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Targeting MicroRNA Precursors in Acute Myeloid Leukemia: A System Biology Approach Using CRISPR/C2c2

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Abstarct

Background: Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy characterized by the rapid proliferation of immature myeloid cells in the bone marrow. This condition arises from genetic mutations in myeloid progenitor cells, leading to disrupted hematopoiesis and an accumulation of leukemic blasts in the bloodstream. Despite advances in treatment modalities, including chemotherapy and targeted therapies, clinical outcomes for AML patients remain suboptimal due to high rates of relapse and treatment resistance. Recent studies have identified specific microRNAs (miRNAs) that play critical roles in cancer metastasis and progression, suggesting that targeting these miRNAs could offer novel therapeutic avenues. The CRISPR-C2c2 system, a RNA-targeting gene editing tool, has emerged as a promising method for precisely modulating miRNA expression. By inhibiting oncogenic miRNAs or restoring tumor-suppressive miRNAs, this approach aims to mitigate AML progression. This study investigates the computational design of crRNAs targeting hsa-miR-301b and hsa-miR-21, both implicated in AML metastasis, utilizing bioinformatics tools to enhance targeting specificity and efficacy.

Methods: In this study mirBase server miRNA sequences were used to design crRNAs targeting AML metastasis. After that UALCAN server was used for expression analysis. Bioinformatics software, specifically the CRISPR-C2c2 system, was used for computational analysis. Structural studies were performed on the crRNAs, and simulation and molecular docking investigations were conducted to improve accuracy.

Results: crRNAs targeting miR-301b and miR-21 exhibit significant structural resemblance with the binding energy seen in the healthy state.

Conclusion: Evaluating crRNAs for RNA-level editing requires more than just sequence-based assessments; including simulation and molecular docking studies can enhance accuracy.

Keywords: AML, miRNA, CRISPR/C2c2, System Biology, Precision Medicine

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Background:

When it comes to the origin, symptoms, and outlook, acute myeloid leukemia (AML) is a varied blood cancer. [1]. Despite current treatments, clinical outcomes in AML patients are generally unsatisfactory [2]. Leukemia, a clonal cancer, can arise from myeloid or lymphoid progenitors in the bone marrow. AML is caused by mutations in myeloid lineage precursor cells and dysregulation of bone marrow infiltration, resulting in the generation of immature myeloid cells and disruption of normal blood cell production. AML is recognized by elevated blast numbers in the bloodstream, with a diagnosis of 20% or greater blasts being considered AML [3]. The main reason for morbidity and mortality in AML patients is reported to be their high vulnerability to life-threatening infections and headaches [4]. Despite progress made in traditional cancer treatments like chemotherapy, radiotherapy, and surgery, cancer mortality has decreased in recent years. Yet, obstacles persist that contribute to the recurrence of cancer cells. These difficulties have resulted in the discovery of cancer treatments that target mutations. While conventional chemotherapy destroys rapidly dividing healthy and cancerous cells using toxic substances, targeted therapies focus on abnormal proteins produced by mutated genes. Malignant and nonmalignant cells typically have contrasting sensitivities due to the absence of tumorigenic mutations in normal cells targeted by drugs. As a result, targeted therapy frequently leads to quick and significant shrinkage of tumors and decreases the risk of toxicity in healthy cells caused by traditional chemotherapy [5].

At present, a new potential genetic manipulation method has surfaced in the form of a gene editing tool known as CRISPR (Cas) system, which stands for clustered regularly interspaced short palindromic repeats [6]. Research has shown that utilizing CRISPR/Cas is a more cost-effective, less prone to contamination, and more precise and reliable method for targeting miRNAs in cancer therapy compared to existing methods [7]. As there is a link between cancer progression and changes in miRNA levels, cancer treatments are being created based on two main strategies: inhibiting the overexpression of specific miRNAs and restoring the activity of miRNAs that act as tumor suppressors. Additionally, small molecule inhibitors, antago miR, and miRNA sponges are designed to target and reduce the levels of overexpressed miRNAs, especially those oncogenic miRNAs highly expressed in cancer cells. Yet, these methods have constraints and require more study to enhance effectiveness and lessen harm. For instance, miRNA sponges obstruct the miRNA seeding area, inhibiting numerous genes by targeting several miRNAs from the same family, while LNA-modified antisense oligonucleotides enhance nuclease resistance and shield the target probe from degradation. They are also unable to be placed at the beginning of the probe or a nearby nucleotide [8]. A newly discovered system called Cas13a (also known as C2c2) is capable of targeting RNA with guidance from RNA [4]. The gene editing systems that target RNA have demonstrated promising potential in treating not only cancer but also other malignant diseases through the manipulation of essential RNA molecules (including mRNAs and non-coding RNAs like microRNAs, lncRNAs, etc) [9]. miRNAs are tiny RNAs (18–24 nucleotides) that regulate mRNA post-transcriptionally, primarily by inhibiting translation or causing degradation when they bind to the 3' untranslated region of the mRNA [10]. miRNAs play a role in controlling various biological functions such as cellular differentiation, proliferation, and apoptosis; thus, they are readily detectable in blood samples and are non-invasive to collect [10]. miRNAs are crucial for cancer advancement as they have been observed to have crucial functions in controlling cancer signaling pathways and improving various factors such as tumor growth, angiogenesis, and metastasis [11, 12].

Although there have been numerous improvements in the CRISPR/Cas system, its application in diagnostics and gene therapy has only begun to be developed in the last few decades. In 2012, it was observed that modifying the guide RNA (gRNA) sequence allows for the customization of CRISPR nucleases, which is advantageous for gene editing [13]. In 2013, researchers managed to modify different cell types with the help of a particular Cas protein known as Cas9 [14]. It is a type II protein, which relies on the guide RNA's capability to attach to double-stranded DNA and then be cut by the Cas9 endonuclease [15]. Type II CRISPR systems, such as Cas9 with RuvC and HNH nuclease domains, and type V systems with a single RuvC domain-containing effectors like Cpf1, C2c1, and C2c3, fall under the Class 2 category.

So far, all systems that have been functionally characterized only focus on DNA, with type III-A and III-B multicomponent systems being the only ones that also target RNA in addition to DNA. Even though the putative class 2 type VI system reportedly has only one effector protein C2c2, which does not share similarities with any established DNA nuclease domain, it does contain two Nucleotide-binding (HEPN) domains from Higher Eukaryotes and Prokaryotes.

Given that every identified HEPN domain is an RNase, likely that C2c2 functions exclusively as an RNA-targeting CRISPR effector. C2c2, with two HEPN domains, indicates it functions as a single-unit endoribonuclease [4]. In 2015, two proteins known as Cas12a and Cas13a, identified for genome editing, were initially designated as Cpf1 and C2c2, respectively [16-18]. Once Cas13a identifies and connects to the specific RNA, it will trigger the ability to cleave other non-targeted RNAs. Nevertheless, the eukaryotic species did not demonstrate the collateral cleavage activity of this system, and there was limited understanding of its underlying mechanism [19]. Up to now, this particular CRISPR-based RNA targeting technology has been utilized in biomedical settings, like identifying particular sequences of viral RNA or tumor RNA circulating in patients [20].

A new approach for regulating post-transcriptional acute myeloid leukemia (AML) metastasis was suggested in this study by utilizing bioinformatics software to target microRNA precursors. Additionally, to enhance the precision and specificity of the CRISPR-C2c2 technique proposed in this research, initial assessments were carried out at the crRNA target design stage, followed by structural investigations utilizing bioinformatics methods. Employing bioinformatics tools, particularly the CRISPR-C2c2 system, plays a crucial role in decreasing costs, time, and trial-and-error frequency.

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Methods Sequence retrieval

The initial miRNA sequences of the target were downloaded from the miRBase server (https://www.mirbase.org) in FASTA format using the keywords "hsa-mir-301b" and "hsa-mir-21" and their corresponding accession numbers, MI0005568 and MI0000077. The miRBase website provides a wide range of data on microRNAs that have been published. This consists of information on their sequences, precursors in biogenesis, coordinates in the genome, surrounding context, citations to pertinent literature, deep sequencing expression data, and annotation from the community. Additionally, miRBase functions as a hub linking users to external sources that offer more details on microRNA genes and sequences. These resources include both predicted and experimentally validated targets of microRNAs (miRNAs) [1].

Expression and Survival Rate of miRNAs

To assess the expression levels of hsa-miR-21 and hsa-miR-301b in Acute Myeloid Leukemia (AML) samples, the UALCAN server (http://ualcan.path.uab.edu) was utilized. This tool allows for the analysis of gene expression data across various cancer types using the Cancer Genome Atlas (TCGA) dataset. The expression levels of the selected microRNAs were compared to normal tissue controls to establish their significance in AML [21, 22].

Secondary structure analysis of hsa-mir-301b and hsa-mir-21

The RNAfold server was used to determine the lowest free energy of secondary structure for hsa-mir-301b and hsa-mir-21 at(http://rna.tbi.univie.ac.at/cgibin/RNAWebSuite/RNAfold.cgi) . Access to the basic secondary structure prediction program on the ViennaRNA web services is provided by the RNAfold server. Basically, the RNAfold algorithm uses dynamic programming and thermodynamic energy parameters to find the minimum free energy (MFE) and the secondary structure of a single RNA molecule. In addition, it computes the partition function of the equilibrium ensemble of all secondary structures, allowing for the prediction of base pair probabilities and reliability measures for the MFE [2].

Design and Simulation of crRNAs Targeting for Enhanced Interaction with CRISPR-C2c2

The CRISPR-RT server (http://bioinfolab.miamioh.edu/CRISPR-RT) was employed to create and simulate crRNAs targeting hsa-miR-301b and hsa-miR-21. This specialized algorithm is designed specifically for the CRISPR-C2c2 method, offering a range of parameters to ensure accurate crRNA design [3]. Key parameters include the size of the matching region, protospacer adjacent motif (PAM) sequence, and the permissible number of mismatches or gaps. By defining these specific parameters, the CRISPR-RT system generates a variety of crRNA sequences based on the input target sequence data [23].

Prediction and Structural Analysis of crRNAs Targeting CRISPR-C2c2 Interaction

The 3D structure of the CRISPR-C2c2 enzyme (Cas13a) was obtained from the Protein Data Bank (https://www.rcsb.org) using the code 5WTK. To forecast the 3D structure of the designed crRNAs and investigate their interactions with the CRISPR-C2c2 enzyme, the RNAComposer server was utilized. RNAComposer provides an efficient platform for the automated prediction of extensive RNA 3D structures, operating on principles of machine learning and leveraging the RNA FRABASE database to link RNA secondary structures with their tertiary configurations [10, 11, 24]. Additionally, the Mfold server was employed to predict the secondary structure and perform energy dot plot analysis for hsa-miR-301b and hsa-miR-21 crRNAs, further enhancing our understanding of their structural dynamics [25].

Molecular docking analysis of hsa-mir-301b and hsa-mir-21 crRNAs

Determining the binding sites of CRISPR-C2c2 with target crRNAs involved a search in the Uniprot database (https://www.uniprot.org/uniprotkb/P0DOC6/entry) for predictions. The HDOCK server at http://hdock.phys.hust.edu.cn was utilized to perform molecular docking simulations with the CRISPR-C2c2 enzyme (cas13a) and simulated crRNAs. This server is specifically created for molecular docking of different types of interactions, such as protein-protein, protein-DNA, and protein-RNA. HDOCK utilizes a worldwide docking strategy to produce intricate structures, removing the requirement for prior knowledge of the binding interface range [26]. The researchers used the Rosetta-Vienna RNP-ΔΔ method (https://rosie.rosettacommons.org/rnp_ddg) to forecast the binding strengths between crRNA and CRISPR-C2c2 enzyme. This was accomplished by combining 3D structure modeling and energetic calculations utilizing RNA secondary structure [27].

Data visualization

The 3D structure of hsa-mir-301b and hsa-mir-21 crRNAs was displayed using the RCSB 3D viewer (http://rcsb.org/3d-view). Furthermore, the Pymol program version 2.5.5 was employed to showcase the 3D configuration of the CRISPR-C2c2 enzyme, highlighting the domain and examining the connections between hsa-mir-301b and hsa-mir-2 crRNAs with the CRISPR C2C2 enzyme.

Results

Sequence retrieval

The microRNAs chosen for this study have been specifically selected due to their significance in multiple signaling pathways linked to AML metastasis, as shown by previous research. To start the analysis, the precursor miRNA sequences for hsa-mir-301b and hsa-mir-21 were acquired in FASTA format from the trusted miRbase database. It is important to mention that adult miRNAs were not considered as appropriate targets because of their small size and the risk of off-target effects. This careful method guarantees that our examination concentrates only on miRNAs with the most significant influence on AML spread, thus improving the precision and dependability of our results. The miRNAs of interest are presented in **Table 1.**

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miRNA	Precursor sequence
hsa-miR-301b	GCCGCAGGUGCUCUGACGAGGUUGCACUACUGUGCUCUGAGAAGCAGUGCAAUGAUAUUGUCAAAGCAUCUGGGACCA
hsa-	UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACAC

Table 1: *miRNAs involved in precursor*

Expression and Survival Analysis of MicroRNAs Using UALCAN

hsa-mir-301b Expression and Survival Profile Based on Patients' Cancer Stages and Overall Survival

The expression analysis of has-miR-301b across different stages of AML indicates that its regulation is most pronounced in M3. Furthermore, the analysis reveals that, except for M6 and M7, expression levels remain consistently high, underscoring the significance of this miRNA **(Fig. 1-A)**. In contrast, the survival rate demonstrates a low expression level across various individuals **(Fig. 1-B)**.

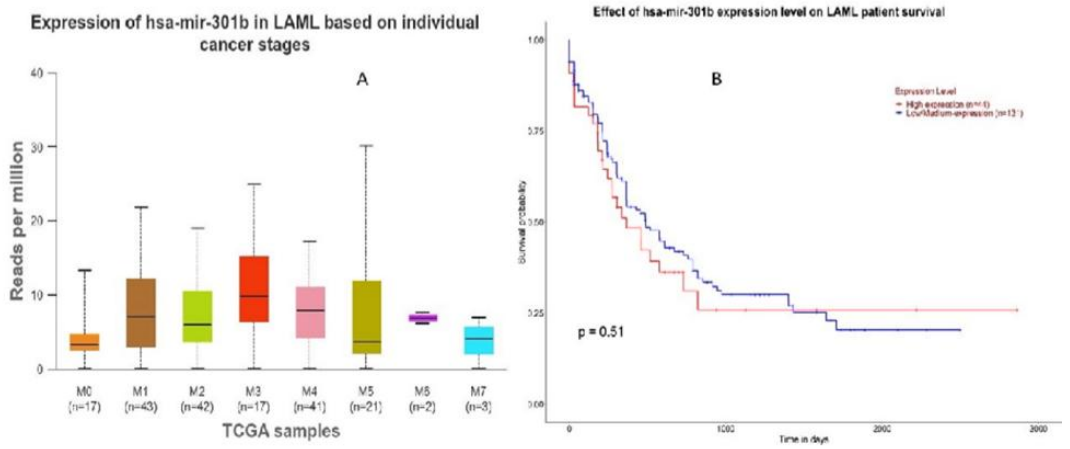
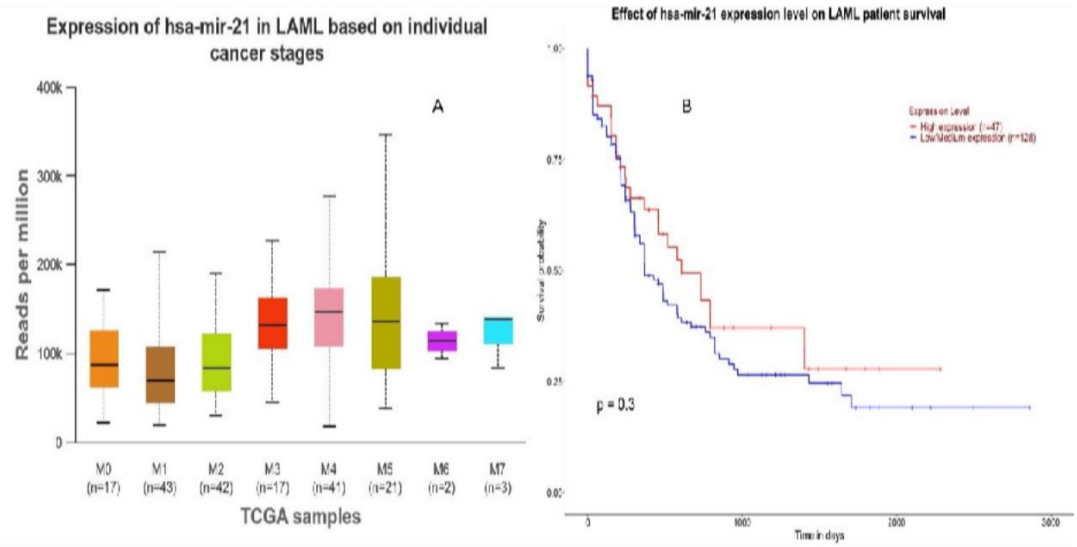


Figure 1: *Role of hsa-mir-301b in Different Cancer Stages (A), Overall Survival rate (B) hsa-mir-21 Expression and Survival Profile Based on Patients' Cancer Stages and Overall Survival*

The survival rate of has-miR-21 exhibits high expression levels over the days **(Fig. 2- B)**, with particularly elevated expression observed in M5 **(Fig. 2- A)**. However, its expression remains consistently high when compared to M6 and M7 **(Fig. 2-A)**.



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Figure 2: *Role of hsa-mir-21 in Different Cancer Stages (A), Overall Survival rate (B)*

Secondary structure analysis of hsa-mir-301b and hsa-mir-21

An analysis of secondary structures was carried out to determine the best areas for predicting the crRNA of the target miRNA in upcoming studies. The RNAfold server was used to forecast thermodynamic characteristics for hsa-mir-301b and hsa-mir-21. As per the RNAfold findings, the thermodynamic ensemble's free energy was measured at -32.53 kcal/mol for hsa-mir-301b and

-36.04 kcal/mol for hsa-mir-21. Significantly, hsa-mir-301b showed a greater variety in its ensemble (5.75) than hsa-mir-2 (3.18), leading to hsa-mir-301b having a lower minimum free energy (MFE) below the MFE chart threshold, unlike hsa-mir-21. Additionally, when examining the entropy of hsa-mir-301b and hsa-mir-21, it was found that the sections of these miRNAs marked by * at the beginning and end are the most ideal for crRNA prediction due to their lower entropy compared to other regions **(Fig. 3a, b)**.

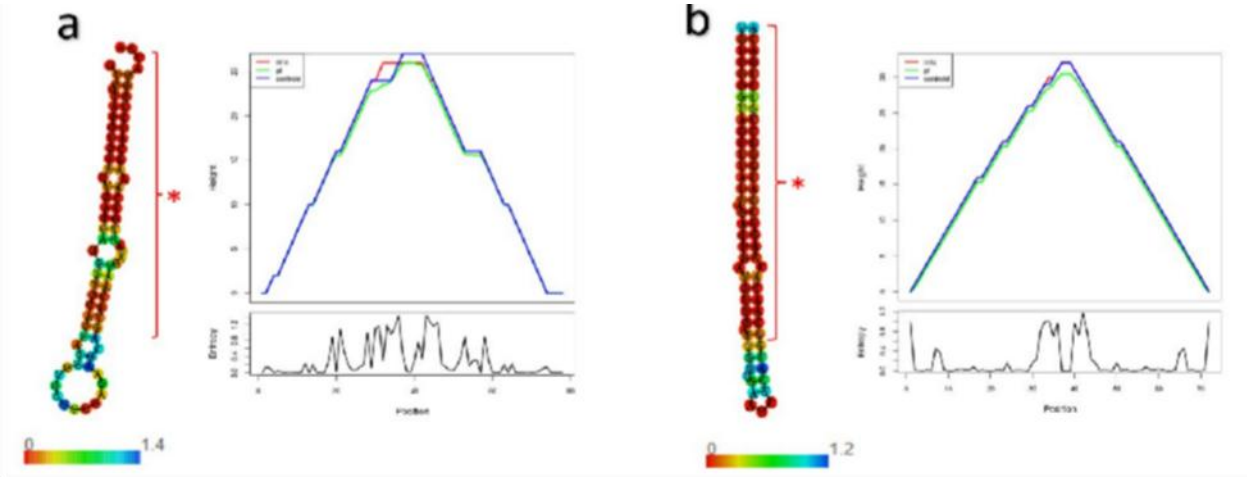


Figure 3: *Secondary structure analysis of target miRNAs by RNAfold. a: RNAfold results of mir-301b, b: RNAfold results of mir-21.*

Prediction of crRNA sequence for MicroRNA Precursors

This study presented a new method for controlling AML metastasis at the post-transcriptional level by utilizing bioinformatics tools and focusing on microRNA precursors. Moreover, in order to improve the accuracy and efficiency of the suggested technique (CRISPR-C2c2), comprehensive examinations were carried out while designing the target crRNA, which involved structure forecasting. The CRISPR-C2c2 system showed significant decreases in expenses, trial frequency, and researcher mistakes. By utilizing the findings from the CRISPR-RT tool, suitable hsa-mir-301b and hsa-mir-2 crRNAs were chosen based on factors like crRNA start and end positions, GC% composition, and the minimum non-target sites in the Genome and Transcriptome. These factors show the specificity of the planned sequence. CRISPR-RT designs crRNAs that are inserted into a scaffold structure, with the designed part targeting the desired region and the scaffold part activating the CRISPR-C2c2 enzyme through its secondary and tertiary structures. The crRNAs that were created for the chosen miRNAs are provided in **Table 2.**

No	miRNAs	Protospacer flanking site (crRNA)	Start	En d	G C %	Transcri pt Target	Gene Targ et
1	hsa- mir-301b	5′- GCCGCAGGUGCUCUGACGAGGUUGCAC UA	1	29	64	1	1
2	hsa- mir- 21	5′- GGCAACACCAGUCGAUGGGCUGUCUGA CA	44	72	61	2	1

Table 2: *crRNAs designed for miRNA precursors using the CRISPR-RT server*

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Structure prediction and Analysis of crRNAs target

The MFold web server was used to predict the secondary structure of hsa-mir-301b and hsa-mir-21 crRNAs from their primary sequences, and the free energy of the target crRNAs was then determined **(Fig. 2a, d)**. The studied crRNAs exhibit excellent stability with a free energy of less than -1.99 kcal/mol. At 37 °C, the Mfold server showed the most accurate free energy calculations for hsa-mir-301b and hsa-mir-21, with values of -9.7 kcal/mol and -6.4 kcal/mol, respectively. Furthermore, the energy dot plot examination of hsa-mir-301b and has-mir-21 crRNAs reveals that hsa-mir-301 crRNA has a more stable configuration compared to has-mir-21 crRNA, potentially enhancing its interaction with CRISPR-C2c2 **(Fig. 4c, f)**. RNA Composer was used to predict the tertiary structure of hsa-mir-301b and hsa-mir-2 crRNAs **(Fig. 4b, e)**.

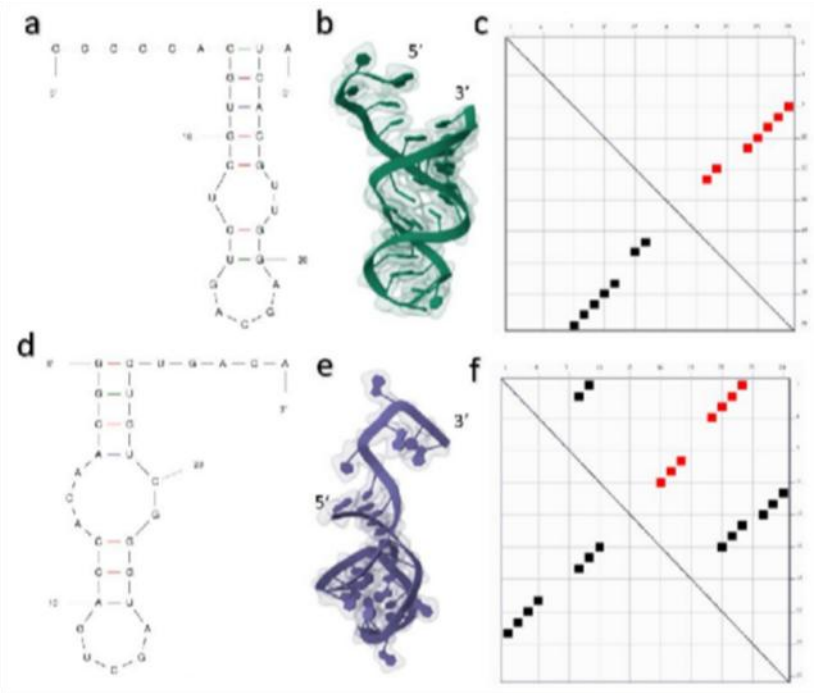


Figure 4: *3D structure prediction and analysis of target crRNAs. a: secondary structure of mir-301b crRNA, b: 3D structure of mir-301b crRNA, c: energy dot plot of mir-301b crRNA. d: secondary structure of mir-21 crRNA, e: 3D structure of mir-21 crRNA, d: energy.*

Detection of CRISPR/C2c2 domains and crRNA binding sites

CRISPR-C2c2 consists of two primary lobes: the crRNA recognition (REC) lobe and the nuclease (NUC) lobe (Fig. 5a). The REC lobe is made up of an N-terminal domain (NTD) and a Helical-1 domain, whereas the NUC lobe includes two HPN domains, a Linker joining them, and a Helical-2 domain. The REC lobe contains a space located between the NTD and Helical-1 regions. The NTD domain, found at the beginning of C2c2, shows the lowest level of conservation among all parts of C2c2. The NTD-facing surface of the Helical-1 domain is positively charged, creating the channel for crRNA binding. The NUC lobe contains two enduring enzymatic regions, HPN1 and HPN2, as well as a Helical-2 region and a Linker. The crRNA binding locations of CRISPR-C2c2 were discovered through prior research and the Uniprot database **(Fig. 5b)**.

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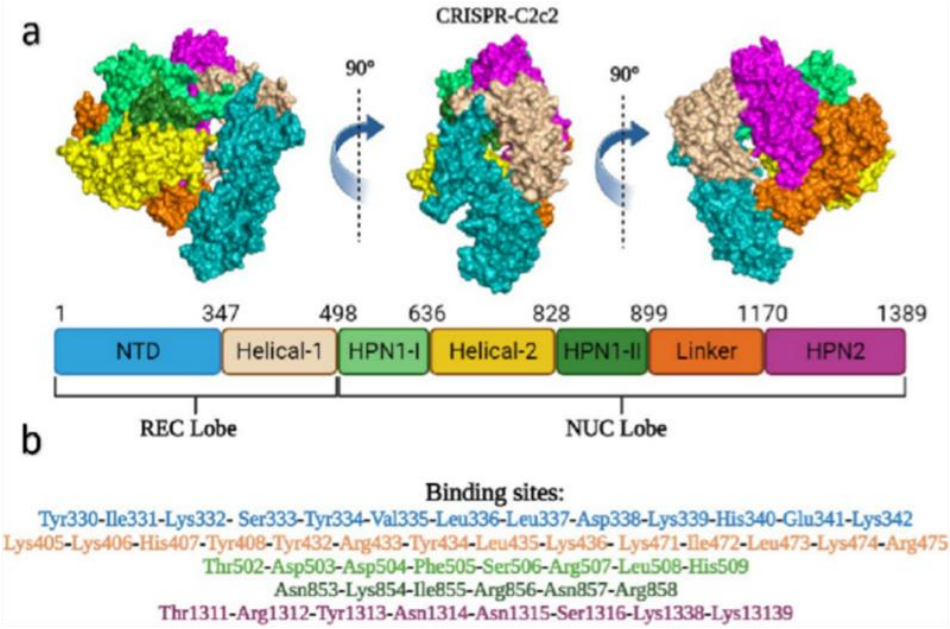


Figure 5: *CRISPR-C2c2 domains and crRNA binding sites. a: CRISPR-C2c2 domains, b: crRNA binding sites.*

Molecular docking analysis of hsa-mir-301b and hsa-mir-21 crRNAs

By utilizing the HDOCK server, 100 docking models were simulated for each target crRNA with the CRISPR-C2c2 complex. The top models of hsa-mir-301b and hsa-mir-21 crRNA were chosen from 100 predicted models using docking score and ligand RMSD (Table 3). Additionally, binding energy was determined by the Rosetta-Vienna RNP-ΔΔ server using the 3D structure of hsa-mir-301b and hsa-mir-21 bound with CRISPR-C2c2.

No	crRNAs	Docking Score	Ligand rmsd (Å)	Binding energy (kcal/mol)	Number of interactions
1	hsa-mir-301b	895	135.62	-9.73	64
2	hsa-mir-21	931.69	15.88	-8.78	51

Table 3: *HDOCK server results based on the interaction between protein CRISPR-C2c2 and crRNA*

The CRISPR-C2c2 docking complex, along with the engineered crRNAs, was assessed and studied utilizing HDock receptor-ligand interface components and Pymol software. According to the findings, the level of interaction was greater between hsa-mir-301b crRNA and CRISPR-C2c2 compared to hsa-mir-21 crRNA complexed with CRISPR-C2c2 **(Table 3, Fig. 6)**. The highest interaction between hsa-mir-301b crRNA and CRISPR-C2c2 occurred in the NTD and Helical-1 domain, whereas for the hsa-mir-21 crRNA complexed with CRISPR-C2c2, the interaction was more prominent in the Helical-1 and HPN2 domains, as shown in Figure 6a, b. According to the analysis of the 3D structure of CRISPR-C2c2 bound to hsa-mir-301b and hsa-mir-21 crRNAs, hsa-mir-301b crRNA was found close to the surface of CRISPR-C2c2 and showed higher interaction with NTD and Helical-1 domains compared to hsa-mir-21 crRNA **(Fig. 4a)**. On the other hand, the hsa-mir-21 crRNA was located inside the CRISPR-C2c2 pocket compared to the hsa- mir-301b crRNA, leading to interaction with the HPN1-II domain in both cases **(Fig. 6b)**.

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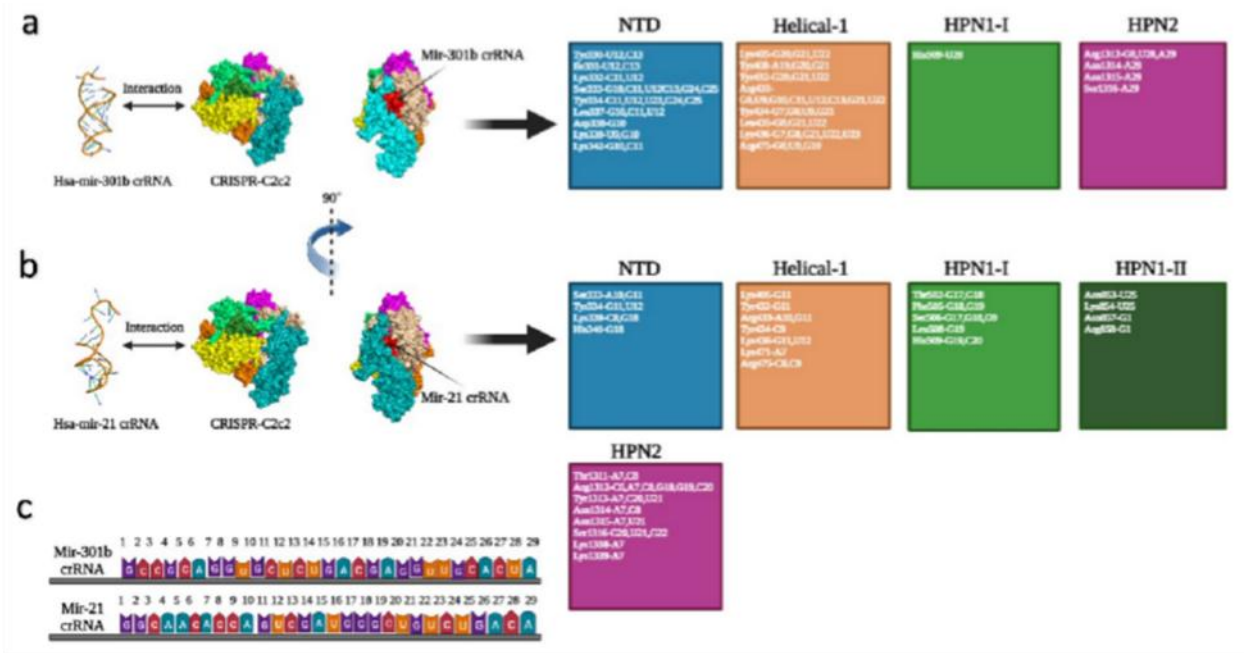


Figure 6: *Molecular docking analysis of mir-301b and mir-21 crRNAs. a: docking analysis of mir-301b crRNA in different domains, b: docking analysis of mir-21 crRNA in different domains, c: numerical sequence of mir-301b and mir-21 crRNAs.*

Pymol software was used for 3D analysis of the interaction between CRISPR-C2c2 and hsa-mir-301b and hsa-mir-21 crRNAs in two key domains - NTD and Helical-1, during the interaction process (**Fig. 7**).

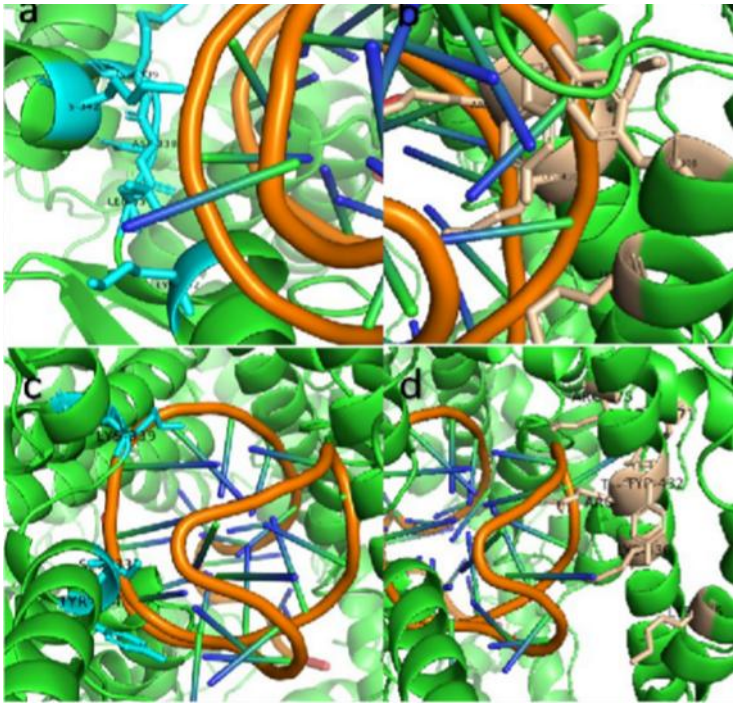


Figure 7: *3D analysis of interaction between CRISPR-C2c2 and target crRNAs. a: interaction NTD domain with mir-301b crRNA, b: interaction Helical-1 domain with mir-301b crRNA, c: interaction NTD domain with mir-21 crRNA, d: interaction Helical-1 domain with mir-21 crRNA.*

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Discussion:

AML makes up approximately 80% of leukemia cases and is the most prevalent type of leukemia seen in adults. AML is defined by mutations in genes linked to blood cell formation. These genetic changes result in the increased growth of immature myeloid cells in the blood and bone marrow, causing problems with red blood cell production and bone marrow function [28]. Frequently, individuals going through AML treatments experience challenging side effects, indicating the necessity to create treatments like gene-targeted therapies that can improve quality of life [29].

In the initial phase of this study, our primary objective was to identify suitable and commonly expressed miRNAs, accomplished through the UALCAN web server [21, 22]. The analysis revealed that the survival rate of has-miR-301b was notably low during disease progression, while has-miR-21 exhibited higher levels in certain individuals. Both miRNAs showed elevated expression primarily during the middle stages of cancer. However, aside from M6 and M7, both maintained relatively high expression levels, potentially linked to their roles in metastasis. The lower expression in M6 and M7 may indicate that metastasis had already occurred in earlier stages of the disease.

The C2c2 system's focus on RNA provides a means to manipulate gene expression at a post-transcriptional level. This is particularly advantageous in systems biology, where understanding.

The dynamic interactions within cellular networks are crucial. By using C2c2 for targeted RNA degradation, researchers can assess the functional roles of specific RNA molecules and their contributions to cellular behavior and pathways. This allows for high-throughput analyses of gene functions and interactions, facilitating the construction of synthetic regulatory networks and enhancing our understanding of cellular responses to various stimuli [30]. In cancer biology, the CRISPR/C2c2 system has been utilized for specific gene knockdown, such as targeting oncogenes or mutant forms of tumor suppressor genes. For instance, studies have demonstrated that C2c2 can effectively reduce the expression of the mutant EGFRvIII in glioma cells, leading to decreased tumor growth and angiogenesis. This specificity is crucial for developing targeted therapies that minimize off-target effects [31, 32]. When compared to RNAi, the CRISPR-Cas13a system shows higher efficiency in suppressing gene expression in bacteria, plants, and mammalian cells [33]. Research conducted by Zhao et al. revealed that the CRISPR-Cas13a system achieves over 90% knockdown efficiency in targeting KRAS-G12D mRNA, without impacting wild-type KRAS mRNA, leading to tumor growth inhibition and apoptosis in pancreatic cancer [34]. Furthermore, it has been demonstrated to be capable of suppressing the proliferation of glioma cells in both laboratory settings and living organisms [35]. In a separate research conducted by Natalia Rivera-Torres et al, on the application of CRISPR in treating AML, they employed CDM, a technique that involves an in vitro gene editing system using CRISPR-directed gene editing to produce expression vectors. These vectors, when combined with a specific donor DNA fragment, can mimic various mutations found in FLT3 (the most commonly mutated gene in AML) in a reliable and accessible manner [36].

Many factors play a role in the development and outcome of AML. Because of the significance of microRNAs in the onset and progression of AML. Recent research has highlighted the importance of miR-301 and miR-21 expression in leukemia. MiRNA-301b is a cancer-promoting miRNA that is upregulated in many types of cancer, such as AML, breast cancer, and colorectal cancer. A recent study by Lu et al. discovered that miR-301b is responsible for promoting proliferation and blocking programmed cell death in AML cells by targeting FOXF2 through the Wnt/β-catenin pathway [37]. Furthermore, elevated levels of miR-301b in bladder cancer cells can result in decreased levels of USP13, ultimately leading to suppression of PTEN, a crucial tumor suppressor protein [38]. Studies show that miR-21 is an important cancer-related microRNA that is increased in various cancer forms such as breast and colorectal cancers. This specific miRNA focuses on various crucial genes, such as PTEN [39] and PDCD4 (protein involved in cell death programming) [40]. A recent research in AML models resulting from activation of HOX transcription factors showed that inhibiting miR-21 and miR-196b could prevent leukemia initiation [41]. Based on the results, numerous studies have examined the crucial functions of miRNA in the AML model [42-44]. The CRISPR/C2c2 system has been used for other roles, such as early detection of cancer [45] and infectious diseases like COVID-19 [46]. In a study by Tan and colleagues, this system was used to detect two important biomarkers, circROBO1 and BRCA1, in breast cancer [47].

Wessels et al. introduce Cas13 RNA Perturb-seq (CaRPool-seq), a novel technique for efficient combinatorial targeting of RNA transcripts in single cells using the CRISPR-Cas13d system. This method enables simultaneous perturbation of multiple genes and integrates with single-cell RNA sequencing for detailed gene expression analysis. The study highlights its advantages over traditional methods, enhancing the understanding of gene interactions and cellular responses [48]. It may seem that this type of CRISPR system has been mostly used for diagnostic purposes for DNA, RNA, or mutations, but various studies have also examined the therapeutic aspect of this system and found it very promising [49]. Although our study is a systems biology approach, other studies have used the CRISPR/C2c2 system in in vitro studies for cancer, such as bladder [50] and Lung [51].

As a result of these investigations, we chose to analyze the effects of miR-301b-3p on AML using bioinformatics resources. Bioinformatics is a critical tool for handling large amounts of biological data. In modern biotechnology, a variety of tools and software are utilized to analyze biological data and address medical and biological inquiries by applying mathematics and statistics.

Our study with high novelty is among the few systems biology studies on AML, and under suitable conditions for validation, it can be very promising.

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Conclusion:

The investigation into the application of the CRISPR-C2c2 system for targeting microRNAs associated with acute myeloid leukemia (AML) metastasis reveals promising advancements in the realm of precision oncology. This study meticulously elucidates the computational methodologies employed to design crRNAs specifically aimed at hsa-miR-301b and hsa-miR-21, both of which are implicated in the progression of AML. By integrating bioinformatics tools with molecular docking and structural analysis, the research not only enhances the specificity of crRNA design but also demonstrates the potential for significant therapeutic implications.

The findings of this study underscore the critical role of computational approaches in refining CRISPR-based interventions for AML. The successful targeting of miRNA precursors through the CRISPR-C2c2 system represents a novel strategy for post-transcriptional regulation, potentially mitigating metastasis and improving clinical outcomes. Future research should focus on translational applications, assessing the efficacy and safety of these targeted therapies in clinical settings. Moreover, as our understanding of miRNA dynamics in cancer biology deepens, the integration of advanced computational techniques will be essential for developing innovative therapeutic modalities that can adapt to the complexities of AML and other malignancies. The ongoing evolution of CRISPR technology holds substantial promise for revolutionizing cancer treatment paradigms, paving the way for more effective and personalized approaches to patient care.

Decelerations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

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Availability of data and materials

The datasets generated and analyzed during the current study are available in the miRBase: the microRNA database, CRISPR-RT(RNA Targeting) server, protein database, HDOCK server, PyMol, RNAComposer, and RNA FRABASE repository, UALCAN Server [(https://www.mirbase.org/, http://bioinfolab.miamioh.edu/CRISPR-RT/, https://www.rcsb.org, http://hdock.phys.hust.edu.cn/, https://pymol.org/2/,https://rnacomposer.cs.put.poznan.pl/ , http://rnafrabase.cs.put.poznan.pl/, http://ualcan.path.uab.edu].

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