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Adiponectin signals through Adiponectin Receptor 1 to reverse imatinib resistance in K562 human chronic myeloid leukemia cells

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ABSTRACT

Adiponectin, a member of adipokines, is a functional ligand for Adiponectin Receptor-1 (AdipoR1) and Adiponectin Receptor-2 (AdipoR2), and has been found to be linked to the risk of CML. Imatinib has undoubtedly revolutionised the management and outcome of chronic myeloid leukemia (CML), however imatinib resistance has been recognized as a major problem in CML therapy. In this study, we first established imatinib-resistant K562 CML cells, and then evaluated the effect of Adiponectin in reversing imatinib resistance. The data presented here demonstrated that Adiponectin was able to reverse K562 resistance to imatinib *in vitro* and *in vivo*. Additional data with molecular approaches suggested that the reversion of Adiponectin in imatinib resistance signals through AdipoR1 but not AdipoR2 to down-regulate Bcr-Abl expression and effect in imatinib-resistant K562 CML cells. Taken together, our data showed that Adiponectin can reverse imatinib resistance in CML, and to a certain extent elucidate the mechanism of Adiponectin reversing imatinib resistance that may provide a new and promising approach in imatinib resistance management in CML therapy.

1. Introduction

Adiponectin, also called GBP-28, apM1, AdipoQ, and Acrp30, has been discovered to be an adipocyte secreted hormone. It is a protein of 244 amino acids and the product of the apM1 gene, which is specifically expressed in human adipocytes [1]. Adiponectin, a member of adipokines, has been found to be an important negative regulator of hematopoiesis and linked mainly to the risk of Chronic myeloid leukemia (CML) [2]. It is well established that Adiponectin has modulated cell proliferation and apoptosis via two distinct receptors: Adiponectin Receptor-1 (AdipoR1) and Adiponectin Receptor-2 (AdipoR2) [3,4]. Moreover, a recent report has revealed that AdipoR1 expression was significantly increased in the mononuclear cells in CML patients, and this increase was similar in newly diagnosed and in imatinib treated CML patients [5], on the other hand, imatinib could increase Adiponectin secretion through the suppression of PI3 kinase signaling [6]. Furthermore, Adiponectin levels are elevated in CML patient plasma after imatinib therapy [7]. Taken together, it is tempting to hypothesize that Adiponectin and its functional receptor AdipoR1 might be involved in regulation of imatinib responding in CML treatment.

CML is a hematopoietic stem cell disorder with an elevated but immature white blood cell count [8]. CML is generally diagnosed by the presence of an abnormal Philadelphia (Ph) chromosome, which results from a translocation between the long arms of chromosomes 9 and 22. This exchange brings the Bcr gene and the proto-oncogene Abl together [9]. The hybrid gene, Bcr-Abl, encodes for a fusion protein with tyrosine kinase activity leading to uncontrolled growth. One of the major achievements in the treatment of CML has been the development of the first tyrosine-kinase inhibitor imatinib mesylate (STI571, Gleevec), a phenylaminopyrimidine derivative. Imatinib directly occupies the ATP-binding pocket of the Abl-kinase domain, and prevents conformation change of the protein into the active form [10], with the subsequent regulation on transcription of several genes involved in the control of cell cycle, cell adhesion, and cytoskeleton organization, leading to apoptosis of target cells [11]. Despite high rates of hematological and cytogenetic responses to therapy, the emergence of resistance to imatinib has been recognized as a major problem in CML treatment [12,13]. So far, mechanisms identified in development of imatinib-resistance include overexpression of Bcr-Abl associated with amplification or mutation of Bcr-Abl, and overexpression of

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the multidrug-resistant P-glycoprotein (MDR-1) [14–16]. Although considerable progress has been made in the elucidation of the mechanisms of resistance, we are still far from understanding the cause of this resistance and the proper solution to reverse imatinib resistance in human CML cells [13].

Involvement of Adiponectin in the regulation of imatinib resistance in human CML cells has not been hitherto examined. Therefore, in this study, we first established imatinib-resistant K562 CML cells, and then evaluated the effect of Adiponectin in reversing imatinib resistance. The data presented here showed that Adiponectin was able to reverse K562 resistance to imatinib *in vitro* and *in vivo*. Additional data with molecular approaches suggested that the reversion of Adiponectin in imatinib resistance signals through AdipoR1 but not AdipoR2 to downregulate Bcr-Abl expression and effect in imatinib-resistant K562 CML cells.

2. Materials and methods

2.1. Cell lines and culture conditions

Human chronic myelogenous leukemia K562 cells (ATCC) were maintained in RPMI 1640 (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone). Cells were cultured at 37 °C in 5% CO_2.

Development of imatinib-resistant K562 cells: cells were exposed to stepwise increasing concentrations of imatinib (Novartis Pharmaceuticals, Switzerland) starting with a concentration of 50 nM. Sub-populations of cells that were able to grow in the presence of 500 nM imatinib, were selected. Then, the inhibitory concentration 50 (IC_{50}) values of imatinib were determined and compared with control sensitive parental cells by MTT assay.

2.2. Cell proliferation assay

Cell proliferation was measured by MTT assay. In brief, cells (5×10^4 cells/well) were plated into 96-well plates containing 100 μL of the growth medium in indicated concentrations of imatinib at 37 °C in 5% CO_2 for 96 h. They were then treated with 5 μL of MTT (5 mg/mL) for 4 h. The reaction was stopped by using 100 μL of 0.1 N HCl in anhydrous isopropanol. Cell growth was evaluated by measuring the absorbance at 570 nm, using an automated plate reader.

2.3. Cell cycle analysis

Cells were fixed in 70% ethanol, for at least 2 h at 4 °C, and stained with 20 μg/mL propidium iodide (PI) containing 10 μg/mL RNase A for 30 min at room temperature. Fluorescence cell analysis was performed with a FACSCalibur (Becton–Dickinson, CA, USA) and sub-G_0/G_1 cell populations were considered apoptosis [17].

2.4. SiRNAs transfection

Human AdipoR1, AdipoR2, and non-targeting (scrambled) siRNAs were synthesized by Qiagen. The sequences of AdipoR1 and AdipoR2 siRNAs were used as previously described [18]. Transfection of K562 cells was performed using Transmessenger Transfection Reagent (Qiagen) as described by the manufacturer.

2.5. Real-time PCR

Total RNA was extracted using Trizol reagent according to the manufacturer's instructions, 1 μg of total RNA was converted to cDNA by SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen, Life Technologies). PCR was performed on ABI Prism 7000 using corresponding primers and SYBR gene PCR Master Mix (Invitrogen). The primer sequences were as follows: AdipoR1-forward (5′-CGGTGGAACTGGCTGAACTG-3′), AdipoR1-reverse (5′-CCGCACCTCCTCCTCTTCTT-3′); AdipoR2-forward (5′-ACGGAGTTGTCAGCACTCAC-3′) AdipoR2-reverse (5′-GCCATCGTCTTGTACCTCAC-3′); Bcr-Abl-forward (5′-GGGAGCAGCAGAAGAAGTGT-3′), Bcr-Abl-reverse (5′-AAAGGTTGGGGTCATTTTCAC-3′) and GAPDH-forward (5′-GAAGGTGAAGGTCGGAGTC-3′), GAPDH-reverse (5′-AGATGGTGATGGGATTTC-3′). Template cDNA was denatured at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The quantification data were analyzed with ABI Prism 7000 SDS software. The cycle time values were normalized to GAPDH of the same sample.

2.6. Western blot

Whole-cell lysates were prepared and subjected to western blot analysis. Equal amounts of the cell lysates were resuspended in 5× Tris–glycine SDS sample buffer, electrophoresed on 8–15% SDS–PAGE, and transferred to nitrocellulose membranes (Amersham Pharmacia). The detection of proteins was performed with anti-CrkL antibody (Cell signaling Technology), anti-Phospho-CrkL (Tyr207) antibody (Cell signaling Technology), anti-c-Abl antibody (Santa Cruz Biotechnology), and anti-β-actin antibody (Cell signaling Technology), followed by corresponding IDRy second antibody. The blots were scanned using an Odyssey Imaging System (LI-COR Bioscience, USA).

2.7. IMR-K562 xenograft model

BALB/c nu/nu female mice (4–6 weeks of age; vital river, China) were used in this study. All animal experiments were done in accordance with protocols approved by the Institutional Authority for Laboratory Animal Care of Wenzhou Medical University.

1.0×10^7 IMR-K562 cells in a total volume of 0.1 mL were injected subcutaneously into the single flanks of the mice. Mice bearing IMR-K562 cell xenografts were divided randomly into nine groups ($n = 8$ in each group) when the bearing tumor reached approximately 20 mm^3, including (a) an equal volume of saline (buffer control); (b) rhAdiponectin (10 mg/kg); (c) rhAdiponectin (50 mg/kg); (d) imatinib (100 mg/kg); (e) imatinib (100 mg/kg) plus rhAdiponectin (10 mg/kg) and (f) imatinib (100 mg/kg) plus rhAdiponectin (50 mg/kg). Recombinant human Adiponectin was from Peprotech.

Imatinib was given orally by gavage and rhAdiponectin were given by intraperitoneal (i.p.) injection.

Treatments began on day 14, when the average tumor volume was 200 mm^3, and were given every day, for a total of ten dosages. Tumor volumes were calculated using the following formula: $\pi ls^2/6$, in which l represents the long diameter of the tumor, and s represents the short diameter.

Tumor inhibition rate of tumor growth was calculated as (1 − average tumor weight of treated group/average tumor weight of saline control group) × 100%.

2.8. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 software. IC_{50} values were calculated by nonlinear regression using the sigmoidal dose–response equation. Statistical analysis of the differences among different treatment modalities was performed using the unpaired Student's t-test. $P < 0.05$ was considered statistically significant.

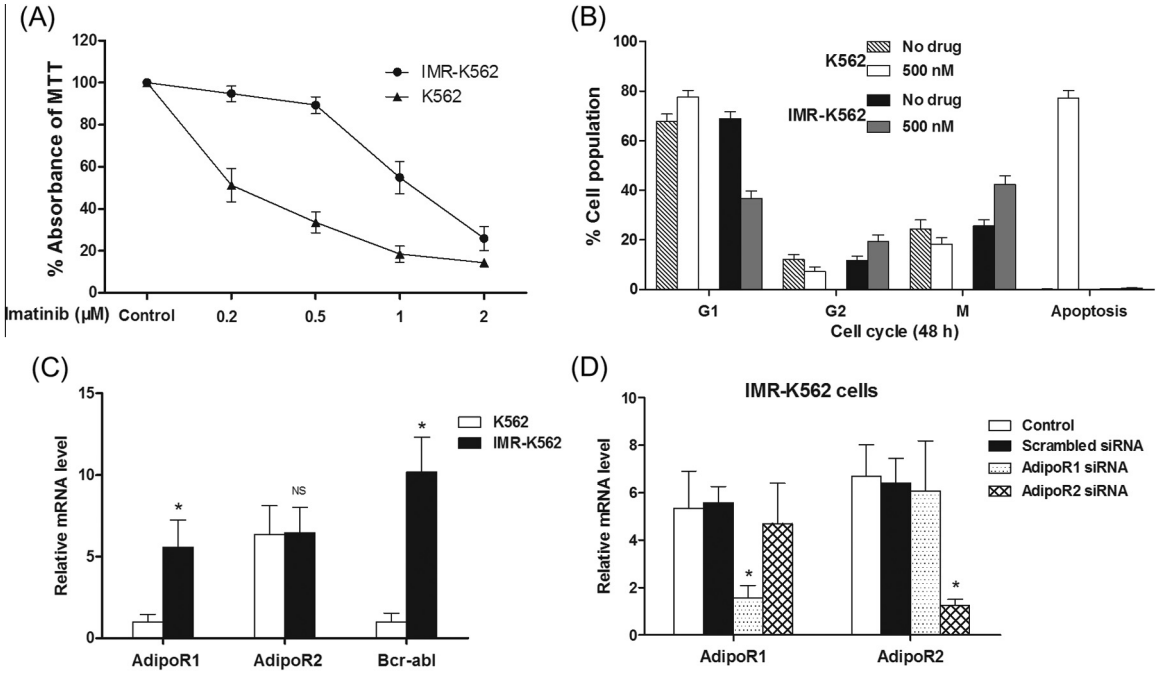

Fig. 1. Characterization of imatinib-resistant K562 human chronic myeloid leukemia cells. (A) IC_{50} of imatinib was determined by MTT assay for IMR-K562 and K562 cells. (B) Effects of imatinib (500 nM for 48 h) on cell cycle profiles of IMR-K562 and K562 cells. (C) Real-time PCR analysis of mRNA expression levels of AdipoR1, AdipoR2 and Bcr-Abl in IMR-K562 and K562 cells. (D) Real-time PCR analysis of specificity and effectiveness of siRNAs for the inhibition of mRNA expression of AdipoR1 and AdipoR2 in IMR-K562 cells. Columns, mean ($n = 3$); bars, SD. $^*P < 0.05$. NS, not significant. Data are representative of three individual experiments.

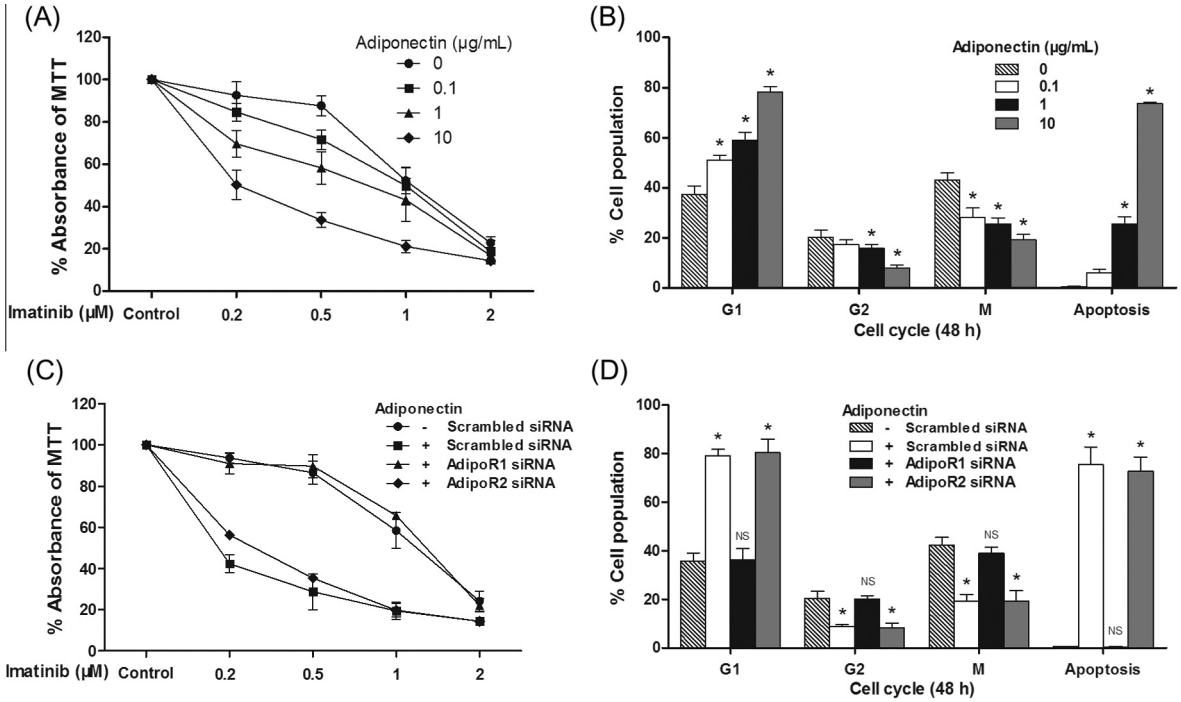

Fig. 2. Adiponectin reverses K562 resistance to imatinib *in vitro*. (A) IC_{50} of imatinib was determined by MTT assay for IMR-K562 cells treated with indicated concentrations of Adiponectin (0 μg/mL: 1100 nM; 0.1 μg/mL: 1000 nM; 1 μg/mL: 600 nM; 10 μg/mL: 210 nM). (B) Effects of imatinib (500 nM for 48 h) on cell cycle profiles of IMR-K562 cells treated with indicated concentrations of Adiponectin. Effects of siRNAs against AdipoR1 and AdipoR2 on the reversion of Adiponectin in imatinib resistance, (C) proliferation of IMR-K562 was assessed by MTT assay, (D) cell cycle profiles of IMR-K562 were examined using FACS. Columns, mean ($n = 3$); bars, SD. $^*P < 0.05$. Data are representative of three individual experiments.

3. Results

3.1. Establishment and characterization of imatinib-resistant K562 human chronic myeloid leukemia cells

It is well established that long term exposure to increasing concentrations of imatinib results in the development of resistance to imatinib in K562 human chronic myeloid leukemia cells [19]. First, the degree of resistance was determined by measuring the IC_{50} values of imatinib at 96 h using MTT assay. As shown in Fig. 1A, K562 cells that survived upon chronic exposure to 500 nM imatinib, which were referred to as IMR-K562, expressed ~6-fold resistance, as compared with their parental sensitive counterparts. The IC_{50} values of imatinib were 210 and 1200 nM for K562 and IMR-K562 cells, respectively. Furthermore, the cell cycle profiles of K562 and IMR-K562 cells revealed that, although exposure to 500 nM imatinib for 48 h caused apoptosis in ~76% of the population in K562 cells, there was no detectable apoptosis in resistant IMR-K562 cells (Fig. 1B). Notably, there were no significant changes in the cell cycle profiles of IMR-K562 cells as compared with K562 cells. In addition, expression levels of AdipoR1, AdipoR2 and Bcr-Abl were examined and the results showed that mRNA expression levels of AdipoR1 and Bcr-Abl were increased 5- and 10-fold in IMR-K562 cells compared with K562 cells respectively, whereas AdipoR2 mRNA level was found to be unchanged (Fig. 1C). To further investigate the functions of AdipoR1 and AdipoR2 in imatinib-resistance, the specificity and effectiveness of siRNAs for the inhibition of transcription were confirmed using real-time PCR (Fig. 1D).

3.2. Adiponectin reverses K562 resistance to imatinib in vitro

The upregulation of AdipoR1 suggested that its functional ligand Adiponectin might play a critical role in resistance to imatinib. To testify the hypothesis, indicated concentrations of Adiponectin were added. Surprisingly, IMR-K562 resistance to imatinib was abolished by Adiponectin in a dose-dependent manner, with the IC_{50} values of imatinib were decreased compared with the control (Fig. 2A). In addition, cell cycle profiles of IMR-K562 cells were corresponded to MTT assay. Detectable apoptosis rates in IMR-K562 cells were increased by Adiponectin, also dose dependently (Fig. 2B). However, these effects were attenuated by silencing AdipoR1 but not AdipoR2 (Fig. 2C and D), indicating that the resensitization effect of Adiponectin to the imatinib resistance in CML cells were via AdipoR1 but not AdipoR2.

3.3. Adiponectin downregulates Bcr-Abl expression and effect in imatinib-resistant K562 CML cells

As demonstrated in Fig. 1C, mRNA expression levels of Bcr-Abl were upregulated in the IMA-K562 compared with K562 cells, suggesting that the resistance mechanism to imatinib might related to the Bcr-Abl amplification. We next tested whether Adiponectin affects the Bcr-Abl expression in IMA-K562 cells. Intriguingly, after

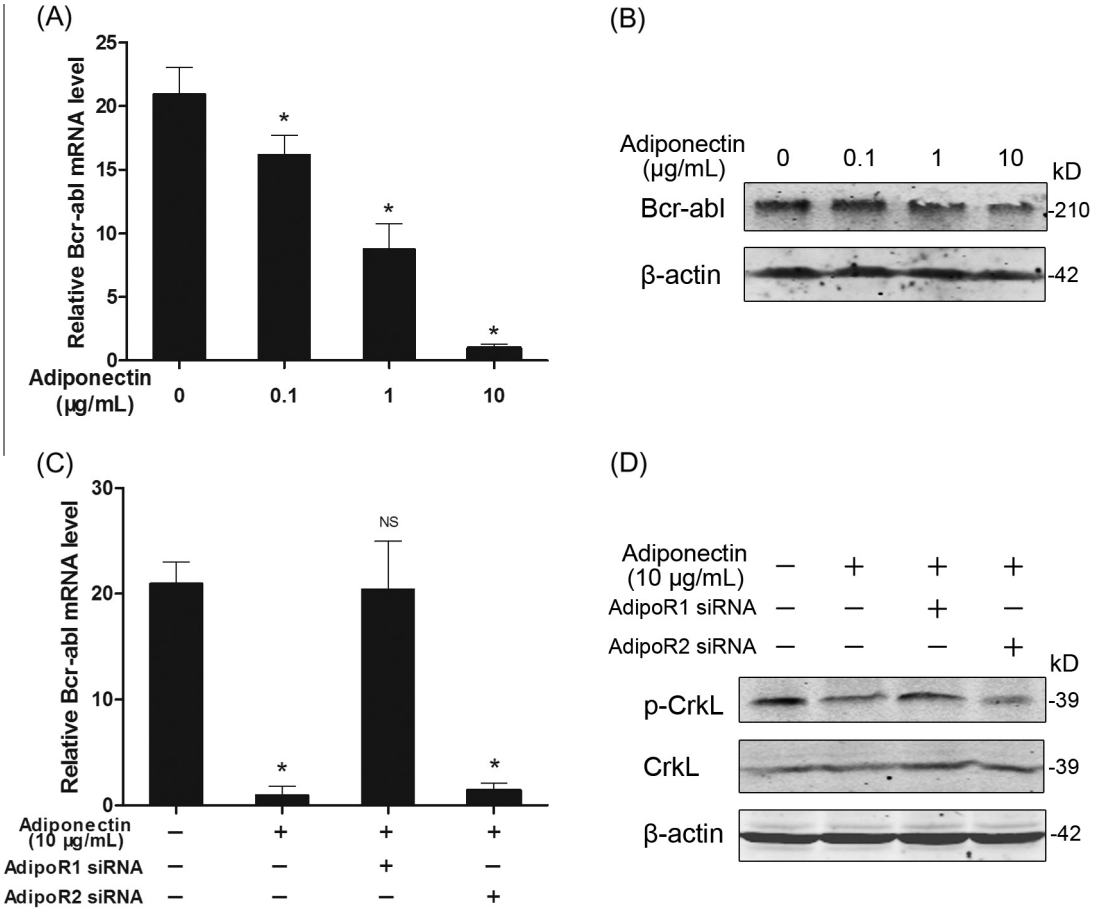

Fig. 3. Adiponectin downregulates Bcr-Abl expression and effect in imatinib-resistant K562 CML cells. Bcr-Abl (A) mRNA and (B) protein levels after treatment with indicated concentrations of Adiponectin for 12 h. (C) Effects of siRNAs against AdipoR1 and AdipoR2 on the Bcr-Abl mRNA expression levels after treatment with 10 μg/mL Adiponectin for 12 h. (D) Effects of siRNAs against AdipoR1 and AdipoR2 on CrkL phosphorylation after treatment with 10 μg/mL Adiponectin for 12 h. Columns, mean ($n = 3$); bars, SD. *$P < 0.05$. NS, not significant. Data are representative of three individual experiments.

12 h in culture with indicated concentrations of Adiponectin, there was no obvious cell death in IMA-K562 cells, however, both mRNA and protein levels of Bcr-Abl were decreased by Adiponectin in a dose-dependent manner (Fig. 3A and B). These data implicated that Adiponectin reversed resistance to imatinib might be linked to the overexpression of Bcr-Abl in IMA-K562 cells. In addition, downregulation effect of Bcr-Abl in mRNA levels by Adiponectin was abolished by silencing AdipoR1 but not AdipoR2 (Fig. 3C). Furthermore, Bcr-Abl tyrosine kinase activity was determined by assessment of tyrosine phosphorylation of the Crk-oncogene-like protein (CrkL), as shown in Fig. 3D, CrkL phosphorylation was inhibited by Adiponectin, which was reversed by silencing AdipoR1 but not AdipoR2 (Fig. 3D).

3.4. Adiponectin reversed IMR-K562 resistance to imatinib in vivo

To assess the potential reversible effect of Adiponectin in imatinib-resistance CML cells, we examined Adiponectin resensitizing the anti-cancer activity of imatinib *in vivo*.

The reversible effects of two doses of Adiponectin (10 and 50 mg/kg i.p. qd) on imatinib-resistance were determined in mice bearing IMR-K562 tumor xenografts. The results presented in Fig. 4A demonstrated that Adiponectin reversed imatinib-resistance in a dose related manner. Antitumor activity of Adiponectin plus imatinib was significantly better than that of the group with imatinib alone, however Adiponectin alone with the doses of 10 or 50 mg/kg showed no signs of antitumor activity. The tumor inhibitory rate of the combined Adiponectin (50 mg/kg) and imatinib (100 mg/kg) therapy was 78.3%, while the inhibitory rate of the single imatinib (100 mg/kg) therapy was 10.67%, and the inhibitory rate of Adiponectin (10 mg/kg) plus imatinib (100 mg/kg) therapy group was 40.8%. Notably, mRNA expression levels of Bcr-Abl in bearing K562 tumors were decreased by Adiponectin in a dose-dependent manner (Fig. 4B), confirming that the mechanism of Adiponectin reversed resistance to imatinib might be linked to the Bcr-Abl downregulation. Taken together, Adiponectin resensitizes imatinib treatment in IMR-K562 xenograft model and the reversible effect was due to modulation of Bcr-Abl expression.

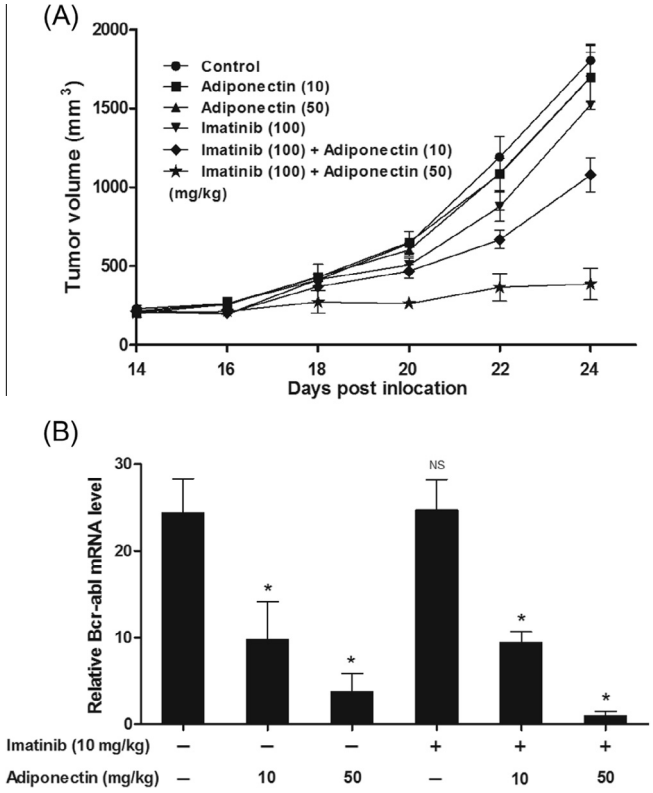

Fig. 4. Adiponectin reverses IMR-K562 resistance to imatinib *in vivo*. (A) Effect of indicated treatments on the volume of IMR-K562 xenograft tumors ($n = 8$ per group). (B) Real-time PCR analysis of Bcr-Abl mRNA expression levels in the IMR-K562 xenograft tumors after indicated treatments, columns, mean ($n = 8$); bars, SD. $^{*}P < 0.05$.

4. Discussion

In this study, we first established imatinib-resistant K562 CML cells which referred to IMR-K562. Continuous exposure to stepwise increasing concentrations of imatinib resulted in the selection of sub-clones resistance to imatinib, which has been used in several studies to derive imatinib-resistant cell lines [15]. Our results showed that imatinib is ineffective in proliferation suppressing and apoptosis induction in IMR-K562 even at the high dose. Furthermore, mRNA expression level of AdipoR1 was strikingly increased in IMR-K562 cells compared with K562 cells, whereas AdipoR2 mRNA level was found to be unchanged, indicating a possible novel mechanism information about imatinib-mediated resistance.

Adiponectin, a functional ligand of AdipoR1, suppressed cell proliferation and induced apoptosis [3,20,21]. Earlier studies reported that Adiponectin serum levels have been inversely associated with childhood acute myeloblastic leukemia. Moreover, a recent study revealed that AdipoR1 expression was significantly increased in imatinib treated CML patients [5]. Correspondently, our results also showed that AdipoR1 but not AdipoR2 was upregulated in imatinib-resistant K562 CML cells compared with parental-sensitive cells. Taken together, Adiponectin and its functional receptor AdipoR1 might play a critical role in imatinib resistance. Therefore, we next assessed whether Adiponectin was involved in imatinib resistance. Interestingly, MTT assay and cell cycle analysis depicted that IMR-K562 cells resensitized to imatinib treatment in the presence of Adiponectin in a dose-dependent manner, that is to say, Adiponectin was able to reverse K562 CML cells resistance to imatinib. What's more, the reversible effect of Adiponectin in imatinib resistance was abolished by silencing AdipoR1 but not AdipoR2, suggesting that AdipoR1 but not AdipoR2 exhibited a central role in Adiponectin signaling in reversing imatinib resistance.

Resistance development is a multifactorial phenomenon in cells exposed to the kinase inhibitor imatinib [22]. Increasing evidences have since described overexpression of Bcr-Abl was the most frequent cause of resistance identified in cell lines which were engineered to develop resistance [15]. Most importantly, amplification of Bcr-Abl was reported in patients with acquired resistance [23,24]. The amplification of the fusion gene can lead to increased Bcr-Abl activity thus overcoming the inhibiting function of imatinib. To investigate possible downstream mechanisms involved in imatinib resistance, the DNA sequence of the ATP-binding site of Bcr-Abl in IMR-K562 and K562 cells was examined by direct sequencing and MDR1 gene expression were analyzed using real-time PCR in these cells, however, neither mutations detected in Bcr-Abl in these cells nor overexpression of MDR1 in IMR-K562 was found (data not shown). The data presented here then showed that the mRNA expression level of Bcr-Abl was strikingly increased in IMR-K562 cells compared with K562 cells. Furthermore, Adiponectin dose dependently reduced Bcr-Abl expression in both mRNA and protein level. In the meanwhile, Adiponectin also attenuated phosphorylated CrkL which is Bcr-Abl substrate, indicating Adiponectin was able to downregulate Bcr-Abl expression but its tyrosine kinase activity. Additionally, these downregulation effect of Bcr-Abl and its tyrosine kinase activity in Adiponectin was abandoned by gene silence of AdipoR1 but not AdipoR2, which was consistent with data mentioned above,

the reversible effect of Adiponectin in imatinib resistance was due to AdipoR1, not AdipoR2. In other words, the reversion of Adiponectin in imatinib resistance signals through AdipoR1 to downregulate Bcr-Abl expression and effect in IMR-K562 cells.

It is reported that Adiponectin is able to suppress NF-κB pathway, and Bcr-Abl fusion oncoprotein in CML activates NF-κB by stimulating its nuclear translocation and also by enhancing its transactivation function [25,26]. Therefore, it is tempting to hypothesize that NF-κB pathway might be involved in Adiponectin downregulating Bcr-Abl expression and effect in IMR-K562 cells.

It should also be noted that our *in vivo* study demonstrated that Adiponectin resensitizes imatinib in IMR-K562 xenograft model in mice. We found that administration of Adiponectin by i.p. injection significantly sensitized IMR-K562 cells to imatinib treatment but yet shows no inhibition in both tumors volume and weight by itself only. Moreover, Real-time PCR demonstrated that Adiponectin combined with imatinib significantly inhibited Bcr-Abl mRNA expression level in the xenograft tumors as compared to imatinib alone. The inhibition rate of Bcr-Abl expression was correlated with the dose of Adiponectin. On the other hand, the tumor inhibition rate of Adiponectin plus imatinib treatment are comparable with the sensitive K562 cells xenograft model treated by single imatinib [27], indicating Adiponectin has the potential to completely reverse the imatinib resistance. Notably, all animals survived Adiponectin combined with imatinib treatment without appreciable adverse effects in terms of body weight loss (data not shown) or other signs of toxicity during the treatment in our *in vivo* study, suggesting that Adiponectin combined with imatinib were well tolerated.

In conclusion, we demonstrate that Adiponectin was able to reverse K562 resistance to imatinib *in vitro* and *in vivo*. This reversion effect of Adiponectin in imatinib resistance is due to the downregulation of Bcr-Abl expression and effect in AdipoR1 dependent way, whereas AdipoR2 was not involved. Our data to some extent elucidate the mechanism of Adiponectin reversing imatinib resistance that may provide a new and promising approach in imatinib resistance management in CML therapy.

Conflict of interest

The authors have no conflict of interest.

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References

[1] K. Maeda, K. Okubo, I. Shimomura, T. Funahashi, Y. Matsuzawa, K. Matsubara, CDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (adipose most abundant gene transcript 1), Biochem. Biophys. Res. Commun. 221 (1996) 286–289.
[2] F. Avcu, A.U. Ural, M.I. Yilmaz, N. Bingol, O. Nevruz, K. Caglar, Association of plasma adiponectin concentrations with chronic lymphocytic leukemia and myeloproliferative diseases, Int. J. Hematol. 83 (2006) 254–258.
[3] T. Yokota, K. Oritani, I. Takahashi, J. Ishikawa, A. Matsuyama, N. Ouchi, S. Kihara, T. Funahashi, A.J. Tenner, Y. Tomiyama, Y. Matsuzawa, Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages, Blood 96 (2000) 1723–1732.
[4] D. Barb, C.J. Williams, A.K. Neuwirth, C.S. Mantzoros, Adiponectin in relation to malignancies: a review of existing basic research and clinical evidence, Am. J. Clin. Nutr. 86 (2007) s858–s866.
[5] K. Ozturk, F. Avcu, A.U. Ural, Aberrant expressions of leptin and adiponectin receptor isoforms in chronic myeloid leukemia patients, Cytokine 57 (2012) 61–67.
[6] S. Fitter, K. Vandyke, S. Gronthos, A.C. Zannettino, Suppression of PDGF-induced PI3 kinase activity by imatinib promotes adipogenesis and adiponectin secretion, J. Mol. Endocrinol. 48 (2012) 229–240.
[7] S. Fitter, K. Vandyke, C.G. Schultz, D. White, T.P. Hughes, A.C. Zannettino, Plasma adiponectin levels are markedly elevated in imatinib-treated chronic myeloid leukemia (CML) patients: a mechanism for improved insulin sensitivity in type 2 diabetic CML patients?, J Clin. Endocrinol. Metab. 95 (2010) 3763–3767.
[8] B.J. Druker, ST1571 (Gleevec (TM)) as a paradigm for cancer therapy, Trends Mol. Med. 8 (2002) S14–S18.
[9] J.D. Rowley, Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining, Nature 243 (1973) 290–293.
[10] T. Schindler, W. Bornmann, P. Pellicena, W.T. Miller, B. Clarkson, J. Kuriyan, Structural mechanism for STI-571 inhibition of abelson tyrosine kinase, Science 289 (2000) 1938–1942.
[11] C.B. Gambacorti-Passerini, R.H. Gunby, R. Piazza, A. Galietta, R. Rostagno, L. Scapozza, Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias, Lancet Oncol. 4 (2003) 75–85.
[12] J.F. Apperley, Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia, Lancet Oncol. 8 (2007) 1018–1029.
[13] J.F. Apperley, Part II: management of resistance to imatinib in chronic myeloid leukaemia, Lancet Oncol. 8 (2007) 1116–1128.
[14] 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.
[15] F.X. Mahon, M.W.N. Deininger, B. Schultheis, J. Chabrol, J. Reiffers, J.M. Goldman, J.V. Melo, Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance, Blood 96 (2000) 1070–1079.
[16] M. Deininger, Resistance to imatinib: mechanisms and management, J. Natl. Compr. Cancer Netw. 3 (2005) 757–768.
[17] B. Ogretmen, D. Schady, J. Usta, R. Wood, J.M. Kraveka, C. Luberto, H. Birbes, Y.A. Hannun, L.M. Obeid, Role of ceramide in mediating the inhibition of telomerase activity in A549 human lung adenocarcinoma cells, J. Biol. Chem. 276 (2001) 24901–24910.
[18] T. Yamauchi, J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N.H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T. Shimizu, R. Nagai, T. Kadowaki, Cloning of adiponectin receptors that mediate antidiabetic metabolic effects, Nature 423 (2003) 762–769.
[19] Y. Baran, A. Salas, C.E. Senkal, U. Gunduz, J. Bielawski, L.M. Obeid, B. Ogretmen, Alterations of ceramide/sphingosine 1-phosphate rheostat involved in the regulation of resistance to imatinib-induced apoptosis in K562 human chronic myeloid leukemia cells, J. Biol. Chem. 282 (2007) 10922–10934.
[20] P.O. Iversen, H. Wiig, Tumor necrosis factor alpha and adiponectin in bone marrow interstitial fluid from patients with acute myeloid leukemia inhibit normal hematopoiesis, Clin. Cancer Res. 11 (2005) 6793–6799.
[21] L. Cong, J. Gasser, J. Zhao, B. Yang, F. Li, A.Z. Zhao, Human adiponectin inhibits cell growth and induces apoptosis in human endometrial carcinoma cells, HEC-1-A and RL95-2, Endocr. Relat. Cancer 14 (2007) 713–720.
[22] C.B. Gambacorti-Passerini, R.H. Gunby, R. Piazza, A. Galietta, R. Rostagno, L. Scapozza, Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias, Lancet Oncol. 4 (2003) 75–85.
[23] 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.
[24] L.J. Campbell, C. Patsouris, K.C. Rayeroux, K. Somana, E.H. Januszewicz, J. Szer, BCR/ABL amplification in chronic myelocytic leukemia blast crisis following imatinib mesylate administration, Cancer Genet. Cytogenet. 139 (2002) 30–33.
[25] N. Ouchi, S. Kihara, Y. Arita, Y. Okamoto, K. Maeda, H. Kuriyama, K. Hotta, M. Nishida, M. Takahashi, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Funahashi, Y. Matsuzawa, Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway, Circulation 102 (2000) 1296–1301.
[26] J.Y. Reuther, G.W. Reuther, D. Cortez, A.M. Pendergast, A.S. Baldwin Jr., A requirement for NF-kappaB activation in Bcr-Abl-mediated transformation, Genes Dev. 12 (1998) 968–981.
[27] L. Shi, Q. Song, Y. Zhang, Y. Lou, Y. Wang, L. Tian, Y. Zheng, D. Ma, X. Ke, Y. Wang, Potent antitumor activities of recombinant human PDCD5 protein in combination with chemotherapy drugs in K562 cells, Biochem. Biophys. Res. Commun. 396 (2010) 224–230.