circCRKL, a circRNA derived from CRKL, regulates BCR-ABL via sponging miR-877-5p to promote chronic myeloid leukemia cell proliferation

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Abstract

Background: The BCR-ABL fusion protein is the key factor that results in the occurrence of chronic myeloid leukemia (CML). Imatinib (IM) is a targeted inhibitor of BCR-ABL to achieve complete remission. However, remission failure occurs due to acquired resistance caused by secondary BCR-ABL mutations, underlining the need for novel BCR-ABL-targeting strategies. Circular RNAs (circRNAs) derived from tumor-related genes have been revealed as possible therapeutic targets for relevant cancers in recent investigations. In CML, the roles of this kind of circRNA are yet obscure.

Methods: Firstly, RT-qPCR was used for determining circCRKL expression level in cell lines and clinical samples, RNase R and Actinomycin D were employed to verify the stability of circCRKL. Then shRNAs were designed to specifically knockdown circCRKL. The function of circCRKL in vitro was investigated using CCK-8, colony formation assay, and flow cytometry, while a CML mouse model was constructed to explore the function in vivo. Finally, a dual-luciferase reporter assay, RNA pull-down, RNA immunoprecipitation, and rescue experiments were conducted to investigate the mechanism of circCRKL functioning.

Results: Here, we determined circCRKL, which derives from CML-relevant gene CRKL, is over-expressed in BCR-ABL+ cells. Then we noticed knocking down circCRKL using shRNA lentivirus dampens the proliferation of BCR-ABL+ cells both in vitro and in vivo, and augments susceptibility of resistant cells to IM. Intriguingly, we observed that circCRKL has a considerable impact on the expression level of BCR-ABL. Mechanistically, circCRKL could behave like a decoy for miR-877-5p to enhance the BCR-ABL level, allowing BCR-ABL+ cells to maintain viability.

Conclusions: Overall, the current study uncovers that circCRKL is specifically expressed and regulates BCR-ABL expression level via decoying miR-877-5p in BCR-ABL+ cells, highlighting that targeting circCRKL along with imatinib treatment could be utilized as a potential therapeutic strategy for CML patients.

Keywords: Chronic myeloid leukemia, circCRKL, Proliferation, BCR-ABL, ceRNA

Background

Chronic myeloid leukemia (CML) was identified as a neoplastic disease of hematopoietic stem cells, arising from a fusion event termed BCR-ABL [1]. The oncogenic fusion protein BCR-ABL with aberrant tyrosine kinase function is encoded by the BCR-ABL [2], leading to constitutively activation of various substrates including...
CRKL, PI3K/AKT, and MAPK signaling pathways, resulting in malignant cell proliferation [3–5]. Tyrosine kinase inhibitor (TKI) imatinib mesylate (IM), which has the benefit of significantly enhancing patient prognoses, has been used extensively in the clinical management of CML individuals [6]. However, individuals receiving long-term IM treatment risk developing resistance since BCR-ABL (the root cause of CML) cannot be entirely eradicated. Imatinib resistant CML patients had a significantly increased risk of relapse after discontinuation [7, 8]. The pathogenesis of CML is well understood, however, the underlying molecular mechanisms during disease development and TKI resistance are required to be clarified, thus providing novel insights for diagnosis and therapy in CML.

In the last few decades, the underlying mechanisms of CML progression and TKI resistance predominantly focused on messenger RNAs (mRNAs) that encode specific proteins [9, 10] or long non-coding RNAs (lncRNAs) [11, 12] or micro RNAs (miRNAs). miR-185, for example, has been uncovered to function as a repressor of PAK6, mediating Imatinib resistance in TKIs resistant leukemic cells, indicating that miR-185 might be a novel target for treating TKIs resistant CML patients [13]. As a result of the rapid advancement of high-throughput sequencing, circular RNAs (circRNAs) have come into view. As a unique kind of regulating non-coding RNAs [14], circRNA lacks the classic cap and poly-A tail structure retained by linear RNAs [15]. A stable circular form renders circRNA more suitable as a molecular target [16]. Accumulating evidence elucidated that circRNAs were extensively engaged in regulating the development of many malignancies, including liver cancer [17], lung cancer [18], and leukemia [19, 20]. Diverse functions of circRNAs are rested with different subcellular localization, including sponging miRNA, acting as a scaffold to decoy RNA binding proteins (RBPs) and translated peptides in the cytoplasm, while mediating transcription of parental genes in the nucleus [14, 21–23].

Amounts of evidence illustrated circRNAs derived from tumor-related parental genes can regulate the onset and progression of malignancies. For example, circMYBL2, produced from MYBL2, which was reported to be linked to leukemia, could promote the advancement of FLT3-ITD-mutant acute myeloid leukemia [20]. In addition, by stimulating the Wnt signal pathway, circ β-catenin, which was generated from CTNNB1, translated a novel β-catenin isofrom and accelerated hepatocellular carcinoma cell growth [24]. In CML, the roles of circRNA are yet obscure. It was suggested that fusion-circRNA (f-circRNA) derived from BCR-ABL participated in regulating the progression of CML [25, 26]. However, few pieces of evidence uncovered that the roles of circRNAs originated from other known genes in BCR-ABL cascades, such as CRKL.

CRKL also termed CRK-like gene, produces an oncogenic protein CRKL that has been pinpointed as a predominant element of tyrosine phosphorylation [27]. It has been suggested that CRKL complexes directly with BCR-ABL via the SH3 domain, triggering signaling cascades such as PI3K-AKT pathway that contribute to leukemic transformation [28]. Besides, CRKL lacking the SH2 domain reduced c-MYC expression level in imatinib-resistant cells harboring BCR-ABL T315I mutation [29]. Recent studies showed that hsa_circ_0001206, produced by the second exon of CRKL pre-mRNA, was a tumor suppressor and restrained cell growth by regulating KLF5 through mediating miR-141 in prostate cancer [30]. Besides, several lines of evidence suggested that circCRKL blocked AML progression through targeting miR-196-5p [31].

In this work, we discovered that circCRKL, a circRNA that originated from the second exon of CRKL, is significantly highly expressed and accelerates the BCR-ABL+ cells proliferation including imatinib-resistant cell line K562/G01. Our data further confirmed that circCRKL modulates BCR-ABL via decoying miR-877-5p to hasten cell proliferation. Therefore, our findings suggested that circCRKL impels CML progression by acting an oncogenic role, and targeting circCRKL might represent a novel therapeutic option for CML patients.

Materials and methods

Cell culture and management

Human chronic myeloid leukemia cell lines (K562, K562/ G01, and KCL22), acute monocytic leukemia cell line (THP-1), human lymphoblastic cell line (TK6), and 293 T were all maintained in Roswell Park Memorial Institut-1640 (RPMI-1640, Gibco, USA) or Dulbecco’s modified eagle medium (DMEM, Gibco, USA) with 10% fetal bovine serum (FBS, Gibco, USA) respectively. All cells were cultivated at 37 °C in a suitable atmosphere containing 5% CO2. The Chinese Academy of Science’s Cell Bank provided all of the cell lines utilized in this work.

The bone marrow samples of newly diagnosed CML patients and normal donors were obtained from the Department of Hematology, the Second Affiliated Hospital of Chongqing Medical University. The information was illustrated in Additional file 3: Table S1. The Human bone marrow mononuclear cells (BMMCs) separation Kit (TBD, China) was used to isolate BMMCs from the samples. Before the trial began, all patients signed informed permission forms. The ethics committee of Chongqing Medical University gave its approval to this investigation.
RNA extraction and RT-qPCR
TRizol reagent (Accurate, China) was used to extract total RNA from patient samples and cultured cells. To reverse transcribe circRNA and messenger RNA (mRNA) into complementary DNA (cDNA), a reverse transcription kit (Accurate, China) was employed. The stem-loop primer was used to reverse transcribe miR-877-5p. The quantitative PCR was carried out through SYBR Green Premix Pro Taq HS qPCR Kit (Accurate, China) following the product's instructions. Actin or U6 was used as RT-qPCR internal parameters. The $2^{-\Delta\DeltaCT}$ method was utilized to examine the relative expression of RNA. All specific primers sequences used in this investigation were listed in Additional file 3: Table S2.

RNase R resistance and Actinomycin D analysis of circCRKL
The 1 μg of total RNA extraction of K562, and K562/G01 cells were digested at 37 °C for 10 min after adding 2U/mg of RNase R (Geneseed, China), then the mixture was hatched at 70 °C for another 10 min to inactivate the RNase R. Subsequently, the relative RNA enrichment was detected through RT-qPCR.

The 5 μg/mL actinomycin D was mixed with K562 and K562/G01 cells and reacted for 4, 8, and 12 h respectively, the total RNA was then extracted. The relative RNA expression was then identified using RT-qPCR.

Fluorescence in situ hybridization (FISH)
The specific circCRKL probe was labeled with cy3 and synthesized by RiboBio (Guangzhou, China). The assay was conducted in accordance with the manufacturer's orders. In brief, K562 and K562/G01 cells were washed and fixed with 4% paraformaldehyde at room temperature (RT) for 10 min before being permeabilized with 0.5% Triton X-100 at 4 °C for 5 min. Then circCRKL probe was hybridized with cells in a dark moist chamber at 37 °C overnight. To stain the nuclei, 4′,6-diamidino-2-phenylindole (DAPI) was used. A confocal microscope was utilized to acquire photos.

Infection of lentivirus
For circCRKL down-regulation, small interfering oligos targeting circCRKL (the sequences are illustrated in Additional file 3: Table S3) were designed and constructed into lentivirus (GeneChem, China), which were subsequently infected into BCR-ABL + and THP-1 cells. Stable transfection was established through culturing cells in RPMI-1640 medium supplemented with 2 μg/ml puromycin (Solarbio, China) for 7 days, and maintained with 1 μg/ml of that for another 7 days.

CCK-8 assay
The cell viability was determined using a CCK-8 kit (TargetMol, China). Cells were seeded at the number of 3000 per well in a 96-well plate and cultured in an incubator for 24 h before receiving treatment of 10 μl CCK-8 reagent for 3 h. Subsequently, the absorbance was assessed at 450 nm. Three duplicates were set in each group.

Colony formation assay
Cells were seeded at a density of 100 per well into a 96-well plate. After being stilling cultured using RPMI-1640 with 10% FBS for 7 days, photos were taken and the number of colonies was counted. At least three duplicates were set in each group.

Flow cytometry assay
For cell cycle assay, at least $1 \times 10^6$ infected K562 and K562/G01 cells were immobilized through pre-cooled 70% ethanol at 4 °C overnight. After that, each sample was left in a lucifugal chamber at RT for 30 min after receiving 500 μl of propidium iodide (PI) staining reagent. Then cell cycle was detected and analyzed through a flow cytometer (CytoFlex, Beckman coulter).

For cell apoptosis assay, at least $5 \times 10^5$ infected K562 and K562/G01 cells were collected and washed, then cells were suspended with 100 μl pre-cooled PBS and treated with 10 μl Annexin V APC and 5 μl DAPI in the dark at RT for 15 min before being determined with a flow cytometer (CytoFlex, Beckman coulter).

RNA Immunoprecipitation (RIP)
K562/G01 cells were used to conducted RIP assay with the BersinBio™ RNA-Binding Protein Immunoprecipitation Kit (BersinBio, China) in accordance with the manufacturer's protocols. In a nutshell, at least $2 \times 10^7$ cells were harvested, washed, and lysed using 900 μl RIP lysis buffer, then cell lysates were added with 4 μg of anti-AGO2 (Abcam, USA) or the equal amounts of anti-IgG (BersinBio) and incubated at 4 °C overnight. The mixture was subsequently added with 40 μl protein A/G magnetic beads and incubated at 4 °C for 2 h. Finally, the RNAs were extracted and purified using phenol–chloroform and the relative abundance of miR-877-5p and circCRKL was examined through RT-qPCR.

Western blot analysis
To lyse cells, a radio immunoprecipitation assay (RIPA) lysing solution including phosphatase and protease inhibitor was employed, and total protein was then extracted and quantified through a bicinchoninic acid (BCA) protein extraction assay kit (Beyotime, China).
For western blot assay, 40 μg of protein samples were loaded and isolated with 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Subsequently, blocking the polyvinylidene fluoride (PVDF) membranes containing protein blots with 5% nonfat milk powder at RT for 2 h before incubating the primary antibody (1:1000) at 4 °C overnight. After that, the membranes were incubated with relevant secondary antibodies (1:5000) at RT for 90 min. Finally, the Bio-Rad Gel Imaging System was used to visualize and analyze the signals.

**Bioinformatics prediction and dual-luciferase reporter assay**

For the prediction of target miRNAs directly cooperating with circCRKL, three online databases including ENCORI...
infected with sh-NC or sh-circCRKL lentivirus. The mice were closely culled at an ethical endpoint. The animal experiments were given approval by the Ethics Committee of Chongqing Medical University.

Statistical analysis
GraphPad Prism 8 (GraphPad, USA) was used for analyzing data in this research. The student's t-test was applied for calculating the main effect in two groups and One-Way ANOVA was used for that in three and more. p-values < 0.05 (*), p < 0.01 (**), p < 0.001 (***), and p < 0.0001 (****) were regarded as statistically significant.

Results
Characterization of circCRKL in CML cell lines and clinical samples
To uncover the hidden role of circCRKL in CML, the circCRKL expression in BMMCs from CML patients (6 cases) and normal donors (3 cases) was detected by RT-qPCR. CircCRKL was substantially expressed in CML patients relative to normal donors, as demonstrated in Fig. 1A. Furthermore, when comparing BCR-ABL+ cell lines (K562, K562/G01, KCL22, and SupB15) to BCR-ABL- cell lines (TK6 and THP-1), it was surprising to find that the circCRKL expression was significantly higher in BCR-ABL+ cell lines (Fig. 1B). The BCR-ABL expression level of cell lines was evaluated by western blot assays (Additional file 1: Figure S1A). These findings suggested that a link may exist between circCRKL and BCR-ABL. Then we conducted a bioinformatics analysis of the CRKL transcript. It was found that circCRKL is a 466-nt circRNA and was back spliced by exon 2 of pre-CRKL, Sanger sequencing was applied to confirm the cyclization site (Fig. 1C). The results of nuclear and cytoplasm RNA separation assays with RT-qPCR uncovered that circCRKL expression level in the cytoplasm was higher than that in the nucleus in CML cells (Fig. 1D, E), which was further supported by fluorescence in situ hybridization (FISH) assays (Fig. 1F). Subsequently, to determine the circular form of circCRKL, the RNase R analysis was performed and the linear CRKL expression diminished dramatically while circCRKL had no changes, indicating that circCRKL was stable in CML cells (Fig. 1G, H). In addition, the half-life of circCRKL and linear CRKL was determined with the treatment of actinomycin D. It was found that circCRKL had a longer half-life compared with that of linear CRKL (Fig. 1I, J). These findings imply that circCRKL, located in the cytoplasm in CML cells
Fig. 2 (See legend on previous page.)
and highly expressed, is a stable circRNA, indicating a potential role in CML.

circCRKL promotes BCR-ABL+ cells proliferation in vitro
To further explore the biological involvement of circCRKL in CML, a loss-of-function test was performed. We constructed circCRKL knockdown CML cells using shRNA specifically targeting the back spliced junction site of circCRKL. The expression of circCRKL was markedly restrained in CML cells, whereas the linear CRKL expression was unaffected (Fig. 2C–F). Flow cytometry assay showed that circCRKL silencing reduced the number of cells in the S phase (Fig. 2G, H). The effect of circCRKL on apoptosis in CML cells was then investigated, and we discovered that the second shRNA of circCRKL silencing engendered cell apoptosis rate increasing (Additional file 1: Figure S1B).

The differential expression of circCRKL in BCR-ABL+ and BCR-ABL− cell lines raises the question of whether circCRKL specifically activates in cells expressing BCR-ABL. Hence, the effects of circCRKL silencing on cell viability were investigated in two other BCR-ABL+ cell lines (KCL22 and SupB15) as well as BCR-ABL− cell lines (THP-1 and TK-6). The knockdown efficiency of circCRKL was confirmed in these cell lines (Additional file 1: Figure S1C). Next, CCK-8 assays showed circCRKL suppression drastically reduced CML cell growth (Fig. 2C–F). Flow cytometry assay showed that circCRKL silencing reduced the number of cells in the S phase (Fig. 2G, H). The effect of circCRKL on apoptosis in CML cells was then investigated, and we discovered that the second shRNA of circCRKL silencing engendered cell apoptosis rate increasing (Additional file 1: Figure S1B).

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circCRKL silencing impaired the oncogenesis of CML cells in vivo
To better investigate the function of circCRKL, a CML mouse model was created. Sh-NC or sh-circCRKL lentivirus-infected K562/G01 cells were delivered intravenously (i.v.) into immunodeficient NOD/SCID mice, respectively. Prior studies have noted that the features of CML mice containing increased white blood cell count and histopathological variation such as bone marrow, liver, and spleen infiltration caused by leukemic cells [32]. As expected, the white blood cell count of the control group mice increased more than that of the knockdown group, but no distinct difference exists (p = 0.0586) (Fig. 3A). In addition, compared with sh-NC group mice, knockdown of circCRKL ameliorated the splenomegaly. However, there was no significant defect for weights of livers in the sh-circCRKL group compared to the NC group (Fig. 3B, C). To evaluate the infiltration of leukemic cells in more depth, we set out to perform Wright's staining in murine bone marrow, liver, and spleen. The results showed severer infiltration in the sh-NC group xenograft models (Fig. 3D), similarly confirmed by hematoxylin/eosin (HE) staining (Fig. 3E). The BCR-ABL level in mouse BM, liver, and spleen was then assessed with immunofluorescence, and the results revealed a much lower level in circCRKL silencing mice (Fig. 3F). Overall, these findings indicated that circCRKL can promote the malignant progression of CML in vivo.

circCRKL regulates BCR-ABL expression and miR-877-5p is a target for both circCRKL and BCR-ABL
Since we discovered that circCRKL is essential in BCR-ABL+ cells, it's critical to figure out how circCRKL and BCR-ABL are linked. Notably, western blot showed a reduction of the BCR-ABL and c-ABL protein levels in circCRKL suppressed cells (Fig. 4A). The BCR-ABL expression level was further examined using an immunofluorescence test, and the results were in concert with those previously mentioned (Fig. 4B). Furthermore, RT-qPCR demonstrated that knocking down circCRKL reduced BCR-ABL mRNA expression (Fig. 4C), suggesting circCRKL may regulate BCR-ABL at the mRNA level. The mechanism by which circCRKL controls BCR-ABL, however, is yet unknown.

Given that circCRKL is predominantly found in the cytoplasm of CML cells, which is a critical prerequisite for circCRKL to function as a miRNA sponge. Therefore, we screened downstream miRNAs of circCRKL from three databases (circInteractome, circBank, and ENCORI) (Fig. 4D). Subsequently, ENCORI and the other two databases TargetScan and miRwalk were applied to predict miRNAs bound to ABL. Then we overlapped miRNAs of circCRKL and ABL (Fig. 4E). Among candidate miRNAs, miR-877-5p is a unique miRNA containing binding sites for both circCRKL and ABL 3’UTR (Fig. 4F), as confirmed through dual-luciferase reporter assays. The wild-type circCRKL reporter’s luciferase activity was drastically diminished when miR-877-5p was ectopically expressed.
Fig. 3 (See legend on previous page.)
however, the mutant reporter's activity was unaffected, demonstrating that circCRKL may interact with miR-877-5p at specific sites (Fig. 4G), and similar results were noticed between miR-877-5p and ABL (Fig. 4H). The connection between circCRKL and miR-877-5p was further confirmed by RNA pull-down assays. The efficiency of the circCRKL probe was testified by RT-qPCR, then as expected, miR-877-5p was found to be specifically enriched
Fig. 5 (See legend on previous page.)
miR-877-5p restrains CML cells proliferation via regulating BCR-ABL

To figure out the biological relevance of miR-877-5p in CML and whether BCR-ABL is controlled by miR-877-5p, the expression level in BMMCs from CML patients was determined (Fig. 5A), then inhibitor or mimics was utilized to knock down or overexpress miR-877-5p, respectively. The effect of inhibitor or mimics on miR-877-5p in CML cells was examined with RT-qPCR (Fig. 5B, C). The BCR-ABL mRNA expression was significantly up-regulated by inhibiting miR-877-5p, whereas overexpressing miR-877-5p markedly decreased BCR-ABL (Fig. 5D). Likewise, western blot demonstrated that miR-877-5p inhibition augmented BCR-ABL protein level, while miR-877-5p overexpression showed an opposite effect (Fig. 5E). Furthermore, CCK-8 (Fig. 5F, G) and colony formation assays (Fig. 5H, I) revealed that miR-877-5p inhibition stimulated CML cells proliferation and miR-877-5p overexpression had an opposite effect. Altogether, these findings revealed miR-877-5p acts as a negative regulator of BCR-ABL.

circCRKL promotes CML cells proliferation via sponging miR-877-5p to modulate BCR-ABL

To further explore whether circCRKL supports CML cells proliferation by controlling miR-877-5p/BCR-ABL axis. A rescue experiment was performed by co-transfecting sh-circCRKL along with miR-877-5p inhibitor. Knockdown of miR-877-5p distinctly restored the restrained growth induced by suppressing circCRKL in CML cells, according to CCK-8 and colony formation assays (Fig. 6A–D). Moreover, miR-877-5p knockdown markedly ameliorated the reduced BCR-ABL level induced by circCRKL suppression (Fig. 6E). In addition, the function of sh-circCRKL in miR-mimic cells was determined by measuring the BCR-ABL protein level in CML cells coupled with circCRKL knockdown. According to the findings, BCR-ABL protein levels were lower in CML cells co-transfected sh-circCRKL along with miR-mimic than that in sh-circCRKL+mimic-NC cells (Fig. 6F). Collectively, these results proved that circCRKL promotes CML cell proliferation through regulating miR-877-5p mediated BCR-ABL.

K562/G01 cells are more sensitive to imatinib when circCRKL is knocked down

Given that the imatinib-resistant cell line K562/G01 had higher levels of circCRKL expression than K562 cells. We speculate that a link may exist between circCRKL and imatinib resistance. The half-maximal inhibitory concentration value (IC-50) of K562 or K562/G01 cells against imatinib was measured using a CCK-8 assay (Additional file 2: Figure S2A). Subsequently, the cell viability of stable knockdown of circCRKL and circCRKL wild-type K562/G01 cells treated with imatinib for 48 h was determined and the IC-50 value was computed. Indeed, the data demonstrated that circCRKL silencing observably reduced cell viability, and the IC-50 value was decreased simultaneously (Additional file 2: Figure S2B). In addition, flow cytometry demonstrated that knocking down circCRKL boosted the apoptosis in K562/G01 cells treated with imatinib (Additional file 2: Figure S2C). We assumed that miR-877-5p might be implicated in imatinib-resistance, hence the impact of suppressing or overexpressing miR-877-5p on the IC-50 value of K562/G01 cells was then investigated. However, no distinct effect on IC-50 value was caught (Additional file 2: Figure S2D). Previous investigations have linked the Wnt pathway with imatinib-resistance [34, 35], the relevant markers including CTNNB1 and c-Myc were detected using a western blot assay, accordingly. The data demonstrated a discernible loss of both CTNNB1 and c-Myc in K562/G01 cells which circCRKL was knocked down (Additional file 2: Figure S2E). Collectively, these findings indicated that circCRKL may regulate the susceptibility of K562/G01 cells to imatinib through mediating the Wnt/β-catenin signaling pathway.

Discussion

**BCR-ABL** is a fusion gene that leads to the occurrence and progression of CML [2, 36]. Despite that tyrosine kinase inhibitors such as imatinib targeting BCR-ABL oncoprotein have ameliorated survival effectively [6], the TKI-resistance and uncontrolled progression prevent part of CML patients from curative benefit [8, 37]. Therefore, novel treatment strategies are supposed to be provided for optimizing the outcomes of CML patients.
Fig. 6  (See legend on previous page.)
During the past decade, the evolution of high-throughput sequencing has brought circRNAs to the forefront. circRNAs have been widely developed for therapeutic targets in diverse diseases due to their stable circular structure. An artificial circRNA scRNA21 was applied to sponge oncogenic miR-21 to restrain gastric cancer cell proliferation [38]. More recently, circRNA has been developed into vaccines for the treatment of SARS-CoV-2 [39]. Accumulating evidence has proved that circRNAs generated by cancer-associated parental genes play a vital role in those cancers [40]. For example, it has conclusively been shown that circMYBL2, derived from AML-related gene MYBL2, exerts a cancerogenic role in leukemia harboring FLT3-ITD mutation [20]. However, few species of investigations on the function of such circRNAs in CML. In this current research, we firstly reported that circCRKL, back spliced from the second exon of pre-CRKL, was highly expressed in BMMCs of CML patients and BCR-ABL+ cell lines. Previous studies revealed that circCRKL was downregulated and mitigated carcinogenesis in prostate cancer and acute myeloid leukemia [29, 30]. The reason circCRKL differential expressed in CML and other cancers could be explained is that circRNAs are cancer-specific and cell type-specific [41]. We hypothesized that circCRKL can function in CML in concert with its parental gene CRKL. As expected, further loss of function assays showed that knockdown of circCRKL dampened CML cells proliferation in cell lines and mouse models, suggesting circCRKL exerts an oncogenic role in CML.

circRNAs located in the cytoplasm can absorb specific miRNAs to control the related target genes, and to mediated tumorigenesis and progression. For example, circDYL sponges miR-145-5p to augment tumor-suppressor TJP1 in Wilm’s tumor [42]. circRNF220 was identified as a key upstream regulator of miR-30a, leading to elevated TJP1 expression level by sponging miR-877-5p, thus facilitating CML cell proliferation (Fig. 6G). Besides, we reveal that circCRKL plays a crucial role in imatinib-resistant cells (Additional file 2: Figure S2), which provides a novel perspective for easing the drug resistance of CML patients. However, extensive investigation to uncover the molecular basis of circCRKL involved in imatinib resistance should be undertaken. Overall, our work suggests that circCRKL can lead the progression of a therapeutic target for CML.

Conclusions

In summary, our studies uncover circCRKL, generated from CML-related gene CRKL, maintains BCR-ABL expression level by sponging miR-877-5p, thus facilitating CML cell proliferation (Fig. 6G). Besides, we reveal that circCRKL plays a crucial role in imatinib-resistant cells (Additional file 2: Figure S2), which provides a novel perspective for easing the drug resistance of CML patients. Additionally, researchers have confirmed miR-877-5p as a positive element in other malignancies [46, 47]. Herein, we firstly reported the involvement of miR-877-5p in CML. The RNA pull-down and dual-luciferase reporter assays illustrate that circCRKL exerts a sponge effect on miR-877-5p. Furthermore, we discovered that in CML, miR-877-5p was downregulated, and miR-877-5p silencing could offset circCRKL knockdown-mediated the proliferation restrain in CML cells. More than that, deep investigations demonstrated that miR-877-5p silencing elevated BCR-ABL expression level, and reverse results were induced by ectopic expressing miR-877-5p. Additionally, rescue experiments demonstrated that inhibition of miR-877-5p ameliorates the reduced BCR-ABL caused by knockdown of circCRKL, which validated the hypothesis we raised. Collectively, we revealed that circCRKL could sponge miR-877-5p to regulate BCR-ABL expression level to maintain the malignancy of CML cells. However, the present research is predominantly at the stage of cell and animal trials, additional studies were required to determine whether the target can be applied to CML individuals.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-022-03586-2.

Additional file 1: Fig. S1. circCRKL is required in BCR-ABL+ cells. A. The BCR-ABL levels in various cell lines were determined with western blot assays. B. Effect of circCRKL suppression on apoptosis in CML cells was assessed by flow cytometry. C. RT-qPCR was performed to detect the efficiency of circCRKL knockdown in KCL22, SupB15, THP-1, and TK-6 cell lines. D-G. CCK8 assays were used to assess the effect of circCRKL suppression on cell viability in KCL22 (D), SupB15 (E), THP-1 (F), and TK-6 (G) cells. *p < 0.05, **p < 0.01 and *** < 0.001.

Abbreviations

CML: Chronic myeloid leukemia; circRNA: Circular RNA; BCR-ABL+: BCR-ABL positive; BCR-ABL−: BCR-ABL negative; miRNA: Micro RNA; TKI: Tyrosine kinase inhibitor; IM: Imatinib; IC50: Half-maximal inhibitory concentration; RPMI1640: Roswell Park Memorial Institute-1640; DMEM: Dulbecco’s modified eagle medium; FBS: Fetal bovine serum; BMMCs: Bone marrow mononuclear cells; FISH: Fluorescence in situ hybridization; RIP: RNA Immunoprecipitation.
Additional file 2: Fig. S2. circCRKL knockdown ameliorates the sensitivity of imatinib-resistant cell line K562/G01. A, The cell survival rate and IC50 value were calculated with a CCK-8 assay after treatment with imatinib for 48 hours at different concentrations in K562 and K562/G01 cells. B, The cell survival rate and IC50 value were calculated with a CCK-8 assay after treatment with imatinib for 48 hours at different concentrations in K562/G01 cells knocked down circCRKL or its normal control. C, The apoptosis rate was measured with flow cytometry 48 hours after treatment with imatinib at 5 μM concentration. D, The cell survival rate and IC50 value were calculated with a CCK-8 assay after treatment with imatinib for 48 hours at different concentrations in K562/G01 cells suppressed or over-expressed miR-877-5p. E, Relative protein levels were determined with western blot assays. *p < 0.05 and ** < 0.01.

Additional file 3: Table S1. Clinical patients information. Table S2. Primers sequences used in RT-qPCR assay. Table S3. The siRNA sequences used for knocking down circCRKL. Table S4. The probe sequences used for RNA pull-down assay.

Acknowledgements
Not applicable.

Author contributions
JMW conceived and designed the investigation. JMW, YL performed the experiment and wrote the original draft. YQF analyzed and visualized the results, and revised the manuscript. GYJ, YHP reviewed the manuscript. WLF supervised the investigation and provided pivotal suggestions on the final version of the manuscript. All authors read and approved the final manuscript.

Funding
This work was funded by the National Natural Science Foundation of China (No. 81772255).

Availability of data and materials
Not applicable.

Declarations
Ethics approval and consent to participate
All animal experiments were carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and were conducted with the approval of the Ethics Committee of Chongqing Medical University.

Consent for publication
All authors read and approved the final manuscript.

Competing interests
The authors declare no potential conflicts of interest.

Received: 29 March 2022 Accepted: 12 August 2022 Published online: 04 September 2022

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