Bioinformatic Tools for the Identification of MicroRNAs Regulating the Transcription Factors in Patients with β-Thalassemia

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ABSTRACT: β-thalassemia is a significant health issue worldwide, with approximately 7% of the world's population having defective hemoglobin genes. MicroRNAs (miRNAs) are short noncoding RNAs regulating gene expression at the post-transcriptional level by targeting multiple gene transcripts. The levels of fetal hemoglobin (HbF) can be increased by regulating the expression of the γ-globin gene using the suppressive effects of miRNAs on several transcription factors such as MYB, BCL11A, GATA1, and KLF. An early step in discovering miRNA:mRNA target interactions is the computational prediction of miRNA targets that can be later validated with wet-lab investigations. This review highlights some commonly employed computational tools such as miRBase, Target scan, DIANA-microT-CDS, miRwalk, miRDB, and micro-TarBase that can be used to predict miRNA targets. Upon comparing the miRNA target prediction tools, 4 main aspects of the miRNA:mRNA target interaction are shown to include a few common features on which most target prediction is based: conservation sites, seed match, free energy, and site accessibility. Understanding these prediction tools' usage will help users select the appropriate tool and interpret the results accurately. This review will, therefore, be helpful to peers to quickly choose a list of the best miRNAs associated with HbF induction. Researchers will obtain significant results using these bioinformatics tools to establish a new important concept in managing β-thalassemia and delivering therapeutic strategies for improving their quality of life.

KEYWORDS: Bioinformatics, beta-thalassemia, hemoglobinopathy, microRNAs, gamma-globin

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Introduction

Hemoglobinopathies are the most common hereditary disorders spread worldwide. They impose a heavy burden on families and the healthcare system in India as they are a major cause of morbidity and mortality.[1] Hemoglobinopathies include thalassemia and sickle cell anemia and its variants such as hemoglobin S, D, and E.[2] Thalassemias are broadly classified into "α-thalassemia" and "β-thalassemia" based on the mutation in the corresponding globin gene, either α or β globins, which are present on chromosomes 16 and 11, respectively. They are inherited in an autosomal recessive manner resulting in an abnormal production of adult hemoglobin (HbA), ineffective erythropoiesis, and shortened red-cell survival.[3,4] The heterozygotic form of thalassemia is asymptomatic, whereas individuals who inherit the defective genes from each parent are homozygotes, expressing life-threatening clinical manifestations. The decrease or absence of production of the β-globin chains of the hemoglobin molecule causes β-thalassemia. More than 200 mutations have been found in the hemoglobin subunit beta (HBB) gene or its immediate flanking region. Most frameshift mutations are caused due to single nucleotide substitutions, nucleotide insertions, or deletions. Occasionally, β-thalassemia is also caused due to gross gene deletion.[5,6] The most common mutations found in patients with β-thalassemia include 29% of CD17 (A>T), 27% of CD 41-42 (-TTCT), 14% IVS-II-654 (C>T), 6% CD26 (G>A), and 3% CD26/CD27.[7] In this review, we shall discuss the bioinformatic approaches to explore the differentially expressed microRNAs (miRNAs) inducing the γ-globin gene in patients with β-thalassemia. For better understanding, we shall begin with different types of thalassemia based on clinical severity, the role of transcription factors involved in the switching of fetal to HbA, biogenesis of miRNAs, and finally, the bioinformatic tools which would assist in the identification of significant miRNAs having a potential to be used as a diagnostic marker or therapeutic target.

Classification of Thalassemia

According to the severity of the clinical phenotype, thalassemia was previously graded as minor, intermediate, or major. Thalassemia major refers to patients with extreme anemia that manifest at a young age and require lifelong blood transfusions and iron chelation therapy. In contrast,

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thalassemia minor relates to asymptomatic individuals with moderate anemia, and a heterozygous genetic mutation.[8] The latter category does not require blood transfusion but may need genetic counseling in the premarital or prenatal stage. Patients with thalassemia intermedia present with a range of clinical severity from mild, moderate, to moderately severe anemia, requiring no blood transfusions to periodic and regular blood transfusions if they experience thalassemia-related complications, such as leg ulcer, pulmonary hypertension, thrombotic events, infection, and endocrine dysfunction.[9] However, over the last decade, there has been a shift to a simpler classification based on the need for blood transfusions as "transfusion-dependent thalassemia" (TDT) and "non-transfusion-dependent thalassemia" (NTDT).[10] Patients with TDT require regular blood transfusions to survive, without which they will suffer from various complications and have a reduced lifespan. It mainly comprises β-thalassemia major, HbE/β-thalassemia, those who survived Hb Bart's, hydrops fetalis, and severe nonendothelial HbH disease. Patients with NTDT may not need regular blood transfusions for survival but may need transfusions on rare occasions during physiologic stress like pregnancy or infection.[11] The NTDT encompasses a broad range of clinical severity, ranging from mild to fairly persistent severe anemia, which can obstruct physiologic processes, including growth and development, leading to various clinical complications later in life. These patients may often need more frequent transfusions later in life due to the disease complications, such as the occurrence of splenomegaly. It mainly comprises minor and intermediate forms of β-thalassemia, intermediate HbE/β-thalassemia, and moderate forms of HbH disease.[12]

Epidemiology

According to World Health Organization (WHO), about 7% of the world population is a carrier of abnormal hemoglobin genes; It is found that 300 000 to 500 000 children are born per annum with hemoglobin disorder, which is highly prevalent in the Middle East, Mediterranean, Central Asia, Southern China, and Indian subcontinents.[13] Approximately 78% of these births occur in low- and middle-income countries.[14] Worldwide, the mortality rate accounts for 3.4% of children below 5 years of age.[4]

According to UNICEF, India encompasses around 35 to 45 million carriers of β-thalassemia; on average, one in every 25 Indians is a carrier of β-thalassemia.[15] Every year, 7500 - 12 000 children are born with a major form of β-thalassemia.[16] Nearly 30 000 children undergo regular blood transfusion, whereas around 3000 patients die yearly due to uncontrolled iron overload before 20 years of age. β-thalassemia is commonly observed in the population of Gujaratis, Sindhis, Bengalis, Muslims, Punjabis, Kolis, Mahars, Gauras, Saraswats, and Lohanas.[14] β-thalassemia carriers are 3% to 15% in North India and 1% to 3% in South India.[17]

The Fetal-to-Adult Globin Switch

Hemoglobin is composed of α-globin and β-globin peptide chains and heme as a prosthetic group needed to carry oxygen. Various β-like globin molecules are generated because the human β-globin locus on chromosome 11 is developmentally controlled. In the early part of the first trimester, an embryonic kind of a β-like globin known as ε-globin is produced inside the yolk sac-derived primitive erythrocyte lineage.[18] The major β-like globin molecule produced from stem cells and progenitor cells in the fetal liver is the γ-globin.[19] The HbF is formed when γ-globin chains combine with α-globin chains to create a stable tetramer.[20] The key hemoglobin in the fetus, HbF (α2γ2), is present between 65% and 90% at birth and typically drops to <2% by 6 to 12 months. The γ-gene is turned off 6 months after childbirth, while the β-gene is turned on, resulting in the production of HbA (α2 β2). After 6 months of age, around 1% of HbF is still generated, but they are distributed unevenly, with some red cells (F cells) expressing more HbF than others.

The HbF levels can also be elevated as a result of a variety of other factors such as hemopoietic stress and genetic defects such as δβ-thalassemia, hereditary persistence of fetal hemoglobin (HPFH), and XmnI polymorphism (−158 C>T) (18). *HBG1 (Aγ)* and *HBG2 (Gγ)* (hemoglobin subunit gamma) are the 2 genes that code for the HbF subunits during the primitive developmental stage, and these genes vary from each other by a single amino acid (glycine [γG] or alanine [γA]). *HBG1* and *HBG2* genes start switching to adult *HBB* genes as the infancy period arrives.[21] According to recent research, elevated HbF levels in patients with β-thalassemia result in asymptomatic clinical outcomes. An increased level of HbF has also been shown to help decrease the disease severity in patients with β-thalassemia. HBS1, MYB, B-celllymphoma/leukemia 11A (BCL11A), KLF1, leukemia/lymphoma-related factor (LRF), and other transcription factors are involved in the synthesis of HbF (Figure 1).[22]

Transcription Factors in Hemoglobin Switching

Transcription factors, including HBS1L-MYB, regulate HbF levels on the LCR (locus control region) of the β-globin locus. The role of MYB on γ-globin expression is still unclear; however, some studies have reported that variation in the level of MYB may lead to elevated levels of HbF.[23,24] BCL11A is a zinc-finger transcription factor that controls HbF to HbA switching and silencing; thus, it represses the HbF gene (*HBG1 and HBG2*).[25] Within the β-globin locus, BCL11A primarily interacts with transcription factors like GATA (globin transcription factor), FOG1 (Zinc finger protein), and NuRD (nucleosome remodeling and deacetylase), a chromatin remodeling complex that includes the LCR region. BCL11A knockdown in cultured human erythroid progenitor cells resulted in overexpression of HbF. This, along with other transcription factors, may help target a therapeutic approach inducing the expression of HbF via modulating the BCL11A activity.[26]

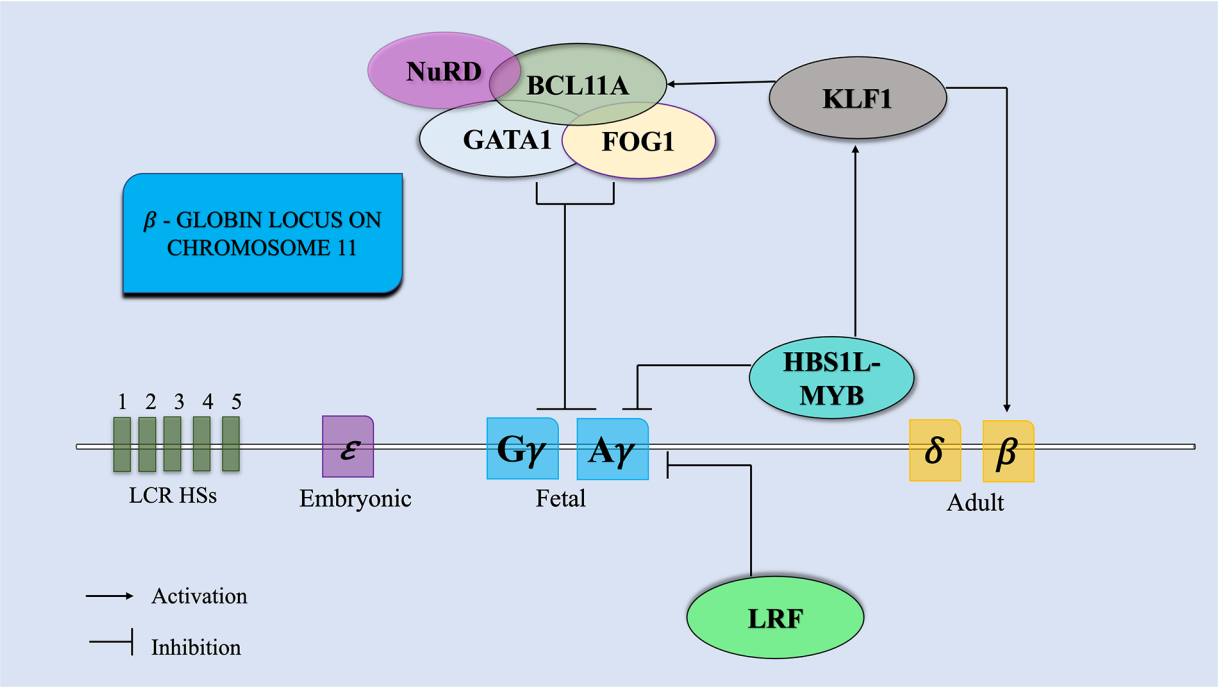

Figure 1. Role of transcription factors in switching the γ- to β-globin gene: HBS1 L: GTP-binding protein-myeloblastosis (HBS1L-MYB) are the transcription factors that regulate HbF levels on the locus control region (LCR) of the β-globin locus. BCL11A mainly interacts with transcription factors such as globin transcription factor (GATA), zinc finger protein (FOG1), and nucleosome remodeling and deacetylase (NuRD), acts as a regulator of HbF to HbA switching; KLF (EKLF1) (erythroid-specific Kruppel-like factor) activates the transcription of BCL11A by binding to its promoter region, thereby mediating the γ-globin to β-globin gene switching. By interacting with the *HBG1* and *HBG2* genes, LRF represses the synthesis of the γ-globin gene and maintains the density of nucleosomes, resulting in the silencing of the γ-globin gene.

KLF (EKLF1) (erythroid-specific Kruppel-like factor) is the principal regulator of adult β-globin gene expression.[27] KLF activates the transcription of BCL11A by binding to its promoter region, thereby mediating the γ-globin to β-globin gene switching. When KLF1 is knocked out, the *BCL11A* gene is not expressed, which increases γ-globin expression. This indicates that KLF1 and BCL11A play an essential role in hemoglobin switching.[28] Normally, KLF1 activates BCL11A, which stops the synthesis of the γ-globin gene, thus switching the HbF to HbA. By interacting with the *HBG1* and *HBG2* genes, LRF suppresses γ-globin gene expression while maintaining the nucleosome density, resulting in the silencing of the γ-globin gene.[29]

Biogenesis of miRNAs

The regulatory role of miRNAs in various cell processes is now well understood. MiRNA-based regulatory mechanisms are classified as epigenetic regulatory mechanisms.[30] The miRNAs play a role in the production and maturation of erythrocytes, the expression of hematological factors, and the regulation of globin gene expression through post-transcriptional gene silencing. The miRNAs are small noncoding single-stranded RNAs (18-25 nucleotides in length) found in eukaryotes. At the post-transcriptional level, they control gene expression by binding to the target mRNA's 3′ untranslated region (3′ UTR). The miRNAs are essential because they induce negative gene regulation via base pairing with complementary sequences leading to mRNA degradation or translational inhibition.[31] The miRNA genes are transcribed by the enzyme RNA polymerase II/III. Pri-miRNAs have a unique hairpin shape that is polyadenylated at the 3′ end and capped at the 5′ end. Subsequently, primary miRNA (pre-miRNA) is processed by the enzyme DiGeorge syndrome critical region 8 (DROSHA/DGCR8). One strand of pri-miRNA is cleaved at the base of the secondary structure by the enzyme DROSHA, which consists of RNase III domains such as RIIIa and RIIIb, respectively. Furthermore, it cuts the single-stranded RNA in the 3′ and 5′ ends, releasing pre-miRNAs, which is 60 to 70 nucleotide in length.[32] Pre-miRNA is transported to the cytoplasm from the nucleus by the enzyme Exportin 5. They are then processed by the RNase III enzyme DICER1, containing 2 catalytic domains, which bind to the pre-terminal miRNA's loop sequence and cleave RNA stem, resulting in the formation of mature 18- to 22-nucleotide-long miRNA; this process is assisted under the influence of transactivation response RNA-binding protein (TRBP) complex . The miRNA duplex is packed into Argonaute (Ago) protein in the RNA-induced silencing complex (RISC) and successively unwound into a single strand in the RISC. One strand acts as a guide strand and forms a silencing complex at 3′ UTR of target mRNA for translational repression (Figure 2).[33,34]

It has been possible to increase the expression of γ-globin gene, thus elevating HbF by using the suppressive effects of particular miRNAs on several transcription factors, such as MYB,

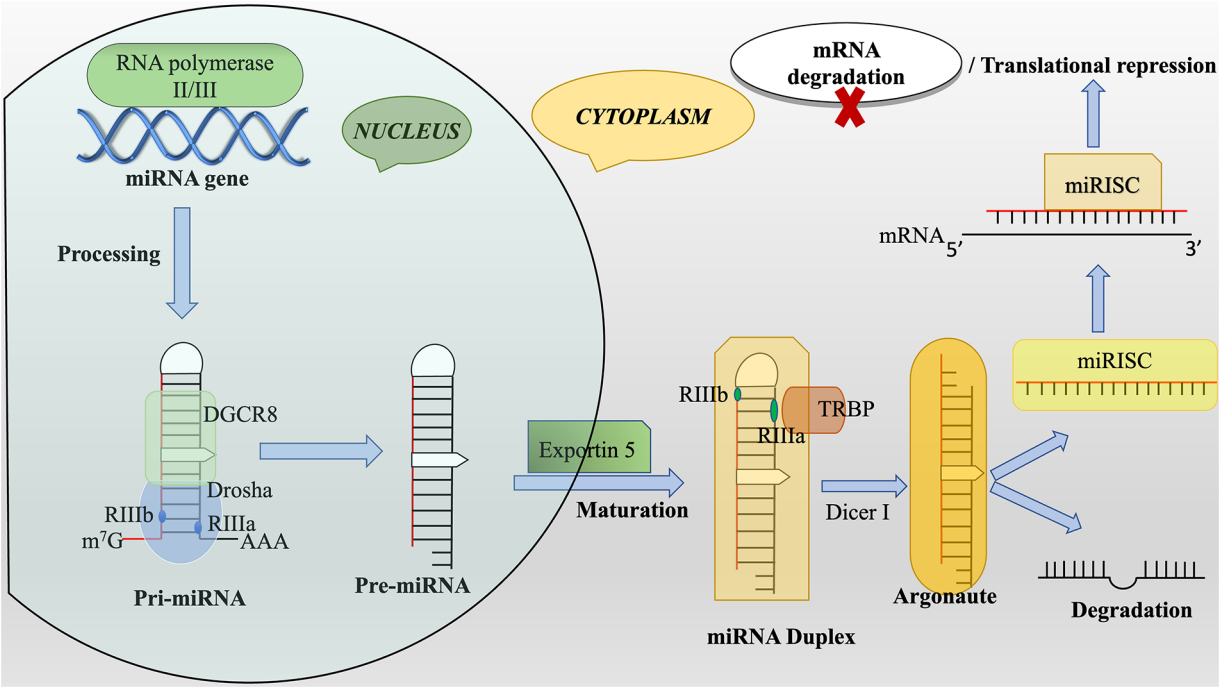

Figure 2. Biosynthesis of miRNAs:RNA polymerase II/III transcribes the miRNA gene to produce pri-miRNA, which is subsequently processed by Drosha to produce pre-miRNA. Pre-miRNAs are carried to the cytoplasm, where the enzyme Dicer acts on them to form mature miRNA duplexes (22 nucleotides). The RISC subsequently packs this into Ago protein, which is then unwound into a single strand in the RISC. One strand acts as a guide strand and results in the formation of a silencing complex at 3′ UTR of target mRNA for translational repression.

BCL11A, GATA1, and KLF. As a result, this method could be used as a novel therapeutic strategy for inducing HbF and reducing clinical complications in patients with β-thalassemia.[35] The results published by Jessica Gasparello et al reported that miRNA controls the expression of transcription factors, resulting in increased expression of the γ-globin gene. Examples are miR-16-1 and miR-15a as targets for MYB,[36] miR-34a, which targets KLF-1,[37] and miR-486-3p, which targets BCL11A.[38] Valentina Lulli et al[38] studied that in erythroid cells, overexpression of miR-486-3p lowered BCL11A levels, which was related to enhanced expression of the γ-globin gene, whereas suppression of miR-486-3p levels raised BCL11A and reduced γ-globin gene expression. Kuo-Ting Sun et al[39] studied using miR-138 mimic and healthy subjects' exosomes which inhibited the BCL11A activity and increased the expression of a γ-globin gene in K562 cell lines. A study on miRNA expression patterns in patients with β-thalassemia reported that more experimental studies need to be carried out to analyze overexpression or knock-outs of differentially expressed miRNA, which can further provide stronger results; hence, this may help in developing novel therapies to induce HbF level and thus reduce the burden of blood transfusion in patients with β-thalassemia.[40]

Based on the above evidence from the literature, it is evident that miRNAs play an important role in the pathophysiology of a variety of diseases, including β-thalassemia; hence, there has been an increased interest in the scientific community to study these molecules in detail as an increased understanding will help us utilize these miRNAs as a diagnostic marker or therapeutic targets. Experimental studies on these miRNAs can be exhaustive, considering the number of miRNAs and the procedural difficulties of performing wet-lab experiments. Bioinformatic databases have become a lifesaver for scientists working on miRNAs as they provide comprehensive knowledge about various significant miRNAs regulating several pathways. To give an exhaustive outline of the current bioinformatics tools, we have elaborated most commonly used tools, and these chosen tools are accessible freely. The most frequently referred database is the miRBase[41]; however, different databases have likewise been created to supplement miRbase, such as MirGeneDB[42] or miRCarta.[43] Although more than 2300 human miRNAs have been discovered, not all of them have been included in these databases.[44]

Preferred tools are beneficial for researchers to get a basic idea about the miRNAs participating in increasing the expression of the γ-globin gene. The miRNAs-based bioinformatics tools generate the database search for microRNAs, target prediction, pathways, and biomarker discovery.[45] To begin with, text mining and bioinformatics meta-information bases like OMIC tools (https://omictools.com/)[46] and newly developed Tools4miRs (https://tools4mirs.org/) can be utilized to get the list of accessible miRNA bioinformatics tools; then, the most frequently used tools which are linked to miRbase can be selected.[47]

Bioinformatics Tools in miRNA Research

To effectively manage thalassemia, it is fundamental to study the role of each miRNA involved and the pathways they participate in. Although enormous practical information is

accessible, it is a considerable task for the researchers to know the role of each miRNA involved in the expression of γ-globin. Before the wet-lab experiments, bioinformatic methods attempt to predict a successful target miRNA. Sankha Subhra Das et al studied the expression patterns of miRNAs in patients with HbE/β-thalassemia, using miRNA polymerase chain reaction assay, 8 miRNAs were found to be upregulated "miR-146a-5p, miR-146b-5p, miR-148b-3p, miR-155-5p, miR-335-5p, miR-192-5p, miR-7-5p, and miR-98-5p" and 4 miRNAs were downregulated "let-7b-5p, let-7a-5p, miR-92a-3p, and miR-320a." Bioinformatic analysis was done using DIANA-MicroT, miRDB, and TargetScan and found that these miRNAs are associated with signaling pathways, such as HIF-1 and MAPK, which may result in an elevated level of HbF expression in patients with HbE/β-thalassemia.[40] Jessica et al studied the role of miR-210 that increases HbF concentration by down-regulating the BCL11A levels. They used miRwalk, an online tool, to find the mRNA base pairing between the miRNA-210-3p coding region and target genes KLF-1, BCL11A, and MYB.[48]

This knowledge allows researchers to understand how miRNAs function in physiological and disease states. This article introduces a few techniques to detect miRNA targets, including various methodologies for miRNA:mRNA interaction recognition. In recent years, the number of miRNA resources has increased, and various bioinformatics databanks have been created to manage miRNA-related data such as miRNA sequence, miRNA target prediction, miRNA expression, analysis of miRNA regulatory networks, metabolic pathways, and exploration of transcription factors are some of the categories.[49]

The principal elements of bioinformatics include investigation of raw data, data processing, and broad biodata mining to give valuable outcomes dependent on "in-silico factual information" and "numerical strategies."[45] The miRNA identification is complicated and needs practical knowledge. Recent technological advances, such as high-throughput sequencing, have made it easier to assess their expression patterns. Some of the computational-based techniques were introduced to study the structural variants, putative genes, and the targets of miRNAs. A web-based tool such as MiRscan, Rfam, and miRNA-Base (miRbase) database was created, which stored miRNA gene sequences from various species.[50]

Characteristics of miRNA target prediction tools include

These tools mainly use the following features: miRNA seed region, conserved site, free energy, and site accessibility.

The seed region

The miRNA's seed region is a highly conserved segment that enables miRNA to be classified into families and species. The seed region is a conserved sequence with 8 nucleotides beginning at the 5′ end and ending toward the 3′ ends of miRNA sequences. Seed match occurs between miRNA and mRNA nucleotide based on the Watson-Crick rule.[51] The insertion of nucleotides at the 3′ end of the miRNA sequence is one factor that allows the strong pairing of the seed region with the target gene composition of adenine (A) and uracil (U) total number of binding sites at the 3′ UTR. The seed region includes 6mer, 7mer-m8, 7mer-A, and 8mer. The miRNA targets can be predicted using seed match, and the following tools can be used: mirSVR, DIANAmicroT, miRanda, and target scan.[52]

Conserved sites

The miRNA-binding sites are preserved across different species; these sites are considered "conserved." The conserved region in miRNA sequence analysis might be anywhere throughout its structure, including the miRNA, 5′ UTR, and 3′ UTR, or a mixture of all three. The seed region of miRNA has more conservation than the non-seed region.[51] The miRNAs' conserved sites can be evaluated using the promoter region and target genes assessment.[52] Evolutionary distance and phylogenetic calculations are considered by the miRNA target prediction tool. The tools mainly include PicTar, microT, TargetScan, DIANA-PITA, and miRanda.[53]

Free energy

To measure the interaction between the miRNA and its target mRNA, minimum free energy (MFEs) must be calculated. During a reaction, the change in free energy is referred to as (ΔG). miRNA:mRNA binding increases when the free energy is low.[54] Negative ΔG reactions have less energy available for future reactions, resulting in a stable interaction between miRNA and mRNA. Free energy can be measured using the Vienna RNA package and is calculated in terms of negative real value and expressed in kcal/mol.[55]

Site accessibility

The ability of miRNA to find and hybridize with an mRNA target is determined by site accessibility. In the first step of the miRNA:mRNA hybridization process, miRNA binds to a short region of mRNA. As the miRNA binds to a target, the secondary structure of the mRNA unfolds. Therefore, the assessed amount of energy expected to make a site available to the miRNA can be used to determine that an mRNA is a miRNA target. The tools mainly include miRDB, TargetScan, microTar Base, and miRanda.[56]

Other Target Sites

In addition to the 3′ UTR target sites, other mRNA sites have been identified as miRNA targets, including coding sequences for mRNAs, 5′ UTR, and open reading frames that can be used by the miRNA target prediction tools.[57]

Search for miRNAs

miRBase

The investigation of miRNAs can be initiated by exploring miRBase, which gives valuable information regarding the properties of each miRNA. It is a public repository for all reported microRNA sequences, which was established in the year 2002. Its initial release included 218 miRNA loci from 5 different species. The most recent release, in September 2018, had 38 589 entries representing hairpin precursors and 48 860 mature miRNAs from 271 species.[41] Hairpin and mature miRNA sequences can be searched and browsed with miRBase.[58] miRBase displays data in 3 categories such as (1) expression pattern of each miRNA, (2) classification of new genes identified with miRNAs, and (3) information on mature and immature miRNAs, including their chromosomal area and structures. miRBase is appropriately connected to different data sets that offer target genes.[59]

To Evaluate Predicted miRNA Targets

Target scan

TargetScan allows users to search for miRNAs by name, gene name, or miRNA families broadly conserved, conserved, or poorly conserved across multiple species.[60] Different transcripts for each gene are generated and further analyzed based on site type, probability of conserved targeting (P_{CT}), context score, percentage of context score, weighted context score, and conserved site for each miRNA. Searching the tool using the miRNA name shows the target gene, and the results can be obtained based on the conserved sites, cumulative weighted context++ score, and aggregate P_{CT}.[61] Target scan uses a model named context-plus plus (context++) score, which considers context scores such as AU content, type of binding site, 3′ UTR region and nearest distance from the 3′ UTR end for binding of miRNAs to its target site.[62] The conservation of a 3′ UTR is evaluated, and then, the k-mer analysis is performed as the 3′ UTR has numerous target sites, and aggregate P_{CT} is produced. In addition, the gene's 3′ UTR creates a link with a conserved seed sequence. This tool can also calculate the free energy of miRNA:mRNA duplex along with the score by finding the number of A and U content.[63]

Diana micro T-CDS

The Diana-microT-CDS is the recent version of the miRNA target prediction system. The most relevant features derived from "photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation" (PAR-CLIP) data are identified using machine learning in the upgraded version. These data allow Diana-micro T-CDS to know the miRNA-binding site at the coding region and 3′ UTR. The accessibility of the 3′ UTR can be predicted using Sfold. The keywords used while searching Diana-micro T-CDS are miRNA, gene name, KEGG description, and Ensembl ID. The resulting output includes the information regarding the binding site, conserved region, context score, predicted target location, and links to Ensembl, PubMed, and miRBase.[64] For miRNA-mRNA base pairing, this tool mainly uses miRNA-recognition elements (MREs) found in the 3′ UTR region of mRNAs. The calculation is based on guanine-uracil (G-U) wobble dinucleotide base pairs and free energy of complementary base pairing to identify MRE. The following parameter includes miRNA-related protein (miRNP), limiting the location and size of nucleotide loops and protrusions between miRNA and its related MRE.[62]

MiRwalk

This tool predicts the target miRNA-binding sites and information on all known genes of humans, rats, and mice.[65] MiRWalk utilizes automated "PubMed text mining searches" to track down data on miRNAs. It is expected to serve as an exhaustive database covering miRNA targets related to their target genes, diseases, pathways, and transcription factors. miRWalk also uses a computational approach to find the complementary regions between miRNA and targeted gene sequences. Many other well-known prediction databases and tools are merged with the results of the miRWalk algorithm, including DIANA-microT-CDS, miRanda-rel2010, DIANA-microTv-4.0, miRmap, mirBridge, miRDB4.0, doRiNA, miRNAMap, PicTar, and TargetScan6.2.[66]

MiRDB

miRDB serves as a repository for predicting the targets of miRNA with data acquired from version 21 of miRbase. MirTarget can be used for target prediction; hence, both conserved and nonconserved targets can be predicted. It uses an supportive vector mechanism (SVM) modeling tool to produce a probability score, which signifies the statistical confidence of the prediction results. miRDB functioning is updated using the MirTarget algorithm and provides target sequences of gene or miRNA for transcriptome-wide prediction of miRNA regulators of gene targets. Searching MiRDB with miRNA name displays a complete sequence that can help get more detailed information about each miRNA. To compare the primary sequence of miRNAs, complete alignment of the entire miRNA sequence can be used, which helps discover the functional genes of particular miRNAs.[67]

microTar base

The miRTarBase was created to provide the most up-to-date and complete information on experimentally validated miRNA-target interactions. It detects miRNA target by base pairing with the seed region complementary to 3′ UTRs of mRNA. This tool predicts the MFE change, seed region, fold change, and miRNA-mRNA interaction of each miRNA molecule.[68] Summarized features of the reviewed databases are

Table 1. Summary of reviewed databases.

DATABASES	FEATURES	LINK	REFERENCES
miRNABase	Public repository and online resource for microRNA sequences and annotation	http://mirbase.org/.	Guo et al[57]
Target Scan	Predicts biological targets of miRNAs	http://www.targetscan.org/.	Kiriakidou et al[61]
Diana microT-CDS	Detects MREs located in both the 3′ UTR and CDS regions	http://www.microrna.gr/microT-CDC	Paraskevopoulou et al[64]
MiRwalk	Predict target sites and miRNA target interactions	http://mirwalk.uni-hd.de/.	Sticht et al[66]
MiRDB	Functional annotation	http://mirdb.org.	Wong and Wang[67]
microTar Base	miRNA target interaction	http://tiger.dbs.nus.edu.sg/microtar/,	Thadani and Tammi[68]

Online databases such as miRNABase, Target Scan, Diana microT-CDS, MiRwalk, MiRDB, and microTar Base, mentioned in the table are commonly used for miRNA target prediction and functional annotations. The latest version of the abovementioned databases can be accessed using the link mentioned in the table.

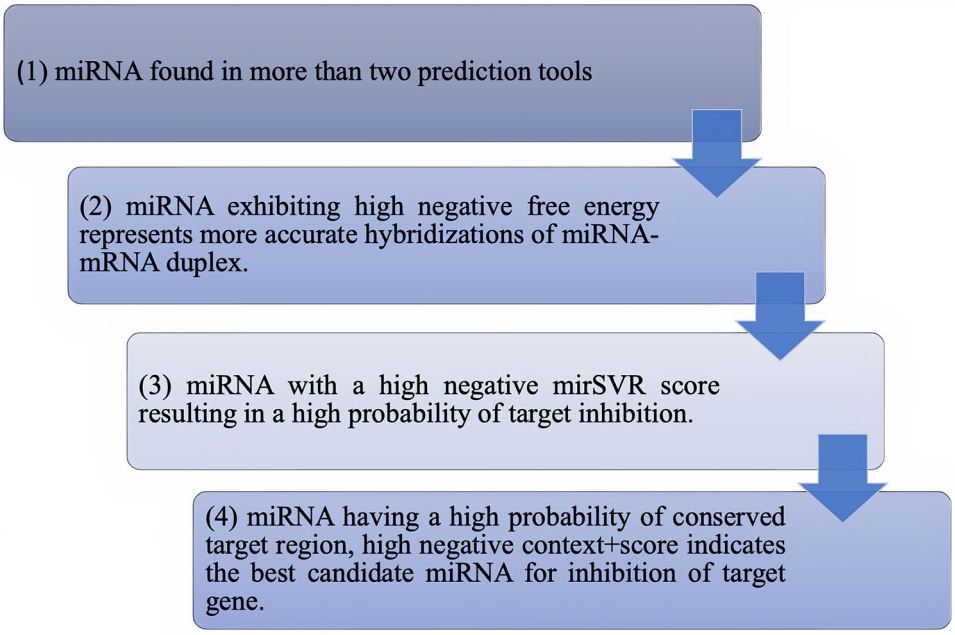

Figure 3. Criteria for selection of miRNAs: Candidate miRNAs may be selected using the criteria shown in the figure.

given in Table 1. Criteria for the selection of miRNAs are shown in Figure 3.[69]

Discussion

In erythroid cells, miRNAs have a role in the maturation, proliferation, regulation, and expression of HbF genes.[70] The role of miRNA in regulating gene expression in β-thalassemia is challenging, and understanding its use as a diagnostic marker or therapeutic target is even more difficult. Earlier reports have shown that miRNA increases the expression of the γ-globin gene in patients with β-thalassemia by regulating the transcription factors.[25]

Bioinformatic tools play a significant role in investigating miRNA and target genes. However, predicting miRNA-mRNA interactions is still challenging because of the lack of knowledge and complexity of collecting data from various tools.[71] There are many miRNA prediction tools available in the database. Most often used bioinformatics tools for predicting miRNAs that mediate the γ-globin production in patients with β-thalassemia are Diana microT-CDS, miRwalk, TargetScan, microTar, and MiRDB. Diana micro T-CDS helps predict functional RNA motifs because of its up-to-date database, mainly for identifying target miRNA. This software is very easy to use, presents good data visualization, and is user-friendly. MirSVR scores, which may be determined based on the 3′ UTR region, conservation of the target region, and the binding energy of miRNA-mRNA interactions, are used in miRwalk to identify miRNA targets genes.[62,64] Target Scan and MiRDB are mainly used for miRNA target prediction by comparing the primary sequences of miRNAs, which help identify functional genes, and it is highly convenient to use.[63,67] microTar predicts the seed region by calculating the MFE of each mRNA molecule, calculating fold change where each seed matches, and finally anticipating the miRNA-mRNA interaction.[68] Besides miRNABase, Target Scan, Diana

microT-CDS, MiRwalk, MiRDB, and microTar Base, the other miRNA target prediction tools include RNAhybrid, starBase, PicTar, TarBase, miRanda, PITA, and SVmicro.[72-76] RNA hybrid is a tool for predicting the miRNA targets in large RNAs by locating the best location to hybridize within a large RNA. This tool predicts putative binding sites, nonoverlapping regions, free energy, and threshold *P* value. A MFEs will be determined when evaluating the target sequences from huge databases. Thus, RNA hybrid helps in finding the target genes that are controlled by the miRNA pathways.[77]

MiRNA prediction is based on complementary sites in 3′ UTR regions that are conserved. It presents the results in the miRNA seed region with accurate complementary sites and then extends to 18- to 24-nucleotide-long sequences representing major interactions. We must consider context score, sites with poor seed pairing, seed match, and MFE value.[53] The selected tools help understand the commonly expressed miRNA and target genes and eliminate false negatives and false-positive results, providing the most reliable and accurate results of the selected miRNAs. The miRNAs with a significant negative free energy indicate that miRNA-mRNA interactions are possible. Therefore, bioinformatic-based identification of miRNA is an alternative approach to help predict novel miRNAs in β-thalassemia. This method is beneficial, inexpensive, and convenient for future studies. To evaluate the "accuracy" and "precision" of the target miRNAs, in vivo and in vitro investigation of gene-targeting miRNAs and miRNA expression is essential.

Conclusion

Thalassemia is most prevalent in India, and treatment modalities are not entirely developed. Many patients with the disease may survive; however, some succumb early. In the current scenario, miRNAs' role in managing β-thalassemia is less explored, but in the near future, miRNA-based therapeutics will give new hope. Bioinformatic approaches in predicting the miRNAs allow researchers to address different aspects of ongoing miRNA research. This review mainly focused on bioinformatics tools such as Diana microT-CDS, miRwalk, TargetScan, microTar, and MiRDB. These bioinformatics tools suggest that the current approaches to identifying the target miRNAs are mainly based on different operational modalities. The miRNA researchers highly accept these tools as they are user-friendly. Combining the results from various bioinformatic tools assists in identifying the candidate miRNAs to be later validated in the wet-lab experiments to induce the synthesis of γ-globin via influencing the transcription factors. It will help researchers establish novel concepts for managing β-thalassemia and deliver therapeutic strategies to improve their quality of life.

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Author Contributions

S.S.K. and R.B.R. performed the literature search and drafted the manuscript, S.M.N. and P.V. critically reviewed the manuscript and provided their intellectual inputs, and A.P. conceptualized the idea critically reviewed the manuscript, and gave the final approval for publication.

REFERENCES

1. Modell B, Darlison M, Birgens H, et al. Epidemiology of haemoglobin disorders in Europe: an overview. *Scand J Clin Lab Invest*. 2007;67:39-69. doi:10.1080/00365510601046557.
2. Weatherall DJ. The evolving spectrum of the epidemiology of thalassemia. *Hematol Oncol Clin North Am*. 2018;32:165-175. doi:10.1016/j.hoc.2017.11.008.
3. Angastiniotis M, Lobitz S. Thalassemias: an overview. *Int J Neonatal Screen*. 2019;5:1-11. doi:10.3390/ijns5010016.
4. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ*. 2008;86:480-487. doi:10.2471/BLT.06.036673.
5. Jha R, Jha S. Beta thalassemia—a review. *J Pathol Nepal*. 2014;4:663-671. doi:10.3126/jpn.v4i8.11609.
6. Thein L, Perrine SP, Leboulch P. Pathophysiology of β Thalassemia. *Hemoglobinopathy*. 2005;1:31-37.
7. Huang TL, Zhang TY, Song CY, et al. Gene mutation spectrum of thalassemia among children in Yunnan Province. *Front Pediatr*. 2020;8:159-155. doi:10.3389/fped.2020.00159.
8. Viprakasit V, Ekwattanakit S. Clinical classification, screening and diagnosis for thalassemia. *Hematol Oncol Clin North Am*. 2018;32:193-211. doi:10.1016/j.hoc.2017.11.006.
9. Musallam KM, Taher AT, Rachmilewitz EA. β-thalassemia intermedia: a clinical perspective. *Cold Spring Harb Perspect Med*. 2012;2:a013482. doi: 10.1101/cshperspect.a013482.
10. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V. *Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT)*. 3rd ed. Nicosia: Thalassaemia International Federation; 2014.
11. Weatherall DJ. The definition and epidemiology of non-transfusion-dependent thalassemia. *Blood Rev*. 2012;26:S3-S6. doi:10.1016/S0268-960X(12)70003-6.
12. Taher AT, Musallam KM, Cappellini MD, Weatherall DJ. Optimal management of β thalassaemia intermedia. *Br J Haematol*. 2011;152:512-523. doi:10.1111/j.1365-2141.2010.08486.x.
13. Williams TN, Weatherall DJ. World distribution, population genetics, and health burden of the hemoglobinopathies. *Cold Spring Harb Perspect Med*. 2012;2:a011692. doi:10.1101/cshperspect.a011692.
14. Mohanty D, Colah RB, Gorakshakar AC, et al. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study. *J Community Genet*. 2013;4:33-42. doi:10.1007/s12687-012-0114-0.
15. Cao A, Kan YW. The prevention of thalassemia. *Cold Spring Harb Perspect Med*. 2013;3:1-15. doi:10.1101/cshperspect.a011775.
16. Faizi N, Kazmi S. Universal health coverage—there is more to it than meets the eye. *J Fam Med Prim Care*. 2017;6:169-170. doi:10.4103/jfmpc.jfmpc_13_17.
17. Singh DM, Dayal P, Dayal P, et al. Clinico-epidemiological profile of thalassemia patients in a tertiary care center. *Pediatr Rev Int J Pediatr Res*. 2019;6:484-488.
18. Sankaran VG, Orkin SH. The switch from fetal to adult hemoglobin. *Cold Spring Harb Perspect Med*. 2013;3:a011643. doi:10.1101/cshperspect.a011643.
19. Olaniyi J, Olaniyi O, Arinola A. Foetal haemoglobin (HbF) status in adult sickle cell anaemia patients in Ibadan, Nigeria. *Ann Ibadan Postgrad Med*. 2011;8:30-33. doi:10.4314/aipm.v8i1.63955.
20. McGrath KE, Frame JM, Fromm GJ, et al. A transient definitive erythroid lineage with unique regulation of the β-globin locus in the mammalian embryo. *Blood*. 2011;117:4600-4608. doi:10.1182/blood-2010-12-325357.
21. Stamatoyannopoulos G. Control of globin gene expression during development and erythroid differentiation. *Exp Hematol*. 2005;33:259-271. doi:10.1016/j.exphem.2004.11.007.
22. Sankaran VG, Weiss MJ. Anemia: progress in molecular mechanisms and therapies. *Nat Med*. 2015;21:221-230. doi:10.1038/nm.3814.
23. Uda M, Galanello R, Sanna S, et al. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of β-thalassemia. *Proc Natl Acad Sci U S A*. 2008;105:1620-1625. doi:10.1073/pnas.0711566105.

24. Lettre G, Sankaran VG, Bezerra MC, et al. DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. *Proc Natl Acad Sci U S A*. 2008;105:11869-11874.
25. Sankaran VG, Menne TF, Xu J, et al. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. *Science*. 2008;322: 1839-1842.
26. Sankaran VG, Xu J, Orkin SH. Advances in the understanding of haemoglobin switching: review. *Br J Haematol*. 2010;149:181-194. doi:10.1111/j.1365-2141. 2010.08105.x.
27. Perkins A, Xu X, Higgs DR, Patrinos GP, Arnaud L, Bieker JJ. Kruppeling erythropoiesis—an unexpected broad spectrum of. *Blood*. 2016;127:1856-1863. doi:10.1182/blood-2016-01-694331.1856.
28. Crispino JD, Weiss MJ. Erythro-megakaryocytic transcription factors associated with hereditary anemia. *Blood*. 2014;123:3080-3088. doi:10.1182/ blood-2014-01-453167.
29. Kang J, Kang Y, Kim YW, You J, Kang J, Kim A. LRF acts as an activator and repressor of the human β-like globin gene transcription in a developmental stage dependent manner. *Biochem Cell Biol*. 2019;97:380-386. doi:10.1139/ bcb-2018-0303.
30. Chuang JC, Jones PA. Epigenetics and microRNAs. *Pediatr Res*. 2007;61:24-29. doi:10.1203/pdr.0b013e3180457684.
31. Wang H, Wang H, Duan X, Liu C, Li Z. Digital quantitative analysis of microRNA in single cell based on ligation-depended polymerase colony (Polony). *Biosens Bioelectron*. 2017;95:146-151. doi:10.1016/j.bios.2017.04.001.
32. Azzouzi I, Schmugge M, Speer O. MicroRNAs as components of regulatory networks controlling erythropoiesis. *Eur J Haematol*. 2012;89:1-9. doi:10.1111/ j.1600-0609.2012.01774.x.
33. Xie M, Li M, Vilborg A, et al. Mammalian 5′-capped microRNA precursors that generate a single microRNA. *Cell*. 2013;155:1568-1580. doi:10.1016/j.cell.2013. 11.027.
34. Cheloufi S, Dos Santos CO, Chong MMW, Hannon GJ. A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. *Nature*. 2010;465:584-589. doi:10.1038/nature09092.
35. Saki N, Abroun S, Soleimani M, et al. MicroRNA expression in β-thalassemia and sickle cell disease: a role in the induction of fetal hemoglobin. *Cell J*. 2016; 17:583-592.
36. Sankaran VG, Menne TF, Šćepanović D, et al. MicroRNA-15a and -16-1 act via MYB to elevate fetal hemoglobin expression in human trisomy 13. *Proc Natl Acad Sci U S A*. 2011;108:1519-1524. doi:10.1073/pnas.1018384108.
37. Ward CM, Li B, Pace BS. Stable expression of miR-34a mediates fetal hemoglobin induction in K562 cells. *Exp Biol Med (Maywood)*. 2016;241:719-729. doi:10.1177/1535370216636725.
38. Lulli V, Romania P, Morsilli O, et al. MicroRNA-486-3p regulates γ-globin expression in human erythroid cells by directly modulating BCL11A. *Plos One*. 2013;8:e60436. doi:10.1371/journal.pone.0060436.
39. Sun KT, Huang YN, Palanisamy K, et al. Reciprocal regulation of γ-globin expression by exo-miRNAs: relevance to γ-globin silencing in β-thalassemia major. *Sci Rep*. 2017;7:202. doi:10.1038/s41598-017-00150-7.
40. Das SS, Das S, Byram PK, et al. MicroRNA expression patterns in HbE/β-thalassemia patients: the passwords to unlock fetal hemoglobin expression in β-hemoglobinopathies. *Blood Cells Mol Dis*. 2021;87:102523. doi:10.1016/j.bcmd. 2020.102523.
41. Kozomara A, Birgaoanu M, Griffiths-Jones S. MiRBase: from microRNA sequences to function. *Nucleic Acids Res*. 2019;47:D155-D162. doi:10.1093/nar/ gky1141.
42. Fromm B, Billipp T, Peck LE, et al. A uniform system for the annotation of vertebrate microRNA genes and the evolution of the human microRNAome. *Annu Rev Genet*. 2015;49:213-242. doi:10.1146/annurev-genet-120213-092023.
43. Backes C, Fehlmann T, Kern F, et al. MiRCarta: a central repository for collecting miRNA candidates. *Nucleic Acids Res*. 2018;46:D160-D167. doi:10.1093/nar/ gkx851.
44. Alles J, Fehlmann T, Fischer U, et al. An estimate of the total number of true human miRNAs. *Nucleic Acids Res*. 2019;47:3353-3364. doi:10.1093/nar/gkz097.
45. Pourteymourfard Tabrizi Z, Jami MS. Selection of suitable bioinformatic tools in micro-RNA research. *Gene Reports*. 2020;21:100893. doi:10.1016/j.genrep. 2020.100893.
46. Henry VJ, Bandrowski AE, Pepin AS, Gonzalez BJ, Desfeux A. OMICtools: an informative directory for multi-omic data analysis. *Database (Oxford)*. 2014;2014:bau069. doi:10.1093/database/bau069.
47. Lukasik A, Wójcikowski M, Zielenkiewicz P. Tools4miRs—one place to gather all the tools for miRNA analysis. *Bioinformatics*. 2016;32:2722-2724. doi:10.1093/ bioinformatics/btw189.
48. Gasparello J, Fabbri E, Bianchi N, et al. BCL11A mRNA targeting by miR-210: a possible network regulating γ-globin gene expression. *Int J Mol Sci*. 2017;18: 2530. doi:10.3390/ijms18122530.
49. Witwer KW. Circulating MicroRNA biomarker studies: pitfalls and potential solutions. *Clin Chem*. 2015;61:56-63. doi:10.1373/clinchem.2014.221341.
50. Shukla V, Varghese VK, Kabekkodu SP, Mallya S, Satyamoorthy K. A compilation of Web-based research tools for miRNA analysis. *Brief Funct Genomics*. 2017;16:249-273. doi:10.1093/bfgp/elw042.
51. Innocenti GM. The time when the "Tomte" of evolution was playing with time. *Behav Brain Sci*. 2001;24:287. doi:10.1017/S0140525X0131395X.
52. Fujiwara T, Yada T. miRNA-target prediction based on transcriptional regulation. *BMC Genomics*. 2013;14:S3. doi:10.1186/1471-2164-14-s2-s3.
53. Betel D, Koppal A, Agius P, Sander C, Leslie C. Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol*. 2010;11:R90. doi:10.1186/gb-2010-11-8-r90.
54. Mathews DH, Sabina J, Zuker M, Turner DH. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *J Mol Biol*. 1999;288:911-940. doi:10.1006/jmbi.1999.2700.
55. Lorenz R, Bernhart SH, Hönerzu Siederdissen C, et al. ViennaRNA package 2.0. *Algorithms Mol Biol*. 2011;6:1-14. doi:10.1186/1748-7188-6-26.
56. Long D, Lee R, Williams P, Chan CY, Ambros V, Ding Y. Potent effect of target structure on microRNA function. *Nat Struct Mol Biol*. 2007;14:287-294. doi:10.1038/nsmb1226.
57. Guo ZW, Xie C, Yang JR, Li JH, Yang JH, Zheng L. MtiBase: a database for decoding microRNA target sites located within CDS and 5′UTR regions from CLIP-Seq and expression profile datasets. *Database (Oxford)*. 2015;2015:bav102. doi:10.1093/database/bav102.
58. Moore AC, Winkjer JS, Tseng TT. Bioinformatics resources for microRNA discovery. *Biomark Insights*. 2016;10:53-58. doi:10.4137/BMI.S29513.
59. Kozomara A, Griffiths-Jones S. MiRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res*. 2014;42:D68-D73. doi:10.1093/ nar/gkt1181.
60. Garcia DM, Baek D, Shin C, Bell GW, Grimson A, Bartel DP. Weak seed-pairing stability and high target-site abundance decrease the proficiency of lsy-6 and other microRNAs. *Nat Struct Mol Biol*. 2012;18:1139-1146. doi:10.1038/nsmb.2115.
61. Kiriakidou M, Nelson PT, Kouranov A, et al. A combined computational-experimental approach predicts human microRNA targets. *Genes Dev*. 2004;18: 1165-1178. doi:10.1101/gad.1184704.
62. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *Elife*. 2015;4:1-38. doi:10.7554/eLife.05005.
63. Friedman JM, Jones PA. MicroRNAs: critical mediators of differentiation, development and disease. *Swiss Med Wkly*. 2009;139:466-472.
64. Paraskevopoulou MD, Georgakilas G, Kostoulas N, et al. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. *Nucleic Acids Res*. 2013;41:W169-W173. doi:10.1093/nar/gkt393.
65. Dweep H, Gretz N. MiRWalk2.0: a comprehensive atlas of microRNA-target interactions. *Nat Methods*. 2015;12:697. doi:10.1038/nmeth.3485.
66. Sticht C, De La Torre C, Parveen A, Gretz N. Mirwalk: an online resource for prediction of microrna binding sites. *Plos One*. 2018;13:e0206239. doi:10.1371/ journal.pone.0206239.
67. Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. *Nucleic Acids Res*. 2015;43:D146-D152. doi:10.1093/ nar/gku1104.
68. Thadani R, Tammi MT. MicroTar: predicting microRNA targets from RNA duplexes. *BMC Bioinformatics*. 2006;7:1-9. doi:10.1186/1471-2105-7-S5-S20.
69. Riffo-Campos ÁL, Riquelme I, Brebi-Mieville P. Tools for sequence-based miRNA target prediction: what to choose? *Int J Mol Sci*. 2016;17:1987. doi:10.3390/ijms17121987.
70. Eltaweel NH, ElKamah GY, Khairat R, Atia HAE, Amr KS. Epigenetic effects toward new insights as potential therapeutic target in B-thalassemia. *J Genet Eng Biotechnol*. 2021;19:51. doi:10.1186/s43141-021-00138-x.
71. Seenprachawong K, Nuchnoi P, Nantasenamat C, Prachayasittikul V, Supokawej A. Computational identification of miRNAs that modulate the differentiation of mesenchymal stem cells to osteoblasts. *PeerJ*. 2016;4:e1976. doi:10.7717/ peerj.1976.
72. Quillet A, Saad C, Ferry G, et al. Improving bioinformatics prediction of microRNA targets by ranks aggregation. *Front Genet*. 2020;10:1330. doi:10.3389/ fgene.2019.01330.
73. Krek A, Grün D, Poy MN, et al. Combinatorial microRNA target predictions. *Nat Genet*. 2005;37:495-500. doi:10.1038/ng1536.
74. Krüger J, Rehmsmeier M. RNAhybrid: microRNA target prediction easy, fast and flexible. *Nucleic Acids Res*. 2006;34:451-454. doi:10.1093/nar/gkl243.
75. Yang JH, Li JH, Shao P, Zhou H, Chen YQ, Qu LH. StarBase: a database for exploring microRNA-mRNA interaction maps from Argonaute CLIP-Seq and Degradome-Seq data. *Nucleic Acids Res*. 2011;39:D202-D209. doi:10.1093/nar/ gkq1056.
76. Sethupathy P, Corda B, Hatzigeorgiou AG. TarBase: a comprehensive database of experimentally supported animal microRNA targets. *RNA*. 2006;12:192-197. doi:10.1261/rna.2239606.
77. Rehmsmeier M, Steffen P, Höchsmann M, Giegerich R. Fast and effective prediction of microRNA/target duplexes. *RNA*. 2004;10:1507-1517. doi:10.1261/ rna.5248604.