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RNA INTERFERENCE:THERAPEUTIC APPLICATION ON COVID 19.

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ABSTRACT It is now believed that control in SARS CoV 2(Covid 19) outbreaks is failure in this time all over the world because we have no effective antiviral drugs and vaccineagainst Covid 19.Hence we required effective,specific, fast,without toxicity to host cell and cost-effective alternative to existing gene targeting technologies both in cell-based and in vivo settings.Only we have one way BIOTECHNOLOGY Method, In which one is RNA interference(RNAi) has been successfully used as a more specific and efficient method for gene silencing.Viruses are formidable targets for drug development. As obligate intracellular parasites that take over the host cellular machinery to further their own agenda, they seem to understand the intimate workings of cellular pathways better than those seeking to develop treatments against them. It is difficult to inactivate a virus without doing harm to the host cell.Only RNAi has become evident that has immense potential in suppression of desired genes.Only in the past decade with the concerted efforts to control the HIV/AIDS epidemic have antiviral drugs come into their own. But nowby targeting viral proteins and pathways unique to the viral life cycle, it has become possible to interfere with viral infection and replication for a few viruses without unacceptable host cell toxicity.However, antiviral drugs have only been developed for a handful of viruses, and none of these antiviral drugs is completely effective.Viral resistance, sequence diversity, and drug toxicity are significant problems for all antiviral therapies. In this article, we discuss recent progress and obstacles to harnessing RNA interference to prevent or treat viral infection.Moreover, there is no treatment for most acute viral infections, such as global pandemics caused by newly emerging infectionsSARS CoV 2(Covid 19) which responsible for global deaths,in the setting of economic development and changing ecology, has made the rapid development of novel antiviral therapies an international priority. It is not surprising, that RNA interference (RNAi)was discovered to work in mammalian cells, researchers honed in on attempts to harness this ancient antiviral mechanism to prevent or treat viral infection. If we are prevent anyone gene translation required for covid 19 life cycle, we are successfully destroy the covid 19.

KEYWORDS :- RNAi; Gene silencing; Antiviral; Dicer, miRNA, siRNA,RISC,cocktail, shRNA.

Introduction:

RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules.

Also known as co-suppression, posttranscriptionalgene silencing (PTGS), and quelling.

Andrew Fire and Craig C. Mello shared the 2006 Nobel Prize in Physiology or Medicine for their work on RNA interference in the nematode worm Caenorhabditiselegans, which they published in 1998.

Two types of small ribonucleic acid (RNA) molecules – microRNA (miRNA) and small interfering RNA (siRNA) – are central to RNA interference and these small RNAs can direct enzyme complexes to degrade messenger RNA (mRNA) molecules and thus decrease their activity by preventing translation, via post-transcriptional gene silencing.

Moreover, transcription can be inhibited via the pre-transcriptional silencing mechanism of RNA interference,through which an enzyme complex catalyzes DNA methylation at genomic positions complementary to complexed siRNA or miRNA.

RNA interference has an important role in defending cells against parasitic nucleotide sequences – viruses and transposons.

It also influences development.

The RNAi pathway is found in many eukaryotes, including animals, and is initiated by the enzyme Dicer, which cleaves long double-stranded RNA (dsRNA) molecules into short double-stranded fragments of ~21 nucleotide siRNAs.
Each siRNA is unwound into two single-stranded RNAs (ssRNAs), the passenger strand and the guide strand. The passenger strand is degraded and the guide strand is incorporated into the RNA-induced silencing complex (RISC).
The most well studied outcome is post-transcriptional gene silencing, which occurs when the guide strand pairs with a complementary sequence in a messenger RNA molecule and induces cleavage by Argonaute 2 (Ago2), the catalytic component of the RISC.

In some organisms, this process spreads systemically, despite the initially limited molar concentrations of siRNA.

RNAi is a valuable research tool, both in cell culture and in living organisms, because synthetic dsRNA introduced into cells can selectively and robustly induce suppression of specific genes of interest.

RNAi may be used for large-scale screens that systematically shut down each gene in the cell, which can help to identify the components necessary for a particular cellular process or an event such as cell division.

The pathway is also used as a practical tool in biotechnology, medicine and insecticides.

Cellular mechanism

RNAi is an RNA-dependent gene silencing process that is controlled by the RNA-induced silencing complex (RISC) andis initiated by short double-stranded RNA molecules in a cell's cytoplasm, where they interact with the catalytic RISCcomponent argonaute. When the dsRNA is exogenous (coming from infection by a virus with an RNA genome orlaboratory manipulations), the RNA is imported directly into the cytoplasm and cleaved to short fragments by Dicer. Theinitiating dsRNA can also be endogenous (originating in the cell), as in pre-microRNAs expressed from RNA-

codinggenes in the genome. The primary transcripts from such genes are first processed to form the characteristic stem-loopstructure of pre-miRNA in the nucleus, then exported to the cytoplasm. Thus, the two dsRNA pathways, exogenous andendogenous, converge at the RISC.Exogenous dsRNA initiates RNAi by activating the ribonuclease protein Dicer, which binds and cleaves doublestrandedRNAs (dsRNAs) in plants, or short hairpin RNAs (shRNAs) in humans, to produce double-stranded fragmentsof 20–25 base pairs with a 2-nucleotide overhang at the 3' end. Bioinformatics studies on the genomes of multipleorganisms suggest this length maximizes target-gene specificity and minimizes non-specific effects. These shortdouble-stranded fragments are called small interfering RNAs (siRNAs). These siRNAs are then separated into singlestrands and integrated into an active RISC, by RISC-Loading Complex (RLC). RLC includes Dicer-2 and R2D2, and iscrucial to unite Ago2 and RISC. TATA-binding protein-associated factor 11 (TAF11) assembles the RLC byfacilitating Dcr-2-R2D2 tetramerization, which increases the binding affinity to siRNA by 10-fold. Association withTAF11 would convert the R2-D2-Initiator (RDI) complex into the RLC.R2D2 carries tandem double-stranded RNA-binding domains to recognize thethermodynamically stable terminus of siRNA duplexes, whereas Dicer-2 theother less stable extremity. Loading is asymmetric: the MID domain of Ago2recognizes the thermodynamically stable end of the siRNA. Therefore, the"passenger" (sense) strand whose 5 end is discarded by MID is ejected, while the saved "guide" (antisense) strand cooperates with AGO to form the RISC.[11]After integration into the RISC, siRNAs base-pair to their target mRNA andcleave it, thereby preventing it from being used as a translation template.Differently from siRNA, a miRNA-loaded RISC complex scans cytoplasmic mRNAs for potential complementarity. Instead of destructive cleavage (byAgo2), miRNAs rather target the 3 untranslated region (UTR) regions ofmRNAs where they typically bind with imperfect complementarity, thusblocking the access of ribosomes for translation.Exogenous dsRNA is detected and bound by an effector protein, known as RDE-4 in C. elegans and R2D2 in Drosophila, that stimulates dicer activity. Themechanism producing this length specificity is unknown and this protein onlybinds long dsRNAs.In C. elegans this initiation response is amplified through the synthesis of apopulation of 'secondary' siRNAs during which the dicer-produced initiating or'primary' siRNAs are used as templates. These 'secondary' siRNAs arestructurally distinct from dicer-produced siRNAs and appear to be produced byan RNA-dependent RNA polymerase (RdRP).

SARS CoV 2(COVID 19) :

Coronaviruses are a group of related viruses that cause diseases in mammals and birds.

In humans, corona viruses cause respiratory tract infections that can be mild, such as some cases of the common cold (among other possible causes, predominantly rhinoviruses), and others that can be lethal, such as SARS, MERS ,and COVID-19.

Symptoms in other species vary: in chickens, they cause an upper respiratory tract disease, while in cows and pigs they cause diarrhea.

There are yet to be vaccines or antiviral drugs to prevent or treat human coronavirus infections.

Morphology

Coronaviruses are large pleomorphic spherical particles withbulbous surface projections. The diameter of the virus particles isaround 120 nm.

The envelope of the virus in electronmicrographs appears as a distinct pair of electron dense shells.

The viral envelope consists of a lipid bilayer where the membrane(M), envelope (E) and spike (S) structural proteins are anchored.

A subset of coronaviruses (specifically the members ofbetacoronavirus subgroup A) also have a shorter spike-like surfaceprotein called hemagglutinin esterase (HE).

Inside the envelope, there is the nucleocapsid, which is formed from multiple copies of the nucleocapsid (N)

protein, which are bound to the positive-sense single-stranded RNA genome in a continuous beads-on-astring type conformation.

The lipid bilayer envelope, membrane proteins, and nucleocapsid protectthe virus when it is outside the host cell.
Genome

Coronaviruses contain a positive-sense, single-stranded RNAgenome.

The genome size for coronaviruses ranges fromapproximately 27 to 34 kilobases.

The genome size is one of thelargest among RNA viruses. The genome has a 5 methylated capand a 3 polyadenylated tail.

The genome organization for a coronavirus is 5 -leader-UTRreplicase/transcriptase-spike (S)-envelope (E)-membrane (M)-nucleocapsid (N)-3 UTR-poly (A) tail.

The open reading frames 1aand 1b, which occupy the first two-thirds of the genome, encode the replicase/transcriptase polyprotein.

The replicase/transcriptasepolyprotein self cleaves to form the nonstructural proteins (nsps).

The later reading frames encode the four major structural proteins: spike, envelope, membrane, and nucleocapsid.

Interspersedbetween these reading frames are the reading frames for the accessory proteins. The number of accessoryproteins and their function is unique depending on the specific coronavirus.

Life cycle

Entry

Infection begins when the viral spike (S) glycoprotein attaches to its complementary host cell receptor.

After attachment, a protease of the host cell cleaves and activates the receptor-attached spike protein.

Depending on the host cell protease available, cleavage and activation allows the virus to enter the host cell by endocytosis or direct fusion of the viral envelop with the host membrane.

On entry into the host cell, the virus particle is uncoated, and itsgenome enters the cell cytoplasm.

The coronavirus RNA genomehas a 5 methylated cap and a 3 polyadenylated tail, which allows the RNA to attach to the host cell's ribosome for translation.

The host ribosome translates the initial overlapping open reading frame of the virus genome and forms a long polyprotein.

The polyprotein has its own proteases which cleave the polyprotein into multiple nonstructural proteins.

Replication

A number of the nonstructural proteins coalesce to form a multi-protein replicase-transcriptase complex (RTC).

The main replicase-transcriptase protein is the RNA-dependent RNA polymerase (RdRp).

It is directly involved in the replication and transcription of RNA from an RNA strand.

The other nonstructural proteins in the complex assist in the replication and transcription process.

The exoribonuclease nonstructural protein, for instance, provides extra fidelity to replication by providing a proof reading function which the RNA-dependent RNA polymerase lacks.

One of the main functions of the complex is to replicate the viral genome.

RdRp directly mediates the synthesis of negative-sense genomic RNA from the positive-sense genomic RNA.

This is followed by the replication of positive-sense genomic RNA from the negative-sense genomic RNA.

The other important function of the complex is to transcribe the viral genome.

RdRp directly mediates the synthesis of negative- sense subgenomic RNA molecules from the positive-sense genomic RNA.

This is followed by the transcription of these negative-sense subgenomic RNA molecules to their corresponding positive-sense mRNAs.

Steps in Replication :

1. Their S protein, Covid-19 bind on cell surface molecules such as the metalloprotease alfa amino peptidase N, can also bind on N-acetyl neuraminic acid that serves as co-receptor.

2. Since Covid 19 have a single positive stranded RNA genome, they can directly produce their proteins and new genomes in the cytoplasm.

At first, the virus synthesize its RNA polymerase that only recognizes and produces viral RNAs.

This enzymes synthesize the minus strand using the positive strand as template.

3. Subsequently, this negative strand serves as template to transcribe smaller subgenomic positive RANs which are used to synthezise all other proteins.

Furthermore, this negative strand serves for replication of new positive stranded RNA genomes.

4. The protein N binds to genomic RNA and the protein M is integrated into the membrane of the endoplasmic reticulum like the envelope proteins S and HE.

After binding, assembled nucleocaspids with helical twisted RNA budd into the ER lumen and are encased with its membrane.

5.These viral progeny are finally transported by golgi vesicles to the cell membrane and are exocytosed into extracellular space.

How RNAi Works

RNAi uses small double-stranded RNAs to silence genes bearing a complementary sequence.

Although endogenous gene silencing operates through multiple mechanisms ,including mRNA cleavage, inhibition of translation, and epigenetic modifications of chromatin, mRNA cleavage is the most efficient mechanism and is the mechanism being harnessed for antiviral therapies.

Small RNAs, either exogenous small interfering RNAs (siRNAs) or endogenous microRNAs, are taken up by a cytoplasmic RNA-induced silencing complex (RISC), which cleaves one strand, leaving the remaining unpaired guide strand to search for mRNAs bearing complementary sequences.

Once recognized, if the target site on the mRNA has nearly perfect complementarity to the guide siRNA, the mRNA is cut by an Argonaute endonuclease in the RISC and then degraded, silencing the expression of the protein it encodes. Typically protein expression is reduced but not completely eliminated.

The RNAi machinery is present in all cells, where it is used to regulate the expression of key genes involved in cell development, differentiation, and survival.

Small RNAs can be readily designed to target any gene, whether an endogenous host gene or foreign viral gene.

For example, for HIV virtually all of the nine HIV gene products have been shown to be capable of being silenced, and siRNAs targeting the viral long terminal repeat are also effective.

RNAi Is an Ancient Antiviral

Defense In organisms that lack an adaptiveimmune response to pathogens, RNAiis an important defense against viral infection.In plants and flatworms, the genes encoding key RNAi pathway endonucleases Dicer and Argonaute have developed into gene families. These families may have evolved to provide molecules dedicated to controlling viral infection and preventing chromosomal insertion of rogue genetic elements, such as transposons, to supplement the activities of other homologous RNAi molecules required for regulating endogenous gene expression. Over 90% of plant viruses use double stranded RNA at some point in their life cycle, making them especially vulnerable to Dicer cleavage and suppression by RNAi. Plant viruses have developed mechanisms to bypass RNAi, most prominently by synthesizing viral proteins that bind and sequester siRNAs; other mechanisms have been described and additional ones are still being uncovered. Although the importance of RNAi in antiviral defense has been clearly shown in plants, worms, and flies, whether or not mammals use RNAi to defend against viruses is still uncertain, but likely. Several mRNAs encoded by a primate retrovirus contain complementary sequences that are targeted by an endogenous microRNA. Moreover viral variants with a mutated target sequence replicate more efficiently.Several examples of mammalian virus strategies for interfering with RNAi have been described, suggesting the viruses are trying to bypass an antiviral host response. One strategy, analogous to the suppression of silencing exhibited by plant viruses, is elaboration of proteins, such as NS1 of influenza and E3L of vaccinia, that bind to and inactivate double-stranded RNAs. Another strategy, employed by adenovirus, is copious expression of a microRNA that binds to and inactivates the microRNA export and Dicer processing machinery.Mammalian viruses also take advantage of RNAi

to carry out their replication strategies. In particular,several herpes viruses encode microRNAs that seem to be involvedin regulating viral latency. Viruses may use microRNAs to regulate sequential patterns of gene expression much like other organisms use microRNAs to regulate key genes that determine progression from one developmental state to another.SV40-encoded microRNAs direct the cleavage of early SV40 transcripts, which encode T antigens. Hepatitis C virus makes use of the microRNA miR-122, which is efficiently expressed in liver cells and matches a sequence in the viral 5 untranslated region, to promote its replication . Since viruses have evolved to exploit intrinsic host molecular pathways to promote their own replication, they will be an important tool for probing how microRNAs regulate gene expression even in uninfected cells. Interfering with either virally encoded or host microRNAs required for efficient viral replication may provide a novel strategy for antiviral therapeutics.A recent study shows the feasibility of inhibiting microRNA function in vivo using cholesterol conjugated 2 -O-methyl siRNAs.

Guiding Principles for Choosing siRNAs to Suppress Viruses

RNAi can be used to suppress viral replication by targeting either viral genes or host genes needed for viral replication.

Silencing viral genes such as viral polymerases or master regulators of viral gene transcription (which are essential for fundamental viral processes), or silencing viral genes that act early in the viral life cycle, may more effectively suppress viral replication than targeting late genes or accessory viral genes that contribute to pathogenesis (but that are not vital for viral replication).

In vitro studies have shown the usefulness of RNAi in vitro to suppress virtually every class of virus, whether a virus uses double stranded, single-positive, or negative strand RNA or DNA for its genome.

Algorithms for siRNA or short hairpin RNA (shRNA) design provide guidance for designing effective sequences, but this process is imperfect (with a success rate of over 60%).

The algorithms do not predict which sequences will be most effective at silencing their target gene.

Nor do they predict which sequences maximize viral inhibition and minimize sequence-specific off target effects arising from unintended silencing of partially homologous genes or from sequence-specific binding to Toll-like receptors, which triggers an inflammatory response.

The best candidate siRNA sequences need to be determined by experiment.

Offtarget silencing of genes with partial homology is impossible to avoid because inhibition of translation may involve complementarity to just a seven-nucleotide stretch of the siRNA.

However, translational inhibition generally has only a small impact on gene expression, can be minimized by avoiding sequences that target important genes, and is unlikely to be an important impediment to the clinical use of RNAi, although clinically signifi cant toxicity from off-target effects will be difficult to predict in advance.

Off-target effects caused by incorporation of the passenger strand,instead of the antisense strand, of the siRNA into RISC can be avoided by designing the siRNA ends to favor RISC uptake by the intended active strand.

Addressing the Obstacles of Viral Sequence Diversity and Escape Mutation

Important obstacles to using RNAi as the active principle for antiviral treatments are the related issues of viral sequence diversity and potential viral escape from gene silencing by sequence mutation.

RNA viruses, such as poliovirus or HIV, may be particularly susceptible to escape mutation because of the low fidelity with which their genomes are replicated .

These problems can be circumvented by silencing host genes required for viral entry or replication.

However, targeting host genes may lead to unacceptable host cell toxicity, unless RNAi can be selectively inducedonly in infected cells or transiently in vulnerable subsets of cells.

This might be achieved by targeting siRNAs exclusively into vulnerable cells or by regulating expression of shRNAs by using an inducible promoter that is activated by a viral gene product.

A recent strategy that uses an antibody fragment fusion protein to bind and deliver siRNAs only into cells bearing the cell surface receptor recognized by the antibody can deliver siRNAs and silence gene expression specifically in HIV-infected cell.

Expression of an shRNA designed to silence a host protein required for HIV replication might be induced only upon HIV infection by using a TAT-dependent promoter .

For HIV infection, the viral coreceptor CCR5 might be an exceptional host gene that can be silenced without toxicity, since individuals homozygous for a genetic mutation that functionally inactivates CCR5 are completely asymptomatic.

Since the morbidity and mortality associated with many viral infections may be secondary to stereotypic overly exuberant inflammatory or immune responses, silencing host genes proximal in these pathways provides another possible type of intervention.

For example, hepatitis B and C are not cytopathic viruses, but trigger hepatitis when activated immune cells expressing FasL infi ltrate the liver, where the infection upregulates the death receptor Fas on hepatocytes, making them prime targets for immunemediated apoptosis.

Silencing Fas in hepatocytes or FasL in immune cells might provide an effective immunemodulating therapy to circumvent chronic liver cell damage.

When viral genes are targeted, the related problems of viral sequence diversity and potential escape mutation can be circumvented by choosing highly conserved sequences whose mutation might result in impaired viral fitness.

This may be possible even for a highly diverse and mutationprone virus, like HIV, in which many short sequences are conserved even at the nucleotide level.

By targeting a highly conserved HIV vif sequence, researchers were able to suppress a variety of primary viral isolates chosen at random to represent fi ve distinct viral clades .

In a recent study by Kumar and colleagues published in PLoS Medicine, a sequence conserved among a variety of fl aviviruses silenced both Japanese encephalitis virus and West Nile virus .

Another approach for preventing viral escape mutation is to combine siRNAs targeting multiple genes, much as effective

drug therapy for HIV requires combining antiviral drugs targeting different steps in HIV replication.

For the encephalitis viruses, Kumar and colleagues' study suggests it might be possible to administer a cocktail of siRNAs that could effectively suppress a variety of likely suspect viruses before a definitive diagnosis is made, thus avoiding a dangerous delay in treatment.

For any particular virus, a cocktail of siRNAs targeting viral and host genes whose products act at different points in the viral life cycle is likely to act synergistically to suppress viral replication more effectively and hence reduce the chance of viral escape mutation .

However, the cellular RNAi machinery has limited capacity since silencing can be suppressed by an excess of irrelevant double-stranded small RNAs, a strategy used by adenovirus for suppressing RNAi .

Similarly, gene therapy vectors that express high amounts of shRNAs can cause toxicity by interfering with the nuclear export of endogenous microRNAs .

Therefore, there is probably a limit to the number of siRNAs that can be effectively incorporated into any treatment.

In Vivo Delivery

The key obstacle to harnessing RNAi as a treatment is getting small RNAs into the cytoplasm of cells in vivo. This can be accomplished either by figuring out a way to introduce small RNAs into cells as small molecule drugs (siRNA) or by using a plasmid or viral vector encoding an shRNA so that it will be processed like the endogenous microRNA precursors.

The small molecule approach is best suited to short-term interventions, while gene therapy can potentially be used for long-term silencing via expression from an integrated transgene.

To treat chronic infections, such as HIV or hepatitis C, a gene therapy strategy may make sense.

However, concerns about safety and controlling gene expression make gene therapy less practical for immediate applications.

Although small RNAs are not taken up by themselves into most cells in the body, the mucosal tissues of the body are an important exception.

siRNAs mixed with a cationic lipid or even by themselves are efficiently taken up by epithelial cells in the lung and vagina .

In the genital tract, siRNAs are taken up deep into the lamina propria and the underlying stroma.

The mechanism of uptake is not understood.

Most viral infections are transmitted through mucosal surfaces, which provides the opportunity to intervene to prevent or treat viral infections during transmission or acute infection.

In mice, local instillation of siRNAs targeting viral genes has shown striking protection against respiratory challenge with infl uenza, parainfl uenza, and respiratory syncytial viruses .

The potential for siRNAs to treat viral infection has also been shown in primates challenged with the SARS coronavirus .

These encouraging animal experiments have led to the recent first phase I study of siRNAs designed to treat viral infection.

This study will begin to test the potential of RNAi for treatment of acute respiratory syncytial virus infection, a leading cause of death and serious morbidity in newborn children.

Another mucosal tissue in which siRNAs have been shown to prevent viral infection is the female genital tract, where siRNAs targeting HSV-2 genes protected mice from lethal HSV- 2 infection .

siRNA penetrated deep into the cervicovaginal tissue, and silencing persisted for over nine days.

Mice were even protected when siRNA administration was delayed until 3 hours after viral challenge.

This study suggests the possibility that a persistent antiviral state might be achieved in the genital tract to prevent sexual transmission, reactivation, or even oncogenesis for a variety of sexually transmitted viruses,

such as HSV-2, HIV, and human papillomavirus.
Topical application of antiviral siRNAs might also be effective at other localized and accessible sites of viral infection, such as the upper respiratory tract, eye (i.e., herpes keratitis), or skin (i.e., warts).

Although therapeutic delivery of siRNAs and silencing is clearly feasible at mucosal surfaces, systemic treatment of viruses that are disseminated or infect tissues deep within the body is more diffi cult because of the challenge of efficiently transducing cells in vivo.

The main impediments to systemic uptake are the difficulty in traversing the cell membrane and the short half-life of intravenously injected siRNAs.

Several potential solutions to the former problem have been described recently, including covalent conjugation of the passenger siRNA strand to a cell-targeting moiety, noncovalent association of siRNAs with cell-targeting molecules, and incorporation of siRNAs into liposomes or other nanoparticles.

The short half-life, which is caused by both rapid renal filtration and endogenous ribonuclease digestion, can be readily addressed by incorporating siRNAs into complexes or particles and by minor chemical modify cations of the RNA backbone.

Early studies showing inhibition of hepatitis viral replication required introducing siRNAs via a highvolume bolus (termed hydrodynamic injection) that causes right-sided heart failure and is not suitable for clinical use.

Although siRNAs can be effectively introduced into an organ, such as the liver or kidney, by selective catheterization of the principal vein draining the tissue , this is invasive and costly.

Similarly, the intracerebral injection approach used to deliver siRNAs targeting flaviviruses is not likely to be used clinically.

Intravenous low-pressure injection of chemically modified siRNAs incorporated into modified liposomes reduced replication of a hepatitis B virus replicon in mice .

This study was the first demonstration of a systemic antiviral effect using a method of injection that is clinically feasible.

It is likely that some of the other approaches developed for systemic siRNA delivery described above will also work for silencing viral infection.

However, so far, achieving a noninvasive method to introduce siRNAs into the brain, as would be needed for treating viral encephalitis,remains an elusive goal.

Five Key Papers on Harnessing RNAi for Antiviral Therapy

Novina CD, Murray MF, Dykxhoorn DM, Beresford PJ, Riess J, et al. (2002) siRNAdirected inhibition of HIV-1 infection.

Nat Med 8: 681–686. One of the initial papers to use RNAi in vitro to inhibit HIV; the paper introduced the concept of inhibiting both viral and host genes to suppress viral entry and replication.

Gitlin L, Karelsky S, Andino R (2002) Short interfering RNA confers intracellular antiviral immunity in human cells. Nature 418: 430–434.

An early paper that showed in vitro inhibition of poliovirus and pointed out the potential problem of viral escape. Bitko V, Musiyenko A, Shulyayeva O,Barik S (2005) Inhibition of respiratory viruses by nasally administered siRNA. Nat Med 11: 50–55. A study in mice that showed effi cient silencing and in vivo effectiveness of nasally administered uncomplexed siRNAs for the clinically important respiratory syncytial virus.

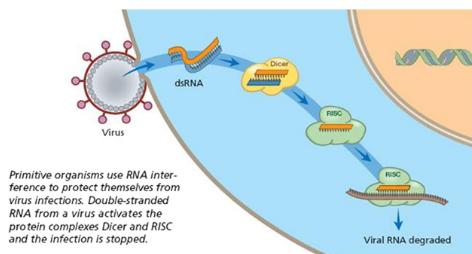
Li BJ, Tang Q, Cheng D, Qin C, Xie FY, et al. (2005) Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque.

Nat Med 11: 944–951. The fi rst study to show therapeutic effi cacy of siRNAs in a nonhuman primate to treat respiratory infection by the SARS coronavirus.

Palliser D, Chowdhury D, Wang QY, Lee SJ, Bronson RT, et al. (2006) An siRNAbased microbicide protects mice from lethal herpes simplex virus 2 infection.

Nature 439: 89–94. A study that shows the potential for vaginal application of siRNAs to prevent or treat sexually transmitted infections.

Abbreviations: RISC, RNA-induced silencing complex; RNAi, RNA interference; shRNA, short hairpin RNA; siRNA, small interfering RNA



Primitive organisms use RNA interference to protect themselves from virus infections. Double-stranded RNA from a virus activates the protein complexes Dicer and RISC and the infection is stopped.

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