Computational analysis to predict role of human microRNAs in Ebola virusgenome

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Abstract**—** *The Ebola virus is one of the most dangerous viruses in Filoviridae family. It causes fatal hemorrhagic fever in both non-human and human primates. The fatality rate is up to ninety percent. There is no effective treatment against EBOV infection so far. By using host microRNAs, we have explored for potential anti-viral therapeutics against EBOV infection, which may down-regulate viral gene expression in order to suppress viral replication. We have identified eight human miRNAs from eight potential hairpin sequences of EBOV genome. Our study provided an interesting hypothesis that those miRNAs are hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054. We suggested that these hairpin sequences could be use as anti-viral therapeutics to quell the replication of EBOV infection in human.*

Keywords— EBOV, microRNA, downregulate, anti-viral, therapeutics, human miRNA.

Abbreviations: Ebola Virus (EBOV), microRNA (miRNA), human microRNAs (hsa-miR).

I. INTRODUCTION

The Ebola virus (EBOV)is already listed as a top categories pathogen by several organizations including WHO, CDC and NIH because fatality rate is up to ninety percent [4,7]. It is a member of **Filoviridae** family that causes fatal hemorrhagic fever including both human and non-human primates [1-3]. Several reports suggested that susceptible hosts can be died by most virulent species within ten days after the appearance of symptoms [4-5]. The first case of Ebola virus was reported in 1976 on the Africa region [6]. Five (5) different species of Ebola viruses have been reported so far. Among these except Reston, REBOV, all 4 are being capable of causing diseases in human [8-9]. As Ebola virus is enveloped, non-segmented, negative-strand RNA viruses and genome size approximately 19 kb in length, it has high fatality rate and easily transmissible [10-11]. The viral genome encodes seven structural proteins and one non-structural protein while viral proteins perform different functions including viral replication [11].

The miRNAs are small non-coding RNA molecule, genomically encoded, usually around twenty-two base pair in length and regulate genes expression at the post-transcriptional level either mRNA degradation or repress translation [12-14]. It is well documented that miRNAs operate their different biological or physiological functions including apoptosis, development, tumorigenesis, stress response, proliferation and fat metabolism [15-16]. Although, current monoclonal antibody (mAb) based therapies are thought to the most efficient against lethal Ebola virus infection in non-human primates. In this research study, we have proposed miRNA-based gene silencing activity as a suitable alternative, in addition to current vaccine mediate treatment.

Viral miRNAs are unique because it regulates both their own gene expression and host gene expression [18]. MiRNA genes are firstly transcribed into primary miRNA. Then they are cleaved by enzymatic activity of the RNase III ribonuclease Drosha into 60-90 base pair long hairpin intermediate known as pre-miRNA [18-20]. By the action of enzyme exportin-5 and ran pre-miRNAs are exported from nucleus to cytoplasm [19]. The pre-miRNAs are further cleaved by Drosha (another RNase III ribonuclease) in the cytoplasm and are formed into a double stranded RNA known as duplex mature RNA [19].RNA-induced silencing complex (RISC) that is one strand (guided strand) of duplex RNA, targets messenger RNA to degrade or repress translational activity [18].

The 3' untranslated region (UTR) of the mRNA and the seed region of miRNA (2-7 bp) has fine complementarity that gives sufficient results in cleavage. On the other hand, faulty complementarity may block translation [18, 21]. Now-a-days,

miRNA is considered as antiviral defense against several viral diseases. As example, miRNAs mediate anti-HCV treatment shows promising effectiveness and safety results in an early stage trial [22]. In this study, we have identified some potential targets of human microRNA on Ebola virus (EBOV) genome by bioinformatically related computerized program. The study will help to understand host pathogen interaction as well as to develop new antiviral therapy against all Ebola virus diseases including fatal hemorrhagic fever.

II. MATERIALS AND METHODS

The miRNA prediction of the EBOV was conducted by using the complete genome sequence of Zaire Ebola virus strain Mayinga (Accession # AF499101.1) obtained from the National Center for Biotechnology Information (NCBI).Figure 1 shows a schematic diagram of the computerized prediction of human miRNAs in EBOV genome. Using a VMir Analyzer program, the viral genome was screened for hairpin-structured miRNA precursors [23-24].The output of VMir Analyzer was visualized using VMir viewer. For cut-off value 60 nt minimum hairpin size, 120 nt maximum hairpin size and 110 minimum hairpin score were used for the filter of sequence.As part of miRNA precursor, 41 sequences with potential hairpin like structures were extracted. Each of the sequence of the candidate miRNA precursors were searched for nucleotide similarity with all human microRNAs by using SEARCH menu of the miRBase database (www.mirbase.org/search.shtml) [17, 25]. Finally, eight (8) sequences were identified as candidate miRNA precursor based on significant sequence similarity with human miRNAs. To ensure effective hybridization between the viral precursor miRNAs and complementary template of the potential human miRNAs were further analyzed by RNA hybrid web server (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) [26]. Finally, the RNA fold web server (http://rna.tbi.univie.ac.at/cgibin/RNAfold.cgi) was used to predict the secondary structure of pre-miRNA [27]

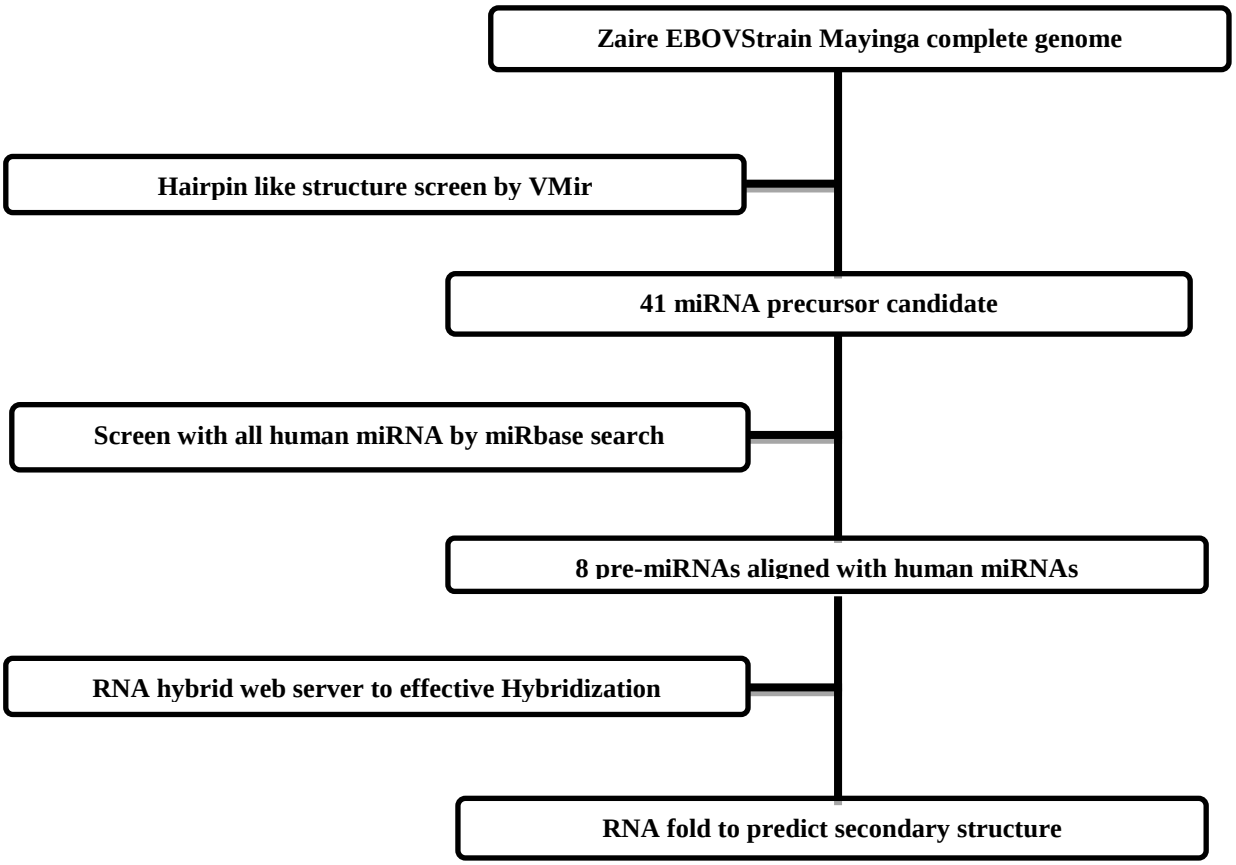


FIGURE 1: Schematic representation of human miRNA prediction on EBOV genome

III. RESULT

3.1 Prediction of precursor miRNA (pre-miRNA) hairpins with VMir

The EBOV genome was screened using VMir analyzer program. VMir viewer program helps to visualize the output of VMir analyzer. This program represents the whole output in graphical manner with sequence length and score. The Figure 2 shows the graphical representation of EBOV precursor miRNAs hairpin. By using default setting, 347 candidate hairpins (Figure

2.a) have been identified. A filter using specific parameters i.e. for cutoff value 60 nt minimum hairpin size, 220 nt maximum hairpin size and 115 minimum hairpin score were applied to avoid bona find hairpin candidate. Finally, 41 pre-miRNA hairpins (Figure 2.b) were extracted as potential hairpins for further analysis.

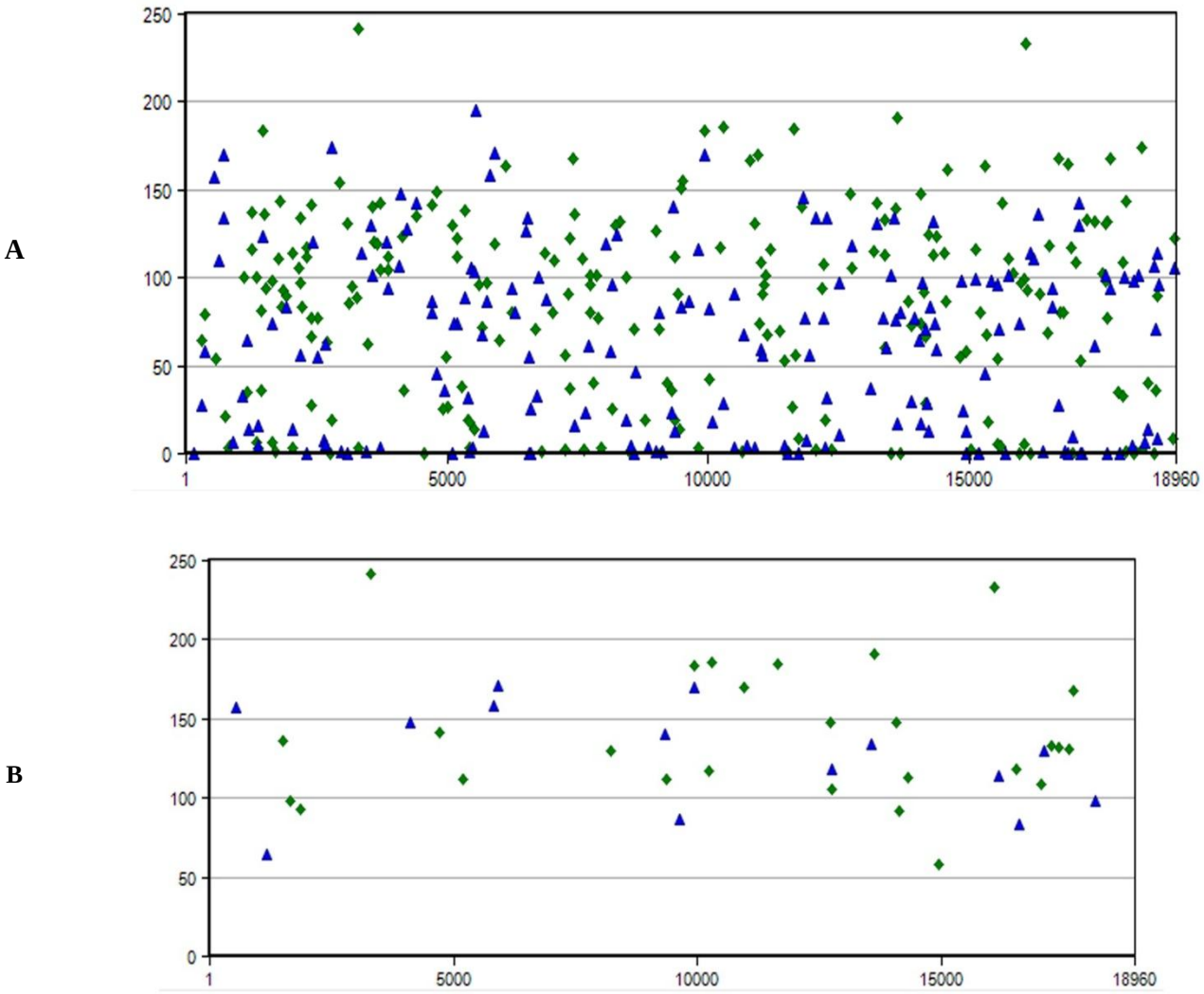


FIGURE 2: Graphical view of VMir analysis of the EBOV genome.
A. All hairpins of pre-miRNAs are showing after default setting. Hairpins are plotted according to location of the viral genome (X axis) and VMir score (Y axis).
B. Customized view of predicted pre-miRNA after faltering (minimum hairpin size: 60, maximum hairpin size: 120, minimum hairpin score: 100 and minimum widow count: 15.

3.2 Prediction of human miRNAs from precursor miRNAs hairpin

The nucleotide similarity with human miRNA of 41 candidate miRNA precursors were searched by using human miRNA filter of SEARCH menu of the miRBase database (www.mirbase.org/search.shtml) [17, 25].The eight (8) sequences were identified as candidate miRNA precursor based on significant sequence similarity with human miRNAs is shown in table 1.Human miRNA having at least 19 bp sequence similarity with candidate miRNA precursor were selected as primary target miRNA [28]. After that, close or closely perfect alignment of those miRNAs seed region (2-7) reside in the 3'-untranslated regions (3'UTR) of the candidate mRNA precursor were potential miRNA targets. Perfect complementary matching between 3' untranslated region (UTR) of the mRNA and the seed region of miRNA (2-7 bp) are important for fruitful cleavage of mRNA or translational repression. Viral precursor miRNA hairpins MD84, MD162, MR68, MR126, MR130, MR152, MR199, and MR215 have shown significant identity with hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054 respectively.

TABLE 1
ALIGNMENTS OF PRECURSOR MIRNAS HAIRPIN SEQUENCES WITH HUMAN MIRNAS.

S. No	Hairpin	Score	Alignments between human microRNA and Ebola virus
1	MD84	140.7	UserSeq 33 uaagaacauugguuccucaa 14 hsa-miR-3915 1 uaagaccaucuuuuccucaa 20
2	MD162	129.2	UserSeq 9 agggagaaggugcuggugc 27 hsa-miR-6760-5p 2 agggagaagguggaagugc 20
3	MR68	111.6	UserSeq 10 uugaaguccuggagugaagucau 32 hsa-miR-4452 1 uugaauucuuggccuuaagugau 23
4	MR126	185.4	UserSeq 55 ugucuagacacucucaguuca 75 hsa-miR-4796-5p 1 ugucuauacucugucacuuua 21
5	MR130	170.2	UserSeq 24 ucggguucuuggagcuccacc 44 hsa-miR-671-3p 1 uccgguucucagggcuccacc 21
6	MR152	147.7	UserSeq 18 guuucacuauguagcacagg 37 hsa-miR-5096 1 guuucaccauguuggucagg 20
7	MR199	118.6	UserSeq 76 ccaaugaugcauggaagaaauu 55 hsa-miR-302c-3p 2 ccacugaaacauggaagcacuu 23
8	MR215	167.6	UserSeq 29 auaacuuaaauguaacucacag 8 hsa-miR-2054 1 auaaauuaaauuuauauuacag 22

3.3 Hybridization between viral precursor miRNAs and human miRNAs

For successful hybridization between target human miRNA and precursor mRNA of EBOV was performed by the RNA hybrid tool (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid) [26]. For microRNA target prediction, RNA hybrid is a tool used widely to find out the minimum free energy hybridization of a long and a short RNA. Pairing energy or minimum free energy (mef) indicating the stability of the hybridization. We allowed -10 kcal/mol pairing energy as cutoff value for selecting potential miRNA. Effective hybridizations were shown in Figure 3.

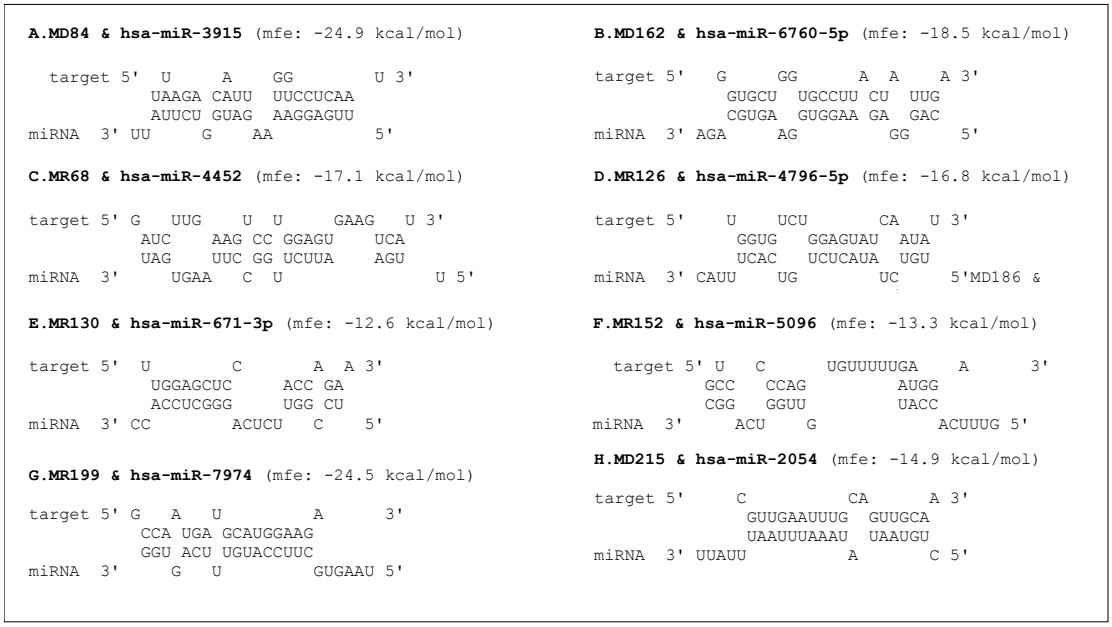


FIGURE 3: Hybridization between microRNA and viral RNAusing RNA hybrid program.

The program finds the energetically most favorable hybridization sites of a miRNA in a large hairpin of viral RNA.

3.4 Prediction of secondary structure of miRNA precursor

By using RNAfold web server (http://rna.tbi.univie.ac.at/cgibin/RNAfold.cgi), secondary structure of pre-miRNA was determined [27]. Only default parameters were in use. To predict the most stable secondary structure, the RNAfold program is widely used. In all cases, folding structures with centroid were depicted.

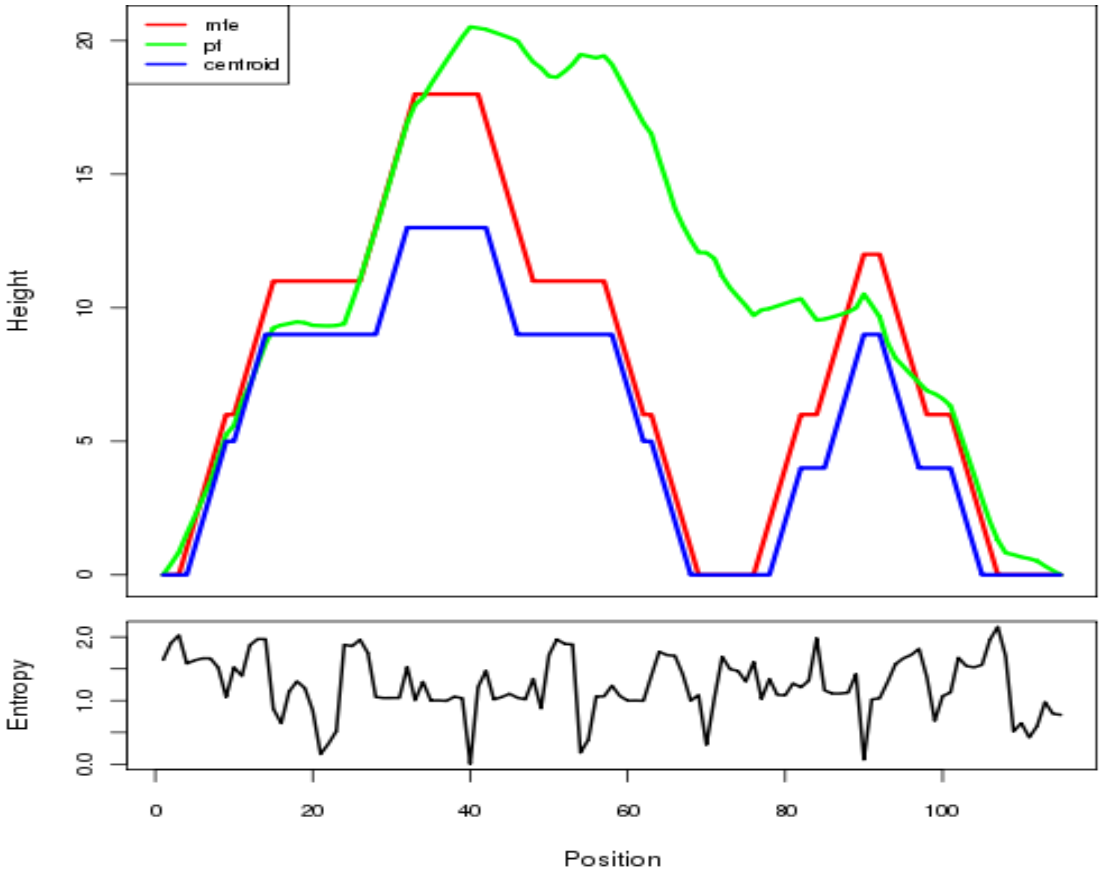
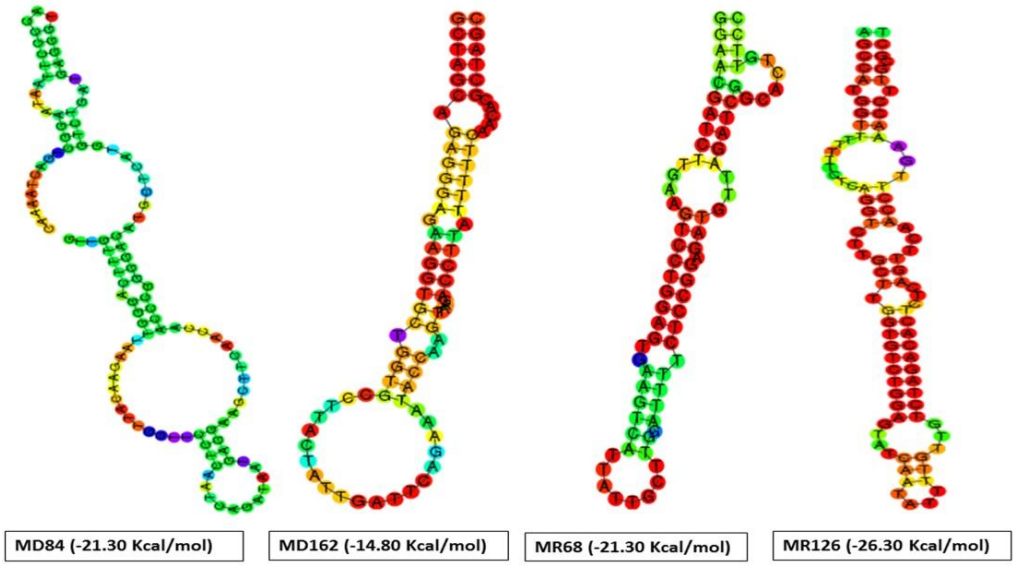


FIGURE 4: Mounting plot of predicted secondary structure of precursor miRNA hairpin

As an example, hairpin MD84 was shown. This plot has shown the minimal free energy structure (red), the thermodynamic ensemble of RNA structures (green), and the centroid structure (blue).



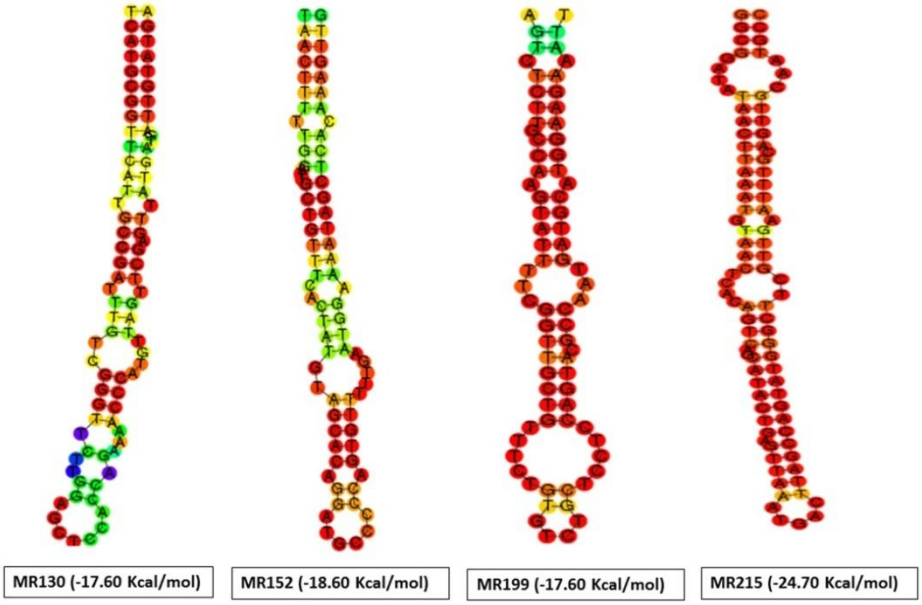


FIGURE 5: Predicated secondary structure of potential hairpins candidate of EBOV Only centroid structures were depicted.

IV. DISCUSSION

In our current investigation, we have identified host miRNAs computationally for EBOV infection in human. This study carried out based on an interesting hypothesis of utilization of host miRNA as a potential post exposure therapy because current evidence suggest that host miRNAs may down-regulated viral gene expression. It has been reported that 3' untranslated region (UTR) of the viral mRNA and the seed region of miRNA (2-7 bp) has perfect complementarity that gives sufficient results in cleavage. On the other hand, imperfect complementarity may block translation [18, 21].Bt the utilization of a series of bioinformatics tools, we predict potential miRNA hairpins candidate for EBOV genome. Viral candidate miRNA hairpins MD84, MD162, MR68, MR126, MR130, MR152, MR199, and MR215 have shown significant identity with hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054 respectively. The RNAhybrid web server ensures the effective hybridization, paring energy, p value and hybridization pattern between viral miRNAs hairpin candidates and selective human miRNAs. RNAfold tools also confirm the potential candidate miRNAs hairpin.

Based on computational analysis including VMir score, effective hybridization, and hybridization pattern and pairing energy, we propose hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054 would be best potential cellular target miRNAs to develop a post exposure therapy.

Although, most of the predicted human miRNAs on EBOV genome functions yet to be discovered but we hypnotize those miRNAs may down-regulate viral gene expression in order to block the replication.

V. CONCLUSION

The candidate potential miRNA targeting EBOV can be predicted by utilizing a series of bioinformatics tools that we have provided in this study. Our computational analysis suggested that miRNAs hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054 can be utilized as some anti-viral therapeutics against EBOV infection in human. Some future studies related this in silico study is also suggested. In order to find out the efficacy of the miRNA against EBOV infection, in vitro studies need to be carried on. To find out the inhibition influence on viral replication by the selected human miRNA, further study should be designed targeting isolation and application of miRNA for the purpose.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

This article does not contain any studies with animals performed by any of the authors.

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