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 (Boulwood 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
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 IncRNAs. Growing data have indicated that IncRNAs play important regulatory roles in the pathological process of tumorigenesis and the development of therapeutic resistance (Lalevee and Feil, 2015). One such IncRNA 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 ratio ≤1%); and imatinib-resistant (IM-R, n=34) with patients those did not receive CMR or MMR (BCR/ABL ratio ≥1%).

**Cell culture and cell transfection**

Human leukemic cell line K562 cells were purchased from ATCC (Manassas, VA, USA), and cultured in RPMI 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% CO2 incubator at 37°C. Imatinib was purchased from Sigma (St. Louis, MO, USA), and imatinib-resistant K562 cells were constructed following the method previously 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 (dT)18 RT primer was used for the reverse transcription of IncRNA. 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’-GCAGCTGCTGCACTAGAGG-3’ (forward); 5’-CGAGTCAAGGAACTGTTTG-3’ (reverse); GAPDH: 5’-GGACCGAGATCCCTCTCAAAAT-3’ (forward); 5’-GGCTGTGTCATACTTCTCATGG-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’-CTCAACTGTGCTGTGAGTCCGCAATTCACTTTGAGTCACAC-3’, (stem-loop-rt primer); 5’-ACACTCCAGCTGCTAGCTATCAGACGTCAGAGGAGTCAACATC-3’, (stem-loop-rt primer); 5’-CTCCTAGTTGGCTGTGGGA-3’, (forward); and 5’-AACGCTTCAGAGAATTTCGGT-3’, (stem-loop-rt primer); 5’-CTCCTAGTTGGCTGTGGGA-3’, (forward); and 5’-AACGCTTCAGAATTTCGGT-3’, (reverse). Human GAPDH and U6 snRNA were used for IncRNA 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 sulfate-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 apoptosis.
Expression of lncRNA MEG3 is greatly reduced in imatinib-resistant (IM-R) and imatinib-sensitive (IM-S) 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.

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

Overexpression of MEG3 in imatinib-resistant K562 cells reduced cell proliferation and induced 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 IC50 value of imatinib in imatinib-resistant K562 cells (p<0.05) (Fig. 3B). 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.

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 IC50 values were determined via CCK-8 assay after 48 hours of imatinib treatment. The IC50 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.
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 IncRNAs 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 IncRNAs 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 IncRNA. 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, IncRNAs and other noncoding RNAs could share common miRNA binding sites (also known as miRNA response elements, MREs) of miRNAs, 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.

REFERENCES

Arora, R., Sharma, M., Monif, T. and Iyer, S. (2016) A multi-centric bioequivalence trial in Ph+ chronic myeloid leukemia patients to assess bioequivalence and safety evaluation of generic imatinib mesylate 400mg tablets. Cancer Res. Treat. 48, 1120-1129.

Barthe, C., Cony-Makhoul, P., Melo, J. V. and Mahon, J. R. (2001) Roots of clinical resistance to STI-571 cancer therapy. Science 293, 2163.

Bellodi, C., Lidonnici, M. R., Hamilton, A., Helgason, G. V., Soliera, A. R., Ronchetti, M., Galavotti, S., Young, K. W., Selmi, T., Yacobbi, R., Van Etten, R. A., Donato, N., Hunter, A., Dinsdale, D., Tirro, E., Vigneri, P., Nicotera, P., Dyer, M. J., Holyoake, T., Salomoni, P. and Calabretta, B. (2009) Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. J. Clin. Invest. 119, 1109-1123.

Boultonwood, J. and Wainscoat, J. S. (2007) Gene silencing by DNA methylation in haematological malignancies. Br. J. Haematol. 138, 3-11.

Hughes, T. P., Saglio, G., Quintas-Cardama, A., Mauro, M. J., Kim, D.
W., Lipton, J. H., Bradley-Garelik, M. B., Utkropec, J. and Hochhaus, A. (2015) BCR-ABL1 mutation development during first-line treatment with dasatinib or imatinib for chronic myeloid leukemia in chronic phase. *Leukemia* **29**, 1832-1838.

Jabbour, E. and Kantarjian, H. (2014) Chronic myeloid leukemia: 2014 update on diagnosis, monitoring, and management. *Am. J. Hematol.* **89**, 547-556.

Pi, P., Diederichs, S., Wang, W., Boing, S., Metzger, R., Schneider, P. M., Tidow, N., Brandt, B., Buerger, H., Bulk, E., Thomas, M., Berdel, W. E., Serve, H. and Muller-Tidow, C. (2003) MALAT-1, a novel noncoding RNA, and thymosin-β4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene* **22**, 8031-8041.

Jurkovicova, D., Lukackova, R., Magyerkova, M., Kulcsar, L., Krivjanska, M., Krivjansky, V. and Chovanec, M. (2015) microRNA expression profiling as supportive diagnostic and therapy prediction tool in chronic myeloid leukemia. *Neoplasma* **62**, 949-958.

Kang, Y., Hodges, A., Ong, E., Roberts, W., Piemarocchi, C. and Pateronostrro, G. (2014) Identification of drug combinations containing imatinib for treatment of BCR-ABL+ leukemias. *PLoS ONE* **9**, e102221.

Lalevée, S. and Feil, R. (2015) Long noncoding RNAs in human disease: emerging mechanisms and therapeutic strategies. *Epigenomics* **7**, 877-879.

Liu, J., Wan, L., Lu, K., Sun, M., Pan, X., Zhang, P., Lu, B., Liu, G. and Wang, Z. (2015) The long noncoding RNA MEG3 contributes to cisplatin resistance of human lung adenocarcinoma. *PLoS ONE* **10**, e0114586.

Liu, Y., Li, Y., Li, N., Teng, W., Wang, M., Zhang, Y. and Xiao, Z. (2016) TGF-β1 promotes scar fibroblasts proliferation and transdifferentiation via up-regulating MicroRNA-21. *Sci. Rep.* **6**, 32231.

Mee, M., Ren, Y., Zhou, X., Yuan, X. B., Han, L., Wang, G. X., Jia, Z., Pu, P. Y., Kang, C. S. and Yao, Z. (2010) Downregulation of miR-21 enhances chemotherapeutic effect of taxol in breast carcinoma cells. *Technol. Cancer Res. Treat.* **9**, 77-86.

Miyoshi, N., Wagatsuma, H., Wakana, S., Shiroishi, T., Nomura, M., Asaka, K., Kohda, T., Surani, M. A., Kaneko-Ishino, T. and Ishino, F. (2000) Identification of an imprinted gene, MEG3/Gt2 and its human homologous MEG3, first mapped on mouse distal chromosome 12 and human chromosome 14. *Genes Cells* **5**, 211-220.

Salizzato, V., Borgo, C., Cesaro, L., Pinna, L. A. and Donella-Deana, A. (2016) Inhibition of protein kinase CK2 by CX-5011 counteracts imatinib-resistance preventing rpS6 phosphorylation in chronic myeloid leukaemia cells: new combined therapeutic strategies. *Onco-target* **7**, 18204-18218.

Salmena, L., Poliseno, L., Tay, Y., Kats, L. and Pandolfi, P. P. (2011) A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* **146**, 353-358.

Schindler, T., Bornmann, W., Pellicena, P., Miller, W. T., Clarkson, B. and Kuriyan, J. (2000) Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* **289**, 1938-1942.

Silveira, R. A., Fachel, A. A., Moreira, Y. B., De Souza, C. A., Costa, F. F., Verjovski-Almeida, S. and Pagnano, K. B. (2014) Protein-coding genes and long noncoding RNAs are differentially expressed in dasatinib-treated chronic myeloid leukemia patients with resistance to imatinib. *Hematology* **19**, 31-41.

Taft, R. J., Pang, K. C., Mercer, T. R., Dinger, M. and Mattick, J. S. (2010) Non-coding RNAs: regulators of disease. *J. Pathol.* **220**, 126-139.

Takahashi, N. and Miura, M. (2011) Therapeutic drug monitoring of imatinib for chronic myeloid leukemia patients in the chronic phase. *Pharmacology* **87**, 241-248.

Tano, K., Mizuno, R., Okada, T., Rakwal, R., Shibato, J., Masuo, Y., Iijiri, K. and Akimitsu, N. (2010) MALAT-1 enhances cell motility of lung adenocarcinoma cells by influencing the expression of motility-related genes. *FEBS Lett.* **584**, 4575-4580.

Taufchi, T. and Ohyashiki, K. (2004) Molecular mechanisms of resistance of leukemia to imatinib mesylate. *Leuk. Res.* **28 Suppl 1**, S39-S45.

Wei, G., Rafiyath, S. and Liu, D. (2010) First-line treatment for chronic myeloid leukemia: dasatinib, nilotinib, or imatinib. *J. Hematol. Oncol.* **3**, 47.

Xia, H. and Hui, K. M. (2014) Mechanism of cancer drug resistance and the involvement of noncoding RNAs. *Curr. Med. Chem.** 21**, 3029-3041.

Xu, G., Zhang, Y., Wei, J., Jia, W., Ge, Z., Zhang, Z. and Liu, X. (2013) MicroRNA-21 promotes hepatocellular carcinoma HepG2 cell proliferation through repression of mitogen-activated protein kinase 3. *BMC cancer* **13**, 469.

Yang, Y., Li, H., Hou, S., Hu, B., Liu, J. and Wang, J. (2013) The non-coding RNA expression profile and the effect of IncRNA AK126698 on cisplatin resistance in non-small-cell lung cancer cell. *PLoS ONE* **8**, e65309.

Zhang, X., Rice, K., Wang, Y., Chen, W., Zhong, Y., Nakayama, Y., Zhou, Y. and Klibanski, A. (2010) Maternally expressed gene 3 (MEG3) noncoding ribonucleic acid isoform structure, expression, and functions. *Endocrinology* **151**, 939-947.

Zhang, X., Zhou, Y., Mehta, K. R., Danila, D. C., Scolavino, S., Johnson, S. R. and Klibanski, A. (2003) A pituitary-derived MEG3 isoform functions as a growth suppressor in tumor cells. *J. Clin. Endocrinol. Metab.* **88**, 519-526.

Zhi, F., Dong, H., Jia, X., Guo, W., Lu, H., Yang, Y., Ju, H., Zhang, X. and Hu, Y. (2013) Functionalized graphene oxide mediated adriamycin delivery and miR-21 gene silencing to overcome tumor multidrug resistance in vitro. *PLoS ONE* **8**, e60034.