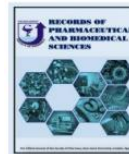




RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES



Small interfering RNA; toward a new antiviral therapy

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Abstract

With the ability of viruses to mutate and introduce a high rate of diversity and continuous resistance to drug development, unique solutions are needed. RNA interference (RNAi) pathway as a natural defense mechanism mediated by plants, invertebrates, and vertebrates displayed a promising gene silencing platform through high sequence-specific technique. Non-coding small interfering RNA (siRNA) as a member of RNAi successfully inhibited many different viruses efficiently and can be designed against any viruses even very small ones. This highly conserved silencing mechanism involved many steps and components to knock down any gene and there are many criteria that influence siRNA efficiency and specificity. RNAi technology attracts the scientific community since its discovery with continuous developments regarding siRNA design, bioinformatics tools, and their delivery to cells. In this mini-review, we discussed the basic pathway of siRNA, their antiviral role, efficient delivery to cells, and the critical factors that affect their efficiency and specificity.

Keywords: RNA interfering, small interfering RNA, gene silencing, non-coding RNA, viruses

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Introduction:

Viruses are intracellular microorganisms that seriously infect all forms of life with a high incidence to mutate and escape from the immune system. Furthermore, viruses depend on host cells to encode their essential elements, which harmed host cells ([Ding and Voinnet 2007](#)). Limited therapeutics against viruses exist with many obstacles, as antiviral therapeutics are always expensive with much research effort and narrow therapeutics window to a specific pathogen, besides the high tendency to resist these therapeutics and initiate drug toxicity ([Ikegami 2012](#)) so, new interventions are required to attack viruses.

RNA interference (RNAi) mechanism evolved previously in response to viral infection in plants and invertebrates by silencing specific genes using non-coding small RNA ([Ding and Voinnet 2007](#)). RNAi depends on 21-30 nucleotides with three

different pathways, small interfering RNA (siRNA), microRNA (miRNA), and Piwi-RNA (piRNA) ([Vaucheret 2006](#)). Small interfering RNA endogenous silencing pathway was applied to mRNA of the specific gene when double-stranded RNA was cleaved by the cytoplasmic DICER endonuclease enzyme into small fragments complementary to the specific query specifically in the middle region ([Levanova and Poranen 2018](#)). The capability of siRNA as an antiviral agent was firstly reported against Respiratory syncytial virus (RSV) which triggered a scientific effort in discovering the potentialities of siRNA against different viruses ([Bitko and Barik 2001](#)). Since the first introduction of siRNA in 1998 ([Fire, Xu et al. 1998](#)), uprising studies directed toward siRNA technology and many different algorithms and bioinformatics tools have been designed and developed to optimize siRNA activity. In this mini-review, the component involved in the siRNA pathway, antiviral application of siRNA against

many viruses, and design criteria for a successful silencing process were our focus points.

1. Pathway and component in siRNA

RNA-endogenous silencing is an uprising antiviral mechanism that regulates gene expression with cellular specific pathways which can be exogenously synthesized and delivered to the cytoplasm of targeted cells as many cellular component involved. RNAi pathway involved many steps and components that are essential for the silencing mechanism. Firstly, a long double-stranded RNA is degraded into 19-23 nucleotides as a short siRNA with 2 overhangs on the 3 prime end by the DICER enzyme ([Bernstein, Caudy et al. 2001](#)). In cytoplasm, this short double-stranded RNA unwinds, and one strand is loaded into RNA-induced silencing complex (RISC) with help of Argonaute protein. This loaded strand is called the guide strand while the passenger strand was degraded. Then this complex search for the complementary sequence where they bind and silence gene expression (Figure 1) ([Hannon and Rossi 2004](#)). The election of guide strand depends on the thermodynamic properties of siRNA duplexes ([Gu, Jin et al. 2011](#)). The remarkable nature of siRNA is the ability to regulate any gene utilizing a high sequence-specific manner, besides interfering with the virus's life cycle in an early event without any toxicity to host cells, so these amazing features announce siRNA as a promising antiviral therapeutics ([Dykxhoorn and Lieberman 2006](#)).

2. Designing an efficient siRNAs

The ideal siRNA is sequence that target an essential event for virus replication with high conservation using many bioinformatics tools, so that, the first step is designing an efficient siRNA. A conserved sequence will be selected by many in silico data bases, any sequence homology will be excluded, and by sequence and structural algorithms a potent siRNA will be selected ([Reynolds, Leake et al. 2004](#), [Tafer 2014](#)). Some essential criteria must be considered in designed sequence, length of siRNA must be 19-23 nucleotides to avoid stimulation of the immune system ([Naito, Yamada et al. 2004](#)). It is preferable to target ORF especially 50- 100 downstream nucleotide ([Fakhr, Zare et al. 2016](#)), and avoid any sequence homology with other genes by using BLAST program to protect siRNA from mediating off-target effects ([Kim 2010](#)). On the level of residues compositions there are many factors that must be considered as GC content, low content caused weak and unspecified binding while high content stabilizes siRNA duplex preventing

unwinding of duplex and their loading in the RISC complex ([Ishizuka, Siomi et al. 2002](#)). The number of A/U residues influences the internal stability of the duplex as more than 5 A/U at the 5 ends of the guide strand is suggested ([Naito, Yamada et al. 2004](#)), presence of U at position 10 enhances activity and efficiency of silencing as RISC spliced mRNA query sequence in 10-11 position ([Elbashir, Martinez et al. 2001](#)). There are many diverse software and online bioinformatics tools that help in designing efficient and specific siRNA with different parameters and algorithms so it is suggestable to use more than one tool to ensure specificity. Reynold rules was and still very efficient in designing a specific siRNA using eight critical rules to design effective siRNA to silence the target gene ([Lakshmi, Umopathy et al. 2019](#)). However none of these software guarantees design, so it must be evaluated to ensure their antiviral activity ([Fakhr, Zare et al. 2016](#)).

3. Delivery of siRNA

The main concept of RNAi is to deliver a small piece of nucleic acid to a target cell to block specific gene expression ([Leonard and Schaffer 2006](#)). There are many challenges that faces siRNA pathway as antiviral therapeutics, firstly, viruses duplicated in a high pace with accumulated mutation especially RNA viruses. Secondly, during viruses life cycles double stranded RNA produced which stimulate immune response mainly in plant viruses rather than animal viruses ([Li, Li et al. 2002](#), [Li, Li et al. 2004](#), [Bennasser, Le et al. 2005](#), [Lecellier, Dunoyer et al. 2005](#)). Delivery of siRNA into targeted cells is the real challenge that faces siRNA as it can be delivered directly to cells which provide rapid but transient silencing activity as in some acute infection as in IVA and SARS- CoV ([Ge, McManus et al. 2003](#), [Ge, Filip et al. 2004](#), [Zheng, Guan et al. 2004](#)), while using vectors mediated a sustained delivery as in chronic viral infection with HIV-1 ([Banerjea, Li et al. 2003](#), [Bennasser, Le et al. 2005](#)). Dose of the delivered siRNA is a critical factor in post- transcription silencing mechanism as high dose stimulate immune response and introduce non- specific effect ([Judge, Sood et al. 2005](#)). To deliver stable siRNA and efficiently uptaken by targeted cells many chemical modification had been developed as addition of 2- omethyl or 2-deoxy or 2- fluor to sugar backbone at position 2 of ribose ring during chemical synthesis, as this modification had no effect on silencing activity but increase plasma stability, reduce off target effect and undetectable by immune system ([Chiu and Rana 2003](#), [Morrissey, Lockridge et al. 2005](#)), two overhang thymidine on both ends of siRNA duplex protect

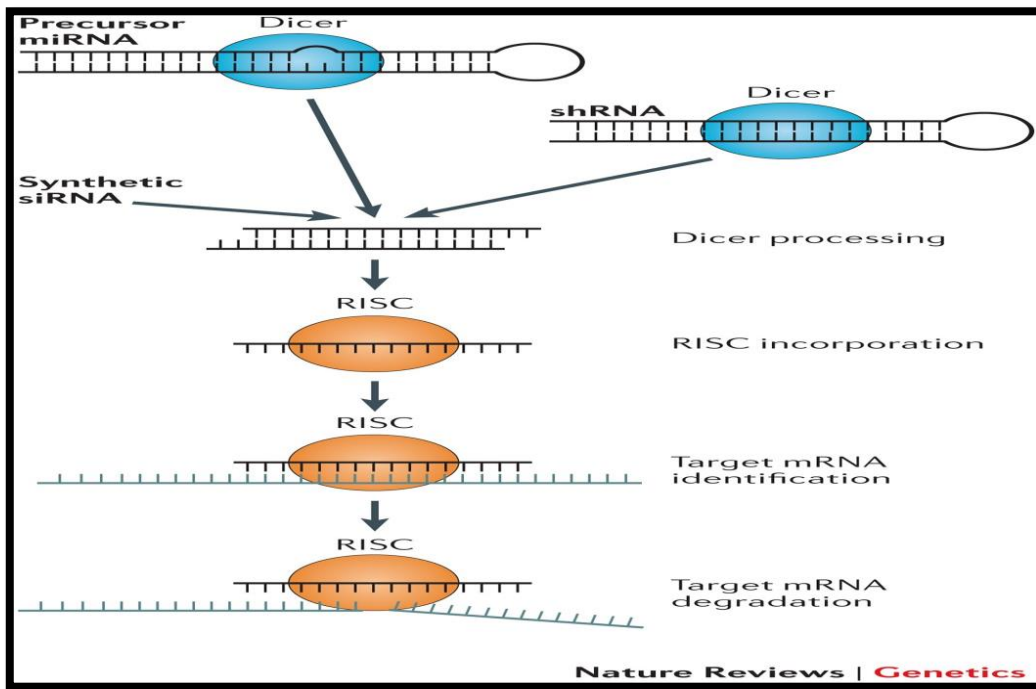


Figure 1: Systemic diagram of RNAi mechanism of action (Wittrup and Lieberman 2015)

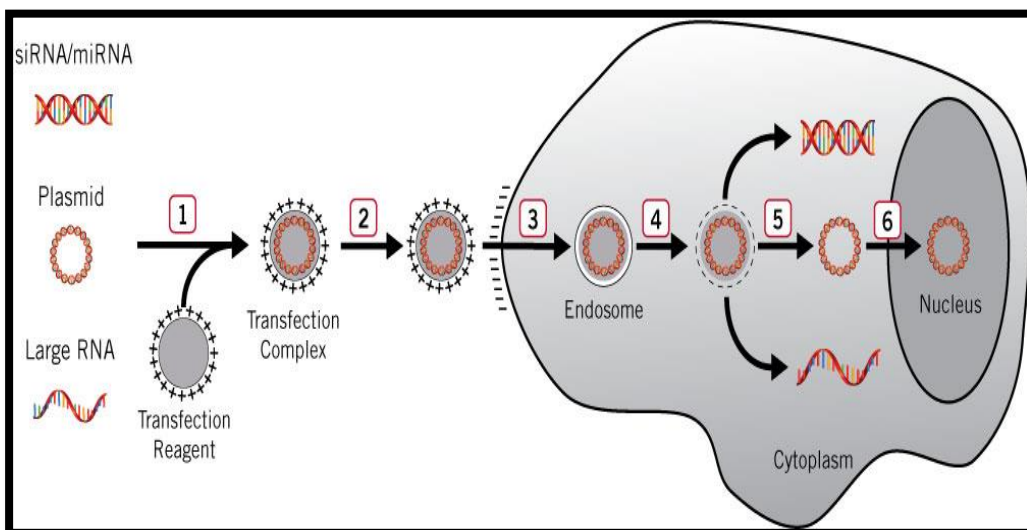


Figure 2: Chemical Transfection into Cells, as 1) represent combination between transfection reagent and nucleic acid to form positively charged transfection complexes. 2) Addition of complexes to cells, and bind to the negatively charged cell surfaces via electrostatic interactions. 3) Cells internalize complexes by endocytosis into membrane vesicles known as endosomes. 4) Destabilizes endosomal membrane by transfection complex 5) Complexes escape from endosomes and release nucleic acid in cytoplasm (siRNA, miRNA, or large RNA are generally active in cytoplasm). 6) DNA must localize to the nucleus, where gene expression cassette is transcribed (Figure Mirus Bio).

siRNA from nuclease degradation and increase stability beside being cost – effective (Elbashir, Harborth et al. 2001, Alvarez, Elbashir et al. 2009). siRNA can be delivered in mammalian cell culture when conjugated with liposomes known as

transfection agent efficiently which highlighted the unexpected outcomes of siRNA pathway against many viruses without wasting time or using costly animal models (Figure 2) (Bitko and Barik 2001).

4. The success of siRNA as antiviral treatments

After the antiviral success of siRNA against RSV ([Bitko and Barik 2001](#)), different viruses had been evaluated as a target for gene silencing. As all viruses used host cell component for expression of their genome, in addition that RISC is targeted only cytoplasmic mRNA, siRNA considered an ideal target for endogenous gene silencing pathway without toxicity to host cells ([Leonard and Schaffer 2006](#)). In arthropod-transmitted viruses, siRNA mediated a promising antiviral potentiality regardless these viruses are positively stranded RNA or negatively stranded RNA as Flaviviridae viruses and Bunyaviridae viruses respectively ([Blair and Olson 2015](#), [Dietrich, Jansen et al. 2017](#)). Presence of high sequence homology between several arboviruses as Togaviruses ([Stollar and Shenk 1973](#), [Karpf, Lenches et al. 1997](#)), Flaviviruses ([Schmaljohn and Blair 1977](#)), Bunyaviruses ([Beaty, Bishop et al. 1983](#), [Elliott and Wilkie 1986](#)) allowing siRNA to target all these viruses with only minor change in siRNA design. siRNA inhibited many viruses as Human immunodeficiency virus (HIV) by targeting all 9 genes ([Dyckhoorn and Lieberman 2006](#)), Hepatitis B virus ([Arbuthnot, Carmona et al. 2005](#)), Influenza A virus ([Tompkins, Lo et al. 2004](#)). For Orthopoxviruses, siRNA greatly inhibited Monkey pox virus replication efficiently at concentration 10 nM ([Alkhalil, Strand et al. 2009](#)). In Human papilloma virus (HPV) siRNA targeting the apoptotic effect and restrain virus transmission ([Butz, Ristriani et al. 2003](#)). Studies against SARS-CoV virus effectively silenced virus replication ([Meng, Lui et al. 2006](#)). SiRNA designed against Ebola virus when the massive outbreak inflicted in 2014 and showing complete abrogation of virus ([Thi, Mire et al. 2015](#)). This unique antiviral activity of siRNA against many different viruses within diverse families due to its ability to target a specific gene sequence by selecting the most conserved region in virus genome that is essential for virus replication.

Conclusion

Antiviral therapeutics that depending on siRNAs proved successful opportunities to be the future antiviral agents against almost all viruses by just modifying any design to be specific for targeted query sequence utilizing query data and following specific criteria. The great intervention in computational tools facilitate designing and target prediction. So this selective gene regulation pathway can be used to display antiviral activity, so we planned to investigate the possible potentialities

of specific siRNA against many viruses to figure out their efficiency and specificity as antiviral and prophylactic therapeutics.

Conflict of interest: The authors declare that they have no competing interests.

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