ARTIGO ORIGINAL
ISSN 1677-5090

https://doi.org/10.9771/cmbio.v22i2.50816

3D-Structural Characterization of MicroRNA Expressed in Leprosy

Caracterização Estrutural em 3D de MicroRNAs Expressos na Hanseníase

Luís Jesuíno de Oliveira Andrade[1]*, Humberto Barreto de Jesus[2], Luís Matos de Oliveira[3], Moema Farias de Oliveira[4], Laura Farias Caricchio Santana[5], Gabriela Correia Matos de Oliveira[6]

[1]Medical Doctor from the Bahiana School of Medicine and Public Health, Master's and PhD in Internal Medicine from the Federal University of Bahia - UFBA, Full Professor of the Medicine Department at the State University of Santa Cruz - UESC; [2]Medical Doctor from the Faculty of Medicine and Master's in Collective Health from the Federal University of Bahia - UFBA, Sanitary Physician at the National Health Foundation in Itabuna; [3]Medical Student at the Bahiana School of Medicine and Public Health - Salvador, Bahia; [4]Nurse from the Catholic University of Salvador, Sanitary Nurse at the Bahia State Health Department; [5]Medical Student at the Faculty of Medicine - FTC, Salvador, Bahia. [6]Medical Doctor from the Faculty of Medicine FTC - Salvador - Bahia, Family Health Program Physician - Bahia

Abstract
Introduction: *Hansen's disease, or leprosy is caused by Mycobacterium leprae (M. leprae), is a major public health problem in developing countries, and affecting the skin and peripheral nerves. However, M. leprae can also affect bone tissue, mucous membranes, liver, eyes, and testicles, producing a variety of clinical phenotypes. MicroRNAs (miRNAs) have been expressed in the various clinical forms of leprosy and could potentially be used for its diagnosis.* **Objective:** *in silico design of the molecular structure of miRNAs expressed in leprosy.* **Methodology:** *we performed a nucleotide sequence search of 17 miRNAs expressed in leprosy, designing in silico the molecular structure of the following miRNAs: miRNA-26a, miRNA-27a, miRNA-27b, miRNA-29c, miRNA-34c, miRNA-92a-1, miRNA-99a-2, miRNA-101-1, miRNA-101-2, miRNA-125b-1, miRNA-196b, miRNA-425-5p, miRNA-452, miRNA-455, miRNA-502, miRNA-539, and miRNA-660. We extracted the nucleotides were from the GenBank of National Center for Biotechnology Information genetic sequence database. We aligned the extracted sequences with the RNA Folding Form, and the three-dimensional molecular structure design was performed with the RNAComposer.* **Results:** *we demonstrate the nucleotide sequences, and molecular structure projection of miRNAs expressed in leprosy, and produces a tutorial on the molecular model of the 17 miRNAs expressed in leprosy through in silico projection processing of their molecular structures.* **Conclusion:** *we demonstrate in silico design of selected molecular structures of 17 miRNAs expressed in leprosy through computational biology.*
Keywords: *Leprosy; miRNA; Biomarkers.*

Resumo
Introdução: a doença de Hansen, ou hanseníase é causada pelo *Mycobacterium leprae* (*M. leprae),* é um grande problema de saúde pública nos países em desenvolvimento e afeta, a pele e os nervos periféricos. Entretanto, o *M. leprae* também pode comprometer o tecido ósseo, membranas mucosas, fígado, olhos e testículos, produzindo uma variedade de fenótipos clínicos. MicroRNAs (miRNAs) têm sido expressos nas várias formas clínicas da hanseníase e podem ser potencialmente utilizados para seu diagnóstico. **Objetivo:** objetivou-se com esse experimento modelar computacionalmente a estrutura molecular dos miRNAs expressos na hanseníase. **Metodologia:** realizou-se como metodologia uma pesquisa das sequências nucleotídicas de 17 miRNAs expressos na hanseníase, desenhando em modelo computacional a estrutura molecular dos seguintes miRNAs: miRNA-26a, miRNA-27a, miRNA-27b, miRNA-29c, miRNA-34c, miRNA-92a-1, miRNA-99a-2, miRNA-101-1, miRNA-101-2, miRNA-125b-1, miRNA-196b, miRNA-425-5p, miRNA-452, miRNA-455, miRNA-502, miRNA-539, e miRNA-660. Extraiu-se os nucleotídeos do banco de dados do GenBank of National Center for Biotechnology Information . Alinhou-se as sequências extraídas com o RNA Folding Form, e o projeto da estrutura molecular tridimensional foi realizado com o RNAComposer. **Resultados:** demonstrou-se como resultados as sequências dos nucleotídeos e a projeção da estrutura molecular dos miRNAs expressos na hanseníase, e produzimos um tutorial sobre o modelo molecular dos 17 miRNAs expressos em hanseníase através do processamento de suas estruturas moleculares em projeção computacional. **Conclusão:** foi demonstrado computacionalmente o projeto de estruturas moleculares selecionadas de 17 miRNAs expressos em hanseníase através da biologia computacional.
Palavras-chave: Hanseníase; microRNAs; Biomarcadores.

INTRODUCTION

Hansen's disease, also known as leprosy, is a major public health problem in developing countries caused by Mycobacterium leprae (M. leprae), and it affects the skin and peripheral nerves. However, M. leprae can also affect bone tissue, mucous membranes, the liver, eyes, and testicles, producing a variety of clinical phenotypes[1]. About 200,000 new cases were reported in 2017 from almost 150 countries, and migration due to globalization tends to expand the number of cases, bringing leprosy to countries where leprosy is not endemic[2].

Corresponding/Correspondente: *Luís Jesuíno de Oliveira Andrade – Departamento de Ciências da Saúde – End: Universidade Estadual de Santa Cruz – UESC – Ilhéus – Bahia – E-mail: luis_jesuino@yahoo.com.br

Leprosy diagnosis relies on the type and number of lesions, nerve involvement, bacillary load, and histopathology examination. Thus, bacilloscopy and biopsy of skin from an active lesion are considered the "gold standard" in leprosy diagnosis[3].

MicroRNAs (miRNAs) are short, non-coding RNAs that range from 19 to 25 nucleotides long, which intervene in the post-transcriptional control of gene expression, altering the stability of the system of translation, and consequently, miRNA degradation or inactivation[4]. miRNAs are relevant elements in the host-pathogen interaction and have been recognized as biological markers of several infectious diseases. Recent information demonstrates that M. leprae controls the miRNA configuration at the leprosy lesion site, which interferes with the host antimicrobial response[5].

Studies and understanding about the function and structure of miRNAs have grown exponentially in recent years. In particular, the use of bioinformatics with the careful examination of nucleotide sequences provides essential elements for molecular patterning. This study aims to computationally construct the molecular structure of 17 miRNAs expressed in leprosy and produce a tutorial on their modeling.

METHODOLOGY

We performed a nucleotide sequence search for 17 miRNAs expressed in leprosy. We designed the in silico molecular structures of the most expressed miRNAs in leprosy lesions based on literature review studies. These included miRNA-26a, miRNA-27a, miRNA-27b, miRNA-29c, miRNA-34c, miRNA-92a-1, miRNA-99a-2, miRNA-101-1, miRNA-101-2, miRNA-125b, miRNA-196b, miRNA-425, miRNA-452, miRNA-455, miRNA-502, miRNA-539, and miRNA-660.

We obtained the nucleotides from the GenBank of National Center for Biotechnology Information genetic sequence database (https://www.ncbi.nlm.nih.gov/). We aligned the extracted sequences with the RNA Folding Form (http://www.unafold.org/), and designed the three-dimensional molecular structures with RNAComposer (https://rnacomposer.cs.put.poznan.pl/). We then created a tutorial on the molecular models of the 17 miRNAs through in silico molecular arrangement.

Nucleotide search and sequence analysis

GenBank is accessible through the electronic address: https://www.ncbi.nlm.nih.gov/genbank/submit/. It offers numerous nucleotide research algorithms for investigating databases of distinct sequences, including the Nucleotide, Genome Survey Sequence (GSS), and Expressed Sequence Tag (EST) databases. These databases contain diverse nucleic acid sequences.

Molecular model construction

Amino acids and proteins have a nucleotide sequence-based constitution and function, and high-resolution structure methods are critical for understanding their structure. Currently, the most precise computational method to produce trustworthy structural models is homology modeling, which is widely used in various biological applications.

Designing the structure of RNA

RNAComposer is an interactive system that automatically predicts RNA three-dimensional (3D) structures. The system is based on the principles of automatic translation and uses the RNA FRABASE database as a dictionary for the RNA's secondary and tertiary structures.

RNAComposer allows you to work with an RNA molecule of up to 500 nucleotide residues, resulting in only one model of the RNA 3D structure. The RNAComposer package is available for free public download from the homepage at: https://rnacomposer.cs.put.poznan.pl/.

According to the Brazilian Research Ethics Committee (CEP), Resolution CNS 510/2016, our study does not require CEP evaluation as it investigates the theoretical understanding of pathologies in clinical practice using bioinformatics tools.

RESULTS

We searched for nucleotide sequences and designed molecular structures for miRNAs expressed in leprosy lesions, including miR-26a, miR-27a, miR-27b, miR-29c, miR-34c, miR-92a-1, miR-99a-2, miR-101-1, miR-101-2, miR-125b-1, miR-196b, miR-425, miR-452, miR-455-3p, miR-502-3p, miR-539, and miR-660. We created a tutorial on the molecular models of these 17 miRNAs expressed in leprosy lesions through in silico molecular arrangement.

Nucleotide sequence of miRNA-26a

To construct the structure of miRNA-26a-1, the nucleotide sequences with the NCBI identifier code AJ421747 were used in FASTA format obtained from GenBank. The Homo sapiens microRNA miR-26a was planned to encode a 22 bp linear ncRNA.

Molecular model of miRNA-26a

The nucleotide sequences of Homo sapiens microRNA 26a (MIR26A) were acquired using the FASTA format sequence UUCAAGUAAUCCAGGAUAGGCU and secondary structure: ….(((.(((...))))..)))); modeling was performed using RNAComposer, optimized and adjusted for alignment between structural templates and miRNA-26a nucleotides. Based on the sequence alignment between the template structure and miRNA-26a nucleotides, a structural model was built for the nucleotide in question. Using RNACom-

poser of comparative nucleotide modeling, we generated a homology model of microRNA 26a (MIR26A), demonstrated in Figure 1-A with a CPK spacefill style structure in pdb format.

Nucleotide sequence of miRNA-27a

The reconstruction of miRNA-27a was performed from a nucleotide sequence archive in FASTA format obtained from the GenBank database with the identifier code NCBI Reference Sequence: NR_029501.1. The miRNA-27a was planned to encode a 78 bp linear ncRNA. All coded sequences selected in FASTA format used the annotation of the NCBI-Graphics.

Molecular model of microRNA 27a

Based on the sequence alignment between the Homo sapiens microRNA 27a nucleotide sequence: CUGAGGAGCAGGGCUUAGCUGCUUGUGAGCAGGGUCCACACCAAGUCGUGUUCACAGUGGCUAAGUUCCGCCCCCCAG, and the secondary structure: (((.((.((.(((((((((((.((((((((.(((....)))......))))))))))))))))))))))).)).)).))), the structural template for microRNA 27a was produced. Assessment tools were used to measure the reliability of the designed structure. Using the RNAComposer comparative nucleotide modeling server, we generated a homology model of the Homo sapiens microRNA 27a with a Cartoon-style structure in pdb format (Figure 1-B).

Nucleotide sequence of miRNA-27b

To construct the structure of the Homo sapiens microRNA 27b, the nucleotide sequences with the NCBI identifier code NR_029665.1 were used in FASTA format obtained from GenBank. The miRNA-27b was planned to encode a 97 bp linear ncRNA.

Molecular model of microRNA 27b

The nucleotide sequences of Homo sapiens microRNA 27b (MIR27B) were acquired using the FASTA format sequence: ACCUCUCUAACAAGGUGCAGAGCUUAGCUGAUUGGUGAACAGUGAUUGGUUUCCGCUUUGUUCACAGUGGCUAAGUUCUGCACCUGAAGAGAAGGU, and secondary structure: (((((((((....((((((((((((....(((((((((....(((...)))..)))))))))..))))))))))))))))))))..))))))).)))); modeling was performed using RNAComposer, optimized and adjusted for alignment between structural templates and microRNA 27b nucleotides. Based on the sequence alignment between the template structure and microRNA nucleotides, a structural model was built for the nucleotide in question. Using RNAComposer of comparative nucleotide modeling, we generated a homology model of Homo sapiens microRNA 27b (MIR27b), demonstrated in Figure 1-C with a CPK spacefill style structure in pdb format.

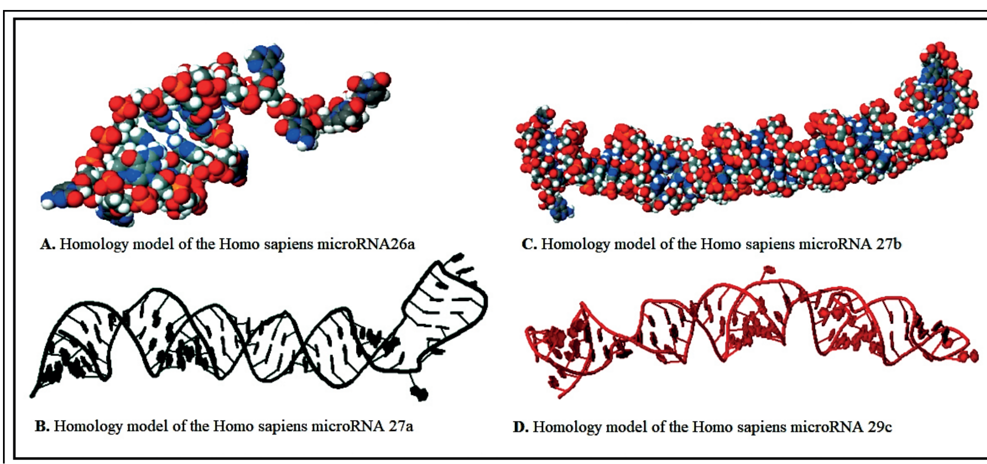
Nucleotide sequence of miRNA-29c

The reconstruction of miRNA-29c was performed from a nucleotide sequence archive in FASTA format obtained from the GenBank database with the identifier code NCBI Reference Sequence: NR_029832.1. The miRNA-29c was planned to encode an 88 bp linear ncRNA. All coded sequences selected in FASTA format used the annotation of the NCBI-Graphics-Homo sapiens microRNA 29c (MIR29c).

Molecular model of microRNA-29c

Based on the sequence alignment between the Homo sapiens microRNA 29c nucleotide sequence: AUCUCUUACACAGGCUGACCGAUUUCUCCUGGUGUU CAGAGUCUGUUUUUGUCUAGCACCAUUUGAAAUCGGUUAUGAUGUAGGGGGA, and the secondary sequence: ((((((((((((... (((((((((((...(((((((.(((...........)))))))))))...)))))))))))))).))))))))))., and the template structure, the structural template for microRNA-29c was produced. Assessment tools were used to measure the reliability of the designed structure. Using the RNAComposer comparative nucleotide modeling server, we generate a homology model of the Homo sapiens microRNA 29c with Cartoon-style structure in pdb format (Figure 1-D).

Figure 1 – *Homology model of the Homo Sapiens microRNA.*



A. Homology model of the Homo sapiens microRNA26a

C. Homology model of the Homo sapiens microRNA 27b

B. Homology model of the Homo sapiens microRNA 27a

D. Homology model of the Homo sapiens microRNA 29c

Source: https://rnacomposer.cs.put.poznan.pl/

Rev. Ciênc. Méd. Biol., Salvador, v. 22, n. 2, p. 188-196, maio./ago. 2023

Nucleotide sequence of miRNA-34c

To construct the structure of miRNA-34c, the nucleotide sequences with the NCBI identifier code NR_029840.1 were used in the FASTA format obtained from GenBank. The miRNA-34c was planned to encode a 77 bp linear ncRNA.

Molecular model of miRNA-34c

The nucleotide sequences of Homo sapiens microRNA 34c (MIR34c) were acquired using the FASTA format sequence: AGUCUAGUUACUAGGCAGUGUAGUUAGCUGAUUGCUAAUAGUACCAAUCACUAACCACACGGCCAGGUAAAAAGAUU and secondary structure: .((((..(((((.(((.((((.(((((.((((((.((....)).)))))))))))).)))).))).)))))..)))). The modeling was performed using RNAComposer, optimized and adjusted for alignment between structural templates and miRNA-34c nucleotide. Based on sequence alignment between the template structure and miRNA-34c nucleotide, a structural model was built for the nucleotide in question. So, using RNAComposer of comparative nucleotide modeling, we generated a homology model of microRNA 34c (MIR34c), demonstrated in Figure 2-A with CPK.

Nucleotide sequence of miRNA-92a-1

The reconstruction of miRNA-92a-1 was performed using a nucleotide sequence archive in FASTA format obtained from the GenBank database with the identifier code NCBI Reference Sequence: NR_029508.1. The miRNA-92a-1 was planned to encode a 78 bp linear ncRNA. All coded sequences selected in the FASTA format used the annotation of the NCBI-Graphics.

Molecular model of microRNA-92a-1

Based on sequence alignment between the Homo sapiens microRNA 92a-1 nucleotide sequence: CUUUCUACACAGGUUGGGAUCGGUUGCAAUGCUGUGUUUCUGUAUGGUAUUGCACUUGUCCCGGCCUGUUGAGUUUGG and secondary structure: ..(((...((((((((((((.(((.((((((((((((......))))))))))))).))))))))))))).))))....., and the template structure, the structural template for microRNA 203a was produced. Assessment tools were used to measure the reliability of the designed structure. Therefore, using RNAComposer comparative nucleotide modeling server, we generated a homology model of the Homo sapiens microRNA 92a-1 (MIR92A1) with the Cartoon-style structure in pdb format (Figure 2-B).

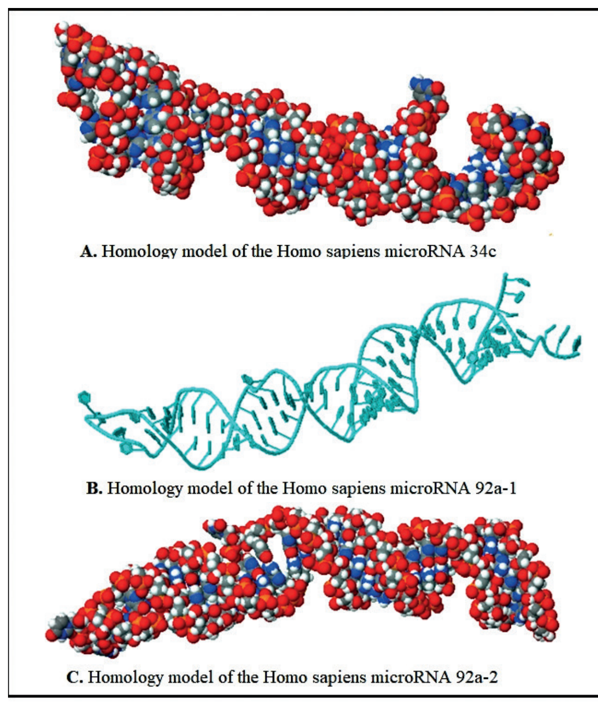
Nucleotide sequence of miRNA-92a-2

To construct the structure of miRNA-92a-2, the nucleotide sequences with the NCBI identifier code NR_029509.1 were used in the FASTA format obtained from GenBank. The miRNA-92a-2 was planned to encode a 75 bp linear ncRNA.

Molecular model of microRNA-92a-2

The nucleotide sequences of Homo sapiens microRNA 92a-2 (MIR92A2), microRNA were acquired using the FASTA format sequence: UCAUCCCUGGGUGGGGAUUUGUUGCAUUACUUGUGUUCUAUAUAAAGUAUUGCACUUGUCCCGGCCUGUGGAAGA, and secondary structure: ((.(((.(((((.(((((..((.(((.((((((...........)))))).))))))..)))))).)))))).)))).)); modeling was performed using RNAComposer, optimized and adjusted for alignment between structural templates and miRNA-92a-2 nucleotide. Based on the sequence alignment between the template structure and miRNA-92a-2 nucleotide, a structural model was built for the nucleotide in question. So, using RNAComposer of comparative nucleotide modeling, we generated a homology model of microRNA 92a-2 (MIR92A2), with the CPK spacefill style structure in pdb format demonstrated in Figure 2-C.

Figure 2 – *Homology model of tht Homo Sapiens microRNA*



Source: https://rnacomposer.cs.put.poznan.pl/

Nucleotide sequence of miRNA-101-1

The reconstruction of miRNA-101-1 was performed using a nucleotide sequence archive in FASTA format obtained from the GenBank database with the identifier code NCBI Reference Sequence: NR_029516.1. The miRNA-101-1 was predicted to encode a 75 bp linear ncRNA. All the coded sequences selected in the FASTA format used the annotation of the NCBI-Graphics.

Molecular model of microRNA-101-1

Based on the sequence alignment between the Homo sapiens microRNA-101-1 nucleotide sequence: UGCCCUGGCUCAGUUAUCACAGUGCUGAUGCU GUCU

AUUCUAAAGGUACAGUACUGUGAUAACUGAAGGAUGG-CA, and the secondary structure ((((...(((((((((((((((((.((((..... .....)))))))))))))))))))).))..)))), and the template structure, the structural template for microRNA-101-1 was produced. Assessment tools were used to measure the reliability of the designed structure. Thus, using the RNAComposer comparative nucleotide modeling server, we generated a homology model of Homo sapiens microRNA 101-1 (MIR101-1), microRNA, with a Cartoon-style structure in pdb format (Figure 3-A).

Nucleotide sequence of miRNA-101-2

To construct the structure of miRNA-101-2, the nucleotide sequences with the NCBI identifier code: NR_029836.1 were used in the FASTA format obtained from GenBank. The miRNA-101-2 was predicted to encode a 79 bp linear ncRNA.

Molecular model of miRNA-101-2

The nucleotide sequences of Homo sapiens microRNA 101-2 (MIR101-2) were obtained using the FASTA format sequence: ACUGUCCUU UUUCGGUUAUCAUG-GUACCGAUGCUGUAUAUCUGAAAGGUACAGUACU-GUGAUAACUGAAGAAUGGUGGU, and the secondary structure: (((.((.((((((((((.((((((((((.....(((((((.........))))))))))))))))))))))))))))))) .)).))); modeling was performed using the RNAComposer, optimized and adjusted for alignment between the structural templates and miRNA-101-2 nucleotide. Based on the sequence alignment between the template structure and miRNA-101-2 nucleotide, a structural model was built for the nucleotide in question. Thus, employing RNAComposer of comparative nucleotide modeling, we generated a homology model of microRNA 101-2 (MIR101-2), with CPK spacefill style structure in pdb format, as demonstrated in Figure 3-B.

Nucleotide sequence of miRNA-125b-1

The reconstruction of miRNA-125b-1 was performed using a nucleotide sequence archive in FASTA format obtained from the GenBank database with the identifier code NCBI Reference Sequence: NR_029671.1. The miRNA-125b-1 was predicted to encode an 88 bp linear ncRNA. All coded sequences selected in FASTA format used the annotation of the NCBI-Graphics.

Molecular model of microRNA 125b-1

Based on the sequence alignment between the Homo sapiens microRNA-125b-1 nucleotide sequence: UGCGCUCCUCUCAGUCCCUGAGACCCUAACUUGUGAU GUUUACCGUUUAAAUCCACGGGUUAGGCUCUUGGGAG-CUGCGAGUCGUGCU, and the secondary structure: .((((.. (((.((((.((((((((.((((((((((((..(((((.....))))).)))))))))))).)))))).)))))).)))..)))). and the template structure, the structural template for microRNA-125b-1 was produced. Assessment tools were used to measure the reliability of the designed structure. Thus, using the RNAComposer comparative nucleotide modeling server, we generated a homology model of Homo sapiens microRNA-125b-1 (MIR125B1), microRNA, with a Cartoon-style structure in pdb format (Figure 3-C).

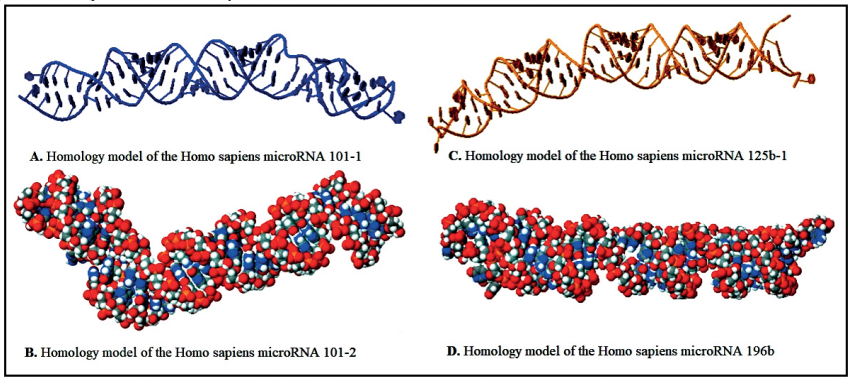
Nucleotide sequence of miRNA-196b

To construct the structure of miRNA-196b, the nucleotide sequences with the NCBI identifier code NR_029911.1 were used, in the FASTA format obtained from the GenBank. The miRNA-196b was planned to encode an 84 bp linear ncRNA.

Molecular model of microRNA 196b

The nucleotide sequences of Homo sapiens microRNA-196b (MIR196B) were acquired employing the FASTA format sequence: ACUGGUCGGUGAUUUAGGU-AGUUUCCUGUUGUUGGGAUCCACCUUUCUCUCGACAG-CACGACACUGCCUUCAUUACUUCAGUUG, and secondary structure: (((((..((((((..(((((((.((.((((((((((.....))))))))))))).)).))))))). .)))))))))..; Modeling was performed employing the RNAComposer, optimized and adjusted for alignment between structural templates and microRNA-196b nucleotide. Based on sequence alignment between the template structure and microRNA nucleotide, a structural model was built for the nucleotide in question. Therefore, using RNAComposer of comparative nucleotide modeling, we generated a homology model of Homo sapiens microRNA-196b (MIR196B), microRNA, with a CPK spacefill style structure in pdb format, demonstrated in Figure 3-D.

Figure 3 – *Homology model of the Homo sapiens microRNA.*



Source: *https//rnacomposer.es.put.poznan.pl/*

Nucleotide sequence of miRNA-425

The reconstruction of miRNA-425 was performed from a nucleotide sequence archive in the FASTA format obtained in the GenBank database with the identifier code NCBI Reference Sequence: NR_029948.1. The miRNA-425 was planned to encode an 87 bp linear ncRNA. All coded sequences selected in FASTA format used the annotation of the NCBI-Graphics-Homo sapiens microRNA 425 (MIR425), microRNA.

Molecular model of microRNA-425

Based on the sequence alignment between the Homo sapiens microRNA-425 nucleotide sequence: GAAAGCGCUUUGGAAUGACACGAUCACUCCCGUUGAGUGGGCACCCGAGAAGCCAUCGGGAAUGUCGUGUCCGCCCAGUGCUCUUUC, and secondary structure: ((((((.((.((((...(((((((.((.(((((.....(((.........)))..)))))).)))))))))...)))).)).))))), and the template structure, the structural template for microRNA-425 were produced. Assessment tools were used to measure the reliability of the designed structure. Therefore, using the RNAComposer comparative nucleotide modeling server, we generate a homology model of the Homo sapiens microRNA-425, with a Cartoon-style structure in pdb format (Figure 4-A).

Nucleotide sequence of miRNA-452

To construct the structure of miRNA-452, the nucleotide sequences with the NCBI identifier code NR_029973.1 were used in the FASTA format obtained from GenBank. The miRNA-452 was planned to encode an 85 bp linear ncRNA.

Molecular model of miRNA-452

The nucleotide sequences of Homo sapiens microRNA-452 (MIR452) were acquired employing FASTA format sequence: GCUAAGCACUUACAACUGUUUGCAGAGGAAACUGAGACUUUGUAACUAUUCUCAGUCUCAUCUGCAAAGAAGUAAGUGCUUUGC, and secondary structure: ((.((((((((((.....(((((((((.((.(((((((((.....))))))))).)).)))))))...)))))))))).)); modeling was performed employing the RNAComposer, optimized and adjusted for alignment between structural templates and miRNA-452 nucleotide. Based on sequence alignment between the template structure and miRNA-452 nucleotide, a structural model was built for the nucleotide in question. Therefore, employing RNAComposer of comparative nucleotide modeling, we generated a homology model of microRNA-452 (MIR452), with CPK spacefill style structure in pdb format demonstrated in Figure 4-B.

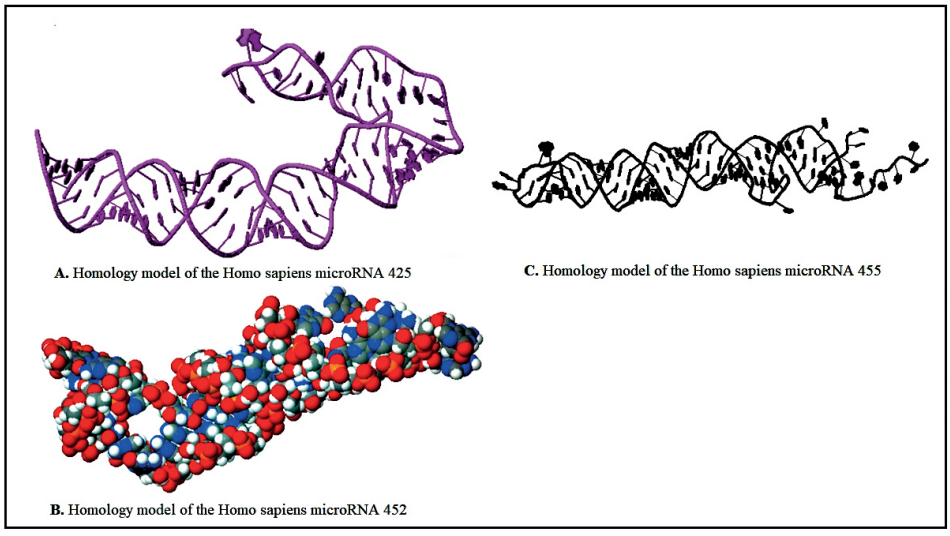
Nucleotide sequence of miRNA-455

The reconstruction of miRNA-455 was performed using a nucleotide sequence archive in FASTA format obtained from the GenBank database with the identifier code NCBI Reference Sequence: NR_030255.1. The miRNA-455 is predicted to encode a 96 bp linear ncRNA. All coded sequences selected in FASTA format used the annotations provided by NCBI Graphics.

Molecular model of microRNA-455

Based on sequence alignment between the Homo sapiens microRNA 455 nucleotide sequence: UCCCUGGCGUGAGGGUAUGUGCCUUUGGACUACAUCGUGGAAGCCAGCACCAUGCAGUCCAUGGGCAUAUACACUUGCCUCAAGGCCUAUGUCAUC, and secondary structure: ..... (((.((((((((((((((((.((((((.(((.(((........))).))))..))))))).)))))))))).....))))))..))).. ....., and the template structure, a structural template for microRNA 455 was produced. Assessment tools were used to measure the reliability of the designed structure. Therefore, using the RNAComposer comparative nucleotide modeling server, we generated a homology model of the Homo sapiens microRNA 455 (MIR455), microRNA, with a cartoon-style structure in pdb format (Figure 4-C).

Figure 4 – *Homology model of the Homo Sapiens microRNA 452*



Source: https://rnacomposer.cs.put.poznan.pl/

Nucleotide sequence of miRNA-502

To construct the structure of miRNA-502, the nucleotide sequences with the NCBI identifier code: NR_030226.1 were used under the FASTA format obtained from GenBank. The miRNA-502 is predicted to encode an 86 bp linear ncRNA.

Molecular model of microRNA-502

Nucleotide sequences of Homo sapiens microRNA 502 (MIR502), were acquired using the FASTA format sequence: UGCUCCCCCUCUCUAAUCCUUGCUAUCUGGGUGCUAGUGCUGGCUCAAUGCAAUGCACCUGGGCAAGGAUUCAGAGAGGGGGAGCU, and secondary structure: .(((((((((((((((((((((...(((((((...(((.........)))..)))))))))))))))))).)))))))))).; modeling was performed using RNAComposer, optimized and adjusted for alignment between structural templates and miRNA-502 nucleotides. Based on the sequence alignment between the template structure and miRNA-502 nucleotides, a structural model was built for the nucleotides in question. Therefore, using RNAComposer for comparative nucleotide modeling, we generated a homology model of microRNA 502 (MIR502), with a CPK space-filling style structure in pdb format demonstrated in Figure 5-A.

Nucleotide sequence of miRNA-539

The reconstruction of miRNA-539 was performed using a nucleotide sequence archive in FASTA format obtained from the GenBank database with the identifier code NCBI Reference Sequence: NR_030256.1. The miRNA-539 is predicted to encode a 78 bp linear ncRNA. All coded sequences selected in FASTA format used the annotations provided by NCBI Graphics.

Molecular model of microRNA-539

Based on the sequence alignment between the Homo sapiens microRNA-539 nucleotide: AUACUUGAGGAGAAAUUAUCCUUGGUGUGUUCGCUUUAUUUAUGAUGAAUCAUACAAGGACAAUUUCUUUUUGAGUAU, and secondary structure: ((((((((((((((((((.((((((.(((((((...(((....))).))).)))))))))).)))))))))))))))))), and the template structure, a structural template for microRNA-539 was produced. Assessment tools were used to measure the reliability of the designed structure. Therefore, using the RNAComposer comparative nucleotide modeling server, we generated a homology model of the Homo sapiens microRNA 539 (MIR539), microRNA, with a cartoon-style structure in pdb format (Figure 5-B).

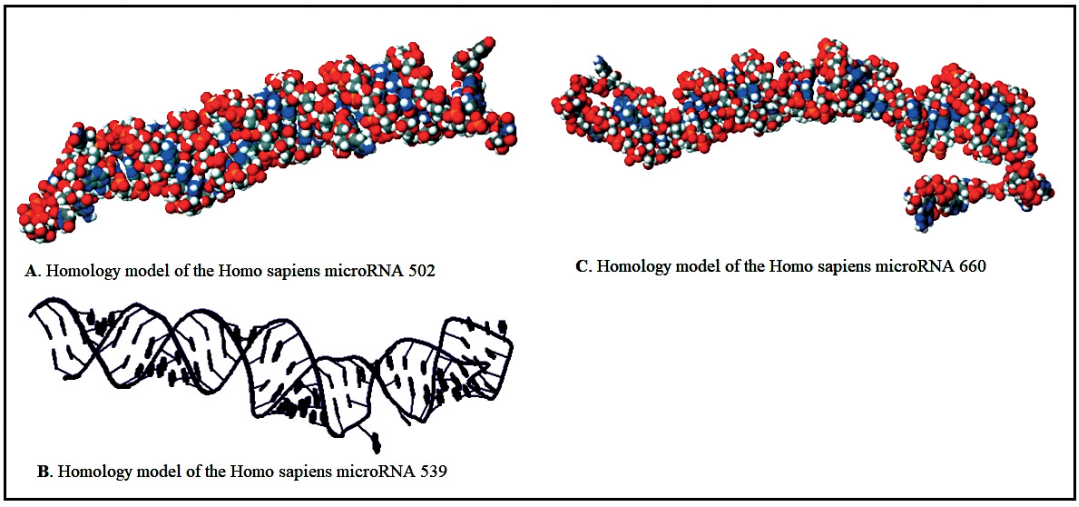
Nucleotide sequence of miRNA-660

To construct the structure of miRNA-660, the nucleotide sequences with the NCBI identifier code: NR_030397.1 were used under the FASTA format obtained from GenBank. The miRNA-660 is predicted to encode a 97 bp linear ncRNA.

Molecular model of miRNA-660

Nucleotide sequences of Homo sapiens microRNA 660 (MIR660) were acquired employing FASTA format sequence: CUGCUCCUUCUCCCAUACCCAUUGCAUAUCGGAGUUGUGAAUUCUCAAAACACCUCCUGUGUGCAUGGAUUACAGGAGGGUGAGCCUUGUCAUCGUG, and secondary structure: ..((((.((((((.....((((.((((((.((((.(((...........))).))))).)))))))))).....)))))).))))..........; modeling was performed employing the RNAComposer, optimized and adjusted for alignment between structural templates and miRNA-660 nucleotide. Based on sequence alignment between the template structure and miRNA-660 nucleotide, a structural model was built for the nucleotide in question. So, employing RNAComposer of comparative nucleotide modeling we generated a homology model of Homo sapiens microRNA 660 (MIR660), microRNA, demonstrated in Figure 5-C.

Figure 5 – Homology model of the Homo sapiens microRNA



Source: https://rnacomposer.cs.put.poznan.pl/

DISCUSSION

The miRNAs have been expressed in a number of infectious and contagious diseases, and have been proposed as potential diagnostic, prognostic and therapeutic markers in leprosy. It has been shown that M. leprae, controls in dysregulation of miRNAs at the site of the infectious lesion in leprosy patients and participating in the host's therapeutic response[6].

The miRNAs are short noncoding RNAs that intervene in the post-transcriptional command of gene manifestation in multicellular organisms, playing a controlling role in multiple physiological and pathophysiological functions[7]. Studies and comprehension about the function and structure of miRNAs have grown significantly in recent years[8]. Our study presents a tutorial on molecular modeling and demonstrates in silico the projection of the molecular structure of 17 miRNAs expressed in leprosy, drawing their molecular structures in silico.

Leprosy is a chronic infectious disease which has M. leprae as the etiological agent. It is a public health problem in the American, African, and Asian continents, especially in developing and poor countries[9]. M. leprae is an intracellular bacillus that has a long incubation time, slow proliferation, and peripheral nerve tropism. Leprosy has three distinct forms: tuberculoid, lepromatous, and an intermediate form[10].

Various microRNAs and their target genes participate in tissue damage in leprosy. Based on a literature review, we address the following miRNAs, which are most frequently expressed in leprosy lesions: miRNA-26a, miRNA-27a, miRNA-27b, miRNA-29c, miRNA-34c, miRNA-92a-1, miRNA-99a-2, miRNA-101-1, miRNA-101-2, miRNA-125b, miRNA-196b, miRNA-425, miRNA-452, miRNA-455, miRNA-502, miRNA-539, and miRNA-660.

The combination of several miRNAs as biomarkers is considered a more efficient option due to the possible overlap in miRNA targeting[11]. A study comparing normal individuals with individuals with leprosy showed that the combination of 17 miRNAs had 80% sensitivity and 91% specificity in discriminating between leprosy and healthy individuals, indicating a high diagnostic potential of the miRNAs described above[12].

With the advancement of bioinformatics, several tools have been developed for each step of miRNA biogenesis, helping researchers in molecular biology investigations[13]. Bioinformatics tools for miRNA design have shown accessibility for experimental evaluations and structure definitions. The computational study of miRNAs is fundamentally based on the performance analysis of their primary and secondary structures[14]. Reviewing the available literature, we found no studies with in silico construction of miRNA-26a, miRNA-27a, miRNA-27b, miRNA-29c, miRNA-34c, miRNA-92a-1, miRNA-99a-2, miRNA-101-1, miRNA-101-2, miRNA-125b, miRNA-196b, miRNA-425, miRNA-452, miRNA-455, miRNA-502, miRNA-539, and miRNA-660 with a 3D structure model.

An alternative method that uses 3D graphical presentation of secondary structures of miRNAs is promising because it creates data matrices based on structural information[15]. In our study, we evaluated the sequence analysis of selected miRNAs and designed 3D structural models. The nucleotide analysis of miRNAs was performed in FASTA format, and the 3D structuring was performed using RNAComposer. To construct the 3D structural models of the miRNAs, we used a strategy of structural analysis by homology with the sequence from the Nucleotide database. The use of molecular models can promote progress in the development of new drugs that facilitate therapy in target organs[16].

CONCLUSION

We demonstrate the in silico design of selected molecular structures of 17 miRNAs expressed in leprosy through computational biology. The function and structure of miRNAs are determined by nucleotide sequences, and early knowledge of these structures can allow identification and localization of binding sites on nucleotides, which can be fundamental for clinical and therapeutic use.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Submetido em: 27/08/2002
Aceito em: 08/06/2023