September - October 2018; 7(5): 3115-3118

International Journal of Research and Development in Pharmacy & Life Science
An International open access peer reviewed journal
ISSN (P): 2393-932X, ISSN (E): 2278-0238
Journal homepage: http://ijrdpl.com

Original Article

Designing of putative siRNA to inhibit dengue virus replication

Om Prakash [1], Bhawana Jain [2] and Amita Jain*[1]

Department of Microbiology, King George's Medical University [1], Lucknow, India

Swami Vivekananda Hospital [2], Lucknow, India

Keywords: Dengue virus; RNA interference; small interfering RNA

Article Information:

Received: June 30, 2018;
Revised: August 10, 2018;
Accepted: September 15, 2018
Available online on:
01.11.2018@http://ijrdpl.com

http://dx.doi.org/10.21276/IJRDPL.2278-0238.2018.7(5).3115-3118

ABSTRACT: Dengue virus is mosquito-borne virus that manifests itself in human infections with dengue fever (DF) to dengue hemorrhagic fever (DHF). DHF can lead to development of dengue shock syndrome (DSS). RNAi works by silencing the target gene expression using siRNA. Hence there arises an urgent need to design potential siRNA against the target sequence of dengue virus gene to control its replication and pathogenicity. This study is aimed to predict self-potential designed siRNA-based therapeutics that might be used for the treatment of dengue virus infection. The prediction of potential siRNA was done by using various computational tools as searching target sequences, multiple sequences alignment, secondary structure prediction, siRNA-target sequence interaction prediction and finally the evaluation of effectiveness of predicted siRNA. Ten pair of potential siRNAs were predicted and designed rationally for silencing five target genes (Capsid, CprM, NS1, NS3 and NS5) of dengue virus used in the study through RNAi technology. The outcomes of this study are ten pair of potential siRNA molecules which might be used as a potential antiviral RNA based therapeutics to suppress the dengue virus replication.

⇑ Corresponding author at:
Amita Jain, Virology Laboratory, Department of Microbiology, King George's Medical University, Lucknow, India
E-mail: amita602002@yahoo.com

INTRODUCTION

Dengue virus (DV) infection is presently a significant worldwide problem [1]. Dengue in human present with a broad spectrum of clinical symptoms that can range from mild fever (DF) to dengue haemorrhagic fever (DHF). DHF can lead to development of dengue shock syndrome (DSS). Dengue virus (causative agent of dengue infection) belongs to *Flaviviridae* family [2]. Its genome is composed of single positive stranded RNA that encodes three structural proteins (nucleocapsid C, membrane associated M and envelope protein E) and seven non-structural proteins (NS1, NS2a, 2b, NS3, NS4a, 4b and NS5). In addition, immature mainly intra cellular virus contains a protein known as a prM (pre-membrane protein) [3].

RNAi (RNA interference) is a natural cellular process which is used to block the translation of target RNA and provides innate defence mechanism against viruses by post-transcriptional gene silencing [4].

RNAi was first discovered in Petunia plant and *Caenorhabditis elegans* and later in mammalian cells by Fire and Mello in 1990. Fire and Mello got Nobel Prize in physiology medicine in 2006 for their discovery of RNAi. Basic principle of RNAi is to silence the specific gene and corresponding protein which it encodes. There are three components of RNAi pathway: Dicer, RISC (RNA induced silencing complex) and Argonaute. RNAi pathway is initiated by an enzyme called Dicer [5]. It is activated in presence of ATP and cleaves the long double stranded RNA molecule into short dsRNA fragment (~20nt) called siRNA. Dicer generates siRNA of different length (21-23nt, 25-27nt and 30nt). This siRNA is unwound in sense and antisense strand in presence of ATP. Antisense strand of siRNA binds with target mRNA strands in presence of RISC and induces cleavage of mRNA with the help of Argonaute protein. RISC has properties to recognize the target gene. Argonaute protein is key component of RISC and responsible for cleavage activity [6]. RNAi generates three types of non-coding RNAs: siRNA, miRNA and piRNA (Piwi – interacting RNAs).

Among them siRNA has wider application as antiviral therapeutic, hence was used for DV (dengue virus) replication inhibition in present study.

Small interfering RNA (siRNA)

siRNA is double stranded RNA molecule with phosphorylated 5' ends and hydroxylated 3' ends with two overhanging nucleotides [7]. It is of 20-25 nucleotides in length. siRNA plays major role in RNAi pathway. Artificially synthesized siRNA can be introduced in cells by transfection. Mechanism of siRNA is dependent on number of siRNA molecules introduced in cells. Numbers of duplexes per cell decreases, as cells divide. Due to this reason inhibition is limited to one to two weeks only.

Aim: A study was planned to design ten pairs of siRNAs against most conserved regions of Capsid, CprM, NS1, NS3 and NS5 dengue genes and to analyze efficacy of designed siRNA to inhibit dengue virus replication *in vitro*.

MATERIALS AND METHODS

Sequence retrieval

The nucleotide sequence of standard strains of all four DV serotypes were retrieved from NCBI database with following accession no. (https://www.ncbi.nlm.nih.gov/genome/?term=dengue+virus) EU081255.1 (D1), FJ467493.1 (D2), KU216209.1 (D3) and KT794007.1 (D4) respectively.

Target selection: Five genes from all four DV serotypes were aligned with the help of Clustal W program. Most conserved and largest segment of sequence were chosen for each gene by siRNA target finder (Ambion USA). These selected sequences worked as targets for respective genes against which siRNA were designed.

Designing of putative siRNAs for each gene: Selection of best possible siRNAs against the chosen target positions for all four DV serotypes were carried out with the help of I-score designer. It includes nine different siRNA designing scores of Ui-Tei, Amarzguioui, Hsieh,Takasaki, s-Biopredsi, i-Score, Reynolds, Katoh and DSIR (https://www.med.nagoyau.ac.jp/neurogenetics/i_Score/i_score.html). To design best possible siRNA, target sequences were also screened using Gene runner, siRNA scales, i-score designer and Reynolds's guidelines. In siRNA designing maximum emphasis was given to complete Reynolds's guidelines as described in Table 1.

Table 1: Representing nucleotide sequence of target, siRNA (sense RNA and antisense RNA), position in genome, dG and Reynold's score

Target sequence	Sense RNA	Antisense RNA	Target gene	Position in genome	No. of Nucleo-tide	No. Amino acid	GC %	Free energy of folding	Free energy of binding with target	Reynolds score
TGCTGAAA CGCGAGAG AAA	UGCUGAA ACGCGAG AGAAAtt	UUUCUCU CGCGUUU CAGCAtt	Capsid	60-401	342	114	43	-1.1	-34.05	6
CCAACAGC AGGGATAT TGA	CCAACAG CAGGGAU AUUGAtt	UCAAUAU CCCUGCU GUUGGtt	CprM	60-899	840	280	43	-0.2	-34.93	8
ACATGGGC TATTGGAT AGA	ACAUGGG CUAUUGG AUAGAtt	UCUAUCC AAUAGCC CAUGUtt	NS1	2385-3440	1056	352	38	0.4	-33.49	7
GCTGACAT GGGCTATT GGA	GCUGACA UGGGCUA UUGGAtt	UCCAAUA GCCCAUG UCAGCtt	NS1	3007-3025	18	6	53	-1.6	-38.04	5
TCATATGG AGGAGGTT GGA	UCAUAUG GAGGAGG UUGGAtt	UCCAACC UCCUCCA UAUGAtt	NS3	4485-6341	1857	619	47	1.5	-35.32	6
TAATGGAT GAGGCTCA TTT	UAAUGGA UGAGGCU CAUUUtt	AAAUGAG CCUCAUC CAUUAtt	NS3	4485-6341	1857	619	37	-1.2	-30.58	6
AACAGTGT GGTTTGTT CCA	AACAGUG UGGUUUG UUCCAtt	UGGAACA AACCACA CUGUUtt	NS3	5592-5610	18	6	42	-1.2	-32.30	5
AGGGGAG GATGGTCA TATT	AGGGGAG GAUGGUC AUAUUtt	AAUAUGA CCAUCCU CCCCUtt	NS5	7819-7837	18	6	47	-0.1	-35.41	8
AGGAGGA CCAGGGCA TGAA	AGGAGGA CCAGGGC AUGAAtt	UUCAUGC CCUGGUC CUCCUtt	NS5	9398-9416	18	6	57	-1.1	-39.92	6
CACCTTTA CCAACATG GAA	CACCUUU ACCAACA UGGAAtt	UUCCAAU GUUGGUA AAGGUGtt	NS5	9399-9417	18	6	42	-1.1	-28.91	5

We considered only those siRNAs having score 5 or more than 5 at Reynolds's scale. After the screening of all essential parameters required for silencing, best siRNA for target was selected from structural and non-structural regions of DV (Table 1). These siRNA were put on BLAST search to ensure specificity for viral regions only. Not for human gene.

Prediction of siRNA secondary structure

RNAi efficiency is usually influenced by the RNA structure of the target sequence/guide strand of siRNA [8]. For every designed siRNA free energy, orientation and ease of target access were also confirmed using **M fold and RNAcofold** webserver.

To get the minimum folding and maximum stability of all possible orientations and free energy **(dG)** was calculated using http://unafold.rna.albany.edu web server. The reason of non-accessibility of target sites by siRNA may be due to secondary or tertiary structures create steric hindrance resulting in different level of positional effects, so we ruled out this reason in present study.

RESULTS AND DISCUSSION

In this study, we have designed ten pair of siRNAs targeting five region/genes of DV which fulfilled all the criteria and algorithms of i-score designer shown in table 1. Designing of siRNA targeting DV gene sequence is done in this study (Figure 1).

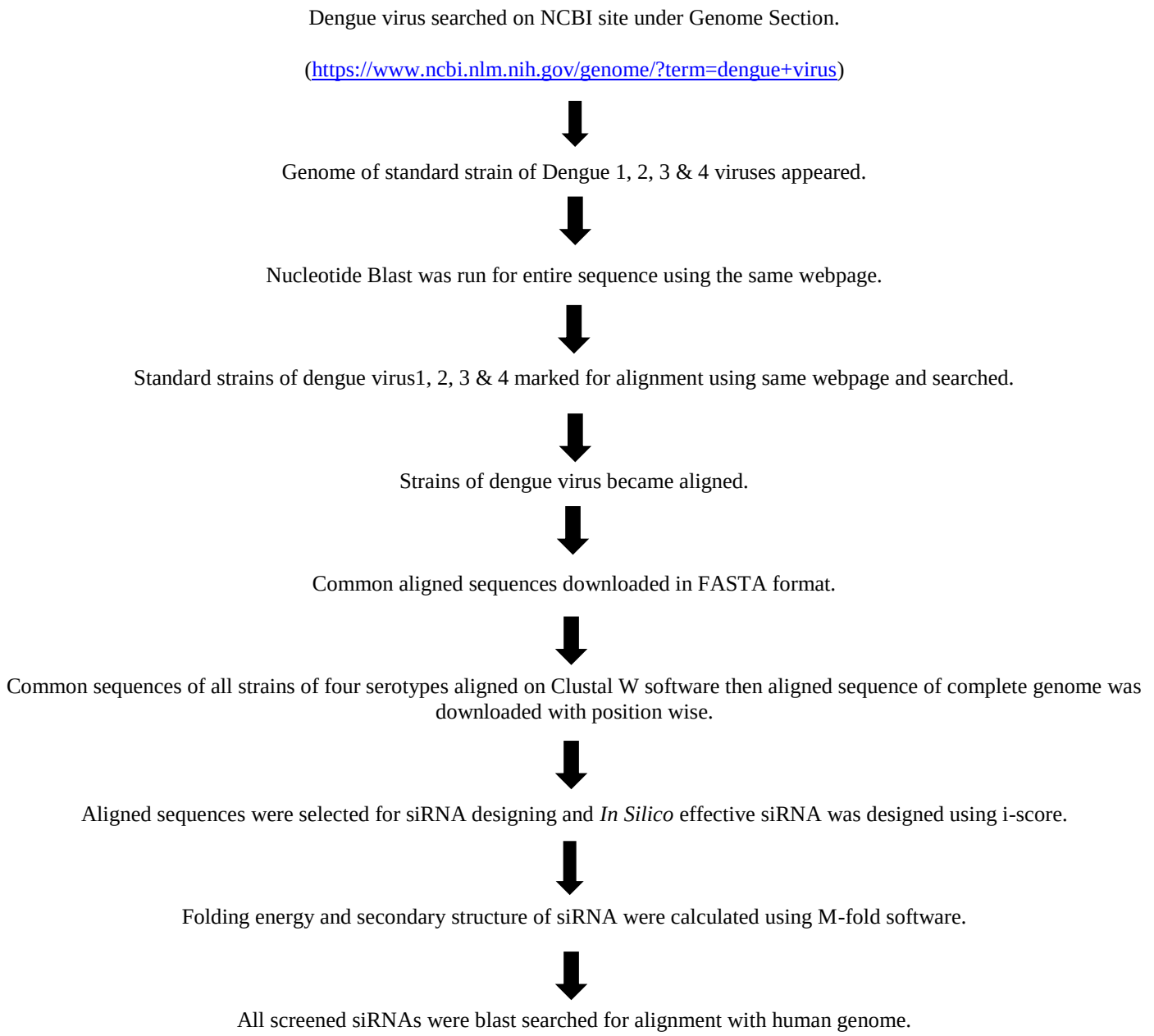

Figure 1: Schematic representation of designing of siRNA against dengue virus serotypes

Off target binding of siRNA is an important factor which needs to be minimized in order to improve specificity of siRNA. Non-designing protocol, to reduce off-target effect, Tm should be less than 21.5◦C [9], which were followed in present study. Blast run was also done against whole genome of human to remove the possibility of off- target silencing.

GC percentage is an important parameter that represents functionality of siRNA (Table1). Studies have shown that 30-50% GC content provides better activity to siRNA than higher G/C content. All siRNA designed in this study, have GC content of appropriate amount. The free energy of RNA folding is a significant parameter which was computed by using m-fold followed by using algorithms for the prediction of siRNA. It has been recommended that a guide strand siRNA must have smallest free energy for their stability [10]. Here, predicted siRNA have minimum folding free energy. Therefore, minimum folding free energy represents the effective siRNA.

Free energy of binding with mRNA was calculated by RNAcofold which is another important parameter for siRNA efficiency [10]. siRNAs designed in present study have smallest RNA-RNA interaction energy for binding. In this computational approach all the parameters and tools used for designing of siRNAs against DV genes fulfil the criteria for best efficiency of RNAi against their target sequence. This study successfully designed ten pairs of siRNAs which fulfil all criteria of siRNA molecules as therapeutic agent. Therefore, authors intended to design the most effective siRNAs targeting various genes of DV, which can be used further for DV inhibition, either singly or in combination. Such approach seems to be quite promising to combat DV, but the result of *in silico* study needs to be approved further by *in vitro* study.

CONCLUSION

This study suggests that designed siRNA can inhibit viral replication and pathogenicity. However experimental approach and validation will be required for supporting this hypothesis.

Conflict of interest Statement: None

ACKNOWLEDGEMENT

Authors acknowledge the Indian Council of Medical Research (ICMR), New Delhi, for providing financial support siRNA project grant no. (VIR/70/2013/ECD-1).

REFERENCES

1. Maria GG, Scott BH, Harvey A, Philippe B, Jeremy F. Dengue: a continuing global threat. *Nat Rev Microbiol.* 2010; 8(12 0): S7–16.
2. Gubler DJ. Dengue and Dengue Hemorrhagic Fever. *Clin Microbiol Rev*. 1998; 11(3):480–496.
3. Erik A. Henchal J. Robert P. The Dengue Viruses. *Clin Microbiol rev*. 1990; 376-396.
4. Obbard DJ, Gordon KHJ, Buck AH, Francis MJ. The evolution of RNAi as a defence against viruses and transposable elements. *Philos Trans R Soc Lond B Biol Sci.* 2009; 364(1513): 99–115.
5. Ronald HM, Plasterk A. Dicers at RISC: The Mechanism of RNAi. *Cell*. 2004; 117:1.
6. Traci M. Hall T. Structure and Function of Argonaute Proteins. *Structure*. 2005; 13:10.
7. Gaglione M, Mercurio ME, Potenza N, Mosca N, Russo A. Synthesis and Gene Silencing Properties of siRNAs Containing Terminal Amide Linkages. *Biomed Res Int.* 2014; 9016-17.
8. Joseph AG, Berger AK, and Walton SP. Impact of target mRNA structure on siRNA silencing efficiency: a large-scale study. *Biotechnol Bioeng*. 2008; 100(4): 744–755.
9. Kumiko Ui-Te. Optimal choice of functional and off-target effect-reduced siRNAs for RNAi therapeutics. *Front Genet.* 2013; 4:107.
10. Ulrike MC, Hakim T, Stephan HB. Thermodynamics of RNA–RNA binding. *Structural bioinformatics*. 2006; 22:10.

How to cite this article:
Om Prakash, Jain B and Jain A. Designing of putative siRNA to inhibit dengue virus replication. *Int. J. Res. Dev. Pharm. L. Sci.* 2018; 7(5): 3115-3118. doi: 10.13040/IJRDPL.2278-0238.7(5).3115-3118