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ORIGINAL ARTICLE

IN SILICO MODELING OF THE MOLECULAR STRUCTURE OF microRNAS MARKERS FOR LIVER FIBROSIS IN HEPATITIS C

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ABSTRACT

Molecular biology looks for evidence that microRNA (miRNAs) plays a relevant function both in the beginning and advanced stages of hepatic fibrosis (HF), and has been proposed as an additional biomarker for HF forecasting in carriers of hepatitis C virus (HCV) infection. The purpose of this study was to develop an *in silico* modeling of the two-dimensional (2D) molecular structure of miRNA markers for HF in carriers of HCV. A search was initially performed for the nucleotide sequence of 6 miRNAs defined as biomarkers for HF, performing a computational simulation of the molecular structure of the following miRNAs: miRNA-182, miRNA-183, miRNA-1260b, miRNA-122-3p, miRNA-378i, and miRNA-214-5p. The nucleotide sequences were chosen in the GenBank of the American National Institutes of Health genetic sequence database. The nucleotide sequence alignment was carried out with a text-based format (FASTA) tool. In the molecular modeling, the structures were built with the RNAstructure, a completely automated miRNAs structure modelling server, available through Web Servers for RNA Secondary Structure Prediction. This study presented the nucleotide sequence and the computational simulation of molecular structures for the following miRNA: miRNA-182, miRNA-183, miRNA-1260b, miRNA-122-3p, miRNA-378i, and miRNA-214-5p. The molecular structure of miRNAs markers for HF in HCV carriers, through computational biology, is essential for designing more efficient optional tools for accurate treatment.

KEY WORDS: Micro-RNA; hepatitis C; hepatic fibrosis; computational biology.

INTRODUCTION

Hepatitis C virus (HCV) infection is recognized as a public health issue, and an estimated 71 million individuals are carriers of chronic hepatitis C infection worldwide. The most contaminated territories are WHO European and Eastern Mediterranean Regions, where the prevalence is 1.5% and 2.3% respectively. In other WHO territories the prevalence of

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HCV infection oscillates from 0.5% to 1.0% (WHO 2017). In HCV infection hepatic fibrosis (HF) is a pathological process due to hepatic stellate cell activation, extracellular matrix producing cells, leading to an imbalance between the deposition and degradation of the extracellular matrix (Jiang et al., 2017).

Molecular biology looks for evidence that microRNA (miRNAs) plays a relevant function both in the beginning and advanced stages of hepatic fibrosis (HF), and has been proposed as an additional biomarker for HF forecasting in carriers of HCV infection (Taherkhani & Farshadpour, 2017). Thus, it is necessary to develop biomarkers for early diagnosis, identifying subjects at risk for developing HF.

The miRNAs are small noncoding RNAs with 19-25 nucleotides that result in a post transcriptional command of the gene expression in multicellular organisms by unbalancing the control in sequences such as translation, leading to target miRNAs silencing or degradation (Boufraqech et al., 2016).

Recently, awareness of the structure and role of miRNA has significantly increased. The bioinformatics programs currently available to construct molecular modeling and analyse nucleotide sequences provide tools for the assembly of miRNA and the understanding of their molecular mechanisms. In recent years, several bioinformatics methods for miRNAs prediction have been developed, providing valuable understanding of the mechanisms of miRNAs transcriptional regulation. The methodological evaluation to forecast inter-miRNAs promoter regions is based on RNA polymerase II binding patterns around Transcription Start Sites (TSSs). Posteriorly, form position-specific scoring matrices to forecast the Transcription Factor Binding Sites (TFBSs) of signal transducer and activators of transcription-1 into genomic regions were created by Wang (Wang et al., 2010). A Support Vector Machine-based model to identify miRNA promoters was defined applying Position Weight Matrix from TRANSFAC® adopting the Match™ program to analyze TFBSs motifs (Chien et al., 2014). The University of California, Santa Cruz Genome Browser includes software such as infinity which demonstrates regulatory networks of miRNAs collecting TSSs positions from miRStart and extracting promoter region sequences of miRNAs, allowing the definition of unknown Transcription Factor-miRNAs regulatory networks (Falcone et al., 2016).

The purpose of this study was to develop *in silico* modeling of the two-dimensional (2D) molecular structure of miRNA markers for HF in carriers of HCV infection, and a tutorial on molecular modeling of 6 miRNAs initially defined as biomarkers for HF prediction.

METHODS

A study was performed on the nucleotide sequence of 6 miRNAs inially defined as biological markers for HF prediction in carriers of HCV infection, based on a review of Medline articles (Van Keuren-Jensen et al., 2016), designing a computational simulation of molecular structures of the following miRNAs: miRNA-182, miRNA-183, miRNA-1260b, miRNA-122-3p, miRNA-378i, and miRNA-214-5p. The nucleotide sequences were selected using GenBank, which is the NIH genetic sequence database. The nucleotides sequence alignment was carried out with a text-based format (FASTA) tool. In molecular modeling, the structures were built with the RNAstructure, a completely automated miRNAs structure modelling server, approachable through Web Servers for RNA Secondary Structure Prediction (http://rna.urmc.rochester.edu/RNAstructureWeb/).

Modeling with RNAstructure

The Predict to Secondary Structure server evaluates the division function, prognosticates the maximum free energy composition, finds structures with maximum presumed precision, and pseudoknots prognostication. This server designs a group of secondary structures, beginning with the lowest free energy structure and adding others with varying probability of accuracy. Other structures are incorporated since the minimum free energy structure might not be the correct one. If shape restrictions are detailed, these are added to the probable annotated structures. Moreover, a second group of shape restrictions, shape annotated structures can be generated. This group of shape structures is distinctive from the probable annotated structure group, and is not probably annotated on its own.

RNAstructure is a software package for RNA secondary structure prediction and appreciation. To access the RNAstructure web servers, a web browser is necessary. After connecting to the web server, a sequence must be followed: go to the browser at http://rna.urmc.rochester.edu/RNAstructureWeb/, the RNAstructure web server. Subsequently the link chosen is “Predict a Secondary Structure Common to Three or More Sequences”. The sequences are inserted applying a multiple sequence FASTA format. Adjust the absolute temperature for structure prediction. Next, options for Multilign and TurboFold can be altered. The calculations are started clicking “Submit Query”. The results of Multilign are displayed in TurboFold. Posteriorly, structures are shown for each sequence.

RESULTS

The nucleotide sequence and the computational simulation were presented for the molecular structure of the following miRNA: miRNA-182, miRNA-183, miRNA-1260b, miRNA-122-3p, miRNA-378i, and miRNA-214-5p.

Nucleotide sequence of miRNA-182 and Molecular model of microRNA-182

The main database used for the construction of the miRNA-182 was the nucleotide sequence file in FASTA format. The full-length nucleotide of miRNA-182 was obtained from the GenBank database using the identifier NCBI: NR_029614.1. The miRNA-182 was predicted to encode a linear110 bp. All coded sequences were chosen and sent as nucleotides in FASTA format, applying the NCBI – Graphics annotation.

Nucleotide sequences of Homo sapiens microRNA-182 were obtained using FASTA format; modeling was conducted using the RNAstructure web server, which was tailored and enhanced for the alignment among microRNA-182 nucleotide and structural templates. Based on a sequence alignment among the microRNA-182 nucleotide and the model structure, a structural template for target nucleotide was produced. Template quality evaluation tools were applied to foresee the confidence of the resulting template. Therefore, applying the RNAstructure web server automatized comparative nucleotide modeling server, a homology template of the Homo sapiens microRNA-182 (MIR182) was developed (Figure 1).

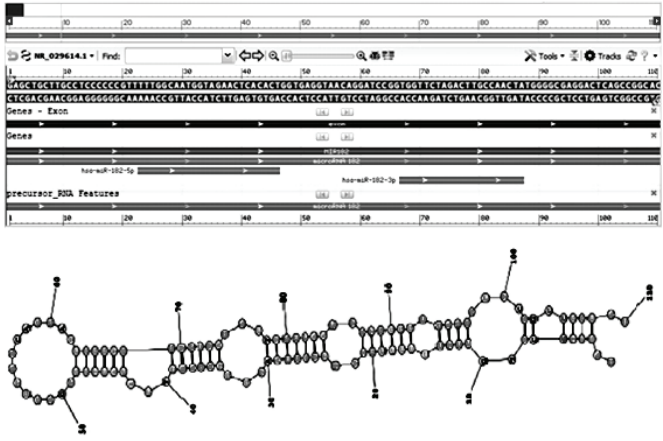

Figure 1. Homo sapiens microRNA-182 (MIR182) – *model-template aligment*.
Source: NCBI Reference Sequence: NR_029614.1
Homology model of the hsa-miRNA-182

Nucleotide sequence of miRNA-183 and Molecular model of miRNA-183

The reconstruction of the miRNA-183 was build from a nucleotide sequence file in FASTA format. The full-length nucleotide of miRNA-183 was obtained from the GenBank database using the identifier NCBI Reference Sequence: NR_029615.1. The miR-183 was predicted to encode a linear 110 bp. All coded sequences were chosen and sent as nucleotides in FASTA format, using the NCBI – Graphics annotation.

A model search with FASTA format was performed against the RNAstructure model library, which was tailored and enhanced for the alignment among Homo sapiens miR-183 (MIR183) nucleotide and structural templates. Based on a sequence alignment among the miR-183 nucleotide and the model structure, a structural template for target nucleotides was produced. Template quality evaluation tools were applied to foresee the confidence of the resulting template. According to the described criteria, a template for the Homo sapiens miR-183 (MIR183) was generated (Figure 2).

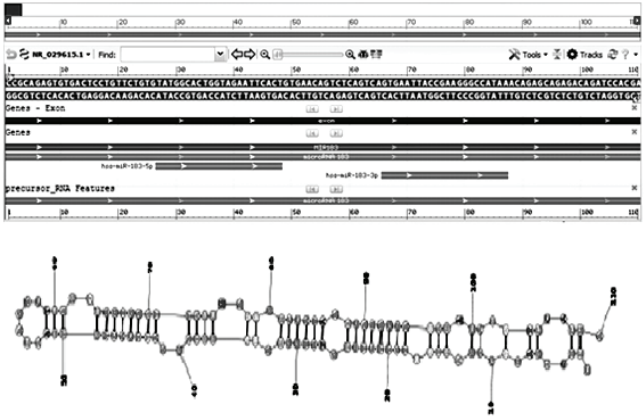

Figure 2. Homo sapiens microRNA-183 (MIR183) – *model-template aligment*.
Source: NCBI Reference Sequence: NR_029615.1
Homology model of the hsa-miRNA-183

Nucleotide sequence of miRNA-1260b and Molecular model of miRNA-1260b

The principal database used for the construction of the miRNA-1260b was the nucleotide sequence file in FASTA format. The full-length nucleotide of miRNA-1260b was obtained from the GenBank database using the identifier Homo sapiens miRNA hsa-miRNA-1260b GenBank: NR_036125.1. The miRNA-1260b was predicted to encode an 89 linear bp transcribed-RNA. All coded sequences were chosen and sent as nucleotides in FASTA format, applying the NCBI – Graphics annotation.

Nucleotide sequences of miRNA-1260b were obtained using FASTA format; modeling was performed using the RNAstructure web server, which were tailored and enhanced for the alignment among Homo sapiens miRNA hsa-miRNA-1260b nucleotide and structural templates. Based on a sequence alignment among the Homo sapiens miRNA-1260b nucleotide and the template structure, a structural template for target nucleotide was produced. Template quality evaluation tools were applied to foresee the confidence of the resulting template. Therefore, applying the RNAstructure web server automated comparative nucleotide modeling server, a homology template of Homo sapiens miRNA-1260b was developed (Figure 3).

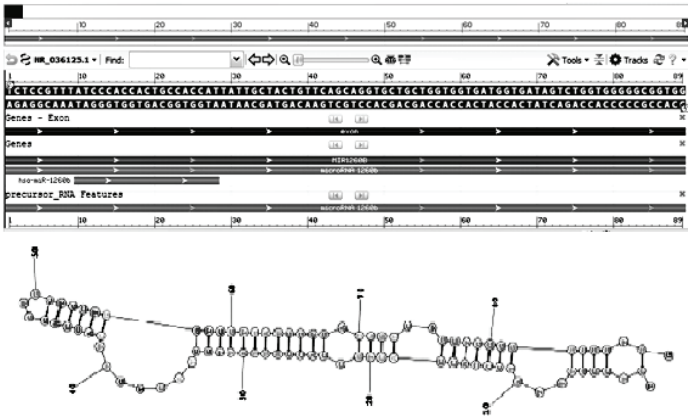

Figure 3. Homo sapiens microRNA-1260b (MIR1260b) – *model-template aligment.*
Source: NCBI Reference Sequence: NR_036125.1
Homology model of the hsa-miRNA-1260b

Nucleotide sequence of miRNA-122-3p and Molecular model of miRNA-122-3p

The reconstruction of the miRNA-122-3p was built from a nucleotide sequence file in FASTA format. The full-length nucleotide of miRNA-122-3p was obtained from the GenBank database using the identifier NCBI Reference Sequence: NR_029667.1. The Homo sapiens micro RNA-122-3p (MIR122-3p), microRNA was predicted to encode a 85 bp linear non-coding RNA, miRNA. All coded sequences were chosen and sent as nucleotides in FASTA format, applying the NCBI - Graphics annotation.

Model research with FASTA format was performed against the RNAstructure template library, which were tailored and enhanced for the alignment of Homo sapiens miRNA-122-3p (MIR122-3p), nucleotide and structural model. Based on a sequence alignment of the miRNA-122-3p nucleotide and the model structure, a structural template for the target nucleotide was produced. Template quality evaluation tools were applied to foresee the confidence of the resulting template. According to the described criteria, a template for the Homo sapiens miRNA-122-3p (MIR122-3p), miRNA was generated (Figure 4).

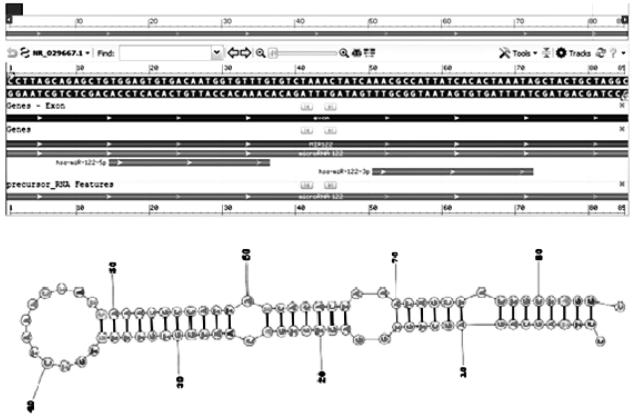

Figure 4. Homo sapiens microRNA-122-3p (MIR122-3p) – *model-template aligment.*
Source: NCBI Reference Sequence: NR_029667.1
Homology model of the hsa-miRNA-122-3p

Nucleotide sequence of miRNA-378i and Molecular model of miRNA-378i

The main database used for the construction of the miRNA-378i was the nucleotide sequence file in FASTA format. The full-length nucleotide of miRNA-378i was obtained from the GenBank database using the identifier Homo sapiens miRNA hsa-miRNA-378i GenBank: NR_039760.1. The miRNA-378i was predicted to encode a 76 linear bp transcribed-RNA. All coded sequences were chosen and sent as nucleotides in FASTA format, applying the NCBI - Graphics annotation.

Nucleotide sequences of miRNA-378i were obtained using FASTA format; modeling was conducted using the RNAstructure web server, which were tailored and enhanced for the alignment of Homo sapiens miRNA hsa- miRNA-378i nucleotide and structural templates. Based on a sequence alignment of the Homo sapiens miRNA-378i nucleotide and the model structure, a structural template for the target nucleotide was produced.

Template quality evaluation tools were applied to foresee the confidence of the resulting template. Therefore, applying the RNAstructure web server automated comparative nucleotide modeling server, developed a homology template of the Homo sapiens miRNA-378i (Figure 5).

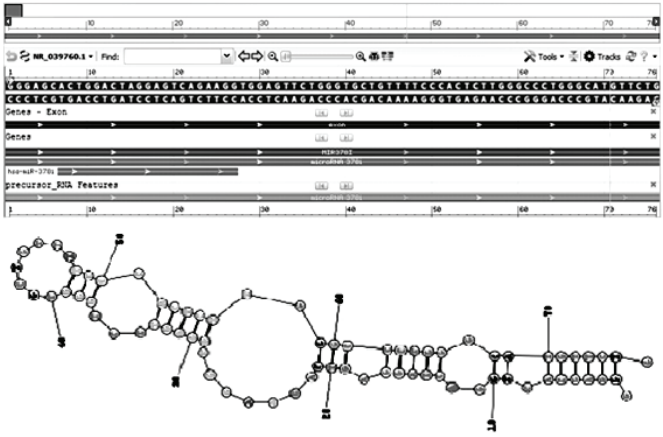

Figure 5. Homo sapiens microRNA-378i (MIR378i) – *model-template aligment.*
Source: NCBI Reference Sequence: NR_039760.1
Homology model of the hsa-miRNA-378i

Nucleotide sequence of miRNA-214-5p and Molecular model of miRNA-214-5p

The reconstruction of the miRNA-214-5p was obtained from a nucleotide sequence file in FASTA format. The full-length nucleotide of miRNA-214-5p was obtained from the GenBank database using the identifier NCBI Reference Sequence: NR_029627.1. The Homo sapiens micro miRNA-214-5p (MIR214-p), microRNA was predicted to encode a 110 linear bp non-coding RNA, miRNA. All coded sequences were chosen and sent as nucleotides in FASTA format, applying the NCBI - Graphics annotation.

Model research with FASTA format was performed against the RNAstructure template library, which were tailored and enhanced for alignment of Homo sapiens miRNA-214-5p (MIR214-5p), nucleotide and structural model. Based on a sequence alignment of the miRNA-214-5p nucleotide and the model structure, a structural template for the target nucleotide was produced. Template quality evaluation tools were applied to foresee the confidence of the resulting template. According to the described criteria, a template for the Homo sapiens miRNA-214-5p (MIR214-5p), miRNA was generated (Figure 6).

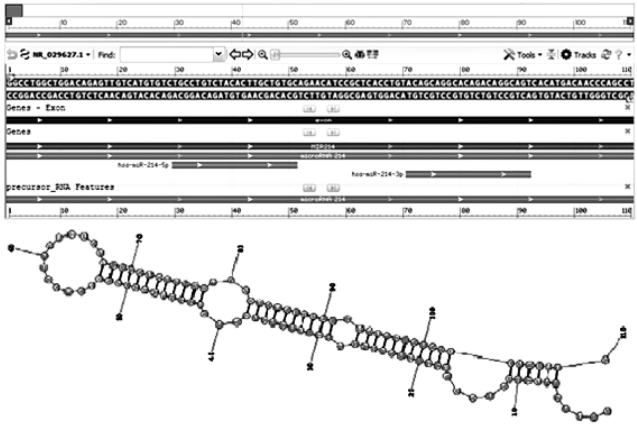

Figure 6. Homo sapiens microRNA-214-5p (MIR214-5p) – *model-template aligment.*
Source: NCBI Reference Sequence: NR_029627.1
Homology model of the hsa-miRNA-214-5p

DISCUSSION

The purpose of this study was to develop a tutorial on molecular modeling of the six miRNAs initially defined as biological markers for HF prediction in carriers of HCV infection. The miRNAs are small non-protein-coding RNA that perform regulatory roles in several physiological and pathophysiological functions (Bartel, 2004). In recent years, the awareness of the structure and role of miRNA has significantly increased.

The miRNAs play a fundamental functional performance in the regulation of physiological and pathological processes in the liver. The evidence demonstrates that microRNAs perform a function in the pathogenesis of HF in HCV infection, being important for diagnosis and treatment (Duan et al., 2013).

Studies demonstrated that serum expression levels of miRNA-182, miRNA-183, miRNA-1260b, miRNA-122-3p, miRNA-378i, and miRNA-214-5p were higher in patients with HF and in carriers of HCV infection, when compared with healthy individuals (Van Keuren-Jensen et al., 2016).

The role and structure of miRNAs are defined by their nucleotide sequences and high resolution structure prediction methods can pinpoint probable connection sites on nucleotides. Most or all binding sites for microRNAs have been detected in30NCRs of target miRNAs (Lewis et al., 2005).

The miRNA-182 located on chromosome 7q32.2, is part of the miRNA-183 family, composite of miR-96, miR-182 and miR-183, and plays an important role in the regulation of the mammalian circadian rhythm, T-cell progression and DNA correction. Moreover, is not usually evident in several malignant tumors, metastasis and more recently has been related to HF in carriers of HCV infection (Duan et al., 2013; Yang et al., 2014). Studies have shown miRNA-182 manifestation quantified in mouse models with alcoholic and non-alcoholic steatohepatitis, as well as increased presence in the advanced HF stage in patients with HCV infection (Dolganiuc et al., 2009).

Technological advances in the post-genomic era have contributed to an expansion in database filing, microarrays and other technological advances which have produced an abundance of information for scientists, and the challenge is to interpret and even to access this information to obtain usable data. Several bioinformatics methods for miRNAs prediction have been developed, providing valuable understanding of the mechanisms of miRNAs transcriptional regulation. The miRNA-182 sequences in the NCBI database were researched applying GenBank database with the identifier NCBI reference sequence, identifying all the nucleotides encoded in miRNA-182 and prognosticating their structure applying domain analysis tools.

Recently, bioinformatics tools for the prediction of miRNAs have gained popularity since experimental studies for defining miRNAs are unusual in their application. Nowadays, *in silico* evaluation of miRNA is based mostly on primary and secondary structure analysis. Revising the literature, no published study was found with a 2D structural model of the miRNA-182. This analysis researched the nucleotide database that is an ensemble of sequences from various databases, including GenBank, RefSeq, TPA and PDB, and no 2D models were found of miRNA-182 built with specific computer software.

In humans the miRNA-183 is located on chromosome 7q32.2, with a 4.2 kb intergenic region between miRNA-96 and miRNA-182 (Kozomara & Griffiths-Jones, 2014). The miRNA-183 has recently been identified to be up-regulated in multiple human malignancies as well as possibly acting as a tumor suppressor (Li et al., 2016). Studies demonstrate that the expression of miRNA-183 is significantly up-regulated in samples of hepatic biopsies in HCV infection patients and HF carriers in stage F3 and F4, when compared with liver biopsies from HF patients in stages F1 and F2 (Van Keuren-Jensen, et al, 2016). The nucleotide analysis of miRNA-183 was obtained in FASTA format in this study, and the 2D modeling applied the RNAstructure web server, which were tailored and enhanced for the alignment of the miRNA-183 and the structural model. Therefore, in order to construct a 2D miRNA-183 structural model the authors utilized the nucleotide database sequence and a structural homology analysis plan. In this analysis only one study was found with a 2D model of miRNA-183, but using the Mfold RNA folding software (Dambal et al., 2015).

The miRNA 1260b is located on chromosome 11q21, and studies show that it may be involved in a variety of cellular activities such as, for example, immune response of dendritic cells, gum disease, renal cancer cell proliferation and invasion in renal cell carcinoma cells, and development of non-small cell lung cancer (Liu et at., 2016). More recently, the miRNA-1260b has been implicated in fibrotic tissue of HCV infection, presenting most substantially down-regulated in HF at an advanced stage (Van Keuren-Jensen et al., 2016). In the medical literature database, no scientific studies were found showing the construction of a 2D structure model of the miRNA-1260b. In the current study, the nucleotide sequence of miRNA-1260b was obtained from the NCBI sequence database, and this sequence was transformed to the FASTA format. The 2D structure of miRNA-1260b was constructed applying the RNAstructure web server. Thus, in the absence of 2D structures for the majority of the sequenced nucleotides, homology modeling experientially shapes the basis for the resolution of the structure.

The miRNA-122-3p is located in chromosome 18q21.31 and been reported to be associated with a number of diseases and pathogenic processes, including lung cancer, rheumatoid arthritis, idiopathic asthenospermia, hepatocellular carcinoma and HCV infection (Zhang, 2017). The miR-122-3p can be considered a marker for HF, since several studies report that it is up or down-regulated in hepatic diseases by other causes, and the level of this miRNA has also been linked with the HCV infection viral load (Henke et al., 2008; Jin et al., 2015). Moreover, the miRNA-122-3p plays a significant role in HCV replication and presents four connection sites in the HCV genome, being involved in the regulation of a number of metabolic pathways in hepatic cells (Hoffmann et al., 2012). Literature analysis did not produce any studies on the production of a secondary structure model of the miRNA-122-3p. A 2D model was designed of the miRNA-122-3p using the RNAstructure web server, underpinned hairpin-shaped stem loop structure incorporated with homology research (Wang et al., 2005). The structure has been predicted and presents a peculiarity, which is a hairpin loop miRNAs structure. Conserved secondary structure is the hallmark of miRNA, and computational advances in this research area allow the *in silico* structure prediction by a web server.

The miRNA-378i is located in chromosome 22q13.2, it belongs to the miR-378 family which presents eleven members (Megiorni et al., 2014). The miR-378 family members impede the activation of activated hepatic stellate cells that play a very important role in HF progression (Lambrecht et al., 2015). A study showed that miR-378i was among the microRNAs majority substantially down-regulated from early to advanced HF (Van Keuren-Jensen et al., 2016). A medical literature revision showed that no studies with 2D structural models of the miRNA-378i were cited. The nucleotide database was also extensively analyzed, including GenBank, RefSeq, TPA and PDB, and an online 2D model of miRNA-378i was built with the RNAstructure programs.

The miRNA-214-5p is located in the human chromosome 1q24.3 (Lee et al., 2009). Studies have demonstrated that the miR-214 expression is up-regulated in an HF progression-dependent way in livers of patients with chronic HCV infection, and the miR-214-5p over-expression performs a primary function in stellate cell activation, in view of the fact that liver stellate cells are fundamental in increasing HF (Iizuka et al., 2012). These studies suggest the role of miR-214 as a possible therapeutic target in HF. Analyzing the medical literature database no scientific studies have been published showing a 2D structure model of the miRNA-214-5p. In this study, the nucleotide sequence of miRNA-214-5p was converted to the FASTA format of the NCBI database, and a 2D structure was built using the RNAstructure web server.

The role and structure of miRNAs are defined by nucleotide sequences, and high resolution structure forecast methods can pinpoint binding links on nucleotides of central significance for application in both clinical and pharmacological aspects.

The molecular composition of the miRNAs biological markers for HF in HCV carriers through computational biology is important for the advancement in choice of the most efficient tools for specific therapy.

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