



LncRNA *CBR3-AS1* is associated with the BCR::ABL1 kinase independent mechanism of tyrosine kinase inhibitor (TKI) resistance in chronic myeloid leukemia patients

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Background: It is difficult for chronic myeloid leukemia (CML) patients with BCR::ABL1 independent drug resistance to achieve optimal efficacy. The aim of this study is to investigate the BCR::ABL1 kinase independent mechanism of tyrosine kinase inhibitor (TKI) resistance in CML patients to develop targeted therapeutic strategy.

Methods: Herein, we analyzed the long non-coding RNA (lncRNA) and messenger RNA (mRNA) expression profiles of patients who achieved sustained deep molecular response (DMR) after TKI treatment and patients with non-DMR using RNA-seq. Furthermore, the differentially expressed lncRNAs and mRNAs were identified. The expression of chosen lncRNA was validated in an expanded cohort, and bioinformatics analysis was performed to analyze the function of selected mRNA.

Results: LncRNA data analysis indicated the diversity lncRNA profiles among healthy individuals, CML patients with non-DMR, and CML patients with DMR. Differential expression analysis and Venn plot of up-regulated lncRNAs in patients with non-DMR (compared with healthy individuals) and down-regulated lncRNAs in patients with DMR (compared to patients with non-DMR) revealed that lncRNA *CBR3-AS1* overexpression might be related to BCR::ABL1 independent TKI resistance of CML patients. The expression of *CBR3-AS1* was then verified in an expanded cohort, suggesting that, compared with control group, there was no statistical difference of *CBR3-AS1* expression in DMR group, whereas, *CBR3-AS1* was up-regulated in non-DMR group. Moreover, the mRNA data analysis of RNA-seq was performed. We considered genes that up-regulated in non-DMR group (compared with control group), down-regulated in DMR group (compared with non-DMR group), showed no statistical difference between control and DMR group as the potential genes that associated with TKI resistance of CML patients. A total of 55 corresponding mRNAs were obtained including *KCNA6*, a target gene of *CBR3-AS1*. Further bioinformatics analysis showed that the major interacted genes of *KCNA6* were enriched in several resistance-associated pathways including interleukin -17 signaling pathway and cyclic adenosine monophosphate signaling pathway.

Conclusions: In conclusion, this work indicates that *CBR3-AS1* might be involved in BCR::ABL1 independent TKI resistance of CML patients through targeting *KCNA6*, providing a novel target for intervention treatment of CML patients with BCR::ABL1 independent TKI resistance.

Keywords: Chronic myeloid leukemia (CML); tyrosine kinase inhibitor resistance (TKI resistance); *CBR3-AS1*; *KCNA6*

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Introduction

Chronic myeloid leukemia (CML) is a group of malignant clonal blood system disease derived from hematopoietic stem cells and is the most prevalent type of chronic leukemia, accounting for approximately 15% of adult leukemia (1). CML occurs due to t(9; 22)(q34; q11) chromosome translocation producing the BCR::ABL1 fusion gene, detected in around 90% of CML patients (2). The fusion protein encoded by BCR::ABL1 fusion gene has robust tyrosine kinase activity, which can activate cell proliferation-related signaling pathways, thus interfering the procedures of the normal cell proliferation and apoptosis, ultimately contributing to the occurrence and progression of CML (2). Tyrosine kinase inhibitor (TKI), represented by imatinib, significantly improves the 5-year overall survival and progression-free survival rate of CML patients to over 90%. Patients who achieve sustained deep molecular response (DMR) after TKI treatment for more than 2 years can try to discontinue the drug, and some patients can achieve long-term treatment free remission, namely, functional cure (3,4). However, TKI resistance contributes to the inability of CML patients to obtain DMR, leading to treatment failure and relapse after remission. Overcoming TKI resistance is still the focus and difficulty of CML clinical research.

Currently, the main causes of TKI resistance are classified into BCR::ABL1 kinase dependent and independent (5). BCR::ABL1 kinase dependent resistance is usually caused by the mutation in the ABL1 gene that

prevents TKI from binding effectively to p210^{bc_r-abl}, and such resistance can be overcome by developing a new generation of TKI. Whereas, the main cause of BCR::ABL1 kinase independent resistance is that the activation of CML cell proliferation and/or self-renewal related signaling pathways is independent of p210^{bc_r-abl} tyrosine kinase activity (6), and the new generation of TKI fails to defeat such resistance. In clinical practice, it is difficult for CML patients with BCR::ABL1 independent drug resistance to achieve optimal efficacy. Thus, it is of great significance to investigate the BCR::ABL1 kinase independent mechanism of TKI resistance in CML patients to develop targeted therapeutic strategy.

Long non-coding RNA (lncRNA) is a class of non-protein-coding RNA sequences with a length of more than 200 nucleotides. Studies have shown that abnormally expressed lncRNAs are associated with pathogenesis and drug resistance of various human diseases, including CML (7,8). As reported by Guo and colleagues, served as a major regulatory molecule in BCR::ABL1 mediated CML, lncRNA *BGL3* promotes apoptosis of CML cells through positive regulation of *PTEN* expression, and ultimately contributes to the progression of CML through (9). In addition, previous studies have demonstrated that both lncRNA *OIP5-AS1* and lncRNA *MALAT1* are involved in the regulation of TKI resistance in CML patients through sponging microRNAs (10,11). lncRNA *MEG3* regulates TKI resistance in CML cells by inhibiting the expression of *miR-21/miR-184* (12). Collectively, these studies mainly focused on investigating the role of lncRNA in BCR::ABL1 dependent TKI resistance and pathogenesis of CML patients.

Herein, we aimed to explore the function of lncRNA in BCR::ABL1 independent TKI resistance of CML patients, demonstrating that *CBR3-AS1* might be involved in BCR::ABL1 independent TKI resistance of CML patients through targeting *KCNA6*. We present this article in accordance with the MDAR reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-24-281/rc>).

Methods

Patients

CML patients diagnosed according to the guidelines for diagnosis and treatment of chronic myelogenous leukemia in China (2020 edition) (4) were enrolled at Department of Hematology, the First Hospital of Putian City in accordance with the Declaration of Helsinki (as

Highlight box

Key findings

- Long non-coding *CBR3-AS1* might be involved in BCR::ABL1 independent tyrosine kinase inhibitor (TKI) resistance of chronic myeloid leukemia (CML) patients through targeting *KCNA6*, and *CBR3-AS1* may be a novel target for intervention treatment of CML patients with BCR::ABL1 independent TKI resistance.

What is known and what is new?

- Long non-coding RNA (lncRNA) showed function in BCR::ABL1 dependent TKI resistance and pathogenesis of CML patients.
- *CBR3-AS1* overexpression is associated with BCR::ABL1 independent TKI resistance of CML patients by targeting *KCNA6*.

What is the implication, and what should change now?

- Further *in vitro* and *in vivo* are needed to validate the function of *CBR3-AS1-KCNA6* in BCR::ABL1 independent TKI resistance of CML patients.

revised in 2013) and approved by the ethics committee of the First Hospital of Putian City (approval No. 2022-027). All eligible participants provided written informed consent. Patients receiving regular and continuous oral administration of TKI for more than 3 months and with $\text{BCR}::\text{ABL}^{\text{IS}} \leq 0.01\%$ (MR4.0) were included in the DMR group. Patients receiving regular and continuous oral administration of TKI for more than 2 years, previously received or being treated with at least one type of second-generation TKI, and with $0.01\% < \text{BCR}::\text{ABL}^{\text{IS}} \leq 1\%$ were included in the non-DMR group. All enrolled patients had no $\text{BCR}::\text{ABL}$ kinase mutations and had not received other drugs that affect the efficacy of TKI. Patients receiving drugs that affect the efficacy of TKI, in combination with other hematological disease, malignant tumor, and severe systemic disease, and with pregnant were excluded. Twelve health individuals were enrolled as control. Once the patients were determined for DMR or non-DMR, the peripheral blood mononuclear cell (PBMC) was collected for analysis.

RNA-sequencing and data analysis

Total RNA was extracted from PBMC using TRIzol (Invitrogen, CA, USA; 15596026). After ribosomal RNA removal, RNA was purified by RNA purification magnetic beads and interrupted using metal ion solution. The first strand of complementary DNA (cDNA) was synthesized in the reverse transcriptase system species by using the fragmented RNA as the template and the random oligonucleotides as the primers. Thereafter, the second stranded of cDNA was synthesized, repaired and A tail was added to the 3' end. After purification and fragment selection (300–500 bp), the products were amplified and enriched by polymerase chain reaction (PCR) to construct library. The obtained raw data in FASTQ format was filtered using fastp (v0.21.0) software (<https://github.com/OpenGene/fastp>) to collect clean data through removing reads containing adapter, containing ploy, and with low quality. Thereafter, the filtered reads were mapped to the reference genome using HISAT2 (v2.1.0) (13). FeatureCounts (v2.0.0) (14) statistics was utilized to calculate the expression of genes, and fragments per kilobase of exon model per million mapped fragments (FPKM) was then used to standardized the expression. Differentially expressed genes were identified using DESeq2 (v1.30.1) with screened conditions as follows: expression difference multiple $|\log_2\text{FoldChange}| > 1$, significant $\text{Padj} \leq 0.05$. Online database genemania was used to analyze the

major interacted proteins of *KCNA6* (<https://genemania.org/search/homo-sapiens/KCNA6>). Gene Ontology (GO) analysis within the molecular function (MF), cellular component (CC), and biological process (BP) categories, as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the major interacted genes of *KCNA6* were performed using R software (v 4.2.1). LncRNAs-messenger RNAs (mRNAs) co-expression pairs were obtained using the weighted gene co-expression network analysis.

Real-time quantitative PCR (RT-qPCR)

RT-qPCR was performed to validate the expression of lncRNA *CBR3-AS1* in PBMC of CML patients. Total RNA was extracted from peripheral blood using Trizol (Ambion, CA, USA; 15596-026) and reverse transcribed into cDNA using HiScript® II Q RT SuperMix for qPCR (+gDNA wiper) (VAZYME, Nanjing, China; R223-01), followed by qPCR amplification using SYBR Green Master Mix (VAZYME, Q111-02). The primer sequences were as follows: lncRNA *CBR3-AS1*, forward, 5'-TCAAGAAGGTGGTGAAGCAGG-3', reverse, 5'-TCAAAGGTGGAGGAGTGGGT-3'; *GAPDH*, forward, 5'-CCTCGATAAGACCGTGAC-3', reverse, 5'-TCTCCCTCTGCTTCCCTA-3'. The relative expression of lncRNA *CBR3-AS1* was calculated using the method of $2^{-\Delta\Delta C_t}$.

Statistical analysis

Enumeration data were described using the number of cases (percentage, %), and measurement data were described as the mean \pm standard deviation or mean (range). The chi-squared test was performed to comparison baseline data between groups. Comparisons of lncRNAs expression among DMR, non-DMR, and health control groups were analyzed using the Mann-Whitney *U* test for non-parametric variables. As for data analysis of RT-qPCR, comparisons between groups were performed using the one-way analysis of variance followed by Tukey's post-hoc test. Statistical significance was defined as P value < 0.05 .

Results

Baseline characteristics of enrolled CML patients

As shown in *Table 1*, a total of 27 CML patients were enrolled in this study, 15 received DMR after TKI

Table 1 Clinical baseline characteristics of CML patients

Characteristics	DMR (n=15)	Non-DMR (n=12)	χ^2	P value
Age, years	42 [31–67]	45 [24–56]	1.025	0.31
Gender			0.000	>0.99
Male	11 [73]	9 [75]		
Female	4 [27]	3 [25]		
First treatment drugs			0.077	0.78
Imatinib	13 [87]	9 [75]		
Replaced with a second generation TKI			1.081	0.58
Dasatinib	7 [47]	6 [50]		
Flumatinib	4 [27]	5 [42]		
Nilotinib	3 [20]	1 [8]		
BCR::ABL ^{IS}	93% [50–271%]	92% [53–191%]	0.439	0.66
Elevated white blood cell	15 [100]	12 [100]	0.000	>0.99
Hemoglobin			0.519	0.47
Normal	4 [27]	1 [8]		
Decreased	11 [73]	11 [92]		
Platelet			2.466	0.29
Elevated	10 [67]	6 [50]		
Normal	4 [27]	6 [50]		
Decreased	1 [7]	0		
ELTS			15.082	0.001
Low risk	10 [67]	1 [8]		
Intermediate risk	3 [20]	1 [8]		
High risk	2 [13]	10 [83]		
SOKAL score			4.182	0.12
Low risk	1 [7]	4 [33]		
Intermediate risk	13 [87]	8 [67]		
High risk	1 [7]	0		

Data are presented as mean [range] or n [%]. CML, chronic myeloid leukemia; DMR, deep molecular response; TKI, tyrosine kinase inhibitor; ELTS, EUTOS long-term survival; EUTOS, European Treatment and Outcome Study.

treatment, with mean age of 42 years (range, 31–67 years), and 12 divided in non-DMR group, with mean age of 45 (range, 24–56 years). Most patients received imatinib as first-line therapy and subsequently switched to second-generation TKI including dasatinib, flumatinib, and nilotinib. All the enrolled patients had elevated white blood cell, and most patients showed decreased hemoglobin. Ten (67%) patients in DMR group showed low-risk, whereas 10 (83%)

patients in non-DMR group showed high-risk according to European Treatment and Outcome Study (EUTOS) long-term survival (ELTS). Most patients in both DMR and non-DMR groups demonstrated intermediate risk according to SOKAL score. The results of statistical difference analysis suggested that there was no statistical difference of included baseline data between two groups (age, $P=0.31$; gender, $P>0.99$; first treatment drugs, $P=0.78$; BCR::ABL^{IS}, $P=0.66$;

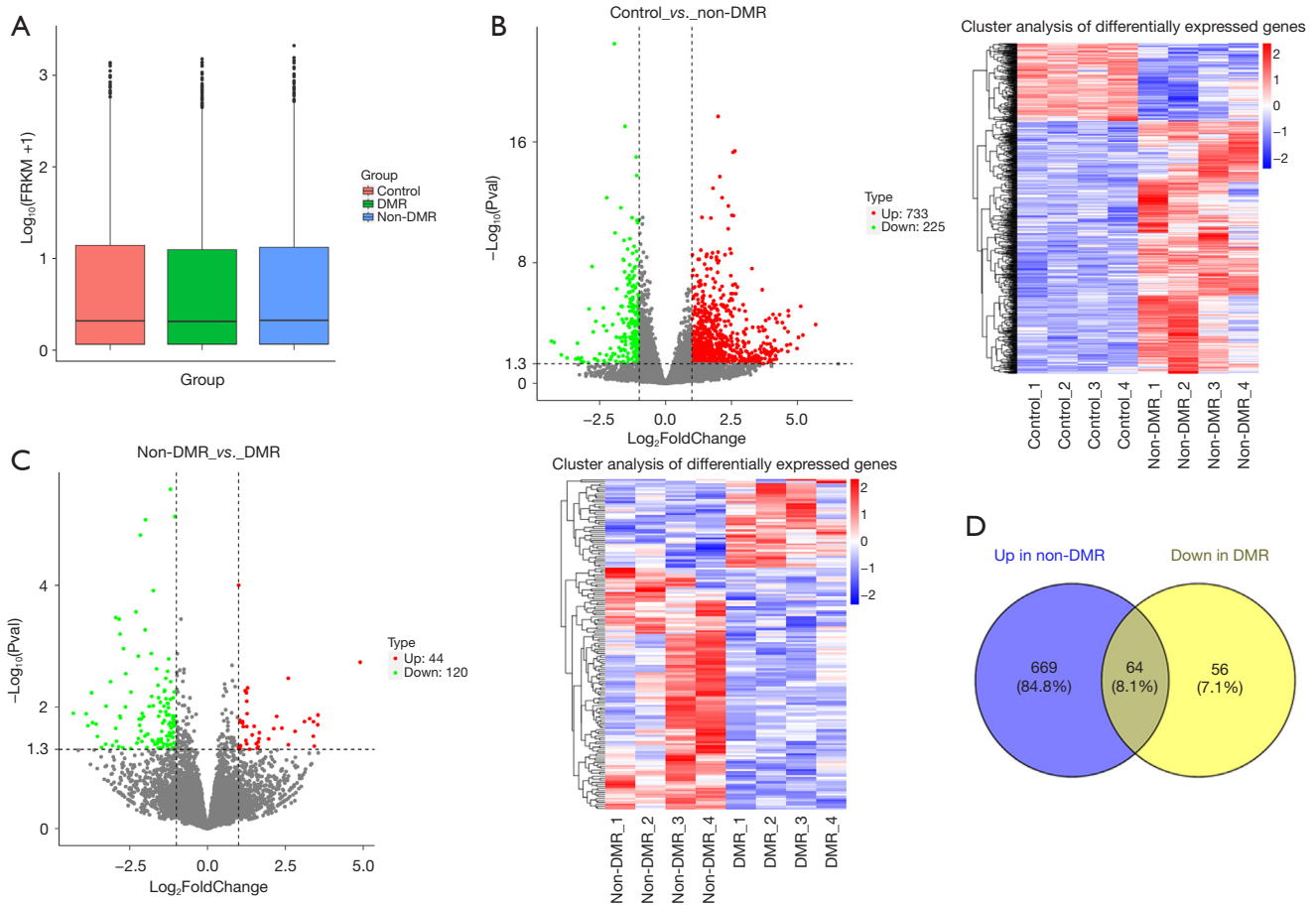


Figure 1 Overview of lncRNA data. (A) FPKM box plot of control, DMR, and non-DMR groups. (B) Volcano plot and heatmap clustering of differential expression lncRNAs between control and non-DMR groups. Each gray dot represents a differentially expressed lncRNA with no statistical significance. (C) Volcano plot and heatmap clustering of differential expression lncRNAs between DMR and non-DMR groups. Each gray dot represents a differentially expressed lncRNA with no statistical significance. (D) Venn plot of up-regulated lncRNAs in non-DMR group (compared with healthy control) and down-regulated lncRNAs in DMR group (compared with non-DMR group). FPKM, fragments per kilobase of exon model per million mapped fragments; lncRNA, long non-coding RNA; DMR, deep molecular response.

hemoglobin, $P=0.47$; platelet, $P=0.29$; SOKAL score, $P=0.12$) except for ELTS ($P=0.001$), indicating that patients with low-risk are easier to obtain DMR.

Overview of lncRNA data

As shown in *Figure 1A*, FPKM box plot revealed the close value of the $\log_{10}(\text{FPKM}+1)$ among control, DMR, and non-DMR groups, indicating the reliability of the data. Differential expression analysis suggested that, compared with healthy controls, 733 lncRNAs were up-regulated and 225 lncRNAs were down-regulated in CML patients with non-DMR. Heatmap clustering clearly revealed a separation

of the differentially expressed lncRNAs between control and non-DMR groups (*Figure 1B*). Additionally, compared with patients in non-DMR group, 44 lncRNAs were up-regulated and 120 lncRNAs were down-regulated of patients in DMR groups. Heatmap clustering revealed a separation of the differentially expressed lncRNAs between DMR and non-DMR groups (*Figure 1C*). Venn plot identified 64 overlapped lncRNAs between up-regulated lncRNAs in non-DMR group (compared with healthy control) and down-regulated lncRNAs in DMR group (compared with non-DMR group) (*Figure 1D*), which was considered as the resistance related genes. Excluding novel genes or informal genes, a total of 16 lncRNAs were obtained, containing, *PLA2G4C-AS1*, *NCF4-*

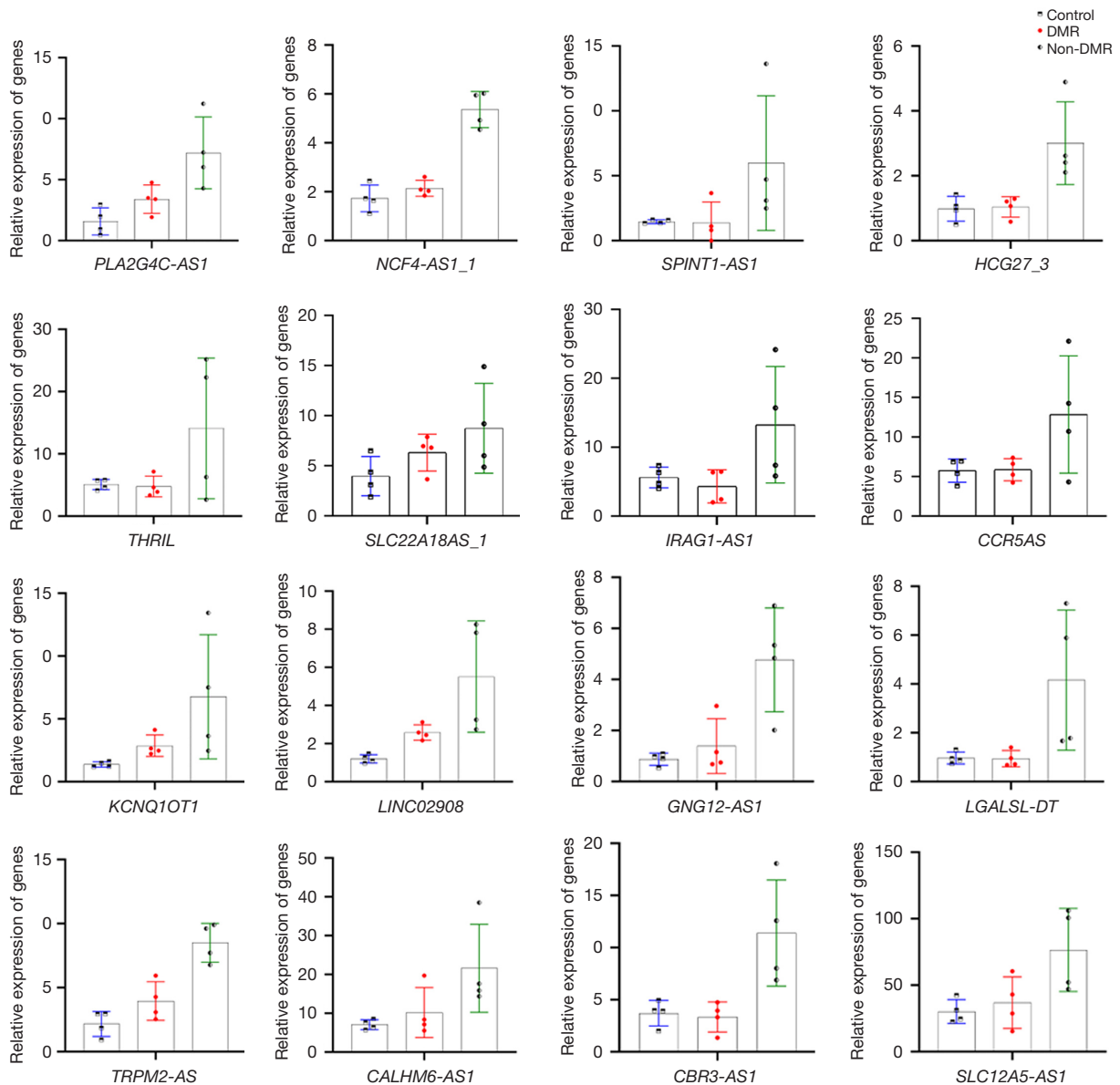


Figure 2 Expression of overlapped lncRNAs between up-regulated lncRNAs in non-DMR group (compared with healthy control) and down-regulated lncRNAs in DMR group (compared with non-DMR group) detected using RNA sequencing. Novel genes or informal genes were excluded. lncRNAs, long non-coding RNAs; DMR, deep molecular response.

AS1_1, SPINT1-AS1, HCG27_3, THRIL, SLC22A18AS_1, IRAG1-AS1, CCR5AS, KCNQ1OT1, LINC02908, GNG12-AS1, LGALSL-DT, TRPM2-AS, CALHM6-AS1, CBR3-AS1, SLC12A5-AS1 (Figure 2).

Expression validation of lncRNA *CBR3-AS1* and lncRNA *TRPM2-AS* in clinical samples

We first excluded genes that had not been reported in

the literature and whose functions were unclear. Genes associated with a variety of tumors but not studied in CML were retained. Then, combined with the expression profiles of genes in the GEPIA database, we finally identified *CBR3-AS1* and *TRPM2-AS* as targets of interest. We first evaluated the expression of lncRNA *CBR3-AS1* and lncRNA *TRPM2-AS* using small samples (five each group) as preliminary experiment, demonstrating that, compared with control group, there was no statistical difference of *CBR3-AS1*

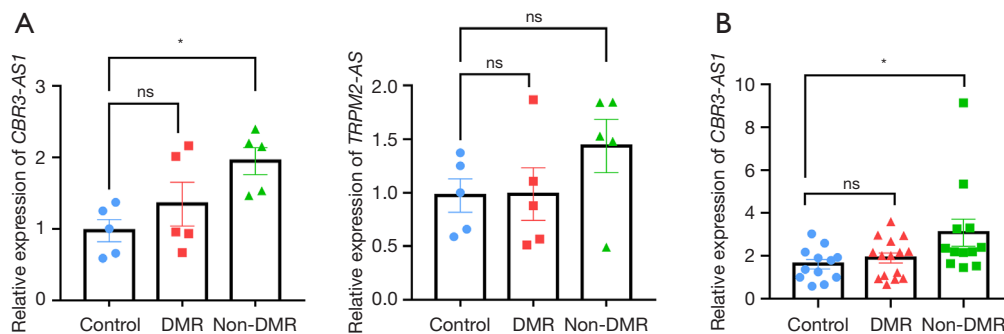


Figure 3 Expression validation of lncRNA *CBR3-AS1* and lncRNA *TRPM2-AS* in clinical samples. (A) Preliminary experiment of *CBR3-AS1* and *TRPM2-AS* expression detection. (B) Detection of *CBR3-AS1* expression in an expanded cohort. ns, no significance. *, $P < 0.05$. lncRNA, long non-coding RNA; DMR, deep molecular response.

expression in DMR group ($P=0.50$), whereas, *CBR3-AS1* was up-regulated in non-DMR group ($P=0.03$) (Figure 3A). However, compared with control group, there was no statistical difference in the expression of *TRPM2-AS* in both DMR ($P=0.99$) and non-DMR group ($P=0.33$) (Figure 3A). Thus, only *CBR3-AS1* was chosen for the further validation in an expanded cohort, demonstrating the similar the results of preliminary experiment (Figure 3B). Collectively, these results suggest that *CBR3-AS1* may be associated with BCR::ABL1 independent TKI resistance of CML patients.

Overview of mRNA data

Furthermore, we analyzed the data of mRNA, showing the close value of the $\log_{10}(\text{FPKM}+1)$ among control, DMR, and non-DMR groups, indicating the reliability of the mRNA data (Figure 4A). Differential expression analysis showed that, compared with control group, 773 mRNAs were up-regulated and 405 mRNA were down-regulated in non-DMR group, whereas 853 mRNAs were up-regulated and 426 mRNA were down-regulated in DMR group; compared with non-DMR group, 89 mRNAs were up-regulated and 113 mRNA were down-regulated in DMR group (Figure 4B). Heatmap clustering showed a separation of the differentially expressed mRNAs between groups (Figure 4B). We considered genes that up-regulated in non-DMR group (compared with control group), down-regulated in DMR group (compared with non-DMR group), showed no statistical difference between control and DMR group as the potential genes that are associated with TKI resistance of CML patients. As shown in Figure 4C, 55 corresponding mRNAs were obtained including *KCNA6*, a target gene of *CBR3-AS1* (available online: <https://cdn.>

amgroups.cn/static/public/10.21037/tcr-24-281-1.xls). Thus, *KCNA6* was selected for the subsequent analysis.

LncRNA CBR3-AS1 might be involved in BCR::ABL1 independent TKI resistance of CML patients through targeting *KCNA6*

Online database genemania was used to analyze the major interacted proteins of *KCNA6*, as shown in Figure 5A. Furthermore, functional enrichment and pathway enrichment analysis of the major interacted proteins were performed using R software. GO functional enrichment analysis demonstrated that the genes were mainly enriched in potassium channel complex term of BP category, voltage-gated cation channel activity of CC category, and transmembrane transporter complex of MF category (Figure 5B). KEGG pathway analysis revealed that the major interacted genes were enriched in several resistance-associated pathways including interleukin (IL)-17 signaling pathway and cyclic adenosine monophosphate (cAMP) signaling pathway (Figure 5C).

Discussion

Our current findings demonstrated the diversity lncRNA profiles among healthy individuals, CML patients with non-DMR, and CML patients with DMR. Differential expression analysis and Venn plot of up-regulated lncRNAs in patients with non-DMR and down-regulated lncRNAs in patients with DMR revealed that lncRNA *CBR3-AS1* overexpression might be related to BCR::ABL1 independent TKI resistance of CML patients. *CBR3-AS1* was initially found to be up-regulated in prostate cancer, which promotes proliferation and suppresses apoptosis of

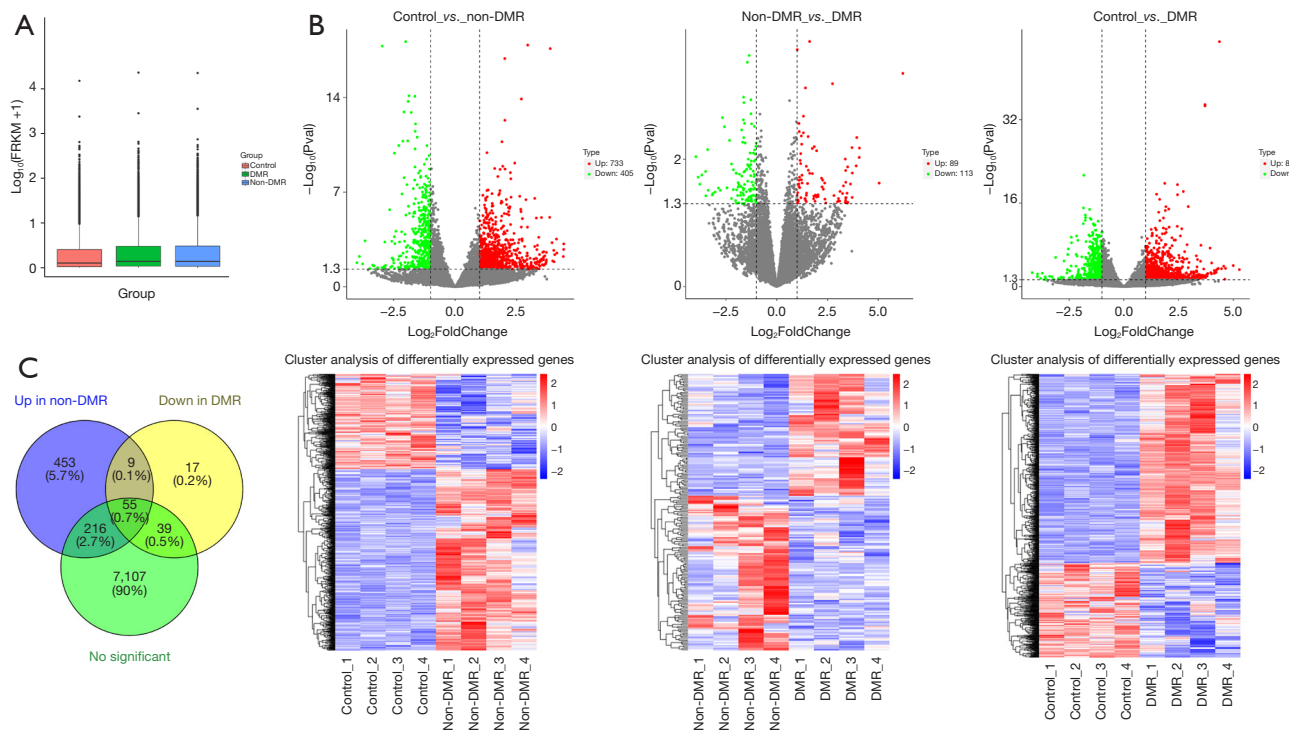


Figure 4 Overview of mRNA data. (A) FPKM box plot of control, DMR, and non-DMR groups. (B) Volcano plot and heatmap clustering of differential expression mRNAs between control and non-DMR groups, non-DMR and DMR groups, as well as control and DMR groups. Each gray dot represents a differentially expressed mRNA with no statistical significance. (C) Venn plot of up-regulated mRNAs in non-DMR group (compared with control group), down-regulated mRNAs in DMR group (compared with non-DMR group), and no statistical difference mRNAs between control and DMR group. FPKM, fragments per kilobase of exon model per million mapped fragments; DMR, deep molecular response; mRNAs, messenger RNAs.

prostate cancer cells (15-17). Subsequent studies discovered the carcinogenesis of *CBR3-AS1* in several human cancers including cervical cancer, osteosarcoma, non-small cell lung cancer, lung adenocarcinoma, and breast cancer (18-22). Recently, the role of *CBR3-AS1* on drug resistance has been uncovered. As reported by Zhang *et al.*, *CBR3-AS1* was obviously overexpressed in breast cancer tissues, which contributed to poor prognosis. *CBR3-AS1* upregulation resulted in chemotherapy resistance of breast cancer by regulating *miR-25-3p* and MEK4/JNK1 signaling pathway (23). Furthermore, we verified the expression of *CBR3-AS1* in an expanded cohort, demonstrating that, compared with control group, there was no statistical difference of *CBR3-AS1* expression in DMR group, whereas, *CBR3-AS1* was up-regulated in non-DMR group, which strengthen our conclusion that *CBR3-AS1* overexpression is associated with BCR::ABL1 independent TKI resistance of CML patients. However, further functional verification experiments *in vitro* and *in vivo* are needed in the follow up study.

To further explore the BCR::ABL1 kinase independent mechanism of TKI resistance in CML patients, we analyzed the mRNA data obtained for RNA-sequencing, indicating the different mRNA profiles among healthy individuals, CML patients with non-DMR, and CML patients with DMR. Subsequent analysis identified 55 mRNAs that may be correlated with TKI resistance in CML patients, including *KCNA6*, a target gene of *CBR3-AS1*. Thus, we hypothesized that *CBR3-AS1* is involved in BCR::ABL1 independent TKI resistance of CML patients through targeting *KCNA6*. Moreover, we performed bioinformatics analysis, and the results showed that the major interacted genes of *KCNA6* were enriched in several resistance-associated pathways including IL-17 signaling pathway and cAMP signaling pathway, which confirmed our hypothesis. IL-17 is a proinflammatory factor that shows an important function in the immune regulation and tumor progression, including tumorigenesis, proliferation, and metastasis (24,25). In addition, IL-17 pathway has been discovered to

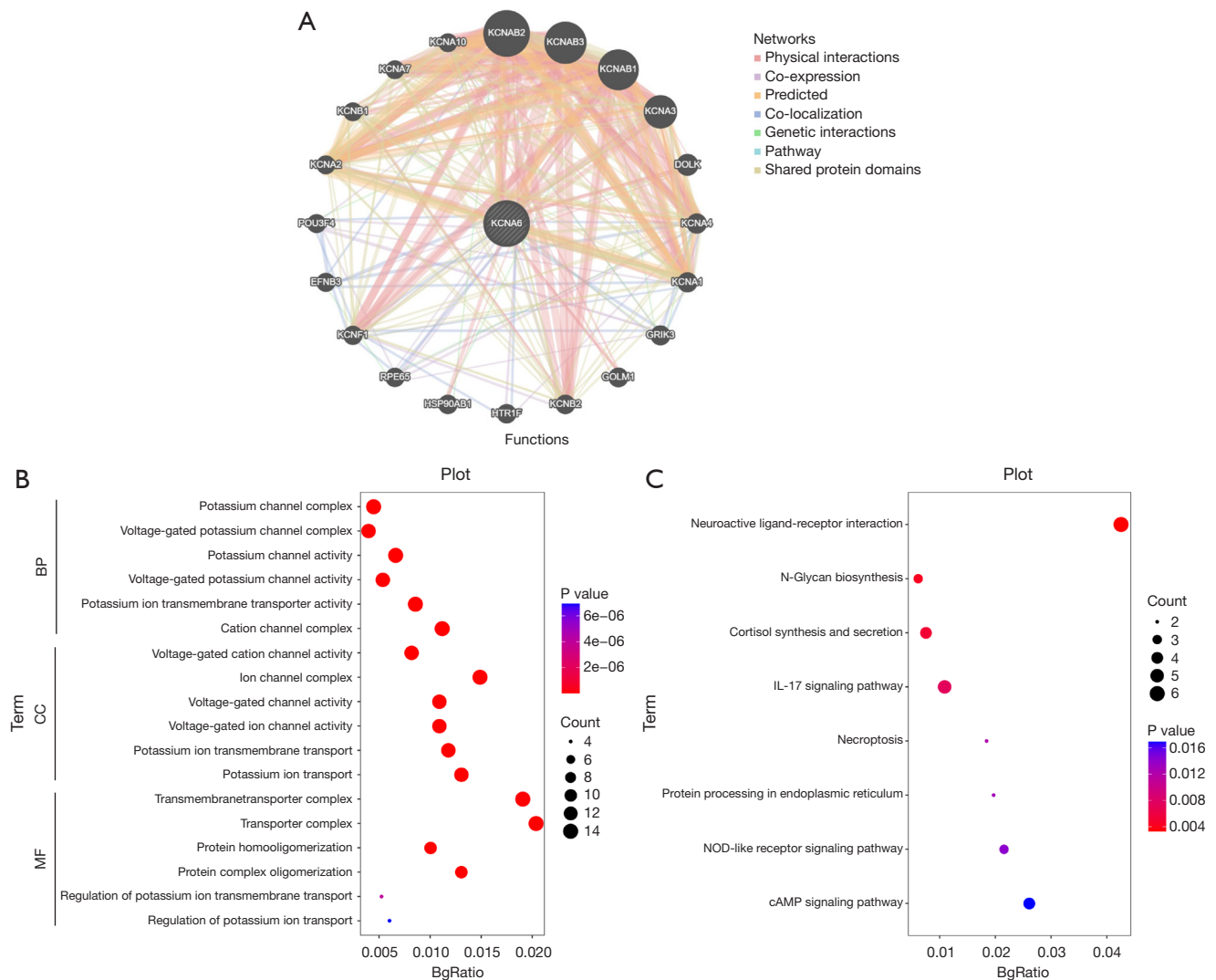


Figure 5 Function prediction of *KCNA6*. (A) Analysis of main interacting proteins of *KCNA6*. (B) Gene Ontology enrichment and (C) Kyoto Encyclopedia of Genes and Genomes pathways enrichment of the major interacted genes of *KCNA6*. MF, molecular function; CC, cellular component; BP, biological process; cAMP, cyclic adenosine monophosphate; NOD, nucleotide oligomerization domain; IL-17, interleukin 17.

be involved in chemotherapy resistance in breast cancer (26), and immune checkpoint blockade sensitivity in pancreatic cancer (27). cAMP is a nucleotide that influences multiple signal transduction pathways, thereby involving in the regulation of numerous cellular BP, including proliferation, differentiation, apoptosis, gene transcription, and protein translation (28). Accumulating evidences also demonstrate the drug resistance modulation of cAMP signaling pathway. As reported by Mishra *et al.*, cAMP signaling inactivation alleviated tamoxifen resistance in estrogen receptor -positive breast cancer (29). Xian *et al.* found that *KCNQ10T1* knockdown enhanced chemosensitivity towards

methotrexate in colorectal cancer through regulating cAMP signaling pathway (30).

Conclusions

In conclusion, the present findings identified lncRNA *CBR3-AS1* as a regulatory gene of BCR::ABL1 independent TKI resistance in CML patients using RNA-seq. The expression of *CBR3-AS1* was then validated using clinical samples. Further mRNA data analysis and bioinformatics analysis demonstrated that *CBR3-AS1* might be involved in BCR::ABL1 independent TKI resistance of CML patients

through targeting *KCNA6*. The limitation of our current study is that we only verified the expression of *CBR3-AS1* in clinical samples, further *in vitro* and *in vivo* will be designed to validate the function of *CBR3-AS1-KCNA6*, as well as IL-17, in BCR::ABL1 independent TKI resistance of CML patients. Collectively, this work investigates the mechanism of TKI resistance in CML patients, providing a new target for the synergy therapy of CML patients.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-24-281/rc>

Data Sharing Statement: Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-24-281/dss>

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-24-281/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the ethics committee of the First Hospital of Putian City (approval No. 2022-027). All eligible participants provided written informed consent.

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