Crimean-Congo Hemorrhagic Fever Virus: Strategies to Combat with an Emerging Threat to Human

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Abstract: Bunyaviridae family consists of vector borne lethal viruses, stands out as the largest virus family with its 350 members. One such virus of this family, Crimean-Congo hemorrhagic fever virus (CCHFV) is transmitted through bites of ixodid ticks or by direct contact with blood from infected animals. Crimean-Congo haemorrhagic fever (CCHF) is a severe disease in humans which is endemic in large parts of the world with a high mortality rate. This virus could also be used as a bioterrorism agent due to its human-to-human transmission with no specific therapy. The pathogenicity factor of CCHFV is unexplored due to the lack of animal models. CCHFV, being an RNA virus, is able to mutate rapidly hence preventing the development of effective therapy against it. Till now ribavirin is the only available drug for supportive treatment but has many side-effects. New technologies like RNA interference have emerged as a solution for epidemics of CCHF. RNAi is a sequence specific approach, has been used successfully against different pathogens. This review focuses on designing and application of RNAi with emphasis on the role of bioinformatics for the anti CCHFV therapeutic development strategy.

Keywords: Crimean-Congo hemorrhagic fever, CCHFV, bunyaviridae, nairoviridae, RNAi, siRNA.

INTRODUCTION

Crimean-Congo hemorrhagic fever virus (CCHFV) is a pathogenic arthropod borne virus. This virus belongs to the Nairovirus genus of Bunyaviridae family which include more than 350 members [1, 2]. Crimean-Congo hemorrhagic fever (CCHF) was reported for the first time in Crimea during an outbreak in 1944. Another viral strain isolated from Congo in 1956, was found to be similar to this strain and thus the virus was given its name Crimean-Congo hemorrhagic fever virus (CCHFV) [3].

Members of this enveloped virus family have a tripartite, single-stranded RNA genome of negative polarity [1, 4, 5, 7-11]. CCHFV causes acute hemorrhagic fever having a high case-fatality rate varying from 10-50% in which most of the deaths occur within 2-15 days from the onset of fever.

The geographic distribution of CCHFV is widespread in Asia, Eastern Europe, the Middle East, Africa, and Russia [6, 12-14]. Despite its prevalence and incidence in large section of the world, very little is known about its pathogenicity due to the lack of specialized laboratory facilities and appropriate animal models. The complete virus genome sequences have been determined from geographically diverse strains of CCHFV but very few protein structures have been reported. Also, these sequenced genomes show high genetic diversity which is another limiting factor for antiviral development [15-17]. The CCHFV is also a potential bioterrorism/biowarfare agent that has been listed in the U.S. as a CDC/NIAID Category C priority pathogen [14]. The epidemic and sporadic cases of CCHFV causes high case-fatality rate. [18]. There is no specific cure for CCHFV infection; patients receive only supportive therapy [19-21]. This highlights the need for a safe and effective antiviral therapy against Crimean-Congo hemorrhagic fever virus.

To bridge the gap between lack of experimental data and antiviral development against CCHFV, RNAi (RNA interference) based inhibitors can be developed, which can interfere with viral replication. This strategy exploits the intrinsic properties of viral genome and down regulates the target RNA. The RNAi specifically and efficiently interacts with the conserved regions within viral transcripts finally leading to the target cleavage [22].

Numerous reasons support the use of RNAi over the other antiviral therapies. RNAi development can begin straightaway after the elucidation of viral genome sequence while antiviral chemotherapeutics require large amount of structural and functional experimental data. The viral replication cycle relying largely on host cell proteins which limit the choice of antiviral targets for chemotherapeutic development. Whereas, RNAi aims small sections of virion nucleic acid which could result in a large number of perspective RNAi targets even in small sized viral genome. Finally, RNAi has no toxicity or immunogenicity towards host and exhibit high degree of specificity, affinity and inhibitory potential against the selected target.

Mechanistically, RNAi begins with the introduction of short double stranded RNA (dsRNA) inside the host cell. These short oligos are then cleaved in to 21-25 nucleotides small interfering RNAs (siRNA) by Dicer, an endora-
bonuclase of RNase III family. Then, these siRNA enter and activates RNA-induced silencing complex (RISC). The siRNA guides the RISC to the target RNAs and cleave the cognate target RNA in a sequence specific manner [23-25].

Although, the structural and biological knowledge of CCHFV is limited but the availability of genome sequences of geographically distinct strains makes RNAi a preferable approach for antiviral therapeutics. In this review, we focus on the epidemiology of CCHFV, scope of siRNA technology for developing the antiviral therapy and different bioinformatics tools for designing siRNAs.

**BUNYAVIRIDAE FAMILY OF VIRUSES**

Bunyaviridae family includes more than 350 virus species which are further grouped into five genera Hantavirus, Nairovirus, Orthobunyavirus, Phlebovirus and Tospovirus Table 1. Unlike all other viruses in the family, Hantaviruses are not transmitted by arthropod vectors [1, 2]. All the five groups of Bunyaviruses differ in their host preferences, antigenic nature and morphological features.

The Nairovirus genus includes dreadful viruses like Crimean-Congo hemorrhagic fever virus, digue virus and nairobi sheep disease virus. The genomic ends of the Nairovirus have repeated redundant sequences, which pair off noncovalently to form closed circular RNA. These ends have nine nucleotides length conserved regions that are UCUCAAAGA at 5’ end and AGAGUUUCU at 3’ end.

**CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS**

CCHFV belongs to Nairovirus group that causes the most dreadful disease among all the other members of Bunyaviridae. CCHFV consists of tripartite, negative sense single stranded RNA genome. The small segment (S) of the genome encodes for the viral nucleocapsid, the medium segment (M) encodes for the two glycoproteins (Gn and Gc) and non-structural proteins (mucin, GP160, GP85, GP38, and NSm), and the large segment (L) encodes a RNA-dependent RNA polymerase (RdRp). The L, M and S segments are encapsidated by nucleoprotein and associate with RdRp resulting in the formation of ribonucleoprotein particles [5, 7, 9, 10, 26, 27]. Fig. (1).

The three genome segments encode for RdRp (L segment), Gn, Gc glycoproteins and nonstructural proteins (M segment) and the nucleoprotein (S segment).

The virions of CCHFV are pleomorphic to spherical in shape with a diameter of approximately 90-100 nm. They have three filamentous, non-segmented, circular nucleocapsids. The ribonucleocapsid has a length of about

<table>
<thead>
<tr>
<th>Genus</th>
<th>Viruses</th>
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<tr>
<td>Hantavirus</td>
<td>Hantaan virus</td>
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<td>Puumal virus</td>
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<td></td>
<td>Sin Nombre virus</td>
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<tr>
<td>Nairovirus</td>
<td>Crimean- Congo hemorrhagic fever virus</td>
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<td></td>
<td>Dugbe Virus</td>
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<td></td>
<td>Nairobi sheep virus</td>
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<td>Orthobunyavirus</td>
<td>Bunyamvera</td>
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<td>La Crosse virus</td>
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<td>Phlebovirus</td>
<td>Rift valley fever virus</td>
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<td>Uukuniemi virus</td>
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<td>Sandfly Sicilian virus</td>
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<tr>
<td>Tospovirus</td>
<td>Tomato Spotted Wilt virus</td>
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Table 1. Classification of Bunyaviridae Family of Viruses

![Fig. (1). The genomic organization of CCHFV.](image-url)
The virus capsid is enveloped by a single 5nm thick lipid bilayer. The envelope proteins form distinctive spike-like small projections ~5-10nm long. Not much is known about the cellular receptors for CCHFV, except nucleolin, which is a cell surface protein.

CCHFV having L, M and S segments of genome encapsulated by a single envelope with glycoproteins GN (Black) and GC (White) protruding from its surface. Tick species, which can be infected with this virus have been identified and include Argas reflexus, Hyalomma anatolicum, Hyalomma detritum, Hyalomma marginatum and Rhipicephalus sanguineus. Among these species, Hyalomma sp. is the main carrier of CCHFV [14, 29, 30]. The presence of the carrier ticks has been reported in Asia, Eastern Europe, the Middle East, Central Africa, South Africa and Madagascar. Ticks carry this virus to small domestic and wild mammals particularly sheep, goat, cattle, hedgehogs, rats and hare. These mammals develop high titers of virus in blood, but tend not to fall ill and act as CCHFV reservoirs [14, 31-34].

**EPIDEMIOLOGY**

CCHFV prevails in an enzootic tick-vertebrate-tick cycle in nature. It has the largest geographical distribution among all tick borne viruses. This virus has been isolated from more than 30 countries in Asia, Africa, South-East and Middle-East Europe [35, 36]. Seven genotypes of CCHFV have been identified on the basis of their sequence analysis Table 2.

CCHF outbreak is more in spring and early summers in endemic countries of Northern Hemisphere, pointing towards its seasonal dependency. This could be associated with high tick density during these seasons. [37-39].

Burt et al. investigated the molecular epidemiology of CCHFV in 2005. Seventy CCHFV isolates from different parts of Africa, the Middle East and Greece were analyzed on the basis of their S segment of the genome. This analysis showed up to 18% differences at genetic level. Based on this genetic analysis the isolates have been categorized into three groups. The group A includes two clades: an African clade and an Asian clade embracing isolates from Pakistan, China, Russia, Iran and Madagascar. Isolates from South and West Africa are included in group B and group C includes a single isolate from Greece [40].

**LIFE CYCLE OF CCHFV**

It is assumed that like other viruses, CCHFV also utilizes surface glycoproteins for its attachment to the host. The cellular receptors for CCHFV have not yet been identified. Different studies have revealed that CCHFV is internalized by clathrin-dependent endocytosis in a pH dependent pathway [41]. Transcription of viral replication proteins takes place within a few hours of infection [41]. This process occurs in the cytoplasm of the host cell mediated by viral RNA dependant RNA polymerase. Negative strand synthesis is shunned during this phase of infection. Then, the virus
follows a cap snatching strategy which is common in bunyaviruses. The viral polymerase cleaves the host mRNA from its 5' ends up to approximately 15 nucleotides to form leaders. These RNA leaders are then used for the transcription of non-polyadenylated mRNA of three RNA segments [42, 43]. The mRNAs are generally shorter than transcription of non-polyadenylated mRNA of three RNA leaders. These RNA leaders are then used for the assembly. All the viral proteins and polymerase assemble at the Golgi cisternae, from where mature virions eventually open out into vesicles. These viral particles are then conveyed to the cell surface and discharged along the secretary pathway [41, 49].

**PATHOGENESIS**

The pathogenesis of CCHF is not well defined. In hemorrhagic fever, viruses disable the host immune response by manipulating the cells that initiate the antiviral response [50, 51]. An endothelial infection has an important role to play in case of CCHF pathogenesis [52, 53]. The endothelial damage leads to hemostatic failure by stimulating platelet aggregation and degranulation. This activates the intrinsic coagulation cascade [54].

In severe cases of CCHFV infection, decline in platelet count, rise in aspartate and alanine aminotransferase (AST and ALT) levels in serum and prolongation of prothrombin and partial thromboplastin (PT and aPTT) times is observed. An endothelial damage contributes to coagulopathy and activates the intrinsic coagulation cascade. This finally leads to hemorrhage due to clotting factor deficiency [55]. Cytokines like IL-10, IL-1, IL-6 and TNF-a play a key role in disease progression [56, 57].

**THERAPY**

The treatment for CCHFV is primarily symptomatic and supportive, as there is no established specific therapy available. [58]. Supportive therapy includes administration of clotting factors, fresh frozen plasma, thrombocyte, albumin and antibiotics [59]. In few cases, methylprednisolone and VAHPs give some relief but their mechanism of action is not clear. In less severe condition, where hemorrhagic symptoms are not present, antipyretics and antibiotics are sufficient for the treatment [60].

Supportive care must begin immediately with the onset of hemorrhagic symptoms.

Ribavirin has been used as a drug for CCHF treatment but is not much effective with reported side effects [20, 61]. The ribavirin is effective only in the early stage of disease. Moreover, ribavirin treatment has lead to hematological and neurological abnormalities in some cases [62-66]. The use of passive antibody transfer treatment is also reported in CCHF patients [67]. The immunoglobulin made from CCHF survivor’s plasma was injected intravenously or intramuscularly. This treatment was tested on small number of patients and needs further validation. It has also been reported that the interferon-induced MxA GTPase exhibit antiviral effect against CCHFV. The recombinant MxA inhibits viral replication by interacting with the viral nucleocapsid protein. This therapy showed side effects and thus was also not carried to the next stage [46, 68].

**PROBLEMS IN ANTIVIRAL DEVELOPMENT**

Bunyaviruses have developed the ways to counteract with the host innate and adaptive immunity. RNA viruses are known for their high mutation rate. This antigenic drift is used by RNA viruses to escape host defense mechanism [15]. The genetic diversity appears to be the result of not only accumulation of mutations but also of frequent RNA segment reassortment and even RNA recombination [69]. Genetic reassortment of the RNA viruses leads to the development of new forms of viral immunogenic proteins. [70]. In Bunyaviridae, the S region codes for nucleoprotein (NP) and non-structural proteins (NS). Mutations in this region have been shown to affect the host innate defense mechanism by suppressing IFN induction. All these modes of frequent genetic variations are limiting factor for development of antiviral chemotherapeutic agents and vaccines against CCHFV. RNAi approach which utilizes sequence specific inhibition can thus be a successful solution for anti CCHFV therapy.

**RNAI APPROACH**

RNA interference is a RNA dependant gene silencing process that plays important role in regulation of gene expression. This mechanism is controlled by the RNA induced silencing complex (RISC). It initiates in the cell’s cytoplasm by the interaction of short double stranded RNA molecules with the argonaute, a catalytic component of RISC [24]. RNAi functions at transcription, post-transcription and translation level and serves as a safeguard for maintaining genome integrity. It protects the host from viral attacks and invasion of mobile genetic elements by degrading them.

**MECHANISM OF RNA INTERFERENCE**

Biological studies have revealed the mechanism of RNA interference inside the cell. This process is divided into two phases, first the formation of double stranded siRNA (initiation phase) and second one leads to the activation of RISC complex (effector phase). Initial step begins with the introduction of long double stranded RNA molecule inside the cell. These dsRNA are recognized by double stranded RNA binding proteins, RDE-4 (C. elegans)/RDE-2 (Drosophila) [71-73]. These dsRNA are cleaved by Dicer, a member of the RNase III family, into small interfering RNAs.
siRNAs) which are 21-25 nucleotides in length, containing a two-nucleotide overhang at the 3’ end of each strand [74, 75].

The microRNAs (miRNA) are genomically encoded noncoding RNAs which help in regulation of gene expression [76-77]. The miRNAs are endogenous to every cell and structurally similar to the siRNA which are produced from exogenous dsRNA. Extensive post-transcriptional modification is required for the maturity of miRNA. The miRNA is expressed as pri-miRNA, which is processed by RNaseIII enzyme Drosha and dsRNA binding protein DGCR8 (digeorge syndrome critical region protein 8 or Pasha in invertebrates) to form pre-miRNA (precursor-miRNA) [78, 79]. The dsRNA portion of this pre-miRNA is cleaved further by Dicer to produce mature miRNA for integration into the RISC complex [80].

Dicer produces double stranded fragments, however only one of the two strands (guide strand) binds the argonaute protein and directs the gene silencing. The choice of this strand is determined by the thermodynamic properties of the duplex [81, 82]. The other strand known as anti-guide strand is degraded during RISC activation. Finally, the guide strand directs the RISC complex for the cleavage of the target mRNA strand complementary to bound siRNA [83, 84] Fig. (3).

The dsRNAs perfectly complementary to target viral mRNA sequence exogenously introduced inside the host cell. These RNAs are recognized as foreign by Dicer which cleaves them into ~21 nt siRNA duplexes. Individual arms of these duplexes are then loaded into RISC where, they are used as templates to target viral genomes and transcripts for degradation.

siRNA BASED ANTIVIRAL APPROACHES

There are several studies which support the application of siRNA technique due to its high efficiency, rapidity and specificity of action against viruses. In last few years siRNA based RNA interference has been shown to inhibit many disease causing viruses like: polio [85, 86], foot-and-mouth disease [87, 88], hepatitis C [89, 90], HIV [91, 92], influenza virus [93, 94], respiratory syncytial virus [95, 96], hepatitis B [97-100], C viruses [101-103], marburg [104] and ebola filoviruses [105, 106]. Human immunodeficiency virus type 1 (HIV-1) was the first primate virus shown to be inhibited by RNA interference (RNAi). The siRNA mediated inhibition of the early and late steps of HIV infection was reported by Jacque. Various regions of HIV genome were targeted to prevent the formation of viral complementary DNA intermediates [91]. Till now, several target against HIV has been identified which include structure protein Gag [107-110], and Env [110, 111], reverse transcriptase Pol [107], accessory proteins (Nef and Vif) [91, 112, 113], and regulatory proteins (Tat and Rev) [114-116]. Although, few studies suggest that HIV-1 can escape from inhibition by mutation of its RNAi target sequence [113, 117, 118]. To overcome this problem, lentiviral vectors containing different siRNA expressing cassettes has been developed, which can simultaneously target multiple sequences [119, 120].

Hepatitis C virus, which has been recognized as a global health problem by WHO, was also studied for probable siRNA targets. The 5’ UTR and the coding sequences of Core, NS3, NS4B and NS5B of HCV have been shown to be sensitive to siRNA [90, 101, 121-124]. In case of hepatitis B virus, P, Pre C/C, Pre S/S, X genes have been employed as targets of RNAi [125-129]. Multiple shRNAs that target DR elements and regions that code for core, polymerase, PreS, S and X proteins have been designed and tested. These shRNAs effectively inhibited HBV replication and also showed synergistic effects with antiviral drug, lamivudine [130].

The prophylactic and therapeutic efficacies of siRNA were observed in case of SARS-CoV infection. Vector based shRNAs against leader sequence [131, 132], 3’-UTR [133], non-structural [133] and structural genes [132, 134-136] of SARS-CoV were synthesized and tested in vitro as well as in vivo with satisfactory results. Antiviral activity of siRNAs was evaluated against hazare virus which is closely related to CCHFV, by targeting the L (polymerase), M (glycoproteins) and S (nucleoprotein) regions of the viral genome [137]. These studies showed that nucleoprotein gene of hazare virus is a prime RNAi target whereas targeting L protein does not have much inhibitory effect. Such studies were also performed in

Fig. (3). Antiviral RNAi pathway.

Antiviral RNAi pathway.
case of rift valley fever virus and La Crosse virus [138, 139]. In case of both HAZV and La Crosse Virus, viral replication was inhibited significantly by targeting the S segment while targeting L and M segment showed a weaker effect.

siRNAs directed against highly conserved regions of the nucleoprotein gene of orthobunyavirus Akabane has shown up to 99% inhibition of viral replication [140]. Three siRNA genes, targeted to the S segment were designed based on most conserved regions among different isolates using various web programs (PILUP and PRETTY) [140]. These targets were located at position 29, 151 and 255 of the S segment. Targets at position 29 and 151 were 100% conserved in all the strains while a substitution of G in place of A at position 255 was observed in two Australian strains RB935 and R7949 of akabane [140].

Nairoviruses like other RNA viruses, show high mutation rate which might contribute to their escape from siRNA inhibition. This could be avoided by targeting the most conserved viral genome regions for siRNA designing. In case of nairoviruses, S region is most conserved among all the three regions. This region has thus emerged as the most potent siRNA target in case of CCHFV. In nairoviral infection, nucleoprotein plays a crucial role in viral replication and transcription therefore targeting it could lead to successful inhibition of CCHFV.

Antiviral effect of siRNAs used in combination with ribavirin has been studied for nairoviruses. This strategy reduces the toxic effects of drug by decreasing its active dose and also prevents the chances of drug resistance. Use of siRNA in combination with ribavirin has shown synergistic effect in case of hazara virus [137].

These experiments demonstrate the ability of siRNA as a promising strategy which could be used as a successful future anti-CCHFV therapy.

**siRNA DESIGNING**

Bioinformatics programs can be used to identify the potential target for RNAi based antiviral development [141, 142]. The siRNA sequence should be designed to give maximum silencing without any off target effects and the suppression of target gene should not have any harmful effect. It is important to select a sequence that is unique to our targeted gene by performing BLAST searches. Even a single mismatch can affect siRNA’s specificity towards the target sequence. All the sequences, which have high similarity with the target gene, should be checked to avoid any undesirable silencing. The selected sequences should display favorable thermodynamic profile for incorporation of guide stand into the RISC [143]. The sfold web server that aid in the selection of favorable sequences can be accessed on the web (e.g., http://sfold.wadsworth.org).

Cao et al. have used bioinformatics and comparative genomics approach to design siRNA against Hepatitis B virus (HBV) [144]. The NCBI databases (http://www.ncbi.nlm.nih.gov) were used to search the genomic regions, transcripts and products in the complete cds of HBV. The Vector NTI program was used to define coding domain of HBV CDS start and end sites in HBV DNA sequence. siRNA was designed using siRNA Target Designer program tool. The BLAST was to search for homologous sequences against appropriate genome databases. [144].

The problem of functional viral variants in siRNAs designing has been taken into account by Méndez-Ortega et al. The siRNAs were designed against a set of naturally occurring changes, A bioperl based algorithm was developed to identify the existing viral sequence variants and their frequencies. This algorithm was successfully used to identify many subdominant and infrequent viral variants for siRNA design against antiretroviral resistant viruses [145].

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<th>Server</th>
<th>Salient Features</th>
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<tr>
<td><a href="http://www.ictvdb.org/index.htm">http://www.ictvdb.org/index.htm</a></td>
<td>ICTVDB: The Universal Virus Database of the International Committee on Taxonomy of Viruses</td>
<td>This database is a research tool for understanding relationship among viruses.</td>
<td>[151, 152]</td>
</tr>
<tr>
<td><a href="http://viralzone.expasy.org/">http://viralzone.expasy.org/</a></td>
<td>ViralZone</td>
<td>This database contains whole list of known virus and provides information on all known virus families/genera</td>
<td>[153]</td>
</tr>
<tr>
<td><a href="http://www.biovirus.org/index.asp">http://www.biovirus.org/index.asp</a></td>
<td>Viral Bioinformatics Resource Center</td>
<td>This database provides informational, analytical tools and resources. It is concerned primarily with viruses encompassing the families Arenaviridae, Bunyaviridae, Flaviviridae, Filoviridae, Paramyxoviridae, Poxviridae, and Togaviridae.</td>
<td></td>
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<tr>
<td><a href="http://bioinfo.emei.in/virgen/virgen.html">http://bioinfo.emei.in/virgen/virgen.html</a></td>
<td>VirGen: a comprehensive viral genome resource</td>
<td>VirGen is an annotated and curated database comprising complete genome sequences of viruses, value-added derived data and data mining tools.</td>
<td>[155]</td>
</tr>
<tr>
<td><a href="http://www.vizier-europe.org/index.php">http://www.vizier-europe.org/index.php</a></td>
<td>VIZIER: consortium</td>
<td>This project target the replication enzymes of RNA viruses for antiviral therapy</td>
<td>[156]</td>
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Table 4. Server/Tools for siRNA

<table>
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<tr>
<td><a href="http://jura.wi.mit.edu/bio/">http://jura.wi.mit.edu/bio/</a></td>
<td>The whitehead siRNA Selection Web Server</td>
<td>design sequence of 21 nt length dsRNA and presents information about uniqueness of 21mers within the genome, thermodynamic stability of the double stranded RNA duplex, GC content, presence of SNPs etc.</td>
<td>[157]</td>
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<tr>
<td><a href="http://sysbio.kribb.re.kr/AsiDesigner/">http://sysbio.kribb.re.kr/AsiDesigner/</a></td>
<td>AsiDesigner</td>
<td>Provides siRNA design capability for alternative splicing for mRNA level gene silencing. Also provides function like designing of common siRNAs for silencing of more than two mRNAs simultaneously, provides scoring scheme to evaluate the performance of designed siRNA.</td>
<td>[158]</td>
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<td><a href="http://ma.urmc.rochester.edu/cgi-bin/server_exe/oligowalk/oligowalk_form.cgi">http://ma.urmc.rochester.edu/cgi-bin/server_exe/oligowalk/oligowalk_form.cgi</a></td>
<td>OligoWalk</td>
<td>Calculates thermodynamic features of sense-antisense hybridization and predicts the free energy changes of oligonucleotides binding to a target RNA.</td>
<td>[159]</td>
</tr>
<tr>
<td><a href="http://www.microsynth.ch/354.0.html">http://www.microsynth.ch/354.0.html</a></td>
<td>MICROSYNTH</td>
<td>This tool is based on Reynolds-criteria with some proprietary extensions to design siRNA.</td>
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<td><a href="http://www.unige.ch/sciences/biologie/bicel/websoft/RNAi.html">http://www.unige.ch/sciences/biologie/bicel/websoft/RNAi.html</a></td>
<td>TROD (T7 RNAi Oligo Designer)</td>
<td>Designing of the DNA oligonucleotides for the synthesis of short interfering RNAs (siRNAs)</td>
<td>[161]</td>
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<tr>
<td><a href="http://informatics-eskitis.griffith.edu.au/siSVM/">http://informatics-eskitis.griffith.edu.au/siSVM/</a></td>
<td>siSVM</td>
<td>SiSVM is a prediction server based on the machine learning algorithm, support vector machine. It includes the common method of siRNA design like motif rules, energy conditions and specificity searching</td>
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<tr>
<td><a href="http://informatics-eskitis.griffith.edu.au/SpecificityServer/">http://informatics-eskitis.griffith.edu.au/SpecificityServer/</a></td>
<td>SpecificityServer</td>
<td>It helps to identify potential non-specific matches to the designed siRNA. Incorporates latest information about non-specific matches.</td>
<td></td>
</tr>
<tr>
<td><a href="http://crdd.osdd.net/servers/virsirnadb/index.php">http://crdd.osdd.net/servers/virsirnadb/index.php</a></td>
<td>VIRsiRNAdb</td>
<td>Database of Viral siRNA/shRNA, contains experimental information of siRNA sequences, target gene, GenBank accession number, design algorithm etc.</td>
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</tbody>
</table>

are the two major web servers available for siRNA designing against viruses. siVirus has information about highly conserved sequences of HIV, SARS, HCV and influenza virus [149]. Whereas CAPSID has wider application as it could be used in designing siRNAs against user-defined sequences [150]. SPEED is a basic tool used for generating libraries of siRNAs for different genes from their cDNA information [162]. These siRNA libraries can ease the work of designing an effective siRNA in short period of time.

The siRNA sequence databases like siRNAdb, HuSiDa has been developed [163, 164]. The siRNA from these databases can be used for analysis and act as prognostic model. siRNAdb has two sets of information, one set contains experimentally verified siRNAs whereas second set contains computationally chosen siRNAs [163]. In addition to these, another siRNA database has also been developed by members of MIT named as MIT/ICBP siRNA Database which has siRNA and shRNA sequences .This database is managed by MIT researchers, NCI researchers and ICBP and CGAP programs. All the submissions to this database are also submitted to NCBI’s probe database.

CONCLUSION

The epidemic outbreaks of CCHFV have been reported in larger section of world and caused hemorrhagic fever leading to massive life loss. Despite of its great pathological significance there is no specific therapy available for this virus. RNAi technique has been used as a tool in therapy for various viruses like HIV, hepatitis B, C and influenza viruses. In case of hazara virus, which has close resemblance to CCHFV, this technique has given significant results. The siRNA against HAZV target the nucleoprotein gene, which is the most conserved region among nairovirus family, has shown promising inhibitory effects. Nowadays, large number of computational tools like siRNA Target Finder is available which help in identifying the targets for siRNAs. Also, web servers like siVirus and CAPSID are useful in specifically designing siRNAs against viruses. In-silico tools like MASS
are also accessible to validate the efficacy of siRNA by simulating in vivo environment. siRNAdb, Husida and MIT/ICBP siRNA Database provide comprehensive and easily accessible information of experimentally and computationally predicted siRNA from diverse organisms. The information from these siRNA databases could be helpful in efficient designing of siRNA. All these features could possibly make RNAi, a potential approach to combat dreadful CCHFV threat.

**ABBREVIATIONS**

CCHFV = Cremian Congo Hemorrhagic Fever Virus  
CCHF = Cremian Congo Hemorrhagic Fever  
L = Large segment  
M = Medium Segment  
S = Small segment  
RdRp = RNA dependant RNA polymerase  
RNAi = RNA interference  
RISC = RNA Induced Silencing Complex  
NS = Non-structural  
NP = Nucleoprotein  
siRNA = Small interfering RNA  
shRNA = Small hairpin RNA  
HAZV = Hazare virus

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflicts of interest.

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Declared none.

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