on chromosome 14q32 and is expressed in many normal tissues (Miyoshi *et al*., 2000). Loss of *MEG3* has been shown in various human cancers, including brain, bladder, bone marrow, breast, colon and liver cancers, and exogenous expression of *MEG3* significantly suppresses the proliferation of human cancer cells (Zhang *et al*., 2003, 2010). These results emphasize the importance of *MEG3* gene as a tumor suppressor in human cancers. However, it is not clear whether *MEG3* gene is involved in the development of drug resistance in cancer chemotherapy. Therefore, in the present study, we explored the differential expression of *MEG3* gene in imatinib sensitive or resistant CML patients and cell line, and further investigated the underlying mechanisms that contribute to the MEG3-mediated imatinib resistance in CML.

MATERIALS AND METHODS

CML patients' recruitment

A total of 68 Chinese patients with CML in chronic-phase (Philadelphia chromosome-positive), including 30 females and 38 males, aged between 47 to 76 who were under the treatment of imatinib (400 mg/day) for more than two years were recruited in the present study. Informed written consent was taken from all the patients participating in this study and the study protocol was approved by the Ethics Committee of the Southwest Medical University (Luzhou, Sichuan, China). The patients were divided into two groups: imatinib-sensitive (IM-S, n=34) with patients who achieved a complete molecular response (CMR) or a major molecular response (MMR) (BCR/ABL:ABL ratio <1%) and imatinib-resistant (IM-R, n=34) with patients those did not receive CMR or MMR (BCR/ABL:ABL ratio ≥1%).

Cell culture and cell transfection

Human leukemic cell line K562 cells were purchased from ATCC (Manassas, VA, USA), and cultured in RMPI 1640 medium, which is supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified 5% CO_2 incubator at 37°C. Imatinib was purchased from Sigma (St. Louis, MO, USA), and imatinib-resistant K562 cells were constructed following the method previous described (Kang *et al*., 2014). K562 cells were transfected with wild type or mutant MEG3 construct using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. MicroRNA-21 (miR-21) mimics or mimic control (GenePharma, Shanghai, China) at a final concentration of 25 nmol/L was transfected into Imatinib-resistant K562 cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

RNA isolation and real-time quantitative PCR

Peripheral blood samples from CML patients or K562 cells were collected and total RNA was extracted using TRIzol reagent (Invitrogen), according to the manufacturer's instructions. cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen) and Oligo (dT18) RT primer was used for the reverse transcription of lncRNA. Real-time quantitative PCR (RT-qPCR) was performed in triplicates with SYBR Green Real-Time PCR Master Mixes (ThermoFisher, Waltham, MA, USA) on an ABI 7500 Fluorescent Quantitative PCR system (Applied Biosystems, Bedford, MA, USA). The following cycling conditions were used: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec. The primers sequence were: MEG3: 5'-GGCAGGATCTGGCATAGAGG-3' (forward); 5'-CGAGTCAGGAAGCAGTGGGTT-3' (reverse); GAPDH: 5'-GGAGCGAGATCCCTCCAAAAT-3' (forward); 5'-GGCTGTTGTCATACTTCTCATGG-3' (reverse). The primers for miR-21 and U6 expression were designed according to previous studies and purchased from Ribo Biotech (Guangzhou, China). For miR-21 (Xu *et al*., 2013), the primers were: 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAACATC-3' (stem-loop RT primer); 5'-ACACTCCAGCTGGCTAGCTTATCAGACTGATG-3' (forward); and 5'-CTCAACTGGTGTCGTGGA-3' (reverse); For U6 (Liu *et al*., 2016), the primers were 5'-AACGCTTCACGAATTTGCGT-3' (stem-loop RT primer); 5'-CTCGCTTCGGCAGCACA-3' (forward); and 5'-AACGCTTCACGAATTTGCGT-3' (reverse). Human GAPDH and U6 snRNA were used for lncRNA and miRNA normalization, respectively.

Western blotting

Cells were harvested and protein concentrations were measured using BCA protein assay kit (Pierce, Rockford, IL, USA). Total protein extracts were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to nitrocellulose membranes. The membranes were blocked with PBS containing 5% skim milk for 2 hours and incubated with human anti-caspase-3 antibody (Cell Signaling Technology, Berkeley, CA, USA), human anti-MRP1 antibody (Abcam, Cambridge, MA, USA), anti-MDR1 (Santa Cruz, CA, USA) or anti-ABCG2 (Santa Cruz) at 4°C overnight. GAPDH (Santa Cruz) was used as internal control. The membrane was washed 3 times with PBST, and incubated with HRP-conjugated secondary antibodies (Santa Cruz) at room temperature for 2 hours. The membrane was then measured for the expression of protein using an enhanced chemiluminescence reagent kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and images were captured using Image Scanner (FujiFilm, Tokyo, Japan).

Cell proliferation assay

Cell proliferation was measured by CCK-8 Cell Counting Kit (Vazyme Biotech Co., Ltd, Nanjing, Jiangsu, China). To test the cell proliferation rate, cells were seeded in 96 well plates in a total volume of 100 μL in triplicate in each experiment. Proliferation rates were determined at 0, 12, 24, 36, 48, 60, 72 hours after transfection. The fluorescence was then measured by a Fluoroskan Ascent™ FL Microplate Fluorometer (Thermo Scientific, Waltham, MA, USA). After subtraction of background, the cell proliferation was calculated as fold change relative to control cells.

Cell apoptosis assay

Annexin V-FITC/PI Apoptosis Detection Kit (KeyGEN Biotech, Nanjing, Jiangsu, China) followed by flow cytometry analysis was used to detect the imatinib (0.5 μM)-induced

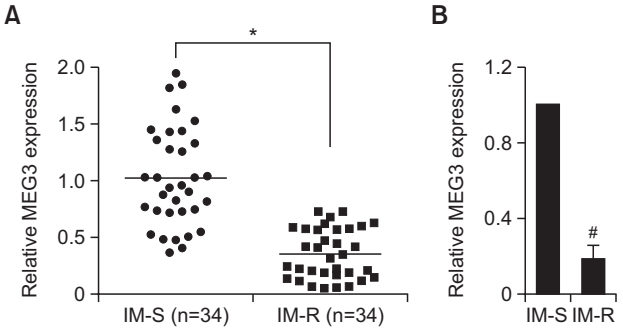

Fig. 1. Expression of MEG3 is reduced in imatinib-resistant human CML patients' peripheral blood cells and K562 cell line. (A) Relative expression of MEG3 in imatinib-resistant (IM-R, n=34) and imatinib-sensitive (IM-S, n=34) human CML patients' peripheral blood cells. *p<0.05. (B) Relative expression of MEG3 in imatinib-resistant (IM-R) and imatinib-sensitive (IM-S) human leukemic K562 cells. At least three biological repeats were performed. $^{\#}p$<0.01.

apoptosis of imatinib-resistant K562 cells. The acquisition and analysis were performed using MoFlow software (Beckman Coulter, Atlanta, GA, USA).

Cytotoxicity assay

In 96 well plates, imatinib-resistant K562 cells were seeded at 1×10^4 cells/well in 100 μL RPMI-1640 medium supplemented with 10% FBS. To detect IC_{50} of imatinib-resistant K562 cells, following MEG3 or vector transfection, imatinib, in the concentration range of 0.1 μM to 10 μM, was added to K562 cells for 48 hours. Next, cells were incubated with 10 μL Cell Counting Kit-8 (CCK-8) each well for 1 hour and absorbance at 450 nm was measured by Microplate Reader (Bio-Tech Company, Winooski, VT, USA). IC_{50} values were calculated with the GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA).

Luciferase reporter assay

Imatinib-resistant K562 cells were co-transfected with pMIR constructs containing the wild type MEG3 or mutant MEG3 construct, along with miR-21 mimics (25 nM or 50 nM) or mimic control (25 nM). Cells were harvested 48 hours post-transfection and Dual Luciferase Assay (Promega, WI, USA) was used to determine the luciferase reporter activities according to the manufacturer's instructions. All transfection assays were carried out in triplicate.

Data presentation and statistical analysis

At least three times of independent experiments were performed for each experiment. All data are presented as mean ± SD and expressed as fold change over control. Student's t-test was used for the comparison between two groups. Values for p<0.05 were considered significant.

RESULTS

Expression of lncRNA MEG3 is greatly reduced in imatinib-resistant CML

We first examined the expression of MEG3 in peripheral blood cells of CML patients from imatinib-sensitive (IM-S) and imatinib-resistant (IM-R) groups. Imatinib-resistant group apparently showed dramatically reduced expression of MEG3 compared to imatinib-sensitive group (p<0.05) (Fig. 1A). Next, we investigated the differential expression of MEG3 in imatinib-resistant or imatinib-sensitive human leukemic K562 cells. Consistently, we found a significant decrease in MEG3 level in imatinib-resistant K562 cells as compared to its sensitive control (p<0.01) (Fig. 1B). These results indicate dysregulation of MEG3 in imatinib-resistant CML patients and cell line,

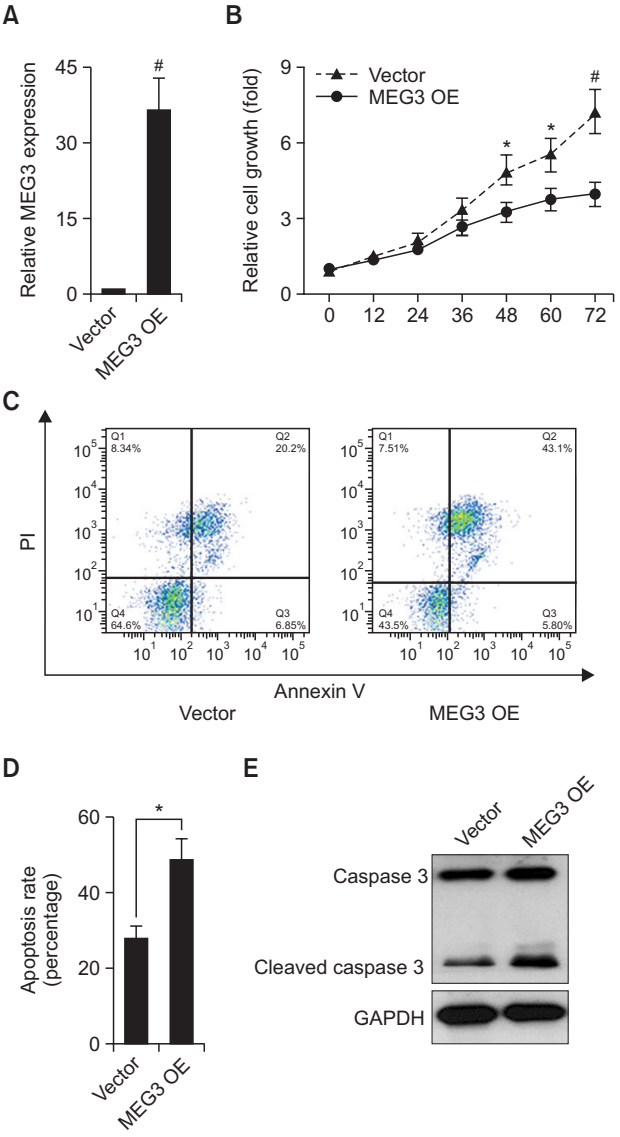

Fig. 2. Overexpression of MEG3 in imatinib-resistant K562 cells reduces cell proliferation and induces apoptosis. (A) Relative expression of MEG3 after overexpression of MEG3 (MEG3 OE) in imatinib-resistant human leukemic K562 cells. (B) CCK-8 assay demonstrates cell proliferation in imatinib-resistant K562 cells at 0, 12, 24, 36, 48, 60, 72 hours after transfection of MEG3 or vector plasmids. *p<0.05; $^{\#}p$<0.01. (C) Representative figure of Annexin V apoptosis analysis in imatinib-resistant K562 cells with or without MEG3 overexpression. (D) Apoptosis rate analysis for Fig. 2C. At least three biological repeats were performed. *p<0.05. (E) Representative western blotting analysis of cleaved caspase 3 in imatinib-resistant K562 cells with or without MEG3 overexpression.

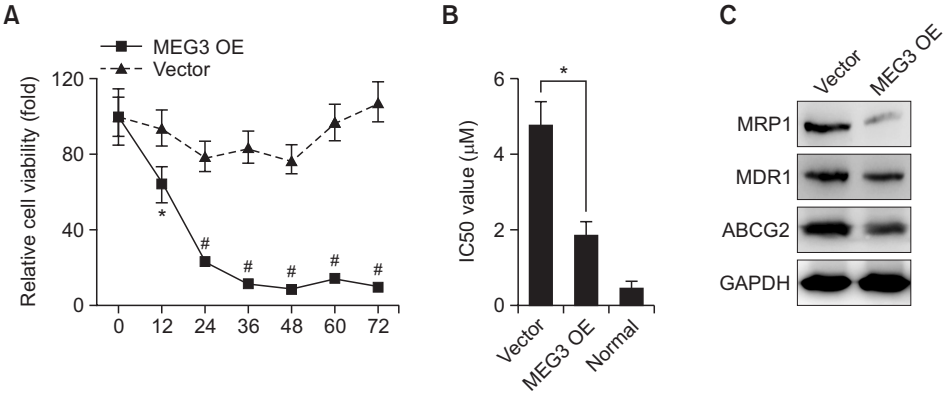

Fig. 3. Overexpression of MEG3 in imatinib-resistant K562 cells increased their sensitivity to imatinib. (A) After transfection of MEG3 or vector plasmids, CCK-8 assay demonstrates cell viability in imatinib-resistant K562 cells at 0, 12, 24, 36, 48, 60, 72 hours after imatinib treatment. *p<0.05; #p<0.01. (B) Imatinib-resistant K562 cells were transfected with MEG3 or vector plasmids, and IC_{50} values were determined via CCK-8 assay after 48 hours of imatinib treatment. The IC_{50} value of imatinib was also calculated in parental K562 cells (normal) as a negative control. *p<0.05. (C) The protein expression of MRP1, MDR1 and ABCG2 in imatinib-resistant K562 cells with or without MEG3 overexpression.

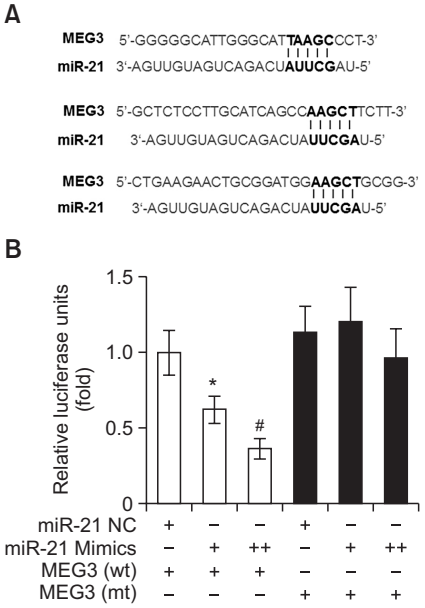

Fig. 4. MEG3 is physically associated with miR-21. (A) Bioinformatic analysis shows the prediction for miR-21 binding sites on MEG3 transcript. The nucleotides in bold are the complementary sequences to miR-21 seed sequences. (B) Luciferase reporter activity in imatinib-resistant K562 cells cotransfected with miR-21 mimics (+:25 nM, ++: 50 nM) or mimic control (+: 25 nM) and luciferase reporters containing wild type MEG3 (wt) or mutant MEG3 (mt) as indicated. Data are presented as the relative ratio of firefly luciferase activity to renilla luciferase activity. *p<0.05; #p<0.01.

suggesting a potential role of MEG3 in imatinib resistance in CML.

Overexpression of MEG3 in imatinib-resistant K562 cells reduces cell proliferation and induces apoptosis

We continued to explore whether exogenous expression of MEG3 in imatinib-resistant K562 cells would have any impact on cell growth and cell death. As shown in Fig. 2, wild type MEG3 construct was transiently transfected into imatinib-resistant K562 cells, and the efficiency of MEG3 overexpression was validated by RT-qPCR analysis (Fig. 2A). 48 hours after transient transfection, the cell proliferation of imatinib-resistant K562 cells with MEG3 overexpression was significantly reduced compared to that of the vector control (Fig. 2B) and this effect remained until 72 hours post transfection via CCK-8 assay. In addition, compared to vector control, MEG3 overexpression dramatically induced the percentage of early and late apoptotic population from 27.05% (vector control) to 48.9% (MEG3 overexpression) in imatinib-resistant K562 cells (p<0.05) (Fig. 2C, 2D). This finding was further confirmed by western blotting analysis, which showed that the amount of cleaved caspase 3 was markedly higher in MEG3-overexpressing K562 cells than the control cells (Fig. 2E). Taken together, these results suggest that MEG3 could exert its biological function through inhibiting cell proliferation and promoting cell apoptosis.

Overexpression of MEG3 in imatinib-resistant K562 cells increased cell sensitivity to imatinib

Next, we examined the effect of MEG3 overexpression on imatinib-induced cytotoxicity in K562 cells via CCK-8 assay. We found that when cells were treated with imatinib (0.5 μM), the cell viability was remarkably decreased in imatinib-resistant K562 cells with MEG3 overexpression as compared with that in vector control (Fig. 3A). Additionally, MEG3 overexpression significantly decreased the IC_{50} of imatinib in imatinib-resistant K562 cells (p<0.05). Compared to the IC_{50} of imatinib in parental K562 cells (normal), we found that MEG3 overexpression, to a great extent, reversed imatinib resistance in imatinib-resistant K562 cells (Fig. 3B). Since it has been reported that one of the major mechanisms for imatinib resistance is the aberrant expression of drug transporters, we also compared the expression of three major multidrug resistant drug transporters, including MRP1, MDR1, and ABCG2, in vector-transfected and MEG3-overexpressing imatinib-resistant K562 cells. Interestingly, the protein levels of MRP1, MDR1 and ABCG2 were significantly decreased in MEG3-overexpressing cells (Fig. 3C), which indicated that MEG3 might improve imatinib

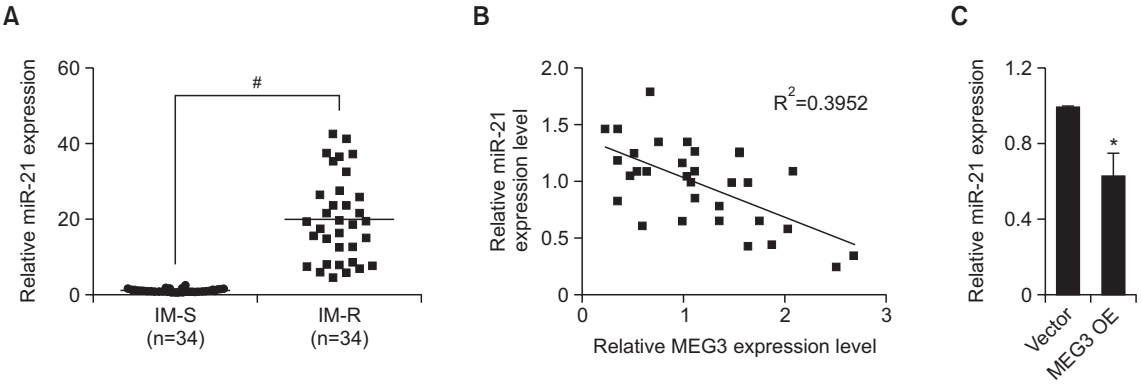

Fig. 5. MEG3 and miR-21 are negatively correlated in CML. (A) Relative expression of miR-21 in imatinib-resistant (IM-R, n=34) and imatinib-sensitive (IM-S, n=34) human CML patients' peripheral blood cells. $^{\#}p<0.01$. (B) Correlation analysis between MEG3 and miR-21 in human CML patients' peripheral blood cells. $R^2=0.3952$. (C) Relative expression of miR-21 in MEG3 or vector transfected imatinib-resistant K562 cells. $^{*}p<0.05$.

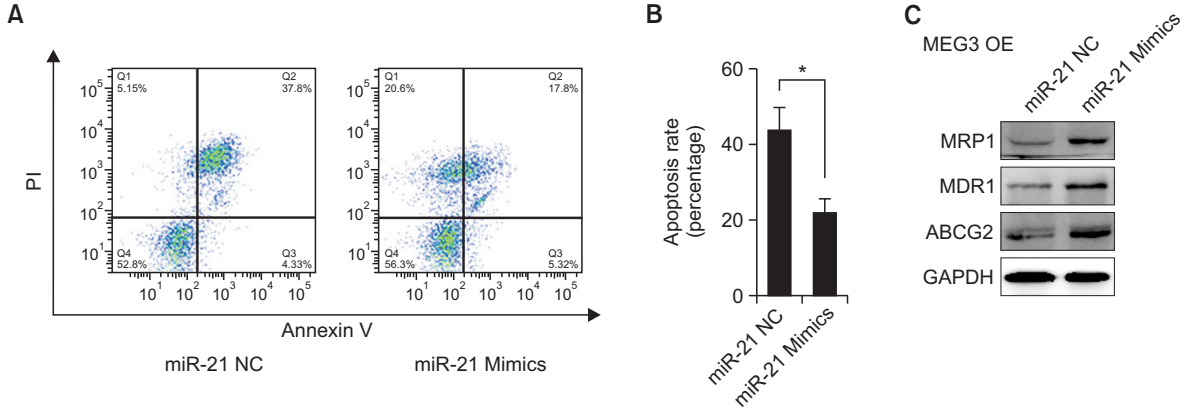

Fig. 6. miR-21 mimics reversed the increased apoptosis and decreased expression of multidrug resistant transporters in MEG3-overexpressing imatinib-resistant K562 cells. (A) MEG3-overexpressing imatinib-resistant K562 cells transfected with mimic controls or miR-21 mimics were subjected to apoptosis analysis. Representative figure is shown. (B) Apoptosis rate analysis for Fig. 6A. At least three biological repeats were performed. $^{*}p<0.05$. (C) The protein expression of MRP1, MDR1 and ABCG2 in MEG3-overexpressing imatinib-resistant K562 cells transfected with miR-21 mimic controls or miR-21 mimics.

resistance in CML through suppressing the overexpression of multidrug transporters.

MEG3 is physically associated with miR-21

Recent studies have suggested competitive endogenous RNAs (ceRNAs), such as lncRNAs, could directly bind to miRNAs through specific miRNA binding sites, therefore preventing the binding of these miRNAs to target mRNA and releasing the mRNA transcripts targeted by those miRNAs (Salmena *et al*., 2011). To determine whether MEG3 could serve as a ceRNA, we searched an open online database lnCeDB (http://gyanxetbeta.com/lncedb/) to look for any miRNAs that could potentially bind to MEG3. Indeed, we found 3 miR-21 binding sites along MEG3 transcripts (Fig. 4A), suggesting that MEG3 could be a ceRNA for miR-21. Furthermore, we constructed two luciferase reporters containing either the wild type MEG3 or a mutant MEG3 with mutations at all 3 predicted miR-21 binding sites. We found that transfection of imatinib-resistant K562 cells with miR-21 mimics greatly reduced the luciferase reporter activities of the wild type MEG3 reporter, but not that of the mutant MEG3 reporter (Fig. 4B), indicating a potential physical interaction between MEG3 and miR-21 via these 3 binding sites.

MEG3 regulates imatinib resistance through suppressing miR-21

Based on the above findings, we continued to investigate the correlation between MEG3 and miR-21 in imatinib-resistant CML. Opposite to our previous findings of MEG3 expression in CML patients (Fig. 1A), we found that the expression of miR-21 in peripheral blood cells of imatinib-resistant CML patients was significantly higher compared to that of imatinib-sensitive CML patients (Fig. 5A). A further correlation analysis disclosed that MEG3 and miR-21 were indeed negatively correlated in CML patients (Fig. 5B). Importantly, when we overexpressed MEG3 in imatinib-resistant K562 cells, we discovered that the expression of miR-21 was greatly decreased (Fig. 5C). Additionally, we found that the cellular apoptosis in MEG3-overexpressing imatinib-resistant K562 cells was reduced from 42.13% when transfected with mimics control

to 23.12% when transfected with miR-21 mimics (Fig. 6A, 6B). Transfection of miR-21 also reversed the expression of multidrug resistant transporters MRP1, MDR1 and ABCG2 in MEG3-overexpressing imatinib-resistant K562 cells (Fig. 6C). Therefore, combined with our previous prediction that MEG3 is a potential ceRNA of miR-21, these results strongly suggest MEG3 regulates imatinib resistance in CML through suppressing miR-21, and subsequently modulating cell proliferation, cell apoptosis and expression of multidrug resistant transporters.

DISCUSSION

LncRNAs have emerged as important regulators in oncogenic and tumor suppressor pathways. For example, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been associated with metastasis and poor prognosis in non-small cell lung cancer (NSCLC) (Ji *et al*., 2003). It is further demonstrated that MALAT1 might increase metastasis through promoting cell motility in lung adenocarcinomas (Tano *et al*., 2010). In addition, several lncRNAs have been characterized to regulate the development of drug resistance in cancer chemotherapy. For instance, through noncoding RNA Expression microarrays, several groups have reported the important roles of lncRNAs in cisplatin resistance in NSCLS, including AK126698, through regulating the canonical Wnt signaling pathway (Yang *et al*., 2013); HOTAIR, through the regulation of p21WAF1/CIP1 (p21) expression (Yang *et al*., 2013); and MEG3, through the control of p53 and Bcl-xl expression (Liu *et al*., 2015). MEG3 is a newly recognized lncRNA. It is widely expressed in normal tissues including the brain, adrenal gland, placenta, pituitary, testes, ovary, pancreas, spleen, mammary gland and liver (Zhang *et al*., 2003). Reduced expression of MEG3 has been reported in a variety of human cancer cell lines, including K562, a human chronic myeloid leukemic cell line. In our current study, we have demonstrated that MEG3 was down regulated in both imatinib-resistant human CML peripheral blood cells and imatinib-resistant K562 cells, and a higher level of MEG3 was associated with reduced proliferation and induced apoptosis of chronic myeloid leukemic cells. Our findings thus confirmed the tumor suppressor properties of MEG3 in CML and for the first time disclosed the potential role of MEG3 in imatinib resistance in CML.

Since we have elucidated the functions of MEG3 in CML chemoresistance, it is important to understand the underlying mechanisms, which would further improve the treatment of CML and potentiate the discovery of novel targets for CML. ceRNA hypothesis is one of the theories that well explain the interactions between different types of RNAs via miRNA. Briefly, lncRNAs and other noncoding RNAs could share common miRNA binding sites (also known as miRNA response elements, MREs) of mRNAs, thus releasing the target mRNAs from these miRNAs and abolishing the downstream effects of these miRNA. In our studies, through bioinformatic analysis, we have found three potential MREs of miR-21 spanning the transcript of MEG3. Overexpression of miR-21 has been implicated in tumorigenesis and chemoresistance, possibly by modulating the expression of specific apoptotic proteins, including Bcl-2. A recent study evaluated expression profiles of common oncogenic and tumor suppressing miRNAs in CML patients and found that miR-21 is significantly up regulated in imatinib-resistant patient compared to imatinib-sensitive patients (Jurkovicova *et al*., 2015). Hence, MEG3 might regulate imatinib resistance through interaction with miR-21. Indeed, we have found in our studies that MEG3 directly binds to miR-21 and their expressions are negatively correlated in CML patients. Most importantly, treatment of miR-21 mimics almost completely reversed the increased apoptosis induced by MEG3 overexpression in imatinib-resistant CML cells. All these results strongly support our hypothesis that MEG3 plays an important role in imatinib resistance in CML through regulating miR-21 and provide the first description that MEG3 serves as a ceRNA for miR-21 in CML.

It has been shown by several groups that inhibition of miR-21 by small interfering RNA could overcome multidrug resistance and restore drug sensitivity in various cancer types (Mei *et al*., 2010; Zhi *et al*., 2013). Interestingly, we have also shown here that overexpression of MEG3 dramatically reduced the expression of three multidrug resistant transporters, including MRP1, MDR1 and ABCG2. In addition, miR-21 mimics could reverse the decreased expression of MRP1, MDR1 and ABCG2 caused by MEG3 overexpression. Therefore, our study has clearly demonstrated the involvement of MEG3 in imatinib resistance in CML, and further elucidated its underlying mechanisms, which is through regulating miR-21, and ultimately modulating cell proliferation, cell apoptosis and expression of multidrug resistance transporters.

Taken together, our research demonstrated that MEG3 is involved in the development of imatinib resistance in CML and possibly contributes to imatinib resistance through regulating miR-21, with cell proliferation, cell apoptosis and expression of multidrug resistant transporters as its downstream effectors. These findings have important impact in our understanding of the mechanisms of imatinib resistance in CML and will potentially facilitate the selection of CML patients who would benefit from imatinib treatments.

CONFLICT OF INTEREST

There are no financial and commercial conflicts of interest should be stated.

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