Biochemical and Biophysical Research Communications 425 (2012) 368–373

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Myc induced miR-144/451 contributes to the acquired imatinib resistance in chronic myelogenous leukemia cell K562

Li Liu *, Sitao Wang, Renan Chen, Yanlan Wu, Bei Zhang, Siyong Huang, Jingyi Zhang, Fang Xiao, Meng Wang, Yingmin Liang *

Department of Hematology, Tangdu Hospital, The Fourth Military Medical University, Xi'an 710038, China

ARTICLE INFO

Article history:
Received 15 July 2012
Available online 27 July 2012

Keywords:
miR-144/451
c-myc
Imatinib resistance
Chronic myelogenous leukemia

ABSTRACT

Imatinib resistance remains the big hurdle for CML therapy. Previous study reveals that c-myc is important for bcr–abl CML cell proliferation, while its role in imatinib resistance is largely unknown. In this study, we first found that c-myc expression is upregulated in imatinib resistant K562R cells, which in turn enhances the expression of miR-144/451. Knockdown of c-myc or restoration of miR-144/451 in the K562R cells sensitizes K562R cells to imatinib therapy. Our study here reveals an regulatory pathway between myc and miR-144/451 and highlights that targeting either myc or miR-144/451 might be valuable for eliminating the imatinib resistant CML cells.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Chronic myelogenous leukemia (CML) is one of the most common leukemias worldwide and more than 90% of CML cases [1] are associated with the presence of the Philadelphia chromosome (Ph+), which is the result of a reciprocal translocation between 9 and 22 chromosomes that fuses Bcr-encoded sequences to a truncated c-Abl. The fusion protein produced has increased protein tyrosine kinase (TK) activity of Abl that is responsible for the malignancy [2]. Imatinib mesylate (Gleevec-2-phenyl amino pyrimidine compound), a specific inhibitor of several TKs, could induce a nearly complete hematologic and cytogenetic remissions in most patients with CML [3]. However, there remains some primary refractory disease and secondary resistance cases in clinical settings. Previous studies have revealed that resistance to imatinib could originate from increased bcr–abl activity due to enhanced bcr–abl gene expression or bcr–abl point mutations [4,5] and activation of other survival pathways, such as lyn activation [6], ERK activation [7], PI3 K activation [8], MDR1 and COX2 induction [9,10].

Previous study revealed that bcr-abl could induce the expression of myc both at mRNA and protein level, and the increased myc in turn promotes the cell proliferation and CML progression by transcribing cyclinD1 [11]. However, whether Myc expression is further upregulated in the imatinib resistant CML cells and whether myc expression is involved in the imatinib resistance is largely unknown.

microRNAs (miRNAs) are found to be an abundant class of endogenous, 19–22 nucleotides long, non-coding, single-stranded RNAs, which bind to the 3′-untranslated region (UTR) of targeted mRNAs and in turn trigger the degradation or translational repression [12]. Multiple studies on the miRNAs and their target messenger RNAs in cancer have demonstrated that some miRNAs act as oncogenes and some others as tumor suppressor genes [13–17]. Among these miRNAs, miR-451 is found to play an important role in CML. Very recently, miR-451 is found to be lowly expressed in CML [18], which are at least partially dependent on BCR-ABL activity [19]. More strikingly, Iraci et al. identified miR-451 as a miRNA with potential to target BCR-ABL kinase [19]. In addition, low miR-451 expression has been associated with higher risk of disease-relapse in gastric cancer patients and is involved in radiation response [20]. Aberrant miR-451 could also confer the drug resistance by promoting the self-renewal ability of colon cancer cells [21]. All of these data suggest that miR451 could play an important role in CML imatinib resistance.

In this study, we first identified that c-myc expression was upregulated in the imatinib resistant K562R cells, which in turn increased the expression of miR-144/451. Furthermore, we demonstrated that restoration of miR-144/451 or knockdown of Myc could sensitize the imatinib resistant cells to apoptosis. Our findings show that myc, miR-144/451 form a regulatory pathway and contribute to the imatinib resistance, and targeting this pathway should be valuable in the development of novel therapies against imatinib resistance in CML.

* Corresponding authors. Address: Department of Hematology, Tangdu Hospital, Baqiao District, Xinshi Road No. 1, Xi'an 710038, China. Fax: +86 2984777842.
E-mail address: liuli029@yahoo.cn (L. Liu).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.098

Table 1
Primers or sequences used in this study.

Gene or primer name	Sequence
pGL3-1000	CC*GGTACC*CAGTGCTAGGCTCTCACTGGAGGT
	GG*AAGCTT*CTGGGGGCACAGGAAGCATGGCTT
ChIP Primer 1	GAAATTTAGGCTGACGGGTA
	GCAGTGGTGGTAGGCAATG
ChIP Primer 2	GAGATCTTAACAGACCCTAG
	CAGCTTCCTGCTCCCTGCTC
ChIP Primer 3	AAAGGCCTTCTAGGGAAAGG
	CATACCGGTCCCTCCCCTGG
P mutant 1	CAGATTCTGGAGATC**CAGTTG**CCAGGACCCTTCCTGCCCT
P mutant 2	CTCCAGTCCCCTTCCCATAACC**CACCTG**GGCTGTGCCTGACCACAGAATC
Myc	CATCCACGAAACTTTGCCCAT
	CGTCCTTGCTCGGGTGTTGTA
GAPDH	GACCTGACCTGCCGTCTA
	AGGAGTGGGTGTCGCTGT
miR-451 forward primer	AAACCGTTACCATTACTGAGTT
miR-144 forward primer	TACAGTATAGATGATGTACT
NC	UUCUCCGAACGUGUCACGUTT
	ACGUGACACGUUCGGAGAATT
miR-451 mimetic	AAACCGUUACCAUUACUGAGTT
	CUCAGUAAUGGUAACGGUUUTT
miR-144 mimetic	UACAGUAUAGAUGAUGUATT
	UACAUCAUCUAUACUGUATT
	CUCAGUAAUGGUAACGGUUUTT

The Restriction sites are indicated with italic letters and underlined, and the mutated sites are indicated with bold letter and underlined.

2. Materials and methods

2.1. Reagents

RPMI medium and fetal bovine serum were purchased from Gibco. Imatinib, MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide), and propidium iodide were from Sigma–Aldrich. FITC Annexin V was from BD Pharmingen™. Nitrocellulose membrane was from Millipore (Bangalore, India). Antibodies against BCR-ABL, pSTAT5, c-Myc and β-actin were from Santa Cruz. All the other chemicals and reagents were purchased from domestic companies and are of molecular biology grade.

2.2. Cell culture and establishment of imatinib resistant K562 cells

Cells were grown in RPMI-1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/ml penicillin, 100 mg/ml streptomycin and 2 mM L-glutamine. K562R cells

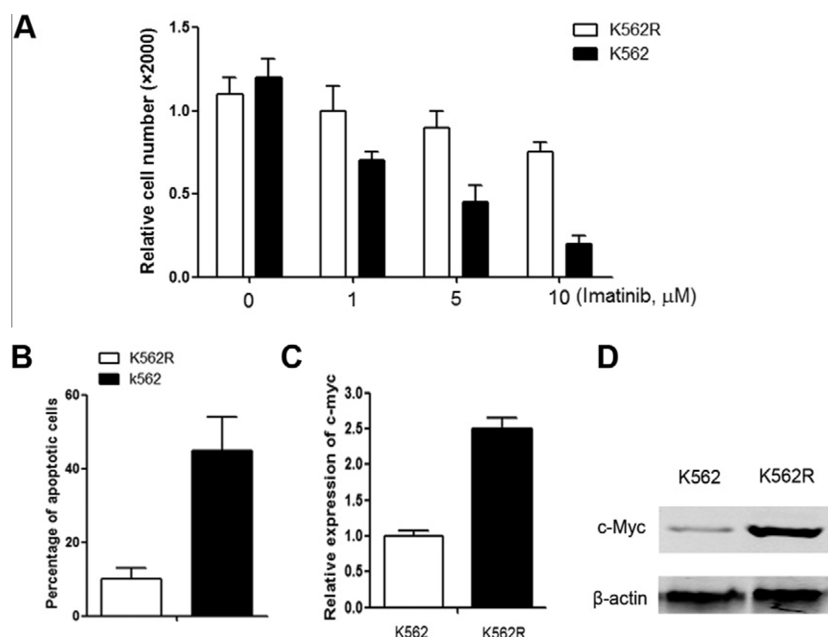

Fig. 1. Increased expression of Myc in imatinib resistant CML cells (A) MTT results of the K562R cells and the parent K562 cells to different dose of imatinib. (B) Apoptotic index of K562R and K562 parent cells to 10 μM imatinib for 24 h. Apoptotic cells were detected by the FITC-AnnexinVFACS. (C) c-myc expression in K562R and parent K562 cells at mRNA by qRT-PCR. GAPDH served as an internal control and relative expression of c-myc was expressed as the $2^{-\Delta\Delta Ct}$ relative to the K562 cells (D) c-myc expression in K562R and parent K562 cells was analyzed by Western blot.

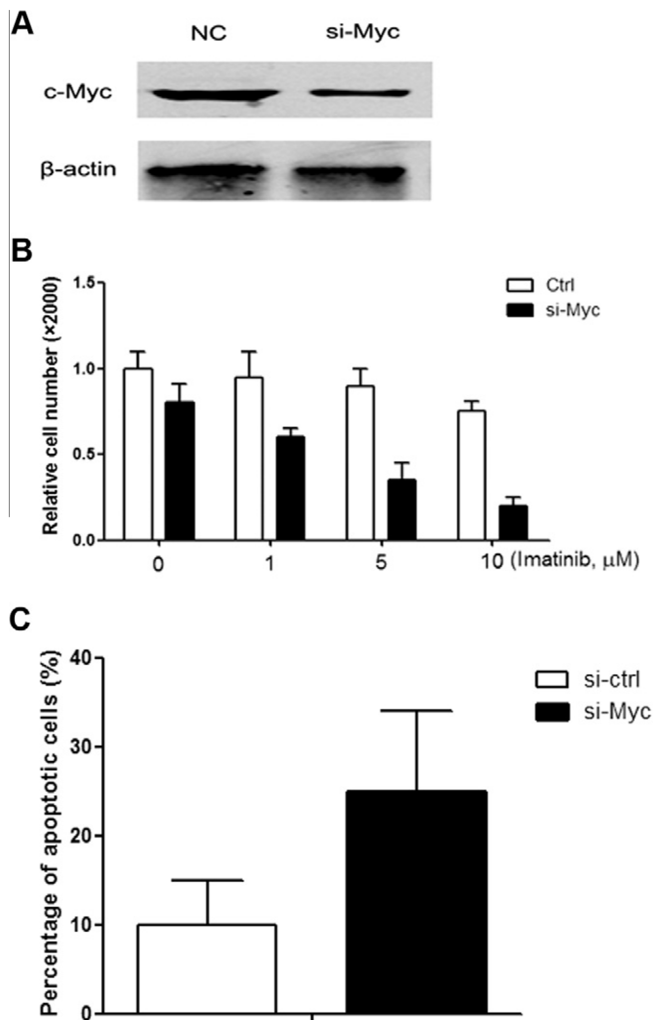

Fig. 2. Increased Myc contributes to imatinib resistance (A)Knockdown efficiency of c-myc in K562R cells examined by Western Blot. K562R cells were transfected with NC or c-myc specific RNAi duplexes and c-myc expression was then analyzed 48 h after transfection by Western Blot. β-actin served as an internal control to insure equal loading. (B) MTT results of the K562R cells with or without Myc knockdown under different doses of imatinib. (C) Apoptotic index of K562R with or without Myc knockdown upon 10 μM imatinib for 24 h. Apoptotic cells were detected by the FITC-AnnexinVFACS.

were established by serial prolonged exposures of K562 cells to increasing concentrations of imatinib starting from 1 nM to 10 nM, 100 nM, to 1 μM as described before [10]. Briefly, cells were cultured at each concentration of imatinib for 1 week. Resistant cells were maintained in RPMI-1640 medium supplemented with 10% FBS and 1 μM imatinib. HEK293 cells were cultured in RPMI-1640 medium supplemented with 10% FBS. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C.

2.3. Facs

Immunofluorescence analysis was performed on a FACSCalibur FACS using CELLQuest software (Becton Dickinson, Mountain View, CA). To determine whether cell death was attributable to apoptosis, whole cells were stained with FITC-conjugated Annexin V and PI (in PBS) according to the manufacturer's protocol. All analyses of whole cells were performed using appropriate scatter gates to exclude cellular debris and aggregated cells.

2.4. MTT assay

Cell viability was determined by MTT assay. 24 h before the assay, K562 and K562R cells (2×10^3 cells/well) were seeded to 96-well culture plate in a final volume of 200 μl and treated as indicated. After treatment, 20 μl of MTT (5 mg/ml of PBS) was added to the medium. After 4 h incubation at 37 °C, plates were centrifuged at 1000 g for 5 min before removing the medium and 100 μl of DMSO was added to each well and plates were agitated for 1 min. Absorbance was read at 570 nm on a multi-well plate reader. Percent inhibition of proliferation was calculated as a fraction of control.

2.5. Real-time quantitative PCR for miRNAs and mRNAs

Total RNA was isolated from cultured cells using the TRizol (Invitrogen), according to the manufacturer's protocol. RT reactions were done using the Qiagen Kit under the instruction of the protocol. Quantitative PCR for target miRNAs was performed using indicated primers (Table 1) and the Sybr green system (Takara). For analysis of the mRNA level of c-myc and GAPDH, mRNA was reverse transcribed by M-MLV and PCR was run using indicated primers (Table 1) and also the SYBR green system (Takara). All reactions were run in triplicate using the ABI7500 system. Mean cycle threshold (*Ct*) values for all miRNAs/mRNAs were quantified by the use of sequence detection system software (SDS, Applied Biosystems). The miRNA/mRNA expression was normalized to U6/GAPDH RNA expression respectively, yielding a ΔCt value. The $\Delta\Delta Ct$ value was then calculated by subtracting the Δ Ct value of the control group. Relative mRNA/miRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.6. Reporter assay

miR-144/451 is encoded by the miR-144/451 cluster. To explore the transcriptional regulation of miR-144/451, a fragment of the miR-144/451 gene promoter (−1000 to + 1 relative to the transcription start site of primiR-144/451) was amplified by PCR using the indicated primers. The amplified fragment was inserted into the pGL3-basic vector (Promega, Madison, WI). This pGL3-1000 vector was used as a template for the cloning of c-myc binding sites mutant reporters using the Multipoint mutagenesis Kit (Takara), with the mutated primer sequence listed in Table 1. These plasmids were then sequenced for confirmation.

Two hundred nanogram of reporter vector and 50 ng internal control pRL-TK vectors were cotransfected with indicated expression vectors in HEK293 cells using Lipofectamine 2000 (Invitrogen). Forty-eight hours after transfection, the luciferase activity was measured by using the dual luciferase reporter assay system (Promega). Fold change of the relative luciferase activity was calculated and expressed as mean ± SE in each group and differences were analyzed.

2.7. Quantitative chromatin immunoprecipitation

K562R cells were used for the chromatin immunoprecipitation (ChIP) assays. Briefly, 5×10^7 cells were cross-linked by 1% formaldehyde, lysed in 400 μl lysis buffer and sonicated to 100–1000 bp fragments. ChIP was conducted with antibodies against c-myc and IgG. Input control DNA or immunoprecipitated DNA was amplified in a 20 μl reaction volume containing 2 μl of the eluted DNA template. We designed three sets of primers targeting different regions in the miR-144/451 promoter (Table 1). Immunoprecipitated fragments and the inputs were amplified by real-time PCR. The results for the immunoprecipitated fragments were calculated and compared with the Ct values obtained for the input sam-

ples in each case. The results are expressed as a percentage of the input.

2.8. Transfection of siMyc, miR-144/451 mimics and antagomir-144/451

siRNA duplexes against control (negative control), c-myc and the miR-144/451 mimics and antagomiR-144/451 were synthesized as described before, with the sequences indicated in Table 1. Transfection of these RNAi duplexes was done as described before [22]. Briefly, cells (1×10^6) were electroporated (330 V, 10 ms) in 100 μl RPMI 1640/10% fetal calf serum (FCS) containing 0.5 μg siRNA duplex in a 4 mm electroporation cuvette using an EPI 2500 gene pulser (Fischer, Heidelberg, Germany). After recovery of 12 h, cells were undergoing further treatment, such as imatinib therapy.

2.9. Statistical analysis

Data were reported as the mean ± S.E. of at least three independent experiments. Statistical analysis of differences was carried out by Student *t* analysis or one-way analysis of variance (ANOVA). A *p*-value of less than 0.05 was considered as significant.

3. Results

3.1. Increased c-Myc expression in imatinib resistant CML cells

To define the essential molecular events involved in the imatinib resistance, we first constructed an imatinib resistant pool K562R from K562 cells. Compared with the parent K562 cells, K562R cells were resistant to imatinib induced cell growth inhibition and apoptosis (Fig 1A and B). Compared with the parental K562 cells, K562R cells displayed an increased c-myc expression both at mRNA and protein level (Fig 1C and D), suggesting that increased c-Myc might contribute to the imatinib resistance. As expected, knockdown of c-Myc (Fig 2A) in K562R cells significantly reduced both the cell proliferation (Fig 2B) and resistance to imatinib (Fig 2C).

3.2. c-Myc reduces the expression of miR-144/451 in K562R cells

To define how the increased c-Myc contributes to the proliferation and imatinib resistance, we focused on the miRNAs. Previous studies suggest that there are many myc repressed miRNAs are potential tumor suppressors [23], among which miR-144 and miR-451 are encoded by the same cluster, suggesting a potent role of

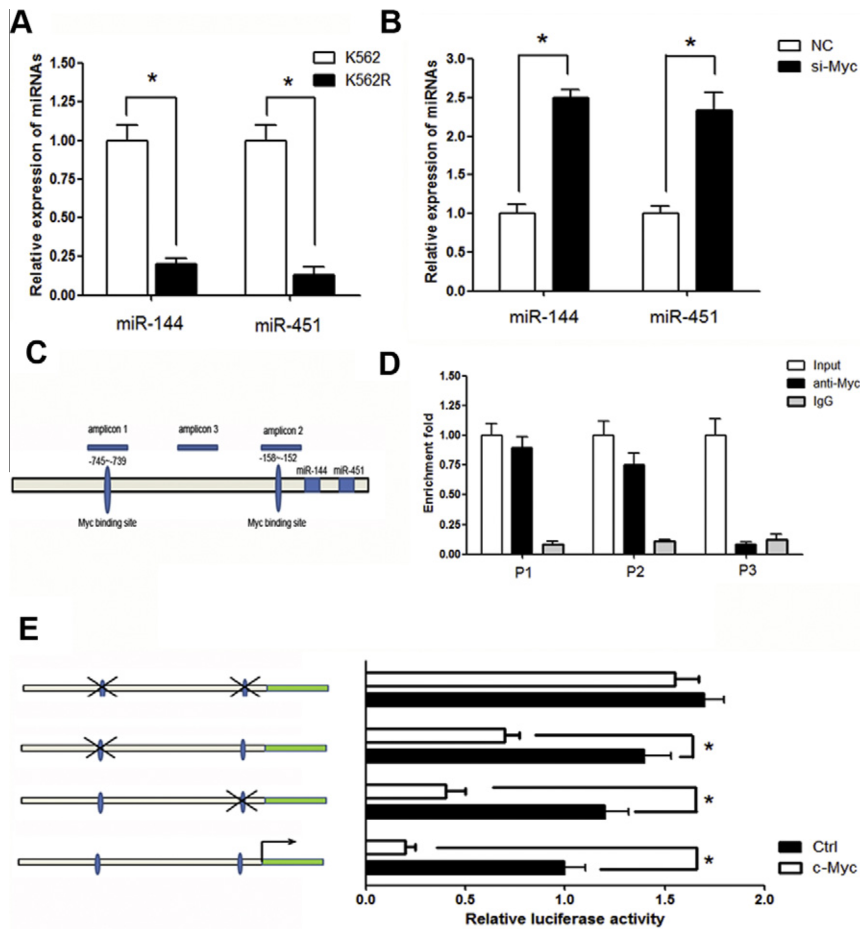

Fig. 3. Increased c-Myc decreases the miR-144/451 expression in K562R cells (A) miRNA specific qRT-PCR analysis of miR-144/451 in K562 and K562R cells. U6 served as an internal control and relative expression of miR-144/451 was expressed as the $2^{-\Delta\Delta Ct}$ relative to the K562 cells. (B) Expression of miR-144/451in K562R cells transfected with NC or c-myc RNAi duplexes. Cells were treated same as above and harvested for RNA analysis 48 h after transfection. (C) Schematic representation of the miR-144/451 gene locus in the chromosome. About 1,500 bp DNA sequence encoding the miRNAs and 5' flanking region was annonated and the consensus sequence for Myc were also indicated above the line, with the amplicons for ChIP assay also noted. (D) Quantitative ChIP analysis of the three loci (amplicon1, 2 and 3) in K562R cells. The relative occupancies of c-myc on the three loci are indicated as vertical bars. As a negative control, ChIP experiments were performed with isotype-matched preimmune IgG. The bar graphs show averages of three independent ChIP experiments. Error bars represent standard deviation. (E) Both the proximal and distal c-myc sites were responsible for c-myc induced activation of the miR-144/451 promoter in HEK293 cells. Mutation of either of the binding sites reduced the responsiveness, while mutation of both the sites nearly blocked the responsiveness. $^*p < 0.05$ ($n = 5$).

this cluster in cancer development. Currently, there is rare reported study about miR-144/451 in CML study. As expected, miRNA specific qRT-PCR analysis revealed that both miR-144 and miR-451 expression in K562R cells were significantly lower than that in the parent K562 cells (Fig 3A). Knockdown of Myc rescued the expression of miR-144/451 in K562R cells (Fig 3B). In silico study further reveals that there are two c-myc binding sites located in the −1000 bp promoter region (relative to the start site of the pre-miR-144) (Fig 3C). ChIP analysis found that there were significant enrichment of the fragment covering the either the distal (−745 ~ −739) or proximal (−158 ~ −152) c-myc responsive elements (amplicon 1 and amplicon 2, Fig 3D). In contrast, there was no significant enrichment of the fragment covering no c-myc responsive element (amplicon 3, Fig 3D). Next, we cloned the −1000 bp promoter region of miR-144/451 and mutated either or both the c-myc binding sites (Fig 3E). Mutation of the c-myc binding sites increased the basal promoter activity mildly in HEK293 cells. Overexpression of c-myc significantly repressed the promoter activity of wildtype reporter and reporters with only one myc binding site mutated (Fig 3E), while the reporter with both the myc binding sites mutated respond weakly to c-myc expression (Fig 3E). All of these suggested that c-myc could interact with both of the putative c-myc responsive elements.

3.3. Restoration of miR-144/451 reverses the resistance of K562R cells to imatinib

From the above data, we tested whether restoration of miR-144/451 might reverse the resistance of K562R cells to imatinib therapy. As expected, transfection of miR-144 and miR-451 either or both could significantly induce the cell growth inhibition, while apoptosis was only moderately induced (Fig 4A and B). Imatinib treatment significantly decreased the cell survival number (Fig 4A) and increased the apoptosis (Fig 4B), which is similar to the parent cell at the concentration of 5 μM imatinib (Fig 1B).

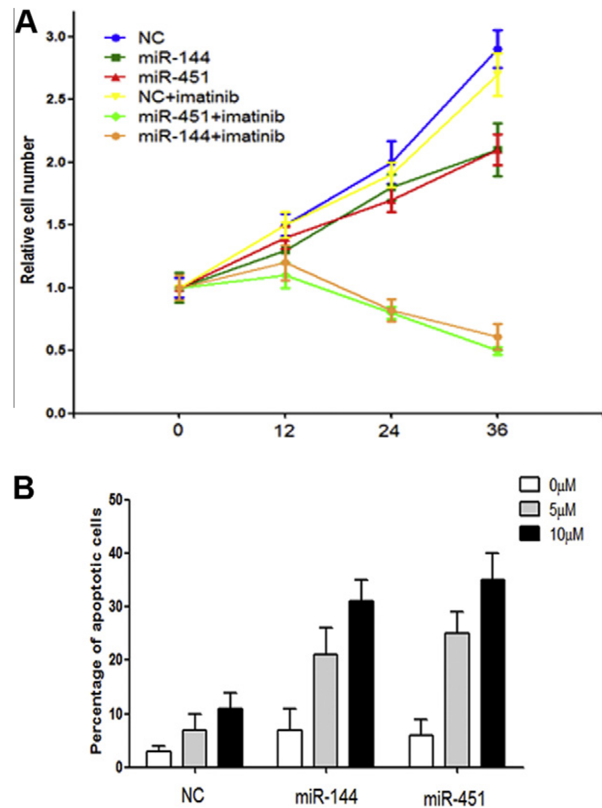

Fig. 4. Restoration of miR-144/451 reverses the resistance of K562R cells to imatinib (A) Cell proliferation rate detected by MTT assay. K562R cells with NC, miR-144, miR-451 transfection were cultured either with or without 5 μM imatinib for additional 12, 24, 36 h before harvest for MTT assay. Relative cell number was compared with the first time point. (B) Percentage of apoptotic cell. K562R cells with NC, miR-144, miR-451 transfection were cultured with or without 5 and 10 μM imatinib for 24 h and apoptotic cells were detected by FACS.

4. Discussion

In this study, by using the imatinib resistant K562 cell K562R, we for the first time identified that miR-144/451 cluster expression was significantly repressed in imatinib resistant cells, which is due to the increased c-myc. Restoration of miR-144/ 451 could sensitize the imatinib resistant cells to apoptosis. Our findings should be valuable in the development of novel therapies that target the imatinib resistance in CML.

Previous studies mainly focus on the role of c-myc on cell proliferation and differentiation, while its role in drug resistance is being realized recently. It has been found that c-myc could suppress BIN1 and thus confer the cancer cells resistance to cisplatin induced cell death [24]. More recently, Porro A et al. demonstrated that c-myc could transcriptionally upregulate the ATP-binding cassette transporter genes in CML CD34+ progenitors cells [25],which is consistent with the idea that leukemia stem cell is resistant to the therapy [26]. Here we show the experimental evidence that c-myc was upregulated in the imatinib resistant CML cells, with the underlying mechanism largely unknown. Wnt signal activation is essential for the survival of CML stem cells [27,28], while c-myc is one of the key targets of Wnt. It is thus highly possible that Wnt could increase the c-myc expression in the imatinib resistant cells.

It is well established that when Myc forming a heterodimer with Max, another basic helix-loop-helix protein [29], this complex can recognize the E-box sequence on the target gene promoter and function as a transcriptional activator. However, accumulating evidence showed that c-myc could interact with Myc-interacting zinc finger protein 1 (Miz-1) and repress the expression of $p15^{Ink4b}$ and $p21^{Cip1}$ [30–32]. Recently, Myc was found to repress wide range of miRNAs by directly binding the promoter of these miRNAs [23]. Here we found that c-myc could directly bind the promoter of miR-144/451. Further study of clarifying whether Miz-1 is included in the complex would shed light on how c-myc represses the expression of miR-144/451.

As to how miR-144/451confer the imatinib resistance, Nerea Bitarte et al. have also demonstrated that miR-451 could negatively regulate the self-renewal, tumorigenicity and chemoresistance of colorectal cancer stem cells, through regulating the ABCB1, COX2 and Wnt pathway [21]. It is thus interesting to test whether restoration of miR-144/451 could also target these molecules in the imatinib resistant cells.

It is important to note that this study was conducted in the K562R cell line, a large scale of clinical study collecting the bone marrow samples from both imatinib responsive and resistant CML patients is needed to further confirm the implication of Myc-miR144/451 pathway in the imatinib resistance at clinical settings.

Taken together, we here define that Myc-miR144/451 pathway might confer the imatinib resistance, and therapeutic targeting this pathway is of great value in combating the CML drug resistance.

Acknowledgements

This study was founded by 863 project of China (2006AA02A111) and the national nature science foundation of China (NSFC C03030202).

References

[1] J.V. Melo, The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype, Blood 88 (1996) 2375–2384.

[2] R. Ren, Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia, Nat. Rev. Cancer 5 (2005) 172–183.

[3] M.H. Cohen, J.R. Johnson, R. Pazdur, U.S. Food and Drug Administration Drug Approval Summary: conversion of imatinib mesylate (STI571; Gleevec) tablets from accelerated approval to full approval, Clin. Cancer Res. 11 (2005) 12–19.

[4] P. le Coutre, E. Tassi, M. Varella-Garcia, R. Barni, L. Mologni, G. Cabrita, E. Marchesi, R. Supino, C. Gambacorti-Passerini, Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification, Blood 95 (2000) 1758–1766.

[5] M.E. Gorre, M. Mohammed, K. Ellwood, N. Hsu, R. Paquette, P.N. Rao, C.L. Sawyers, Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification, Science 293 (2001) 876–880.

[6] Y. Dai, M. Rahmani, S.J. Corey, P. Dent, S. Grant, A Bcr/Abl-independent, Lyn-dependent form of imatinib mesylate (STI-571) resistance is associated with altered expression of Bcl-2, J. Biol. Chem. 279 (2004) 34227–34239.

[7] T. Nambu, N. Araki, A. Nakagawa, A. Kuniyasu, T. Kawaguchi, A. Hamada, H. Saito, Contribution of BCR-ABL-independent activation of ERK1/2 to acquired imatinib resistance in K562 chronic myeloid leukemia cells, Cancer Sci. 101 (2010) 137–142.

[8] H. Quentmeier, S. Eberth, J. Romani, M. Zaborski, H.G. Drexler, BCR-ABL1-independent PI3Kinase activation causing imatinib-resistance, J. Hematol. Oncol. 4 (2011) 6.

[9] A.M. Kalle, S. Sachchidanand, R. Pallu, Bcr-Abl-independent mechanism of resistance to imatinib in K562 cells: Induction of cyclooxygenase-2 (COX-2) by histone deacetylases (HDACs), Leuk. Res. 34 (2010) 1132–1138.

[10] K.M. Arunasree, K.R. Roy, K. Anilkumar, A. Aparna, G.V. Reddy, P. Reddanna, Imatinib-resistant K562 cells are more sensitive to celecoxib, a selective COX-2 inhibitor: role of COX-2 and MDR-1, Leuk. Res. 32 (2008) 855–864.

[11] S. Xie, H. Lin, T. Sun, R.B. Arlinghaus, Jak2 is involved in c-Myc induction by Bcr-Abl, Oncogene 21 (2002) 7137–7146.

[12] V. Ambros, The functions of animal microRNAs, Nature 431 (2004) 350–355.

[13] G.A. Calin, C.M. Croce, MicroRNA signatures in human cancers, Nat. Rev. Cancer 6 (2006) 857–866.

[14] H. Osada, T. Takahashi, MicroRNAs in biological processes and carcinogenesis, Carcinogenesis 28 (2007) 2–12.

[15] B. Zhang, X. Pan, G.P. Cobb, T.A. Anderson, MicroRNAs as oncogenes and tumor suppressors, Dev. Biol. 302 (2007) 1–12.

[16] J. Lu, G. Getz, E.A. Miska, E. Alvarez-Saavedra, J. Lamb, D. Peck, A. Sweet-Cordero, B.L. Ebert, R.H. Mak, A.A. Ferrando, J.R. Downing, T. Jacks, H.R. Horvitz, T.R. Golub, MicroRNA expression profiles classify human cancers, Nature 435 (2005) 834–838.

[17] X.S. Wang, J.W. Zhang, The microRNAs involved in human myeloid differentiation and myelogenous/myeloblastic leukemia, J. Cell. Mol. Med. 12 (2008) 1445–1455.

[18] K. Machova Polakova, T. Lopotova, H. Klamova, P. Burda, M. Trneny, T. Stopka, J. Moravcova, Expression patterns of microRNAs associated with CML phases and their disease related targets, Mol. Cancer 10 (2011) 41.

[19] T. Lopotova, M. Zackova, H. Klamova, J. Moravcova, MicroRNA-451 in chronic myeloid leukemia: miR-451-BCR-ABL regulatory loop?, Leuk Res. 35 (2011) 974–977.

[20] E. Bandres, N. Bitarte, F. Arias, J. Agorreta, P. Fortes, X. Agirre, R. Zarate, J.A. Diaz-Gonzalez, N. Ramirez, J.J. Sola, P. Jimenez, J. Rodriguez, J. Garcia-Foncillas, MicroRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells, Clin. Cancer Res. 15 (2009) 2281–2290.

[21] N. Bitarte, E. Bandres, V. Boni, R. Zarate, J. Rodriguez, M. Gonzalez-Huarriz, I. Lopez, J.J. Sola, M.M. Alonso, P. Fortes, J. Garcia-Foncillas, Microrna-451 is Involved in the Self-Renewal, Tumorigenicity and Chemoresistance of Colorectal Cancer Stem Cells, Stem Cells (2011).

[22] M. Scherr, K. Battmer, T. Winkler, O. Heidenreich, A. Ganser, M. Eder, Specific inhibition of bcr-abl gene expression by small interfering RNA, Blood 101 (2003) 1566–1569.

[23] T.C. Chang, D. Yu, Y.S. Lee, E.A. Wentzel, D.E. Arking, K.M. West, C.V. Dang, A. Thomas-Tikhonenko, J.T. Mendell, Widespread microRNA repression by Myc contributes to tumorigenesis, Nat. Genet. 40 (2008) 43–50.

[24] S. Pyndiah, S. Tanida, K.M. Ahmed, E.K. Cassimere, C. Choe, D. Sakamuro, c-MYC suppresses BIN1 to release poly(ADP-ribose) polymerase 1: a mechanism by which cancer cells acquire cisplatin resistance, Sci. Signal. 4 (2011) ra19.

[25] A. Porro, N. Iraci, S. Soverini, D. Diolaiti, S. Gherardi, C. Terragna, S. Durante, E. Valli, T. Kalebic, R. Bernardoni, C. Perrod, M. Haber, M.D. Norris, M. Baccarani, G. Martinelli, G. Perini, C-MYC oncoprotein dictates transcriptional profiles of ATP-binding cassette transporter genes in chronic myelogenous leukemia CD34+ hematopoietic progenitor cells, Mol. Cancer Res. 9 (2011) 1054–1066.

[26] L. Liu, R. Chen, S. Huang, Y. Wu, G. Li, Q. Liu, D. Yin, Y. Liang, Knockdown of SOD1 sensitizes the CD34+ CML cells to imatinib therapy, Med. Oncol. 28 (2011) 835–839.

[27] M. Pehlivan, Z. Sercan, H.O. Sercan, SFRP1 promoter methylation is associated with persistent Philadelphia chromosome in chronic myeloid leukemia, Leuk. Res. 33 (2009) 1062–1067.

[28] Y. Hu, Y. Chen, L. Douglas, S. Li, Beta-Catenin is essential for survival of leukemic stem cells insensitive to kinase inhibition in mice with BCR-ABL-induced chronic myeloid leukemia, Leukemia 23 (2009) 109–116.

[29] R. Alex, O. Sozeri, S. Meyer, R. Dildrop, Determination of the DNA sequence recognized by the bHLH-zip domain of the N-Myc protein, Nucleic Acids Res. 20 (1992) 2257–2263.

[30] S. Herold, M. Wanzel, V. Beuger, C. Frohme, D. Beul, T. Hillukkala, J. Syvaoja, H.P. Saluz, F. Haenel, M. Eilers, Negative regulation of the mammalian UV response by Myc through association with Miz-1, Mol. Cell 10 (2002) 509–521.

[31] J. Seoane, H.V. Le, J. Massague, Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage, Nature 419 (2002) 729–734.

[32] P. Staller, K. Peukert, A. Kiermaier, J. Seoane, J. Lukas, H. Karsunky, T. Moroy, J. Bartek, J. Massague, F. Hanel, M. Eilers, Repression of p15INK4b expression by Myc through association with Miz-1, Nat. Cell Biol. 3 (2001) 392–399.