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RNA Interference (RNAi) Technology, a New Tool for Therapy

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Abstract

RNA interference (RNAi) is a biological process where RNA inhibits gene expression or translation by silencing targeted mRNA. The mechanism depends on double-stranded RNA (dsRNA) which makes a post transcriptional gene silencing. Small interfering RNA (siRNA) is a powerful hopeful tool in functional genomics and after showing good marker for use in future therapy, it is employing to make silencing for gene expression in mammals. siRNA is used as a protector from infection and inhibit the accessory genes and expression of antigen. Control of normal gene expression consider the main mechanism of RNAi, which its function as a therapeutic tool for a wide range of diseases, including metabolic disorders, infectious diseases and cancer. RNAi technology is still new, and its biological action on siRNA-mediated knockdown of gene expression is not yet fully understood. The development of systems for effective transfer to the target cells was one major challenge in siRNA-based therapy. Over the last few years, a number of tools were designed for DNA and RNA for gene therapy, and widespread efforts are now located into rising clinical applications of siRNAs in a range of diseases.

Keywords: *RNA Interference (RNAi) Technology; mRNA; Double-Stranded RNA (dsRNA); DNA*

Introduction

RNAi has massive potential in suppression of desired genes since the discovery of RNAi and its regulatory potentials. RNAi is now known as stable, efficient, precise and better than antisense technology for gene suppression [1]. Andrew Fire and Craig Mello shared the 2006 Nobel Prize in Physiology and Medicine for their work on RNA interference in nematode worm *Caenorhabditis elegans*. RNAi, an ancient defense pathway, is a denominator for the post transcriptional gene silencing (PTGS) phenomenon detected in a variety of species such as animals, plants and fungi [2]. The mechanism happens at the levels of transcription, post-transcription and translation. Owing to its direct effects on the translation, RNAi can also cause increase of gene expression [3]. There are two types of small ribonucleic acid (sRNA), small interfering RNA (siRNA) and micro-RNA (miRNA) which are central to RNA interference.

Principles and mechanisms of RNAi

The specific degradation of the target mRNA is the RNAi phenomenon where dsRNA suppresses the expression of a target protein. RNAs is a nucleic acid present in all living cells and acts as a messenger carrying instructions from DNA for controlling the synthesis of proteins [4]. Small interfering RNA (siRNA) are short pieces of double-stranded (dsRNA), about 21 nucleotides long, with 3' overhangs

(2 nucleotides) at each end which interfere with the protein translation. RNAi promotes the degradation of mRNA at exact sequences. siRNA stops the production of definite proteins based on the nucleotide sequences of their matching mRNA. This mechanism is called RNA interference (RNAi) and may be referred to as siRNA knockdown or siRNA silencing [5].

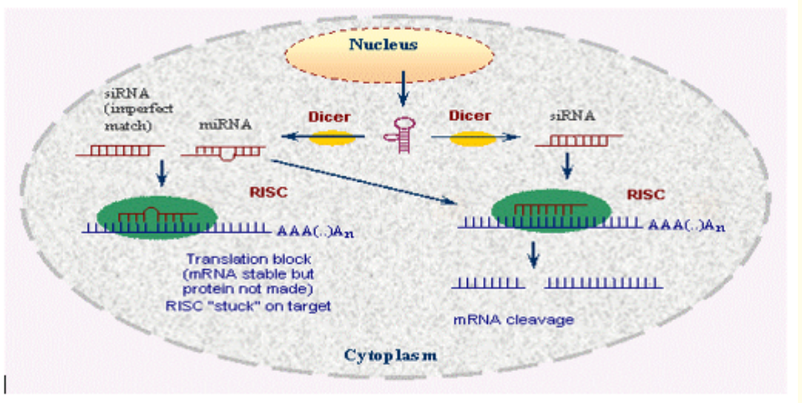
siRNAs must have phosphate groups at the 5' end in order to have activity so it is important not to block the 5' end of the antisense strand with any modifications other than the phosphate group. However, transfected unmodified 5′OH ends are rapidly phosphorylated by cellular kinases, indicating not to phosphorylate synthetic siRNAs.

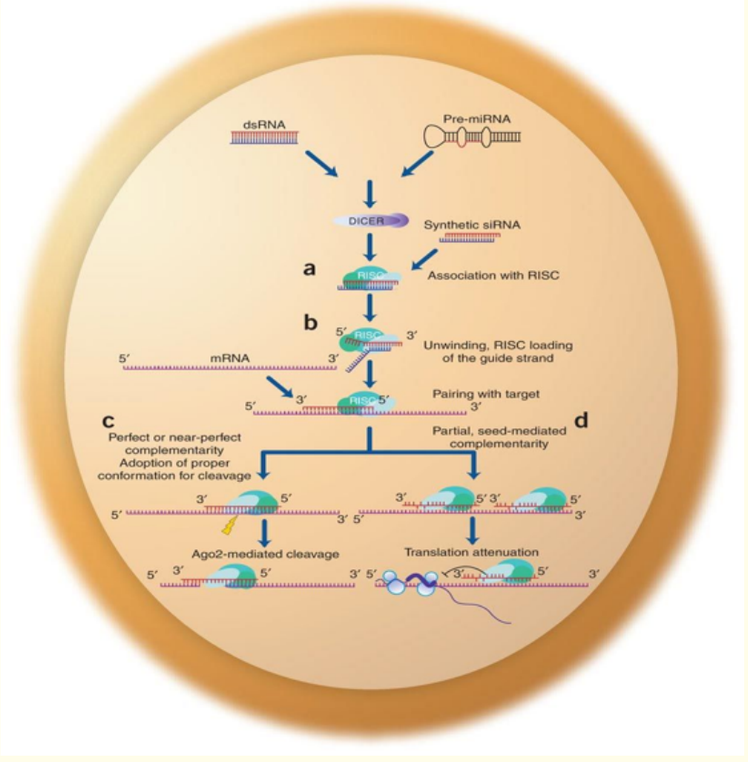
siRNAs are created from dsRNA or hairpin looped RNA (shRNA) which, after entering a cell is split by an RNase III-like enzyme, called Dicer. The siRNA is incorporating into a protein complex called RNAi-induced silencing complex (RISC). RISC search an appropriate target mRNA and siRNA then unwinds and the antisense strand leads degradation of the complementary strand of mRNA by means of a combination of endo- and exonuclease enzymes. Exogenous synthetic siRNAs or endogenously expressed siRNAs combined into the RNA-induced silencing complex (RISC) (Figure 1).

Two strands are formed after splitting of single-stranded RNAs (ssRNAs), the guide strand and the passenger strand. The guide strand is combined into the RNA-induced silencing complex (RISC) while the passenger strand is broken [4]. Outcome of gene silencing is well-studied when the guide strand combines with a complementary sequence in a mRNA molecule. RISC unwinds the duplex siRNA, which then combines to mRNAs by means of its unwound antisense strand that allow a high matching of sequence complementarities to the siRNA [4]. The Cleavage of the definite mRNA starts at a single site 10 nt upstream of the siRNA-target mRNA duplex [5].

Small interfering RNAs (siRNAs) and micro-RNAs (miRNAs) are generated through RNAi pathway via treating of longer dsRNA and stem loop precursors. Dicer enzymes show a critical role in the creation of these two RNAi [6]. They can cut either long dsRNAs or stem-loop precursors (shRNA) to siRNAs and miRNAs in ATP-dependent manner.

Easy two models for the RNAi pathway





Substrates for the RNAi pathway can be derived from a range of sources (e.g. long dsRNA, pre-miRNAs or chemically synthesized siRNAs).

Chemically synthesized molecules can be designed to optimize each of the steps associated with the RNAi mechanism as in figure:

(a) For highly functional siRNAs, association with RISC involves selection of the antisense targeting strand, a process that can be ensured by including differential end thermodynamic bias during siRNA design as well as other parameters usually calculated by functionality scoring.

(b) Limiting GC content and incorporating region-specific flexibility are predicted to greatly enhance duplex unwinding. Loaded RISC can silence genes by two mechanisms.

(c) In the case of perfect complementarity, the target is cleaved by an AgO_2-mediated mechanism.

(d) siRNA specificity can be enhanced at this step by including a sequence alignment (BLAST) filter and eliminating all candidate siRNAs that have perfect or near-perfect complementarity with unintended (unplanned) targets. siRNAs can also induce moderate levels of gene knockdown by seed-mediated translation attenuation. This process of off-targeting can be minimized by including design parameters that select siRNAs with low 3' UTR seed complement frequencies.

RNAi pathway is building on two steps, each involving ribonuclease enzyme. The first step, the trigger RNA (either miRNA primary transcript or dsRNA) is processed into siRNA by the RNase II (Dicer and Drosha). Second step, siRNAs are encumbered into effector RISC complex (RNA-induced silencing complex). The siRNA is unwinding during RISC gathering and ssRNA hybridizes with mRNA target.

By other description for RNAi mechanism and according to Fischer and James (2004), there are two steps in RNAi, initiation and effector step [7]. During initiation step, Dicer binds with high affinity to dsRNA containing 2 nucleotide (nt) 3' overhangs and cuts long dsRNA into siRNAs duplexes. Dicer enzyme cleaves dsRNA into either siRNAs or microRNAs (miRNAs) from endogenous stem loop precursors [8]. siRNA duplexes are fused or incorporated into RISC complex in the effector step. The phosphorylation of siRNA 5'-terminal is essential for entrance into RISC [9]. Ratio of RISC containing the antisense or sense strands of siRNAs are affected by the thermodynamic stability of the first few base pairs of siRNA [9]. The active RISC then objects the homologous transcript by base pairing interactions and cuts the mRNA between the 10th and 11th nucleotide from the 5' terminus of the siRNAs [8]. Base-pairing (partially) of miRNA with 5' or 3' ends of mRNAs leads to a suppression of translation where miRNA match completely complementary to its target mRNA that lead to the degradation of similar mRNA. Degradation of the targeted mRNA by the RNase H enzyme Argonaute that is called Slicer lead to gene silencing. Any mismatches in siRNA/mRNA coupling, the mRNA will not cleave. Like targeted effects, off-target effects (OTEs) are dose dependent. Therefore, it is important to establish dose response profiles for all siRNAs in use and always use the lowest concentration of siRNA that will provide adequate target knockdown.

siRNAs design

siRNAs, the directors that direct RNA interference (RNAi), offer a powerful tool to diminish the expression of a single gene in living cells [10]. The sequence of siRNA plays important role in silencing of the gene expression [11]. The localization of siRNA inside a particular gene exon structure is more important for potency than the location of siRNA inside the entire gene, 50 - 100 nt downstream of the start codon (ATG) is the favorite location of mRNA gene target [11].

The stability of siRNAs depends on its thermodynamic stability that has a significant effect on their potency. More A/U rich lead to less stable for siRNA efficiency toward the 5' end of the guide strand while relatively high Tm lead to more stability with more G/C rich toward the 5' end of passenger strand. Definite sequence characteristics should be avoided in the guide strand to maintain siRNA specificity such

as homopolymeric runs and greater segments of G/C bases. The site accessibility and secondary structure of the target are important factors in the action of siRNAs. So, it is very important to monitor all applicant siRNAs for homology to other targets and eliminate those with significant matching [10].

RNA interference is causing by 3 prime untranslated regions (3'UTRs) of mRNAs that contain regulatory sequences for post-transcriptionally.

3'UTRs contain binding sites for mRNAs and for regulatory proteins. siRNAs can decline gene expression of various mRNAs through binding to specific sites within the 3'-UTR which lead to either inhibiting translation or directly initiating degradation of the transcript. The silencer regions in 3'-UTR bind to repressor proteins leading to inhibition of the expression of mRNA.

Although overhang composition has no impact on cleavage product sizes, nucleotide sequence does play a role in determining Dicer efficiency. Overhangs that contain a C and A in the penultimate and terminal positions (respectively) are processed most efficiently, while those containing an A in the penultimate position and a U in the terminal position are less optimal substrates [12].

Some studies of substrates with varying 3′ overhang lengths showed that the number of nucleotides in the overhang was a critical factor in Dicer specificity. Substrates with overhang lengths of 1, 2, and 3 nt showed concomitant shifts in the primary Dicer cleavage position. Increasing the overhang length further reduced the diversity of cleavage products generating primarily a 21-nt product. Thus, when the overhang length is 0 - 3 nt it appears as though Dicer cleaves dsRNA by "counting" a distance of approximately 23 nt from the 3′ end of the overhang to cleave both strands. In cases where the overhang length extends beyond 3 nt, Dicer no longer uses the 3′ end of the overhang to determine cleavage position.

According to Annaleen., *et al*. (2005), RNA end structure, particularly 3′ overhang length, plays a critical role in determining the position and efficiency of Dicer cleavage [12].

This knowledge has a profound implication for siRNA and shRNA design. shRNA expression by the Pol III promoter results in shRNA termini with variable overhang lengths (1 - 5 Us). This variation drastically affects the specificity of Dicer cleavage and consequently the functionality of cleaved siRNA. DNA-directed RNA interference (ddRNAi) uses DNA templates to synthesize si/shRNA *in vivo*.

ddRNAi depends on U6 or H1 [RNA polymerase III], or U1 [RNA polymerase II] promoters for the expression of siRNA target sequences that have been transfected into mammalian cells [13].

si/shRNA target sequences can be generated by PCR, creating "expression cassettes" that can be transfected directly into cells or cloned into expression vectors [14].

ddRNAi technology involves inserting a DNA construct into a cell, triggering the production of double stranded RNA (dsRNA), which is then cleaved into small interfering RNA (siRNA) as part of the RNAi process, resulting in the destruction of the target mRNA and knocking-down or silencing the expression of the target gene.

Work scheme for siRNA design and selection

To identify optimal sequences for siRNA targeting, it is necessary to delineate target gene sequence space and build a representative candidate list.

In molecular genetics, an open reading frame (ORF) is the part of a reading frame that has the potential to be translated. An ORF is a continuous stretch of codons that contain a start codon (usually AUG) and a stop codon (usually UAA, UAG or UGA). An ATG codon within

the ORF (not necessarily the first) may indicate where translation starts. The transcription termination site is located after the ORF, beyond the translation stop codon.

Using *BRCA1* gene (with 11 variants) as an example, there are 9 stages for RNAi design:

- Stage 1 (identification stage): The sequence is first identified. There are 3,623 19-mer sequences associated with the 5' UTR, ORF and 3' UTR of the shortest variant for *BRCA1* gene.
- Stage 2 (the build stage): Sequences that contain SNPs are outside the ORF and are not present in all of the variants are eliminated, leaving 1,124 sequences.
- Stage 3 (filtration stage): These remaining sequences are then filtered to remove those with attributes that are detrimental to functionality.

  Candidate sequences with degenerate base symbols, known toxic motifs, repetitive bases, excessively high or low GC content, miRNA seed matches and internal complementary stretches are eliminated from further consideration. This significantly reduces the remaining sequence space to 468 sequences (12.9% of the original).
- Stage 4 (scoring stage): The outstanding sequences are scored (using an algorithm that judges sequences on the basis of thermodynamic asymmetry, possible fold-over structures and position-specific nucleotide preferences) and sorted or arranged in descending order of score. As scoring does not eliminate sequences, the current number of sequences (468) is carried through to the next stage.
- Stage 5 (cropped or harvesting stage): To minimize sequence alignment time, the list is cropped or harvested to focus assessment on those sequences having the top 50 scores (1.38% of total). In the majority of cases, the top 50 scores generally provide a sufficient number of target sites to develop multiple highly functional siRNAs.
- Stage 6: In the *BLAST* analysis, sequences that have perfect or near-perfect complementarity to unintended or unplanned targets are eliminated from further consideration. In the case of *BRCA1*, BLAST eliminates only two candidates from the top 50 sequences (48 total target sites remaining).
- Stage 7 (the Pick stage): Four siRNAs that optimally balance functionality and specificity are selected for synthesis.
- Stage 8: The list of candidates might be further narrowed by selecting sequences with low seed complement frequencies in a 3' UTR of target genome.

For safe time and efforts and with the advent of automated design tools, highly functional siRNAs can now be designed in few seconds. In contrast, manual siRNA design or design unaided by automated computational methods can take from hours to days to complete, depending on the size of the transcript, the number of variants, conservation across family members and the tools available for non-automated analysis.

MicroRNA (miRNA) and siRNA

Non-coding RNAs encoded miRNAs and regulate gene expression during development. RNAi phenomenon includes the endogenously miRNAs as well as foreign dsRNA that makes trigger silencing. Before reaching maturity of miRNA, Mature miRNAs are architecturally

similar to siRNAs created from exogenous dsRNA [11]. Longer RNA-coding gene deliver miRNA as a primary transcript known as a *pri-miRNA.* By the micro-processor complex, miRNA is handled in the nucleus to a 70-nucleotide stem-loop structure called a *pre-miRNA*. miRNA and siRNA share the same downstream cellular machinery where dsRNA (portion of pre-miRNA) is cut by Dicer to produce the mature miRNA molecule. Then miRNA incorporated into the RISC complex [15].

siRNAs which is derived from long dsRNA precursors differ from miRNAs in that miRNAs naturally have partial base pairing to a target and inhibit the translation of many different mRNAs with similar sequences while siRNAs typically induce mRNA cleavage only in a single specific target [16].

Challenge of RNAi

Immune response occurred when a mammal cell is injected with dsRNA such as a siRNA. The introduction of a siRNA may cause unplanned off targeting. Nonspecific actions occur because of high dose of siRNA due to activation of innate immune responses. There are many challenges for RNAi as follow:

1. **Getting into the target:** One of the major problems is getting the silencing molecules to where they are needed.

2. **Distribution:** Different methods are using to crack this problem. The Nobel Prize winners used RNA interference molecules (RNAi) to switch off genes those are very similar to molecules produced naturally by cells. Another approach is to try different molecules that might be better at spreading around and entering living cells.

   Antisense oligonucleotides (ASOs) are similar to RNAi molecules but are slightly simpler and are not produced naturally by cells. Its principle is the same by sticking to the mRNA message molecule and prevent the cell from using it to build proteins.

3. **Switching off the gene:** Turn off the gene successfully is the key of a gene silencing treatment.

4. **Choosing the right target and side-effects:** STK33, a protein kinase was one of the most infamous examples of such a misidentified target. Researchers identified it in 2009 as essential for the viability of human cancer cells harboring a mutant version of a known oncogene. STK33 was later shown to not be essential at all before it had attracted considerable pharmaceutical industry attention as a potential drug target. Efficiency and specificity are the key issues for designing siRNAs, and it is of great importance to study possible off-target effects in the siRNA design. Gene silencing treatments might still have side effects but researchers are still working to design the best molecules with the lowest risk of serious side effects and only the safest will be applied for human trials.

Advantages of RNAi (siRNA)

siRNA have many advantages if compared with other conventional drugs.

First, because of target mRNA and siRNA are sequences-specific and complementary so select target sites will much easier and more flexible. Second, within 24 h and only sub stoichiometric dose of siRNA is enough for gene silencing to make reduction of homologous mRNA. Third, the degradation of definite mRNA because of siRNAs can result in cells of different species. Fourth, cell control mechanisms are not affected by siRNAs. The selective destruction of only interested transcript is ensured by the high homology of siRNA to the target region of transcription. siRNAs remain inert within cells without suitable targets. Fifth, siRNAs can silence gene stably.

Therapeutic applications in genetic diseases and gene function

The idea of RNAi therapeutics has paying attention some of the bright minds and it is one of the most highly advanced technology in biotechnology fields. RNAi is a mechanism for controlling normal gene expression, whose duties as a potential therapeutic negotiator for

a wide range of disorders [17]. Current therapies involving pharmacological and biological drugs are not suitable to target mutant genes selectively due to structural indifference of the normal variant of their targets from the disease-causing mutant ones. Seyhan, (2011) have concluded that RNAi and other RNA targeting drugs still hold their promise as the only drugs that provide an opportunity to target genes with SNP mutations found in dominant negative disorders, genes specific to pathogenic tumor cells, and genes that are critical for mediating the pathology of various other diseases [18].

A promising lead toward using RNAi for the treatment of genetic diseases has been provided by preliminary studies demonstrating how single nucleotide polymorphisms (SNPs) in mutant allele transcripts can be used as selective targets for RNAi.

SNPs are very often found in mutant allele transcripts and represent potential selective targets. Systematic analyses of siRNAs in which the polymorphic nucleotide is complementary to the mid region of the siRNA provides siRNA/SNP combination that is highly selective [19]. According to Chang., *et al*. (2012) clinical trials with RNAi have now begin and adapted versions of siRNA that are focusing against disease causing genes are being established, some of which are tested in clinical trials [20].

Post-Transcriptional Gene Silencing (PTGS) or RNAi is a preserved biological response to dsRNA that regulates the expression of protein-coding genes and facilitates fighting to both exogenous pathogenic nucleic acids and endogenous parasitic. This regular mechanism for sequence-specific gene silencing update experimental biology and may have significant practical applications in therapeutic interference, agriculture, functional genomics, and other areas. A particularly influential tool like RNAi for gene silencing allows functional suggestion of a single gene in a given phenotype [9]. Currently, identified genes are under most of the applications to get an overview of their cellular functions [20].

The use of cDNA clones as a starting material for siRNAs production and efficient RNAi libraries construction techniques will lead to whole genome phenotyping screening that will be well- matched with the cytotoxicity studies necessary for the molecular division of the fighting mechanisms [21]. The identification and validation of targets that map chemically tractable gene families is now recognized as a fundamental challenge to the pharmaceutical industry.

RNAi and siRNA have taken center stage as effective biologically relevant mechanisms for gene silencing. One of the key footraces for drug target discovery or validation directly in animal disease models has been the lack of effectiveness of *in vivo* nucleic acid delivery methods. Recently, researchers have utilized siRNA approach for the validation of several drug targets.

RNAi is a powerful technology with tremendous potential for functional genomic analysis, drug discovery and therapeutic applications. siRNA technologies are already widely used as a tool for reverse genetics in mammalian cells and their potential therapeutic applications [21-23]. The introduction of chemically synthesized siRNAs into mammalian cells has been shown to be an effective mechanism for the suppression of cancer that confers a degree of protection to the host cells [24].

Researchers make estimation about gene function based on expression outlines in different samples. Other suggestions of gene function in mammal are developed using similar searches with genes whose tasks are known in model animals. In many cases, testing the accurateness of these ideas can be done using siRNAs.

According to Al-Khalili., *et al*. (2003) who have concluded that glucose uptake increase with increasing of cell surface content of glucose transporter through treatment with GLUT1- siRNA [25].

Other study by Chen and Barritt (2003) used siRNAs with transient receptor potential canonical 1 (TRPC1) gene in liver cells. The TRPC1 gene was believed to be activated by reduction of cellular storage and/or an intracellular messenger. TRPC1- siRNA decreased inflow of $Ca2^{+}$, Mn^{2+}, and ATP and increased cell volume and in hypotonic solutions [26].

RNAi in gene regulation and antiviral responses

RNAi is regarded as a natural defense mechanism against mobile endogenous transposons and invasion by exogenous viruses, which have dsRNA as an intermediate product. With this defense mechanism, organisms maintain genetic integrity and hinder infection [27].

For many applications, it may be complicated to introduce short dsRNAs directly into cells. However, many groups have now shown that appropriately designed DNA molecules containing inverted repeat sequences can be transcribed into RNA molecules that form RNA hairpins [28].

If the sequences are chosen correctly, these are processed by the Dicer nuclease to form siRNAs. Thus, all the methods derived for delivering genes into cells in principle can be used to deliver siRNAs as well. This makes the application of RNAi therapy for the prevention and treatment of viral infection convenient.

siRNA-based prophylaxis are widely used in investigation of viral diseases. siRNAs can prevent the replication of Hepatitis C and HIV as shown in number of studies [29,30]. However, there are important issues and concerns about the therapeutic application of this technology, including difficulties with delivery and uncertainty about potential toxicity that needs to be solved.

The HCV genome is a positive-strand RNA molecule with a single open reading frame encoding a polyprotein that is processed post-translationally to produce at least ten proteins.

Wilson., *et al*. (2003) have suggested that, small inhibitory RNAs (siRNA) targeting the internal ribosome entry site (IRES) and non-structural protein NS3 and NS5b encoding mRNAs were shown to inhibit HCV replicon function in cell culture [31].

Furthermore, anti-HCV siRNAs were shown to ‘‘cure’’ Huh cells bearing persistently replicating HCV replicons.

Delivery of the siRNAs or vectors that carry siRNA expression cassettes is the major challenge for treatment of HCV. The method of delivery used in a number of *in vivo* studies, hydrodynamic intravenous injection, is not possible for the treatment of human hepatitis. Delivery is a problem that must be solved for any therapeutic application of RNAi.

A recent report demonstrates that it is possible to introduce genetic material into hepatocytes using catheters or even localized hydrodynamic procedures [32].

Kapadia., *et al*. (2003) have showed that HCV RNA replication and protein expression are inhibited by using *NS3* and *NS5B* siRNAs in Huh-7 cells. According to their study, inhibition of HCV RNA replication occurred within 2 days of siRNA transfection. The efficient transfection observed in these experiments with stability of the siRNAs reveal the potential application of RNAi to prevent the viral infection [33].

HIV was the first infectious agent targeted by RNAi, it may be owing to the fact that the life cycle of HIV is well understood as is its pattern of gene expression. Moreover, inhibition of HIV replication has been achieved in numerous human cell lines and primary cells including T lymphocytes and hematopoietic stem cell derived macrophages.

Despite the success of *in vitro* RNAi-mediated inhibition of HIV-1, for future clinical applications, targeting the virus directly represents a substantial challenge since the high viral mutation rate will certainly lead to escape mutants.

Delivery of siRNAs or shRNA encoding genetic units to HIV infected cells is also a challenging problem. The target cells are primarily T lymphocytes, monocytes and macrophages. Since synthetic siRNAs will not persist for long periods in cells, delivery would have to be

done repetitively for years to effectively treat the infection. Systemic delivery of siRNAs to T lymphocytes is a major barrier and probably not feasible [15].

There are additional challenges for using siRNAs in the treatment of HIV-1 infection, including validating the approach in a relevant animal model and preventing the emergence of variants resistant to treatment because of the high sequence diversity of the virus.

RNAi for cancer therapy

The use of RNAi for cancer therapeutics could transform treatment of this devastating disease. The strong appeal of RNAi in therapeutics is the potency and specificity with which gene expression can be inhibited. What makes RNAi so exciting to many researchers is its potential for knocking out a protein without harming the cell. By comparison, chemotherapy invariably kills tumors by destroying cancerous cells as well as healthy cells nearby.

Animal models are widely used to investigate the therapeutic efficiency of RNAi. *In vivo* utilization of siRNA was effectively performed by targeting the colorectal cancer associated gene beta catenin. Decreased proliferation and diminished invasiveness were observed following siRNA-mediated silencing of this gene in human colon cancer cells.

Additionally, when treated cancer cells were placed in a nude mouse, prolonged survival was seen compared with mice receiving unmanipulated tumors.

Similarly, silencing the oncogene H-ras led to the inhibition of *in vivo* tumor growth of human ovarian cancer in a SCID mouse model [27]. The challenges for cancer are similar to those faced for other diseases that include finding good targets, delivery and minimizing toxicity. Perhaps the most significant work utilized transferrin containing nano-particles to target Ewing's sarcoma cells in a mouse xenograph model [34]. This study demonstrated the feasibility of using non-lipid based nano-particles for the targeted delivery of siRNAs in a cancer model and provides a powerful proof of principle for systemic delivery of siRNAs to a metastatic cancer.

Interestingly, Olivier., *et al*. (2008) cited that RNAi might also be exploited to silence pathways that facilitate the effects of traditional cancer drugs. This includes targeting of the multidrug resistance gene (MDR1) for re-sensitization to chemotherapy and silencing of double strand break repair enzymes for enhanced effects of radio and chemotherapy [35].

Cyclin E expression were declined by using siRNAs according to study of Li., *et al*. (2004) in HCC cells, inhibition of HCC tumor growth were induced in nude mice where cyclin E siRNA promoted apoptosis and decreased cell proliferation [28]. Recently, Zoheir., *et al*. (2016) have concluded that silencing of IQGAP1 gene by using IQGAP1-RNAi vector showed vital roles in the apoptosis of HepG2 cells and dropped their proliferative and invasive capacities [36]. Although the trials are in their early phase, the promises they are showing now are only indicative of the potential RNAi has for future therapeutic process.

There has been progress over the last 4 years in terms of different delivery system technologies and the movement of RNAi therapeutics from pre-clinical into human trials. With the first substantial patient data from RNAi based therapies on the near-term horizon and new studies likely to begin soon, it is too early to dismiss RNAi therapeutics for cancer treatment.

Some scientists are evaluating siRNAs as therapeutic agents while others are manipulating siRNAs in their drug development methods [37]. If it is realized, using of siRNAs can make a possibility to target any gene for therapeutic involvement.

RNAi trail is active in rodents and that siRNAs are recognized and effective in different tissues [38]. Seok., *et al*. (2017) have described siRNA problems such as off-target effects caused by canonical and noncanonical interactions. Their studies have focused on several ge-

nome-wide methods and chemical alterations for the prevention and evaluation off-target suppression to simplify the use of RNAi with safe specificity [39].

According to study of Wild and Tabrizi (2017), an intrathecally delivered antisense oligonucleotide that aims to lower Huntingtin is now well into its first human clinical trial, with other antisense oligonucleotides expected to enter trials in the next 1 - 2 years and virally delivered RNA interference and zinc finger transcriptional repressors in advanced testing in animal models [40].

In the near future, number of potential diseases will be the target of RNAi technology such as viral diseases, genetic diseases and cancer. Off-target effects, unsafe delivery methods and toxicity are the major difficulties of RNAi and have to overcome before using it as a conventional drug.

RNAi will find a place alongside of many conventional approaches in the treatment of diseases, although it is unclear as how long we have to wait to see the first RNAi based drug. There is great hope that in the near future RNAi based treatment for diseases such as Huntington's disease, HIV, cancer and other genetic based or infectious diseases will be available.

Conclusion

RNAi is considered as a gene-specific therapeutic option for controlling disease. For the potential of RNAi as a gene therapy approach, careful assessments are required for controlling cell mechanism. The challenge of successful siRNA based drugs in the near future is to develop efficient and safe means to deliver siRNAs into cells interested. With the beginning of RNAi library in mammals and the modification of techniques to silence gene, siRNA-based drugs will surely make great advances in the prevention and treatment of diseases. Despite the proliferation of promising studies for RNAi-based drugs, some concern has been raised regarding the safety of RNA interference, especially the potential for "off-target" effects, in which a gene with a coincidentally similar sequence to the targeted gene is also repressed.

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