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RNAi: VIRAL THERAPEUTICS

Clinical Research

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ABSTRACT

RNA interference or RNAi is a natural biological response in eukaryotic cells as their antiviral defense mechanisms. RNAi protects a range of organisms by gene silencing or down regulating protein expression. RNAi induces the activation of ribonucleases targeting degradation of RNA molecules encoding proteins. Genomic alterations have also been observed due to RNAi mechanisms. RNAi has been an important part of biomedical research in terms of elimination and control of viral pathogens. Also RNAi intermediates as small interfering RNAs (siRNAs) are noted to aid antiviral immunity. RNAi has also been involved in targeted transfer and activation of certain molecules which help in treatment of certain clinical disorders. This technique has been used in many applications owed to its adaptabilities. RNAi is resilient over other techniques of gene editing attributed to its dose-dependent applications providing the user a better control. The highly efficient results of RNAi have paved its way for its application in viral infection diagnosis, control and treatment.

KEYWORDS

RNAi, short interfering RNA, siRNA, RNA in viral therapeutics

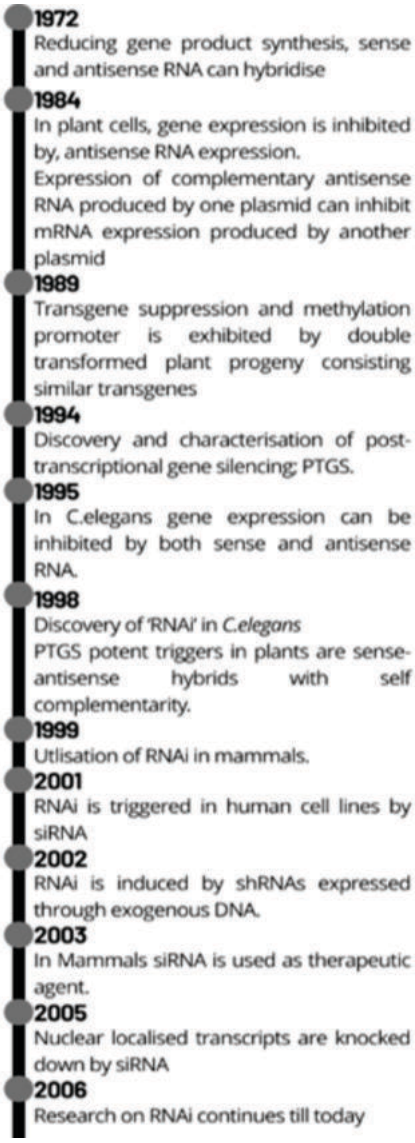

Figure 1. History of discoveries forming current understanding of RNAi

1. INTRODUCTION

Viruses, being obligate parasites, completely depend on host cells' complex metabolic and biosynthetic machinery for propagation which in the long co-evolutionary run gives rise to defense mechanisms in the host body. Generally, cells do not produce double stranded RNA (dsRNA) during nucleic acid metabolism, RNase enzymes like Dicer and Drosha that target dsRNA cleaving, have very easily been generated in host cells as retaliation resulting in the formation of short-interfering RNAs. The naturally occurring antiviral defense is also responsible for transposon silencing, gene regulation and chromosomal modifications [2] which have been termed as 'RNA interference' by Nobel Prize awardees Andrew Fire and Craig Mello. RNA interference has been reported to be used in functional genomics, and varied therapeutic applications. [3]

RNA interference (RNAi) is a conserved biological response to double-stranded RNA, also termed as "post-transcriptional gene silencing", mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, and regulates the expression of protein-coding genes.[3] Functionally, dsRNAs can trigger silencing of complementary strands of messenger RNA thus inhibiting protein formation and gene expression. RNAi has been cultivated to manipulate gene expression experimentally and to probe gene function on a whole-genome scale. [4] Environmental RNAi (eRNAi) is sequence specific regulation of endogenous genes by exogenous dsRNA [5] facilitating significant technological advances in fields of functional genomics and agriculture [6] which includes control of pests and diseases caused by pathogenic bacteria, fungi, and viruses, for improvisation in crop yields and to generate desired plants with novel traits (PNTs). [7]

2. RNAi based therapeutics against viruses

Mammalian cells have evolutionarily conserved RNAi machinery which can be targeted as a means for antiviral defense. In plants, fungi and invertebrates short interfering RNAs (siRNAs) derived from dsRNA viruses facilitate the natural antiviral defense. [8,9] The controversy of RNAi as a functional pathway in mammals arises from the fact that dsRNAs longer than 30 basepairs trigger an interferon response [10,11] which can terminate natural RNAi. However, exogenous siRNAs can be used to initiate RNAi, first demonstrated in HeLa cells and human embryonic kidney cells against the respiratory syncytial virus, [12] and thereafter numerous other studies have highlighted the use of RNAi. The technique remains a viable option for severe viral infections which are without any adequate treatment or effective vaccines. It's potential as an alternative therapy for patients with infections from HIV and HBV.

Table 1: Different virus families causing diseases targeted by RNAi.

Sr. No.	Virus Family	Cause	Target and results
1	Papillomaviridae	Taxa consists of carcinogenic non enveloped DNA viruses. Viral oncogenes type 16 and 18 can cause cervical cancer.	SiRNA developed, targeting viral oncogenes for cervical cancer in HeLa cell lines, resulting in cell death of cancer cells and low off-target effects. [13,14]
2	Polyomaviridae	Cause Merkel cell carcinoma. Merkel cell polyomavirus T antigen (T.Ag) protein is involved in replication and viral infection. Polyomavirus BK (BKV) induces malignant transformation.	siRNAs targeting BKV T-Ag restrained its expression in pRPc cell lines. Blocking of T-Ag resulted in retarded growth of BKV transformed cellls and suppressed tumorigenecity. [15]
3	Poxviridae	Vaccinia virus (VACV) infects vertebrates and invertebrates disrupting host defense mechanisms like Interferon with it's E3L protein.	E3L-specific siRNAs inhibited viral replication by 98% compared to control infection and suppressed both early and late gene expressions of VACV. [16,17]
4	Hepadnaviridae	Hepatitis B virus causes liver cirrhosis and hepatocellular carcinoma.	siRNA against the surface antigen region reduces viral load and secretion of viral antigens in mice. [18]
5	Herpesviridae	Herpes simplex virus glycoprotein E promotes cell to cell spread and immune system evasion.	siRNAs targeting glycoprotein E suppressed its expression and function in HaCaT cells. [19]
6	Reoviridae	Viral nonstructural proteins μNS, σNS and μ2 play key roles in forming viral inclusions for replication and assembly	Plasmid based vectors expressing siRNAs target these proteins in the T3D strain of reovirus showed inhibiting of several steps in replication in 293T cells. [20]
7	Arenaviridae	Cause fatal hemorrhagic fever. Lymphocytic choriomeningitis virus model is used to understand arenavirus pathogenesis.	SiRNAs formulated against L polymerase and Z viral mRNAs halt viral growth in HEK 293T host cells. [21]
8	Paramyxoviridae	RSV (respiratory syncytial virus) causes lung infections in humans.	siRNAs targeting RSV P gene in BALB/c mice model results in reduced lung pathogenesis and pulmonary inflammation. Also generates a strong antiviral reaction when later given RSV. [22]
9	Rhabdoviridae	Rabies virus causes fatal zoonotic disease in humans and animals.	siRNAs against rabies virus nucleoprotein mRNA decreased virus titers in BHK-21 cells. [23]
10	Bunyavirales	John Cunningham virus (JCV) causes demyelinating progressive multifocal leukoencephalopathy disease.	siRNAs targeted at agnoprotein of JCV showed effective inhibition of viral infection in nude mice. [24]
11	Filoviridae	Ebola causes severe human disease.	siRNA for Zaire ebola virus nucleoprotein decreased viral titers in 293T cells. [25]
12	Orthomyxoviridae	Influenza virus causes prevalent infections in susceptible humans despite vaccines.	siRNAs specific for nucleocapsid and transcriptase have been shown to be potent in inhibiting influenza virus production in cell lines and chicken embryos. [26]
13	Picornaviridae	Coxsackievirus and poliovirus are two of the well.known viruses in this group	Treatment with siRNAs significantly decreased cell death in parallel with a reduction in coxsackievirus B3 replication in HeLa cells. Also, the efficient coxsackievirus B3–specific siRNA displayed antiviral activity in other related enteroviruses such as CVB1, CVB5, CVB6, coxsackievirus A9, and Echo6 in HeLa cells. [27]
14	Togaviridae	Chikungunya virus causes chikungunya fever.	siRNA developed for conserved segments of nsP3 and E1 mRNAs of the pathogen were able to drastically reduce the virus titer in Vero cells. [28]
15	Coronaviridae	Severe acute respiratory syndrome (SARS.CoV) causes viral fever and alveoli damage in humans.	siRNAs targeting the 3′ untranslated regions (UTRs) of the virus stops the replication of SARS.CoV in Vero.E6 cells. [29]
16	Hepeviridae	Hepatitis E virus causes high mortality in pregnant women.	siRNAs developed against helicase and replicase genes of the virus found effective in stopping virus replication in A549 as well as HepG2 cells. [30,31]
17	Flaviviridae	Dengue virus causes severe disease and is a global public health threat.	siRNA directed against the conserved 5′ cyclization sequence segment of the DENV genome effectively decreased the viral titers of multiple DENV strains in mice. [32]
18	Retroviridae	HIV causes AIDS in humans.	siRNAs can inhibit HIV.1 growth by aiming the genes for the host CD4 cell receptor, or the viral Gag and Nef proteins in Magi.CCR5, HeLa.CD4, and H9 T cells. [33]

3. Synthesis of RNAi

There are different approaches and methods reported for the formation and development of synthetic molecules which function as siRNA or shRNA for therapeutic or other use in RNAi. A few methods have been listed further.

3.1. Chemical Synthesis uses automated solid-phase synthesis to obtain single-stranded RNAs (ssRNAs) which are then hybridized to a duplex to form dsRNA. The method is expensive but produces siRNA of defined sequence and ultrapure quality.

3.2. Enzymatic Generation employs enzymes like viral polymerase T7 DNA-dependent RNA polymerase (DdRp) for target recognition and annealing in a PCR reaction. The siRNA pools produced are fast, cheap but lack accurate complementarity to target sequence which may or may not evoke notable off-target effects and immune response. [34]

3.3. In vivo Expression From siRNA Expression Vector includes a plasmid or viral vector with a cassette encompassing Promoter region, DNA sequence which would translate to shRNA of desired sequence and transcription stop signal. The shRNA would be cleaved by Dicer into siRNAs. [35]

Chemical Modifications of siRNA can efficiently promote stability, reduce recognition and immunogenic reactions against it and improve delivery of it in-vitro. Immunogenic responses are elicited by dsRNA binding proteins such as cytosolic dsRNA-dependent protein kinase (PKR), pattern recognition receptors (PRRs), retinoic acid-inducible gene I (RIG I)-like receptors (RLRs) and endosomal receptors, Toll-like receptor (TLR) 3, 7, 8, and 9. [36] Backbone modifications like replacing phosphodiester with phosphotriester facilitates cellular uptake, increased in-vivo half life and delivery but in turn has reduced biological activity. [37] Sugar modifications which replace the 2'-hydroxyl group with less nucleophilic group, increases nuclease

resistance and decreases immunogenicity but too many can cause issues with RNA-induced silencing complex (RISC) loading. [38] Several other modifications and their potentials have also been discussed in published literature. [39]

4. Mechanism of Action

Mechanism of RNAi includes sequence specific degradation of host mRNA via cytoplasmic delivery of dsRNA identical to the target sequence. The effector molecule for RNAi is RISC, responsible for gene knockdown, made from proteins of Argonaute family bound to a guide RNA. [40] Majority of RNAi applications are mediated by use of chemically synthesized double-stranded siRNA or vector based short hairpin RNA (shRNA) as the guide RNA for inhibition. There are also miRNAs which arise genomically and function as guide RNA for natural antiviral defense, regulating processes like embryonic development, cell differentiation and proliferation, cell death and organismal response to stress. Currently, its clinical use as biomarkers and in disease diagnostics is rapidly developing. [41] All three types of RNAi use RISC for post-transcriptional gene silencing and have the potential to target select mRNA, but have different efficiencies and specificities of gene knockdown. [42]

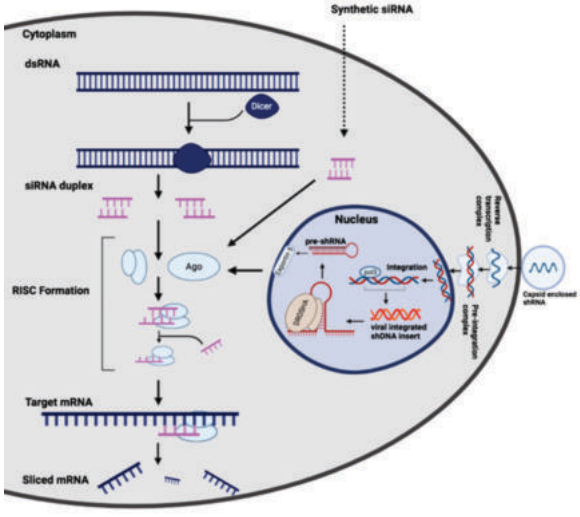

Figure 2: Mechanism of RNAi

4.1. siRNA

Some endogenous origins like centromeres, transposons, other repetitive sequences and more are observed for siRNA but clinical applications are based on chemically produced siRNAs. [43] The engineered siRNAs are directly delivered into the cell cytosol where they are cleaved by an RNAse III enzyme (Dicer), forming 21-nucleotides (nt) or 22-nt dsRNA fragments bearing 3′-overhangs of 2-nt on either strand. Dicer, which contains a catalytic, helicase and PAZ domains is required for both RISC formation as well as the entry of siRNA into RISC. [44] This process may be helped by dsRNA binding proteins (dsRBP) such as TAR RNA-binding protein (TRBP). [40] The double-stranded siRNAs of appropriate size are then selectively assembled into RISC where argonaute 2 cleaves the duplex so that one strand (guide) bound to Argonaute to direct silencing and other strand (passenger) is discarded. Strand retention is based on relative thermodynamic stability of duplex's 5' ends. [43]

4.2. shRNA

Exogenous viral or plasmid vectors encoding a shRNA construct are transfected into the cell that translocates to the nucleus and transcribes into a hairpin loop structure called pri-shRNA. Drosha, a nuclease converts pri- into pre-shRNA. A specialized RAN-GTP dependent nuclear membrane protein called exportin-5 (Exp5) transfers pre-shRNA to the cytoplasm, where Dicer processes it to functional siRNA which can be incorporated into a RISC to cleave the target mRNA. [45] In humans, the Ago2 protein associates with Dicer-HIV transactivating response RNA-binding protein (TRBP) to form RISC-loading complex (RLC). TRBP stabilizes RLC and ensures the proper orientation of the siRNA following its loading, hence implicating it as a major influence in the selection and incorporation of the guide strand in RISC. Thus, shRNAs are converted into siRNAs by the same RNAi machinery that processes miRNAs. [46]

siRNA vs shRNA: Clinically, both siRNA and shRNA are valuable with their distinct pros and cons. siRNA acts directly in cytosol while shRNA begins functioning from nucleus, and the latter requires an additional transport mechanism for delivery. This can be difficult for quiescent cells with low nuclear envelope permeability. Additionally, molecular weight of siRNA is 100 times lower than shRNA and proves to be easy for delivery as well as chemical modifications. [42] On the contrary, viral delivery system of shRNA provides an opportunity to be integrated into host genome, being passed down to daughter cells when target cells divide and thus providing permanent or long-term knockdown of target mRNA.

4.3. RISC

During RNAi pathway, siRNA guide strand directs RISC to complementary RNA targets, which are further degraded. RNA is degraded by PIWI domain of 'Ago' protein. The slicing activity is very precise: phosphodiester linkage between the target nucleotides that are base paired to siRNA residues 10 and 11 (from the 5′ end) is cleaved to generate products with 5′-monophosphate and 3′-hydroxyl termini. After initial cut, cellular exonucleases attack fragments to complete the degradative process. The target dissociates from siRNA after cleavage, freeing RISC to cleave additional targets. [43]

5. Clinical Applications

Sars-CoV-2 (Severe acute respiratory syndrome coronavirus 2) virus is known to cause respiratory illness, predominantly exposed in 2019, causing severe illness, high rate of mortalities and pandemic spread of the same. Sars-CoV and Sars-Cov-2, at nucleotide level, carry high and efficient homology. Proteins of Sars-CoV are homologous at 95% with Sars-CoV-2 proteins, which is crucial to determine evolutionary similarity. In 2003, research demonstrated a successful method of RNAi in therapy against Sars-CoV. Stated that Sars-CoV-2 sequences at a whole-genome level, are found to be 79% identical to Sars-CoV, hence, RNAi might be used as a mode of therapy in Sars-CoV-2 as well. Majorly, four regions of Sars-Cov were targeted while studying the effect of RNAi on Sars-Cov, TRS (transcription regulating sequence), S protein-coding sequence, leader sequence and 3' UTR. [47] This research discovered that RNA transcripts and viral antigens were heavily reduced due to S gene specific targeted siRNAs, which was supported further by the following studies discovering that siRNAs carried promising therapeutic and prophylactic activities against Sars-CoV, as evidenced by reduced fever and acute diffuse alveoli damage. Two siRNA duplexes (siSC2 and siSC5) were used to target ORF1b and spike protein-coding genome region. [47] Reconsidering the fact that Sars-CoV and Sars-CoV-2 carry high sequence similarity at whole-genome level, siSC2 and siSC5 duplexes might be utilized for the management of better clinical and therapeutic outcomes for Sars-CoV-2. However, at present there is no sufficient research evidence and further research needs to be conducted for the same.

5.1. Hepatitis B virus host restricting factor

Hepatitis B Virus is one of the chronic disease; leading to terminal liver disease. Chronic hepatitis B can be treated with approved treatments like, 5' nucleoside/ nucleotide analogs and limited type-1 interferon, NA and IFNs, respectively. IFNs is permitted to fraction of patients after a finite therapy. It suppresses HBV replication, this method is applicable for their immunomodulatory activity potential, along with innate response restoration and also through the means of antiviral IFN-stimulated gene products. [48] Myxovirus resistance proteins denoted as (Mx), are vividly adapted and studied ISGs (IFN-stimulated gene) as humans carry MX1 and MX2 genes, encoding MxA and MxB proteins, respectively. Crystallographic data of MX1 and MX2 shows structural similarity, and ~63% of similar amino acid sequence. In primary human hepatocytes (PHH) and liver progenitor cells (HepaRG), Mx1 and Mx2 proteins have shown to be ISGs, which curates an anti-HBV role. MX2 severs a better inhibition than the four widely known restriction factors viz., A3G, SAMHD1, HNRNPU and MOV10. Similarly, lentiviral shRNA have been used as vectors to target MX2 RNA sites (-A, -B) showed 90% of MX2 suppression in Huh-7 cells. sh-RNA group in negative control showed that in untreated cells, pc/ pg RNA was reduced to 18.6% by IFN-a. [49] When, PHH cells were knockdown by MX2, anti-HBV effects of IFN-a were found weak by 12-22%, therefore, MX2 can contribute considerably to anti-HBV RNA activity using IFN-a. [48]

5.2. RNAi-based therapeutic gene silencing (adenoviral vector)

The systemic administration of adenoviruses is hepatotoxic and this can be utilized for delivering RNAi activators that are expressed, resulting in the silencing of the disease causing genes in the liver. Attributed the toxic nature of adenoviruses, an innate and adaptive

immune response would be initiated in the host, leading to the application of Helper-Dependent adenoviruses being used. These Helper-Dependent adenoviruses lack viral protein encoding sequences and carry advantages which can allocate transgene elements. [50] However, vectors can be more functional if extra cassettes are included in these viruses. A 2008 study mentioned using this approach for Cys-X3-Cys chemokine ligand silencing to prevent acute liver injury caused by adenoviruses. [50,51] Similarly, a research in 2009 confirmed RNAi expression cassettes of Helper-Dependent adenoviruses, where shRNAs targeted insulin-responsive SREBP-1 gene, resulting in 90% sustained SREBP-1 gene knockdown. This technique was used for therapeutic management of type-2 diabetes mouse model with an observation after 3 weeks of Helper-Dependent adenoviruses administration. [50,52]

6. RNAi Applications using Microbes

RNAi using microbial mediation can be applicable as a potential delivery method for the transfer of dsRNA from live microbes into the host organisms. Microorganisms can produce dsRNA in close vicinity of the host cells facilitating the uptake of dsRNA resulting in down regulating the expression of the target gene. Microbial RNAi can be classified as exogenous technology for dsRNA delivery and is generally cost and time efficient. Microbial mediated RNAi has an added advantage of partial resistance to be silenced by RNA-directed DNA methylation. [53] RNAi formation was reported to be induced in C. elegans by feeding it with dsRNAs derived from an engineered recombinant E. coli. A successful RNAi was obtained in five different target mRNA of Colorado potato beetle were designated to the dsRNAs produced from the engineered E. coli. RNase III-deficient E. coli strains were transformed from plasmids to produce dsRNA, ingested by the Colorado potato beetle, which led to a massive rate of mortality and significant weight loss. Equivalently, dsRNA delivery of microbes worked prominently in suppressing serious house cricket pathogen, densovirus as well. [52,54]

7. RNAi Applications in Transgenic Plants

Nematodes, bacteria, and viruses classifying as plant pests are used as targets for successful transgenic plant-mediated RNAi. This technique was well experimented on corn root-worms. Transformed cotton plants carrying V-ATPase subunit-A dsRNA, led to a massive reduction in corn rootworm pests. However, bollworm larvae growth was expressed in plants transformed with P450 gene CYP6AE14 dsRNA, [54] this is crucial in the case of tolerating cotton gossypol. Therefore, decreasing CYP6AE14 mRNA leads to a reduction of larval growth.

8. Future applications

8.1. Attacking HIV-1 RNA: RNAi versus CRISPR-Cas systems

HIV-1 (Human immunodeficiency virus type 1) is effectively controlled and not cured by potent antiviral drugs. Durable strategies to protect target T-cells against HIV-1 infection are stable gene therapy, one of them being clustered regularly interspaced short palindromic repeats (CRISPR-Cas) based on RNA Interference (RNAi) are delivered through lentivrial vectors. Using combinational therapy methodology, combination of multiple inhibitors can be used for different approaches.[55] RNAi attack could use multiple shRNAs as a combination for different approaches, whereas for CRISPR-Cas tested sgRNA combinations along with supportive cellular HIV-1 replication factors can be considered. Since, a new addition to CRISPR-cas tool box denotes specificity to RNA targets, combination of RNA-targeting RNAi strategies can also be considered. Considering when human cells contain an abundance of integrated HIV-1 proviruses, CRISPR-Cas strategies may lead to complete genome segment removal or can cause genome instability and cell death. However, cells infected by a single virus may not cause a serious concern, but the HIV-1 co-injection will exist. [55,56]

8.2. Adenovirus vector-based RNAi therapy for HCV infections

InVivo administration of adenovirus hepatocytes carry natural targeting, which serves well to deliver RNAi expression cassettes that targets virus infections that are liver specific. [55] Similar to HBV, HCV infection is widely known for global health problems, this infection places individuals at verge of cirrhosis and HCC. In HCV, 5' NTR is responsible for initiating HCV ORF translation. Cytoplasmic cycle of the HCV RNA can be considered for RNAi-based treatment. HCV infection in vivo models are limited to chimpanzees and mice that are immunodeficient, human hepatocyte grafted. Host dependent factor silencing can also be useful factor to counter HCV resistant strains. [52]

9. CONCLUSION

RNAi has vast applications in different fields. It has been researched a lot as a potential infection control tool in recent times. RNAi demonstrated a therapeutic efficiency in varied clinical applications and opened up newer avenues. RNAi might also be opted for microbial mediated delivery for gene silencing. It has also emerged as a vast aptitude both in plant and animal pest management in an environment friendly manner.

REFERENCES

1. Montgomery M. RNA interference: historical overview and significance. Methods Mol Biol. 2004;265:3-21. doi: 10.1385/1-59259-775-0:003. PMID: 15103066
2. Wilson RC, Doudna JA. Molecular mechanisms of RNA interference. Annu Rev Biophys. 2013;42:217-39. doi: 10.1146/annurev-biophys-083012-130404. PMID: 23654304; PMCID: PMC5895182.
3. Hannon GJ. RNA interference. Nature. 2002 Jul 11;418(6894):244-51. doi: 10.1038/418244a. PMID: 12110901.
4. Castanotto D, Rossi JJ. The promises and pitfalls of RNA-interference-based therapeutics. Nature. 2009 Jan 22;457(7228):426-33. doi: 10.1038/nature07758. PMID: 19158789; PMCID: PMC2702667.
5. Whangbo JS, Hunter CP. Environmental RNA interference. Trends Genet. 2008 Jun;24(6):297-305. doi: 10.1016/j.tig.2008.03.007. Epub 2008 Apr 29. PMID: 18450316.
6. Ivashuta S, Zhang Y, Wiggins BE, Ramaseshadri P, Segers GC, Johnson S, Meyer SE, Kerstetter RA, McNulty BC, Bolognesi R, Heck GR. Environmental RNAi in herbivorous insects. RNA. 2015 May;21(5):840-50. doi: 10.1261/rna.048116.114. Epub 2015 Mar 23. PMID: 25802407; PMCID: PMC4408792.
7. Fletcher SJ, Reeves PT, Hoang BT, Mitter N. A Perspective on RNAi-Based Biopesticides. Front Plant Sci. 2020 Feb 12;11:51. doi: 10.3389/fpls.2020.00051. PMID: 32117388; PMCID: PMC7028687.
8. Shabalina S. A., Koonin E. V. (2008). Origins and evolution of eukaryotic RNA interference. Trends Ecol. Evol. 23, 578–587. 10.1016/j.tree.2008.06.005.
9. Ding S. W., Li H., Lu R., Li F., Li W. X. (2004). RNA silencing: a conserved antiviral immunity of plants and animals. Virus Res. 102, 109–115. 10.1016/j.virusres.2004.01.021
10. Minks M. A., West D. K., Benvin S., Baglioni C. (1979). Structural requirements of double-stranded RNA for the activation of 2',5'-oligo(A) polymerase and protein kinase of interferon-treated HeLa cells. J. Biol. Chem. 254, 10180–10183.
11. Elbashir S. M., Harborth J., Lendeckel W., Yalcin A., Weber K., Tuschl T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411, 494–498. 10.1038/35078107.
12. Bitko V., Barik S. (2001). Phenotypic silencing of cytoplasmic genes using sequence-specific double-stranded short interfering RNA and its application in the reverse genetics of wild type negative-strand RNA viruses. BMC Microbiol. 1:34.
13. Butz K, Ristriani T, Hengstermann A, Denk C, Scheffner M, Hoppe.Seyler F. siRNA targeting of the viral E6 oncogene efficiently kills human papillomavirus.positive cancer cells. Oncogene. 2003;22:5938.5945. 10.1038/sj.onc.1206894.
14. Chang JT, Kuo TF, Chen YJ, et al. Highly potent and specific siRNAs against E6 or E7 genes of HPV16. or HPV18.infected cervical cancers. Cancer Gene Ther. 2010;17:827.836. 10.1038/cgt2010.38.
15. Sabbioni S, Callegari E, Spizzo R, et al. Anticancer activity of an adenoviral vector expressing short hairpin RNA against BK virus T.ag. Cancer Gene Ther. 2007;14:297.305. 10.1038/sj.cgt.7701014.
16. Dave RS, McGettigan JP, Qureshi T, Schnell MJ, Nunnari G, Pomerantz RJ. siRNA targeting vaccinia virus double.stranded RNA binding protein [E3L] exerts potent antiviral effects. Virology. 2006;348:489.497. 10.1016/jvirol200601.013.
17. Lantermann M, Schwantes A, Sliva K, Sutter G, Schnierle BS. Vaccinia virus double.stranded RNA.binding protein E3 does not interfere with siRNA.mediated gene silencing in mammalian cells. Virus Res. 2007;126:1.8. 10.1016/j.virusres.2007.01.009.
18. Giladi H, Ketzinel.Gilad M, Rivkin L, Felig Y, Nussbaum O, Galun E. Small interfering RNA inhibits hepatitis B virus replication in mice. Mol Ther. 2003;8:769.776.
19. Bhuyan PK, Kariko K, Capodici J, et al. Short interfering RNA.mediated inhibition of herpes simplex virus type 1 gene expression and function during infection of human keratinocytes. J Virol. 2004;78:10276.10281. 10.1128/JVI.78.19.10276-10281.2004.
20. Kobayashi T, Chappell JD, Danthi P, Dermody TS. Gene.specific inhibition of reovirus replication by RNA interference. J Virol. 2006;80:9053.9063. 10.1128/JVI.00276-06.
21. Sanchez AB, Perez M, Cornu T, de la Torre JC. RNA interference.mediated virus clearance from cells both acutely and chronically infected with the prototypic arenavirus lymphocytic choriomeningitis virus. J Virol. 2005;79:11071.11081. 10.1128/JVI791711071-11081.2005.
22. Zhang W, Tripp RA. RNA interference inhibits respiratory syncytial virus replication and disease pathogenesis without inhibiting priming of the memory immune response. J Virol. 2008;82:12221.12231. 10.1128/JVI.01557-08.
23. Brandao PE, Castilho JG, Fahl W, et al. Short.interfering RNAs as antivirals against rabies. Braz J Infect Dis. 2007;11:224.225.
24. Matoba T, Orba Y, Suzuki T, et al. An siRNA against JC virus (JCV) agnoprotein inhibits JCV infection in JCV.producing cells inoculated in nude mice. Neuropathology. 2008;28:286.294. 10.1111/j.1440-1789.2007.00878.x.
25. Groseth A, Hoenen T, Alimonti JB, et al. In vitro evaluation of antisense RNA efficacy against filovirus infection, by use of reverse genetics. J Infect Dis. 2007;196 Suppl 2: S382.389. 10.1086/520604.
26. Ge Q, Eisen HN, Chen J. Use of siRNAs to prevent and treat influenza virus infection. Virus Res. 2004;102:37.42. 10.1016/j.virusres.2004.01.013.
27. Ahn J, Jun ES, Lee HS, et al. A small interfering RNA targeting coxsackievirus B3 protects permissive HeLa cells from viral challenge. J Virol. 2005;79:8620.8624. 10.1128/JVI79138620-8624.2005.
28. Dash PK, Tiwari M, Santhosh SR, Parida M, Lakshmana Rao PV. RNA interference mediated inhibition of Chikungunya virus replication in mammalian cells. Biochem Biophys Res Commun. 2008;376:718.722. 10.1016/j.bbrc.2008.09.040.
29. Wu CJ, Huang HW, Liu CY, Hong CF, Chan YL. Inhibition of SARS.CoV replication by siRNA. Antiviral Res. 2005;65:45.48. 10.1016/j.antiviral.2004.09.005.
30. Kumar A, Panda SK, Durgapal H, Acharya SK, Rehman S, Kar UK. Inhibition of hepatitis E virus replication using short hairpin RNA (shRNA). Antiviral Res. 2010;85:541.550. 10.1016/j.antiviral.2010.01.005.
31. Huang F, Hua X, Yang S, Yuan C, Zhang W. Effective inhibition of hepatitis E virus replication in A549 cells and piglets by RNA interference (RNAi) targeting RNA.dependent RNA polymerase. Antiviral Res. 2009;83:274.281. 10.1016/j.antiviral.2009.06.008.
32. Stein DA, Perry ST, Buck MD, et al. Inhibition of dengue virus infections in cell cultures and in AG129 mice by a small interfering RNA targeting a highly conserved sequence. J

Virol. 2011;85:10154.10166. 10.1128/JVI.05298-11.
33. Novina CD, Murray MF, Dykxhoorn DM, Beresford PJ, Riess J, Lee SK, Collman RG, Lieberman J, Shankar P, Sharp PA. siRNA-directed inhibition of HIV-1 infection. Nat Med. 2002 Jul;8(7):681-6. doi: 10.1038/nm725. Epub 2002 Jun 3. PMID: 12042777.
34. Leavanova A., and Poranen, M. M. (2018). RNA Interference as a Prospective Tool for the Control of Human Viral Infections. Frontiers in Microbiology, 9. doi:10.3389/fmicb.2018.02151.
35. Brummelkamp, T. R., Bernards, R., and Agami, R. (2002). A system for stable expression of short interfering RNAs in mammalian cells. Science 296, 550–553. doi: 10.1126/science.1068999.
36. Heidel JD, Hu S, Liu XF, Triche TJ, Davis ME. Lack of interferon response in animals to naked siRNAs. Nat Biotechnol. 2004 Dec;22(12):1579-82. doi: 10.1038/nbt1038. Epub 2004 Nov 21. PMID: 15558046.
37. Meade BR, Gogoi K, Hamil AS, Palm-Apergi C, van den Berg A, Hagopian JC, Springer AD, Eguchi A, Kacsinta AD, Dowdy CF, Presente A, Lönn P, Kaulich M, Yoshioka N, Gros E, Cui XS, Dowdy SF. Efficient delivery of RNAi prodrugs containing reversible charge-neutralizing phosphotriester backbone modifications. Nat Biotechnol. 2014 Dec;32(12):1256-61. doi: 10.1038/nbt.3078. Epub 2014 Nov 17. PMID: 25402614; PMCID: PMC4378643.
38. Mehta, A., Michler, T., Merkel, O. M., siRNA Therapeutics against Respiratory Viral Infections—What Have We Learned for Potential COVID-19 Therapies?. Adv. Healthcare Mater. 2021, 10, 2001650.
39. Selvam, C., Mutisya, D., Prakash, S., Ranganna, K., & Thilagavathi, R. (2017). Therapeutic potential of chemically modified siRNA: Recent trends. Chemical Biology & Drug Design, 90(5), 665–678. doi:10.1111/cbdd.12993.
40. Wilson RC, Doudna JA. Molecular mechanisms of RNA interference. Annu Rev Biophys. 2013;42:217-39. doi: 10.1146/annurev-biophys-083012-130404. PMID: 23654304; PMCID: PMC5895182.
41. Lam, J. K. W., Chow, M. Y. T., Zhang, Y., & Leung, S. W. S. (2015). siRNA Versus miRNA as Therapeutics for Gene Silencing. Molecular Therapy - Nucleic Acids, 4, e252. doi:10.1038/mtna.2015.23.
42. Wang, J., Lu, Z., Wientjes, M. G., & Au, J. L.-S. (2010). Delivery of siRNA Therapeutics: Barriers and Carriers. The AAPS Journal, 12(4), 492–503. doi:10.1208/s12248-010-9210-4.
43. Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. Cell. 2009 Feb 20;136(4):642-55. doi: 10.1016/j.cell.2009.01.035. PMID: 19239886; PMCID: PMC2675692.
44. Lee YS, Nakahara K, Pham JW, Kim K, He Z, Sontheimer EJ, et al. Distinct roles for Drosophila Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. Cell. 2004;117:69–81.
45. O'Keefe, E.P. (2013). siRNAs and shRNAs: Tools for Protein Knockdown by Gene Silencing. Materials and Methods, 3.
46. Singh, S., Narang, A. S., & Mahato, R. I. (2011). Subcellular Fate and Off-Target Effects of siRNA, shRNA, and miRNA. Pharmaceutical Research, 28(12), 2996–3015. doi:10.1007/s11095-011-0608-1.
47. Wang YX, Niklasch M, Liu T, Wang Y, Shi B, Yuan W, Baumert TF, Yuan Z, Tong S, Nassal M, Wen YM. Interferon-inducible MX2 is a host restriction factor of hepatitis B virus replication. J Hepatol. 2020 May;72(5):865-876. doi: 10.1016/j.jhep.2019.12.009. Epub 2019 Dec 18. PMID: 31863794.
48. Gripon P, Diot C, Thézé N, Fourel I, Loreal O, Brechot C, Guguen-Guillouzo C. Hepatitis B virus infection of adult human hepatocytes cultured in the presence of dimethyl sulfoxide. J Virol. 1988 Nov;62(11):4136-43. doi: 10.1128/JVI.62.11.4136-4143.1988. PMID: 3172341; PMCID: PMC253845.
49. Herrera-Carrillo E, Berkhout B. Attacking HIV-1 RNA versus DNA by sequence-specific approaches: RNAi versus CRISPR-Cas. Biochem Soc Trans. 2016 Oct 15;44(5):1355-1365. doi: 10.1042/BST20160060. PMID: 27911718.
50. Brégnard C, Pacini G, Danos O, Basmaciogullari S. Suboptimal provirus expression explains apparent nonrandom cell coinfection with HIV-1. J Virol. 2012 Aug;86(16):8810-20. doi: 10.1128/JVI.00831-12. Epub 2012 Jun 13. PMID: 22696639; PMCID: PMC3421764.
51. Mowa MB, Crowther C, Arbuthnot P. Therapeutic potential of adenoviral vectors for delivery of expressed RNAi activators. Expert Opin Drug Deliv. 2010 Dec;7(12):1373-85. doi: 10.1517/17425247.2010.533655. Epub 2010 Nov 13. PMID: 21073358.
52. Chen Q, Wei H, Sun R, Zhang J, Tian Z. Therapeutic RNA silencing of Cys-X3-Cys chemokine ligand 1 gene prevents mice from adenovirus vector-induced acute liver injury. Hepatology. 2008 Feb;47(2):648-58. doi: 10.1002/hep.21993. PMID: 18098313.
53. Goodfellow S, Zhang D, Wang MB, Zhang R. Bacterium-Mediated RNA Interference: Potential Application in Plant Protection. Plants (Basel). 2019 Dec 5;8(12):572. doi: 10.3390/plants8120572. PMID: 31817412; PMCID: PMC6963952.
54. Ruiz R, Witting SR, Saxena R, Morral N. Robust hepatic gene silencing for functional studies using helper-dependent adenoviral vectors. Hum Gene Ther. 2009 Jan;20(1):87-94. doi: 10.1089/hum.2008.081. PMID: 18828727; PMCID: PMC2855247.
55. Wieland SF, Chisari FV. Stealth and cunning: hepatitis B and hepatitis C viruses. J Virol. 2005 Aug;79(15):9369-80. doi: 10.1128/JVI.79.15.9369-9380.2005. PMID: 16014900; PMCID: PMC1181548.
56. Donia A, Bokhari H. RNA interference as a promising treatment against SARS-CoV-2. Int Microbiol. 2021 Jan;24(1):123-124. doi: 10.1007/s10123-020-00146-w. Epub 2020 Sep 1. PMID: 32875426; PMCID: PMC7462657.