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Potential treatment for chronic myeloid leukemia using microRNA: in silico comparison between plants and human microRNAs in targeting *BCR-ABL1* gene

Syarifah Faezah Syed Mohamad and Marjanu Hikmah Elias*

Abstract

Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the expression of the *BCR-ABL1* fusion gene. Tyrosine kinase inhibitors (TKI) are used to treat CML, but mutations in the tyrosine kinase domain contribute to CML chemo-resistance. Therefore, finding alternative molecular-targeted therapy is important for the comprehensive treatment of CML. MicroRNAs (miRNA) are small non-coding regulatory RNAs which suppress the expression of their target genes by binding to the 3' untranslated region (3'UTR) of the target mRNA. Hypothetically, the miRNA-mRNA interaction would suppress *BCR-ABL1* expression and consequently reduce and inhibit CML cell proliferation. Thus, our objective was to determine the target interaction of human and plant miRNAs targeting the 3'UTR region of *BCR-ABL1* in terms of miRNA binding conformity, protein interaction network, and pathways using *in silico* analysis. The 3'UTR sequence of *BCR-ABL1* is obtained from Ensembl Genome Browser while the binding conformity was determined using the PsRNATarget Analysis Server, RNA22, Target Rank Server, and DIANA TOOLS. Protein-protein interaction network and pathway analysis are determined using STRING, Cytoscape, and KEGG pathway analysis.

Results: Five plants and five human miRNAs show strong binding conformity with 3'UTR of *BCR-ABL1*. The strongest binding conformity was shown by *Oryza sativa*'s Osa-miR1858a and osa-miR1858b with -24.4 kcal/mol folding energy and a p value of 0.0077. Meanwhile, in human miRNA, the hsa-miR-891a-3p shows the highest miTG score of 0.99 with -12 kcal/mol folding energy and a p value of 0.037. Apart from *ABL1*, osa-miR1858a/osa-miR1858b and hsa-miR891a-3p also target other 720 and 645 genes, respectively. The interaction network of Osa-miR1858a/osa-miR1858b and hsa-miR891a-3p identifies nineteen and twelve *ABL1*'s immediate neighboring proteins, respectively. The pathways analysis focuses on the RAS, MAPK, CML, and hematopoietic cell lineage pathway.

Conclusion: Both plant and human miRNAs tested in this study could be a potential therapeutic prospect in CML treatment, but thermodynamically, osa-miR1858a/osa-miR1858b binding to *ABL1* is more favorable. However, it is important to carry out more research in vitro and in vivo and clinical studies to assess its efficacy as a targeted therapy for CML.

Keywords: *BCR-ABL1*, Chronic myeloid leukemia, Cross-kingdom, *In silico*, MicroRNA, 3' untranslated region

* Correspondence: marjanuhikmah@usim.edu.my

Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, Putra Nilai, Persiaran Ilmu, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia

Background

Chronic myeloid leukemia (CML) is a type of cancer that affects blood and bone marrow cells. It is a clonal disorder of multipotential hematopoietic stem cells that excessively induces proliferation of immature and mature myeloid cells [1]. CML patients are characterized by the accumulation of Philadelphia chromosome-positive (Ph⁺) myeloid cells. Ph chromosome is the result of a reciprocal translocation between Abelson (ABL) proto-oncogene on chromosome 9 and the breakpoint cluster area (BCR) on chromosome 22, t(9;22) (q34;q11) [2, 3]. This translocation generates the *BCR-ABL1* fusion gene, which is the key cause of CML by the development of highly active tyrosine kinase [4]. As the frontline treatment of CML, TKIs are used against the kinase activity of the BCR-ABL protein. However, because TKI resistance in some CML patients are due to tyrosine kinase domain (TKD) mutations, alternative treatments targeting other than TKD are needed [5–7]. Hence, miRNA is proposed as an alternative treatment for CML. This is due to the association between miRNA expression and the onset of TKI resistance [4]. MicroRNAs are small non-coding regulatory RNAs which suppress the expression of their target genes. It reduces the stability of messenger RNA (mRNA) and thus inhibits translation [8]. miRNA comprised 20 to 24 nucleotides that are involved in gene regulation and cell signaling. In cancer cells, miRNAs can modulate multiple dysregulated genes or signaling pathways. This is because gene regulation is controlled by the incomplete complementary binding between the seed sequence of miRNA with the 3' untranslated region (3'UTR) of mRNA. This enables miRNA to simultaneously control the expression of many target genes [9]. Certain miRNAs like hsa-miR-21 and hsa-miR-17-92 clusters are elevated in cancer cells. Meanwhile, other cancer-associated miRNA families such as the hsa-let-7 and hsa-miR-34 with hsa-miR-34 are downregulated [10, 11]. These outcomes suggested that miRNAs play a very important role in promoting or preventing cancer. miRNAs can be manipulated as cancer therapeutic agents using miRNA restoration agents known as miRNA mimics. These mimics are used to compensate for the loss of some miRNA [12, 13]. Meanwhile, another approach is by using the miRNA-inhibiting agents, the antagomiR which functions in blocking the aberrantly overexpressed miRNA. Despite the controversy of cross-kingdom inhibition of cancer cells by plant miRNA, there is a report which indicated that synthetic mimic of plant miRNAs of ath-miR159a (from *Arabidopsis thaliana*), gma-miR159a-3p, and gma-miR159e-3p (from *Glycine max*) was able to inhibit the proliferation of breast cancer cells [14]. Meanwhile, another study confirmed the ability of mol-miR168 (from *Moringa oleifera*) to downregulate the protein

expression of SIRT1, when its mimic was transfected into the human hepatoma cell line G2 (HEPG2) cells [15]. Therefore, to understand the RNA silencing mechanism, this study utilized *in silico* analysis in predicting the miRNA-mRNA interactions. The current study aimed to predict miRNAs that targeted the 3'UTR of the *BCR-ABL1* gene and compare their binding affinity, target genes annotation, pathways involved, and protein-protein interaction in relation to CML pathogenesis.

Method

Sequence of *BCR-ABL1* and miRNAs

The 3'UTR of *BCR-ABL1* gene sequence was obtained from the Ensembl Genome Browser (<https://www.ensembl.org>) [16]. miRNA sequences of the plants were obtained from the Plant Small RNA Target (PsRNATarget) Database (<http://plantgrn.noble.org/psRNATarget/>) [17]. Human miRNA sequences were obtained from miRbase (<http://www.mirbase.org/>) [18].

Predicting miRNA-mRNA target interaction in plants

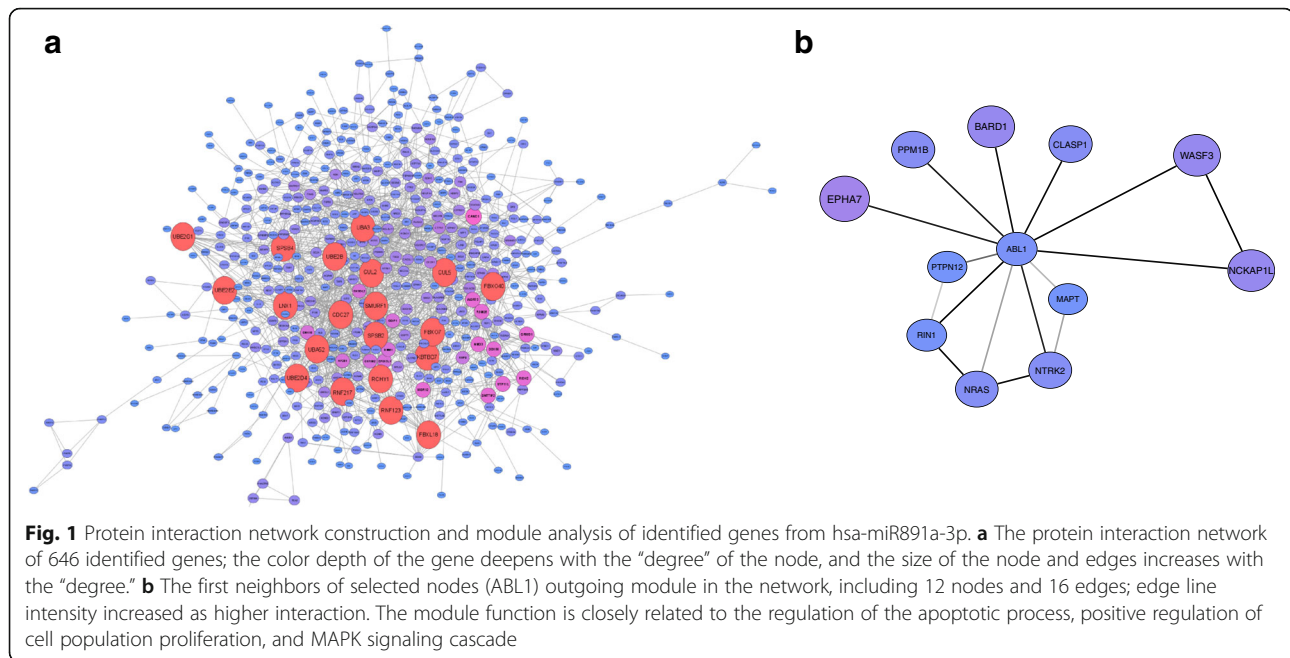
The PsRNATarget and Schema V2 criteria were used in predicting the interaction. The 3'UTR of the *BCR-ABL1* sequence was imported into the software, and miRNAs were selected based on expectation equal to or less than 5. Subsequently, RNA22 (<https://cm.jefferson.edu/rna22/Interactive/>) was used to determine miRNA folding energy, *p* value, and heteroduplex structure by inserting miRNA sequences and single target sequence [19]. Next, miRNA sequences were inserted into Target Rank (<http://genes.mit.edu/targetrank/>) to obtain the list of target genes [20].

Predicting miRNA-mRNA target interaction in human

Screening of human miRNAs targeting 3'UTR of *ABL1* was performed using DIANA TOOLS, with the microT-CDS [21] and miTG scores being set at more than 0.9. Target genes of each selected miRNA were also identified using microT-CDS with the threshold set at 0.7. Predicted miRNAs were later assessed based on their folding energy, *p* value, and predicted target site using the RNA22 software.

Protein-protein interaction (PPI) network

The interactions between genes could be analyzed quickly by constructing the protein interaction network. STRING which is an online website (<http://string-db.org>) was used to build the interactive networks of target genes. By restricting the condition to "human species," networks were constructed, and the "TSV" format file of the gene interaction relationship was downloaded, and then the file is imported into the Cytoscape software (<http://www.cytoscape.org/>) for further analysis and clustering [22]. The Cytoscape MCODE plug-in was



The first neighbors of selected nodes of the ABL1 module were then chosen for further analysis. This module had 12 nodes and 16 edges (Fig. 1b). Pathway enrichment analysis revealed that these nodes were primarily enriched in pathways such as RAS and MAPK signaling pathways. In addition, the encoded proteins were involved mainly in multiple cancer pathways specific to various signaling pathways, including the ErbB signaling pathway (hsa04012), CML (hsa05220), and microRNAs in cancer pathways (hsa05206). The genes that were involved in the pathways were NTRK2, RIN1, NRAS, PPM1B, MAPT, and ABL1. The Gene Ontology of biological processes is emphasized on the cell population proliferation, regulation of apoptotic process, and MAPK cascade (Table 2). Meanwhile, Osa-miR1858a and osa-miR1858b protein networks showed 588 nodes and 1925 edges (Fig. 2a) from 721 genes. The first neighbors of selected nodes of ABL1 displayed a module of 19 nodes and 39 edges (Fig. 2b). The pathway analysis exhibited an enriched pathway of hematopoietic cell lineage and CML. Furthermore, these nodes were also involved with pathways such as ErbB, RAS (hsa04014), Jak-Stat (hsa04630), and Wnt signaling pathways (hsa04310). The genes that were included in the pathways are CDL, STAT5B, BCR, SHC4, ABL1, RAC2, CCND2, PDGFB, XIAP, PAK2, CD5, and TFRC. Moreover, Gene Ontology of biological process focused on the cell population proliferation, hemopoiesis, regulation of apoptotic process, and regulation of MAPK cascade.

Discussion

CML is a myeloid clonal proliferation characterized by the translocation of the *BCR-ABL1* gene in the Philadelphia

chromosome. TKI is preferred as the frontline treatment to treat CML. However, in approximately 30% of CML patients, TKI resistance has become a challenging problem [23]. Therefore, finding the molecular targets for CML treatment is of primary importance to solve this problem. Thus, the current research aimed to determine and evaluate miRNAs from plants and humans that targeted the 3' UTR of the *BCR-ABL1* gene in CML in terms of folding energy, gene annotation, and gene enrichment analysis. It is known that miRNA expression is often altered in diseases, especially in cancers. Either acting as an oncogenic or a tumor-suppressive agent, miRNA had gained the researcher's interest to be used as an alternative therapeutic agent to enhance the current treatment for cancer. Furthermore, cross-kingdom studies of miRNAs between plant and animal kingdoms are intensely explored in recent days. Therefore, this research employed the *in silico* analysis to predict the specific plant and human miRNAs that can target 3'UTR of the human *BCR-ABL1* gene in CML. This is because a cross-kingdom interaction is possible due to the presence of various conserved regions in the human and plant genome [14, 24]. In addition, due to the highly conserved region in the composition of miRNAs processing machinery, the biogenesis and working mechanism of miRNAs in plants showed a similar molecular and development roles as in humans [25]. Besides, it was found that sequences of diverse endogenous plant miRNAs were complementary to the components of the genomes and transcriptomes of humans and animals [26].

The present study predicted plant miRNAs by using the psRNATarget. psRNATarget is a platform that was developed to identify plant small RNA (sRNA) targets by

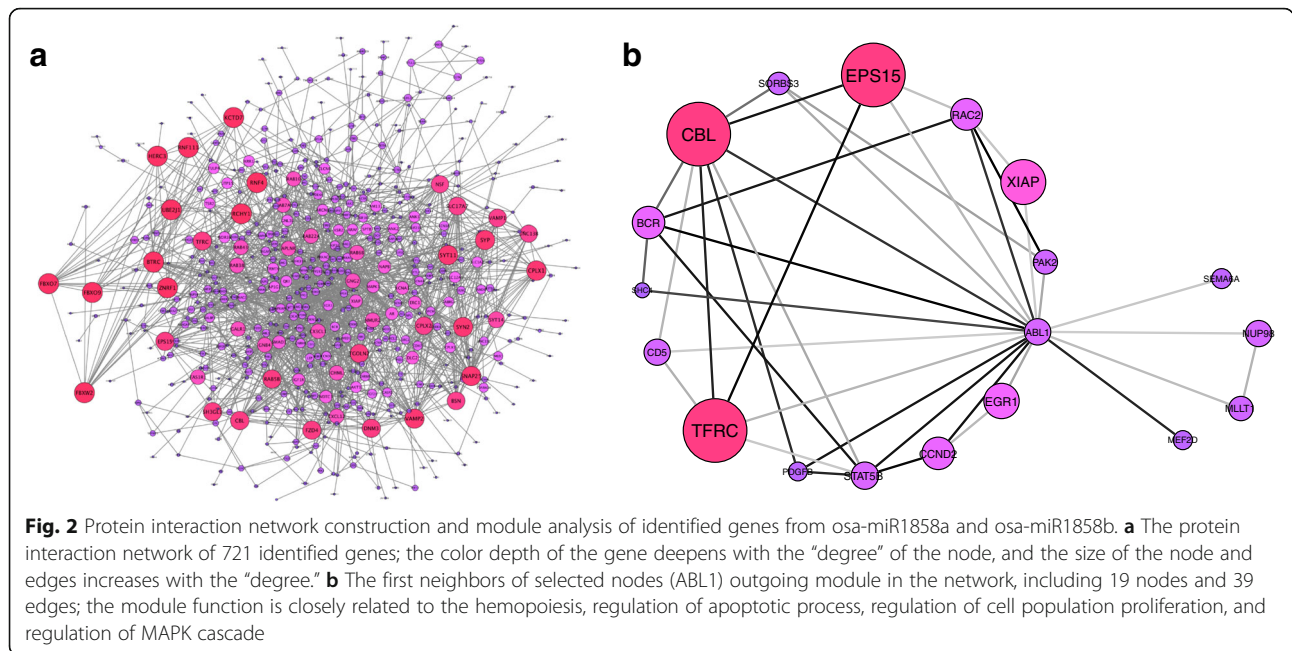


Table 2 The first neighbors of selected nodes (ABL1) of *osa-miR1858a*, *osa-miR1858b*, and *hsa-miR891a-3p* target genes, pathways, and Gene Ontology

miRNA	Target genes	Pathways involved	Biological process involved
Hsa-miR891a-3p	NRAS PTPN12 MAPT NTRK2 PPM1B NCKAP1L EPHA7 WASF3 BARD1 RIN1 CLASP1 ABL1	<ul style="list-style-type: none"> • RAS signaling • MAPK signaling • Chronic myeloid leukemia • ErbB signaling • MicroRNAs in cancer 	<ul style="list-style-type: none"> • Regulation of apoptotic process • Positive regulation of cell population proliferation • Regulation of MAPK cascade
Osa-miR1858a and osa-miR1858b	CCND2 NUP98 EPS15 TFRC SEMA6A XIAP PAK2 MEF2D RAC2 SHC4 STAT5B MLLT1 ABL1 CBL BCR SORBS3 EGR1 CD5 PDGFB	<ul style="list-style-type: none"> • Chronic myeloid leukemia • Pathways in cancer • ErbB signaling • RAS signaling • MicroRNA in cancer • Jak-Stat signaling • MAPK signaling • Hematopoietic cell lineage • Wnt signaling 	<ul style="list-style-type: none"> • Regulation of cell population proliferation • Regulation of apoptotic process • Hemopoiesis • Regulation of MAPK cascade

analyzing complementary matching between sRNA and target mRNA sequences. It employed a predefined grading schema that evaluated the target site accessibility between miRNA and mRNA [27]. The analysis was continued by exporting miRNA sequences into another platform including RNA22 and Target Rank for further validation. These platforms were specifically designed to predict the interaction between miRNA and mRNA in humans. Positive results from RNA22 and Target Rank indicated that the selected plant miRNAs were capable of binding with 3'UTR of *BCR-ABL1* in humans. From the *in silico* analysis, the current study predicted five plants and five human miRNAs that have binding conformity with 3'UTR of the *BCR-ABL1* gene. These miRNAs were selected based on their folding energy, p value, psRNA Target score, and miTG score. For plant miRNAs, *osa-mir1858a* and *osa-mir1858b* showed the most promising results. They have the lowest folding energy of -24.4 kcal/mol and a significant p value of 0.007. Thus, the binding between *osa-mir1858a* and *osa-mir1858b* and 3'UTR of *BCR-ABL1* was much favorable. Nevertheless, in human miRNAs, it was observed that *hsa-miR891a-3p* showed the highest miTG score of 0.99 with -12 kcal/mol folding energy and a p value of 0.037. Therefore, binding of *hsa-miR891a-3p* towards 3'UTR of *BCR-ABL1* was less favorable compared to *osa-mir1858a* and *osa-mir1858b* towards 3'UTR of *BCR-ABL1*. A miRNA can have multiple targets, and one mRNA can be targeted by many miRNAs [28]. This is observed in Table 1, showing that *hsa-miR891a-3p*, *osa-mir1858a*, and *osa-mir1858b* have numerous target genes despite *ABL1*. miRNAs can have multiple targets mainly due to the partial complementary between miRNA and its target mRNA which is known as the seed pairing [29, 30]. Because of this feature, *in silico* tools can predict thousands of miRNA-regulated genes. Recently, studies have found that *hsa-miR-891a* and *hsa-miR-891b* can be used as prognostic biomarker and therapeutic target in different types of cancer such as lung cancer [31], breast cancer [32], colorectal cancer [33], and gastrointestinal stromal tumor [34]. In concordance with the present study, *hsa-miR891a-3p*, *osa-mir1858a*, and *osa-mir1858b* were also predicted to have another 645 and 720 targets genes, respectively. Thus, it is important to analyze all the target genes of *hsa-miR891a-3p*, *osa-mir1858a*, and *osa-mir1858b*. Further analysis on the protein-protein interaction network and KEGG pathway showed that *hsa-miR891a-3p*, *osa-mir1858a*, and *osa-mir1858b* were all involved in the MAPK signaling pathway. The MAPK signaling pathway is very critical for cell existence, and its signaling cascade plays a central role in the pathogenesis of CML [35]. In addition, fundamental cellular processes such as cell proliferation and cell survival are regulated by this

pathway [36, 37]. Moreover, the RAS/MAPK signaling can also lead to the survival of positive BCR-ABL cells under imatinib pressure [38]. Previous findings suggested that apoptosis could be triggered by the inhibition of MAPK signaling in the K562 cell line, and the use of MAPK-specific inhibitors showed cooperative effects with imatinib in CML cells [39]. Furthermore, among the targeted genes of *osa-mir1858a* and *osa-mir1858b*, the STAT5 gene was one of the important targets. This is because STAT5 promoted imatinib resistance by significantly being involved in the activation of hematopoietic cells transformation by p210Bcr/Abl [40]. The activation of this gene would speed up cell division and inhibit DNA repair, which thus causes genomic instability such as the blast crisis of CML [40, 41]. In addition, the current Gene Ontology analysis also showed that STAT5, CCND2, and PDGFB were involved in the JAK/STAT pathway. This finding is supported by a previous study which indicated that STAT5 could regulate the transcription factor and modulate cytokine receptors and growth factors [42]. Therefore, this pathway is crucial in mediating cytokine signaling by promoting the growth of leukemic cells [40]. According to the KEGG pathway enrichment analysis of *osa-mir1858a* and *osa-mir1858b*, the pathways are concentrated on the hematopoietic cell lineage and CML pathway. miRNAs are important regulators involved in many cell functions including the hematopoietic cell lineages. It has been reported that the miRNA expression is regulated during hematopoietic differentiation, suggesting miRNA involvement in the process [43]. Thirteen miRNAs with differential expression in peripheral blood cells have been documented in a previous study, and these hematopoietic-specific miRNAs are essential for blood cells to function properly. In addition, another study also observed that the differently expressed miRNAs allowed the cell-specific transgene expression in human hematopoietic cell lineages [44]. Apart from that, *osa-mir1858a* and *osa-mir1858b* additionally involved in the ErbB, Jak-Stat, and Wnt pathways. The Wnt pathway plays an important function during the growth of normal hematopoietic stem cells (HSC) and leukemic stem cells (LSC). Activation of the Wnt/beta-catenin pathway plays a pivotal part in maintaining the self-renewal potential in HSCs. In CML, the *BCR-ABL1* oncogene stimulates the β -catenin within the blast stage thus encouraging the LSC reestablishment and repressing imatinib's activity [45]. In addition, another study demonstrated that the Wnt-3a pathway could promote the proliferation and survival of acute lymphoid leukemia cells [46]. Nevertheless, target gene annotation results observed that *osa-mir1858a* and *osa-mir1858b* targeted both the BCR and *ABL1* genes which perhaps might help in further reduction of CML cell proliferation. Therefore, the determination of miRNA's target pathways

and genes that are involved in imatinib resistance could be a crucial data that might perhaps be manipulated as alternative treatments for CML therapy.

Conclusion

In summary, Osa-miR1858a/Osa-miR1858b showed the strongest binding conformity to 3'UTR of *BCR-ABL1* with -24.4 kcal/mol folding energy. Nevertheless, hsa-miR891a-3p was chosen based on an excellent miTG score of 0.99 with -12 kcal/mol folding energy. The interaction network of Osa-miR1858a/osa-miR1858b and hsa-miR891a-3p identified 19 and 12 *ABL1*'s immediate neighboring protein, respectively. These proteins were involved in the RAS, MAPK, CML, and hematopoietic cell lineage pathway. miRNA application as a biomarker is being widely used. Therefore, the determination of plants and human miRNA determination using *in silico* analysis that could be used as the molecular targeted therapy for CML was our main objective in the current study. By using *in silico* analysis, this study successfully predicted miRNAs from plants and humans that were able to bind with 3'UTR of *BCR-ABL1*. Theoretically, these miRNAs are capable of suppressing *BCR-ABL1* expression, thus reducing CML cell proliferation. Therefore, it is predicted that these miRNAs could inhibit the *BCR-ABL1* by binding to the 3'UTR and would be the best potential molecular targeted therapy for CML. *In silico* study is an informative and powerful tool in helping the researcher to form an estimation of miRNA-mRNA target interaction. For future study, *in vitro* work could be carried out using CML cells to validate the Osa-miR1858a/osa-miR1858b and hsa-miR891a-3p capability in inhibiting CML cell proliferation by targeting 3'UTR of the *BCR-ABL1* gene.

Abbreviations

3'UTR: 3' untranslated region; CML: Chronic myeloid leukemia; HSc: Hematopoietic stem cells; KEGG: Kyoto Encyclopedia of Genes and Genomes; LSC: Leukemic stem cells; MAPK: Mitogen-activated protein kinase; miRNA: MicroRNA; mRNA: Messenger RNA; sRNA: Small RNA; TKD: Tyrosine kinase domain; TKI: Tyrosine kinase inhibitor

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Authors' contributions

All authors have contributed substantially and equally to the analysis and reporting of this study. SFSM analyzed and interpreted the data regarding the miRNA binding site and was the major contributor in writing the manuscript. MHE performed the plant miRNA analysis and revised and finalized the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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