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THERAPEUTIC APPLICATIONS OF SI RNA: A REVIEW

Nitesh Kumar*

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A. H., Rewa – 486 001 (M. P.), India.

ABSTRACT

RNA interference is the process of gene silencing that plays important role in development and maintenance of genome by targeting and degrading the specific complementary mRNA by means of 20-25 nucleotides double stranded RNA molecules referred as small interfering RNA or SI RNA. Small interfering RNA (siRNA), also known as short interfering RNA or silencing RNA, are a class of 20-25 nucleotide-long double-stranded RNA molecules that play a variety of roles in biology. It is involved in the RNA interference (RNAi) pathway where the siRNA interferes with the expression of a specific gene. In the RNAi pathway, siRNAs also act in RNAi-related pathways, e.g. as an antiviral mechanism or in shaping the chromatin structure of a genome, the complexity of these pathways is only now being elucidated. Si RNA therapeutics opens up an exciting new approach for the treatment of many disease conditions. Molecular biologists also working in the area of molecular pharmaceutics, to ensure that this technology reaches its full potential. The excellent biological activity of siRNA has also been tested for therapeutic drugs. siRNA as a drug promises several advantages over traditional drugs, offering new types of medicines that have a very high target selectivity and that are effective at a low dose as nanomolar or subnanomolar concentrations, with low toxicity due to metabolism to natural nucleotide components.

Keywords: RNA interference, Small interfering RNA (siRNA), Gene silencing, Therapeutic applications

INTRODUCTION

RNAi is the major biological discovery of the decades. This has proven to be an invaluable means for investigating the different gene expression and their manipulation for utilizing the applications of si RNA technology in research field and drug development .This discovery of RNA interference has revolutionized the studies of gene functions which are responsible for various kind of disease in human and animals. RNAi is a highly conserved gene silencing mechanism in which double stranded RNA serves as a signal to trigger the degradation of homologous mRNA and representing a novel therapeutic strategy allowing the knockdown of any pathologically relevant target gene[1]. Recent studies reported that the regulation of gene expression by silencing the

endogenous genes in which ds RNA complex with and destroy a target messenger m RNA the targeting and destruction of m RNA has been demonstrated to be highly specific and more selective [1]. The no. of experiments and studies have been shown that exogenously administered ds RNA 21 -23 –mer ds RNA or si-RNA is equally more efficient potential tools for elucidating the functions of gene and also novel therapeutics modality.

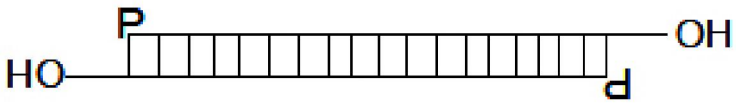
Si RNA therapeutics is realizing the potential of RNA interference by means of potent and stable si RNA compound that can be delivered into cells resulting in the silencing of genes and various harmful and pathological viruses responsible for human and animal disease [2]. The leading scientist from the world observing the si RNA therapeutics as at the forefront effort to discover the RNAi based therapies and leverages the vast potential of siRNA therapeutics and technology to ultimately treat the patients from the normal and incurable diseases whose solution of treatments are still the present question.

HISTORY

In 2006 Andrew Z. Fire and Craig C. Mello were awarded the Nobel Prize for their discovery of RNA interference (RNAi). This pathway is involved in cellular defense against viral invasion and transposon expansion and represents a unique form of post-transcriptional gene silencing [1]. It is also a cost-effective molecular biology tool for the determination of gene function, signaling pathway analysis, RNAi mechanistic studies and target validation and shows tremendous potential for diagnostics and therapeutics [3].

Si RNAs were first discovered by David Baulcombe's group in Norwich, England, as part of post-transcriptional gene silencing (PTGS) in plants and published their findings in Science in a paper titled "A species of small antisense RNA in posttranscriptional gene silencing in plants." Shortly thereafter in 2001, synthetic si RNAs were then shown to be able to induce RNAi in mammalian cells by Thomas Tuschl and colleagues in a paper, "Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells." published in Nature and Genes & Development. This discovery led to a surge in interest in harnessing RNAi for biomedical research and drug development.

STRUCTURE



Schematic representation of a siRNA molecule: a ~19-21basepair RNA core duplex that is followed by a 2 nucleotide 3' overhang on each strand. OH: 3' hydroxyl; P: 5' phosphate.

Each strand has a 5' phosphate group and a 3' hydroxyl (-OH) group. This structure is the result of processing by Dicer, an enzyme that converts either long dsRNAs or hairpin RNAs into siRNAs. siRNAs can also be exogenously (artificially) introduced into cells by various transfection methods to bring about the specific knockdown of a gene of interest. Essentially any gene of which the sequence is known can thus be targeted based on sequence complementarity with an appropriately tailored siRNA. This has made siRNAs an important tool for gene function and drug target validation studies in the post-genomic era.

GENERAL DESIGN GUIDELINES

Selection of si RNA target sites in a variety of different organisms based on the following guidelines. Corresponding si RNAs can then be chemically synthesized, created by in vitro transcription, or expressed from a vector or PCR product.

1]. Find 21 nt sequences in the target mRNA that begin with an AA dinucleotide.

According to Elbashir *et al* [4] ***siRNAs*** with 3' overhanging UU dinucleotides are the most effective.

2]. Select 2-4 target sequences.

Typically more than half of randomly designed siRNAs provide at least a 50% reduction in target mRNA levels and approximately 1 of 4 siRNAs provide a 75-95% reduction. Selection of target sites among the sequences identified in Step 1 based on the following guidelines:

A] siRNAs with 30-50% GC content are more active than those with a higher G/C content.

B] Avoid stretches of > 4 T's or A's in the target sequence.

C] Since some regions of mRNA may be either highly structured or bound by regulatory proteins, select siRNA target sites at different positions along the length of the gene sequence.

D] Compare the potential target sites to the appropriate genome database (human, mouse, rat, etc.) and eliminate from consideration any target sequences with more than 16-17 contiguous base pairs of homology to other coding sequences.

E] Use of BLAST to eliminate any target sequences with significant homology to other coding sequences.

3]. Design appropriate controls and test 3-4 si RNA sequences

A complete siRNA experiment should include a number of controls to ensure the validity of the data.

1] A negative control siRNA with the same nucleotide composition, which lacks significant sequence homology to the genome.

2] dditional siRNA sequences targeting the same mRNA.

METHODS OF PREPARING siRNA

There are several methods for preparing siRNA, such as

In vitro Chemical synthesis,

In the chemical synthesis high quality, chemically synthesized siRNAs can be obtained on a custom basis and the large yield of high purity siRNA. This method is best for Studies that require large amounts of a defined siRNA sequence and not suitable for screening siRNA sequences (cost prohibitive), long term studies which are most expensive.The chemical synthesis of RNA is more difficult than that of DNA because the 2' OH group of RNA nucleotides must be protected during synthesis. Additional steps in the synthesis process introduce 2'-OH protecting groups into monomers and remove them once RNA is assembled.

According to Katoh *et al* [5] a simple, rapid, practical and cost-effective method for preparing active siRNA derived from short hairpin (sh) RNA which is transcribed from a single-stranded synthetic DNA template using T7 RNA polymerase. This method doesn't require any sequence-limitation in the selection of the target region of genes. This

method also demonstrates efficient silencing of several genes by the transcribed siRNAs obtained.

At industrial level the chemical synthesis of siRNA also combines high-throughput RNA synthesis and high-throughput purification. HPP Grade siRNA duplexes have excellent yields and a reproducible purity of >90%. An integrated tracking system monitors siRNA production from the data entry of an mRNA target sequence to chemical synthesis in 96-well plates, quality control (QC), and final processing and packaging. siRNA is synthesized in 96-well format and is purified by automated, high-throughput HPP purification.

In vitro transcription

This method is relative cost per gene as moderate which requires little hands on time also relative ease of transfection. In vitro transcription is best for screening siRNA sequences or when the price of chemical siRNA synthesis is an obstacle but its not suitable for long term studies or studies that require large amounts of a single siRNA sequence. In vitro transcription using T7 RNA polymerase requires that the first 2 nucleotides of the RNA transcript be GG or GA to ensure efficient synthesis. Requiring a GG or GA at the 5' ends of both the sense and antisense strands of an siRNA in addition to the required 3' terminal UU greatly reduces the number of potential target sites for siRNA experiments.

This constraint essentially eliminates in vitro transcription as a viable option for preparing siRNAs.

Production of siRNAs by in vitro transcription is a useful method that it is simple, effective, and inexpensive. Commonly used in vitro transcription promoters use guanine as the transcription start nucleotide. This method is, therefore, restricted to generating siRNAs with target sequences of 5′-NNGN17C. The modified in vitro transcription method, in which pre-siRNAs containing the 5′ overhanging single-stranded leader sequence are first synthesized and then a DNA oligonucleotide complementary to the leader sequence is added to form a RNA-DNA hybrid, which is removed using RNase H to obtain desired siRNAs. Using siRNAs prepared with this method and successfully inhibited the expression of both exogenous and endogenous genes [6].

In Vivo

In vivo, there is no need to work directly with RNA synthesis of sirna will be proceed by either by expression in cells from an siRNA expression plasmid or viral vector and by expression in cells from a PCR-derived siRNA expression cassette.

siRNA expression vectors

RNA polymerase III (pol III), Human U6 promoters, Mouse U6 promoters, the human H1 promoter

RNA pol III was chosen to drive siRNA expression because it naturally expresses relatively large amounts of small RNAs in mammalian cells and it terminates transcription upon incorporating a string of 3–6 uridines.

This method is more effective than synthetic siRNA it is very stable and easy to handle; Stable cell line and Inducible system can be established. Once a DNA construct is made, there will have unlimited supply of siRNA in this method. It is cost-effective and takes a lot of time and trouble to make the DNA constructs. This method is best for long term and other studies in which antibiotic selection of siRNA containing cells is desired and not suitable for screening siRNA sequences since it is time and labor intensive with vectors. The pharmaceutical company like BioVision's GeneBlocker pGB siRNA expression vectors are designed to provide efficient suppression of a target gene in cultured mammalian cells and in vivo. The pGB vector has been optimized for suppressing expression of target genes using the human U6 promotor (a RNA polymerase III promotor) which generates large amounts of siRNA in mammalian cells. The pGB vector provides neomycin resistance marker for the selection of stable cell lines, permitting long term suppression of the target gene. This method of BioVision offers siRNA vectors targeting to important Apoptosis genes and the negative control siRNA vector and pGB cloning vector for cloning.

PCR expression cassettes

This is best methods which rapidly prepare siRNA expression cassettes by PCR, No cloning, plasmid preps or sequencing necessary for PCR expression cassettes; quickly test different siRNA sequences and promoters before cloning into a vector. It avoids costly siRNA synthesis. RNA interference (RNAi) is a process in which double-stranded

RNA (dsRNA) induces the postranscriptional degradation of homologous transcripts. RNAi can be initiated by exposing cells to dsRNA either via transfection or endogenous expression. In mammalian systems, the sequence-specific RNAi effect has been observed by expression of 21-23 base transcripts capable of forming duplexes, or via expression of short hairpin RNAs. Daniela and Rossi [7] describe a facile PCR based strategy for rapid synthesis of siRNA expression units and their testing in mammalian cells. The siRNA expression constructs are constructed by PCR, and the PCR products are directly transfected into mammalian cells resulting in functional expression of siRNAs. This approach should prove useful for identification of optimal siRNA-target combinations and for multiplexing siRNA expression in mammalian cells. Placing the recognition site of an active siRNA into a structured mRNA region has abrogated the siRNA activity. Therefore, a successful gene-targeting project may require the design of many distinct siRNAs at a high cost. The potential design rules, cost-effective strategies for producing siRNAs by T7 RNA polymerase, and expression cassettes for in vivo testing are studied[8].

VECTORS

The synthetic siRNA molecules must be transported into the cells before they can function in RNAi, successful delivery of siRNA is of central importance and must require delivery mechanism as vehicle. These delivery vehicles must protect the siRNA from nucleases in the serum or extracellular media, enhance siRNA transport across the cell membrane and guide the siRNA to its proper location through interactions with the intracellular trafficking machinery. While naked siRNA molecules have been shown to enter cells, significantly more siRNA can be delivered using carrier vehicles [9, 10]. Both viral and nonviral vectors deliver siRNA into cells, although viral vectors are limited to delivering siRNA-expressing constructs such as shRNA.

Non viral vectors

Commercially available cationic lipids such as Oligofectamine can effectively deliver siRNA molecules into cells in vitro with transfection efficiencies approaching 90% [4] However, the high toxicity of cationic lipids limits their use for systemic delivery in vivo. Recent studies showed that cyclodextrin-containing polycations (CDPs) can

achieve safe and effective systemic delivery of siRNA in mice [11]. The recent experiment recorded the nonviral delivery of siRNA using cationic lipids or polymers [12].

Both viral and nonviral vectors are being assessed for siRNA delivery. An alternative to viral vectors is the use of nonviral lipid and polymer-based vectors. While immortalized cell lines can be successfully transfected with nonviral vectors, to date efficient transfection of primary cells has been poor. Therefore finite cell lines or freshly isolated primary cells may be more suitable targets for viral vectors [13]. However, ongoing research into the transfection of primary cells and whole organisms with siRNA using nonviral transfection agents has produced some promising results.

DNA and siRNA are negatively charged, as is the surface of the cell. Therefore using positively charged lipid and polymer based transfection agents can aid in their introduction into the cell by complexing and protecting the negatively charged siRNA and enhancing interactions with the cell surface.

TABLE 1: VECTORS USED TO DELIVER SIRNA TO AIRWAY CELLS IN CULTURE

Transfection reagent	Cell type	mRNA target	Reference
Lipofectamine 2000	Glc-82	GFP	[14]
Oligofectamine	H1703	DVL-3	[15]
TransIT-TKO	CL1-0/CL1-5	Axl	[16]
Lipofectamine	Calu-3	c-erbB2	[17]
Fugene6	A549	Caveolin-1	[18]
sIMPORTER	NHTBE	RAR/RXR	[19]
Dharmafect 1	NCI H1299	RASSF1C	[20]
Lipofectamine 2000	BEAS2B	NFkB (p65)	[21]

Both lipid and polymer based transfection agents have been used successfully to introduce genetic material to cells in culture as well as systemically and locally to animal models [3, 22]. Several commercial lipids have been available for many years for intracellular DNA delivery. However, effective systems for DNA delivery are not always optimal for the delivery of siRNA into cells. A number of transfection agents are currently available that are capable of delivering siRNA intracellularly to cell lines .Some of these products have been available for some time, such as Invitrogen's Lipofectamine

and Lipofectamine 2000, while others have been developed more recently such as Upstates sIMPORTER. More transfection reagents are being produced which are specifically designed to transfect cells with siRNA, including Mirus's TranIT-TKO and Dharmacon's Dharmafect. Novagen has amine- and lipid-based reagents in a single formulation called RiboJuice siRNA Transfection Reagent, which is designed to target a wide range of mammalian cell lines, including lung cells.

Viral Vectors

Viral vectors have been developed for efficient delivery of siRNA into a range of mammalian cells. Genetic material inserted into the vector can encode for shRNA which efficiently blocks production of a specific protein. The various viral vectors has been developed for successful delivery of siRNA such as retrovirus, lentivirus, adenovirus etc.

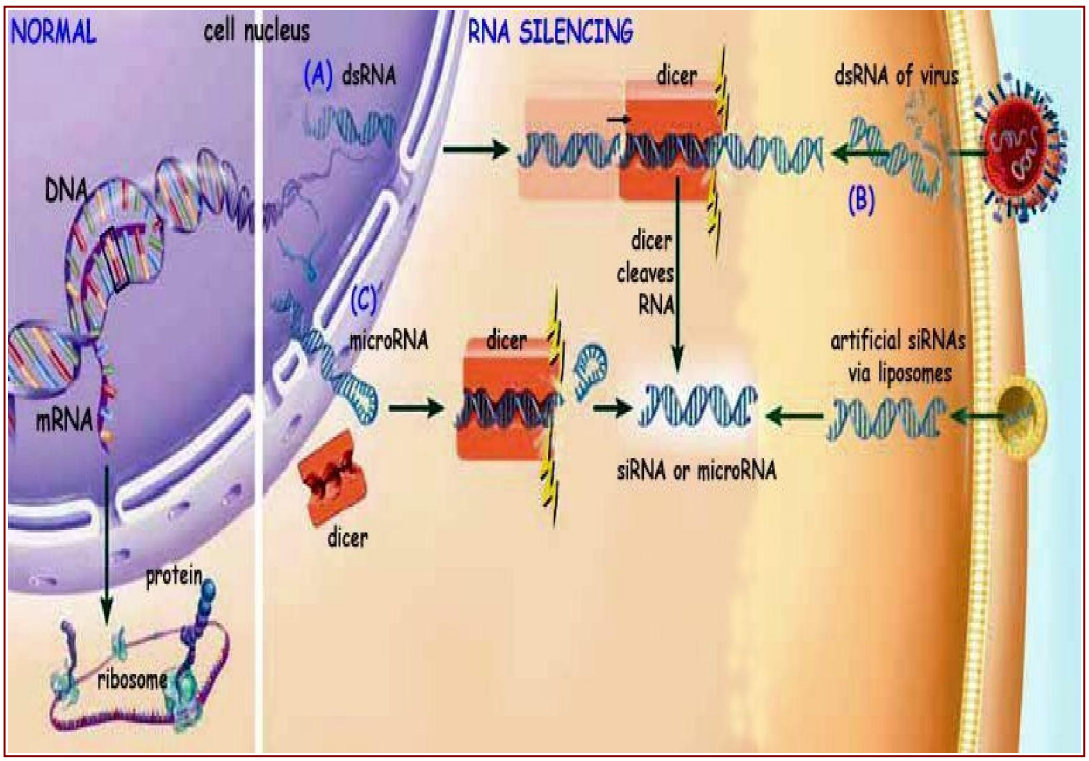


Figure 1
Viral Delivery of siRNA

Retroviral vectors have been designed to produce siRNA driven by either U6 or H1-RNA promoters for efficient, uniform delivery and immediate selection of stable

knockdown in cells. Retroviral systems are effective in most cell lines including primary cells [23].

Adenovirus vectors have been demonstrated to mediate gene silencing in an in vitro lung model [24] and to induce RNAi in a range of animal tissues [25].

Lentiviral vectors portend a promising system to deliver antiviral genes for treating viral infections such as HIV-1 as they are capable of stably transducing both dividing and nondividing cells. Since siRNAs are small nucleic acid reagents, they are unlikely to elicit an immune response and genes encoding these siRNAs can be easily manipulated and delivered by lentiviral vectors to target cells. As such, lentiviral vectors expressing siRNAs represent a potential therapeutic approach for the treatment of viral infections such as HIV-1. This review will focus on the development of lentiviral based delivery, and the potential therapeutic use of si RNAs in treating viral infections [26]. Viral based delivery has several disadvantages, however. Immune response to viruses not only impedes gene delivery but can cause severe complications for the patient. Recent well-documented cases, such as the death of Jesse Gelsinger due to complications associated with an adenoviral vector, highlight this problem [27]. Some viral vectors, e.g., lentivirus, may insert their genome at a seemingly random location in the host chromosome, thereby disturbing gene function [28].

MECHANISM OF ACTION OF SI RNA

In pathogenic cells and viruses

Long double-stranded RNAs (dsRNAs; typically >200 nt) can be used to silence the expression of target genes in a variety of organisms and cell types (e.g., worms, fruit flies, and plants).After administration the long dsRNAs enter a cellular pathway that is commonly referred to as the RNA interference (RNAi) pathway. First, the dsRNAs get processed into 20-25 nucleotide (nt) small interfering RNAs (siRNAs) by an RNase III-like enzyme called Dicer (initiation step). Then, the siRNAs assemble into (RISCs), the endoribonuclease-containing complexes known as RNA-induced silencing complexes unwinding in the process. The siRNA strands subsequently guide the RISCs to complementary RNA molecules, where they cleave and destroy the cognate RNA (effecter step). Cleavage of cognate RNA takes place near the middle of the region bound

by the siRNA strand. In mammalian cells, introduction of long dsRNA (>30 nt) initiates a potent antiviral response, exemplified by nonspecific inhibition of protein synthesis and RNA degradation. The mammalian antiviral response can be bypassed, however, by the introduction or expression of siRNAs.

In Viruses

RNAi is an evolutionarily conserved process observed in the majority of eurkaryotes studied. The functional unit of the RNAi pathway is small interfering RNA or siRNA, which can be specifically synthesized and introduced into a cell to induce gene silencing. It differs from other silencing technologies such as antisense oligonucleotides. In nature, RNAi is initiated when the cell encounters ectopic double stranded RNA (dsRNA), e.g., viral RNA, transposon or microRNA (miRNA). In the cytoplasm the RNase III-like protein dicer cleaves dsRNA from miRNAs or replicating viruses into siRNAs of 19−25 bases in length. The siRNA is then incorporated into the multiprotein RNA-induced silencing complex (RISC), which unwinds the duplex producing two strands; one strand (passenger) is discarded while the other can independently guide targeted mRNA recognition. The binding of siRNA results in a site-specific cleavage of the mRNA thereby silencing the message. The released cleavage products are degraded, and the siRNA:RISC complex is free to find another mRNA target in viral cell. Degrading mRNA results in a profound reduction in the levels of the corresponding protein. RNAi is therefore a highly promising therapeutic approach for diseases where aberrant protein production is a problem.

The advantages of siRNA over other gene/antisense therapies are a robust, potent, specific inhibition, a site of action for siRNAs in the cytoplasm and the diminished risk of toxic effects e.g., immune responses. It is important to note however that siRNA, and endogenously expressed hairpin siRNA, greater than 30 bp can induce a nonspecific innate immune response involving the activation of interferon [2, 8]. However, siRNA is more sensitive to degradation than DNA and may not be completely encapsulated by cationic agents or may form complexes that are too large for efficient gene silencing [3].

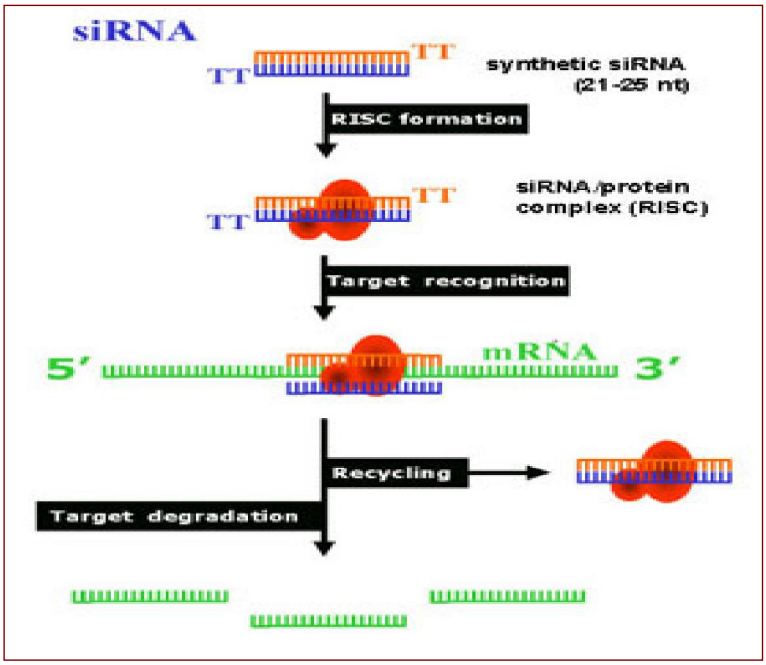


Figure 2
Mechanism of action of siRNA

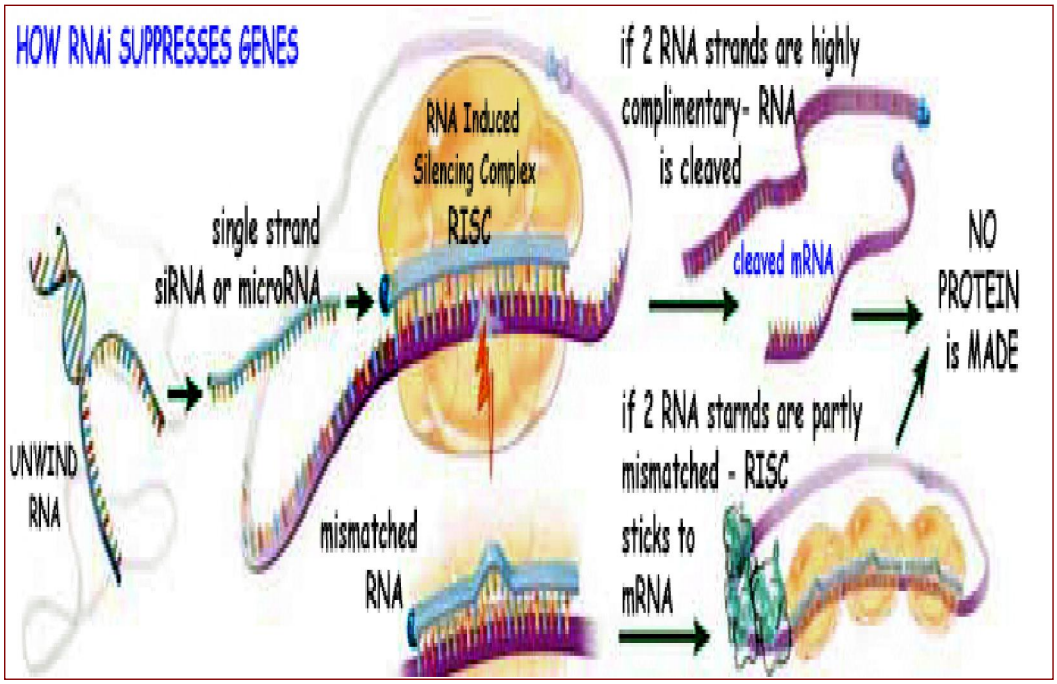


Figure 3
Diagrammatic Mechanism of siRNA

In Cancer cells

Small interfering RNAs (siRNAs) are expected to have a medical application in human as well as animal therapy as drugs with a high specificity for their molecular target mRNAs. RecQL1 DNA helicase in the human RecQ helicase family participates in DNA repair and recombination pathways in the cell cycle of replication. Silencing the RecQL1 expression by RecQL1-siRNA induces mitotic death in vitro specifically in growing cancer cells. By contrast, the same RecQL1 silencing does not affect the growth of normal cells, emphasizing that RecQL1 helicase is an ideal molecular target for cancer therapy. The local and systemic administration of RecQL1-siRNA mixed with polyethyleneimine polymer or cationic liposomes prevented cancer cell proliferation in vivo in mouse models of cancer without noticeable adverse effects [29]. The results indicate that RecQL1-siRNA in a complex with a cationic polymer is a very promising anticancer drug candidate and that in particular, RecQL1-siRNA formulated with a cationic liposome has an enormous potential to be used by intravenous injection for therapy specific for liver cancers, including metastasized cancers from the colon and pancreas.

TRANSFECTION

The transient or stable introduction of exogenous molecules and genetic material like DNA or RNA, into cultured mammalian cells, the process called transfection. It is commonly utilized in biological laboratories for studying gene function, modulation of gene expression, biochemical mapping, mutational analysis, and protein production. Researchers use various carrier molecules to enable non-viral gene delivery of plasmid DNA (pDNA), messenger RNA (mRNA), short interfering RNA (siRNA), and microRNA (miRNA) into cancer cell lines and primary cells.

In Vivo siRNA Transfection

In animals and plants, RNA interference is part of an immune response to viruses or injection of foreign genetic materials into the cellular structure. Because of the robust and selective effect RNAi has on gene expression, it becomes an invaluable research tool.RNAi has been used for in vivo target validation studies using animal models. Therefore, successful, potent RNAi experimentation is dependent upon the highly

efficient delivery of the siRNA into cells by transfection of stable and functional siRNA molecules in combination with transfection reagents which are developed and optimized specifically for use with siRNA transfection and allow transfection in the presence of serum without lowering transfection efficiency or reducing cell viability and cytotoxicity. The major challenge in performing RNAi studies in vivo is the effective, directed delivery of functional siRNA, shRNA, and miRNA molecules into specific tissues. ALTOGEN® in vivo transfection reagents could be conjugated with siRNA and administered intratumorally (i/t) or systemically via intravenous (i/v) tail vein injection in order to provide directed gene silencing in specific tissues, including liver, pancreas, kidney, and tumors. Selective knockdown could be seen as early as 24 hours after injection.

PHARMACOKINETIC ASPECTS OF SI RNA

Small interfering RNA (siRNA) has excellent pharmacological features and is expected to be used for therapeutic drug development, however, the pharmacokinetic study of new RNA technology needs to be established so that extremely small amounts (less than 1 pmol) of siRNA can be detected in organs of experimental animals and in human blood.

Systemic administration, absorption, biodistribution and excretion of si RNA

Systemic administration of siRNA complex to different experimental animals have be examined by different scientists by different routes of administration and their protocols along with results found in scientific literature, some of them also studied the absorbtion, biodistribution and excretion of the administered si RNA molecules alone or in complex form.

As study conducted by Abe *et al* [30] in which the RecQL1–siRNA/LIC-101 complex was injected intravenously into mice at the dose rate of (50 μg siRNA/mouse). The LIC-101 liposome consists of 2-O-(2-diethylaminoethyl)-carbamoyl-1, 3-O-dioleylglycerol and egg phosphatidylcholine for detection of siRNA administered to cells and animals by using a fluorescence intensity distribution analysis polarization system. si RNA can also administered direct into the tumorous tissues as administered si RNA by intratumor route and studied the systemic administration of optimized aptamer-siRNA

chimeras promotes regression of PSMA-expressing tumors [31]. In another study, RNAi therapy was examined for efficient regulation of viral replication by siRNA in a non-human primate surrogate model for hepatitis C virus. Yokotaa *et al* [32] performed administration into marmosets of cationic liposome-encapsulated siRNA (CL-siRNA) for GB virus B (GBV-B), which is most closely related to hepatitis C virus, repressed GBV-B replication in a dose-dependent manner. They administered CL-siRNA at dose rate of 5 mg/kg completely inhibited the viral replication. These results suggested the feasibility of systemic administration of CL-siRNA as an antiviral strategy.

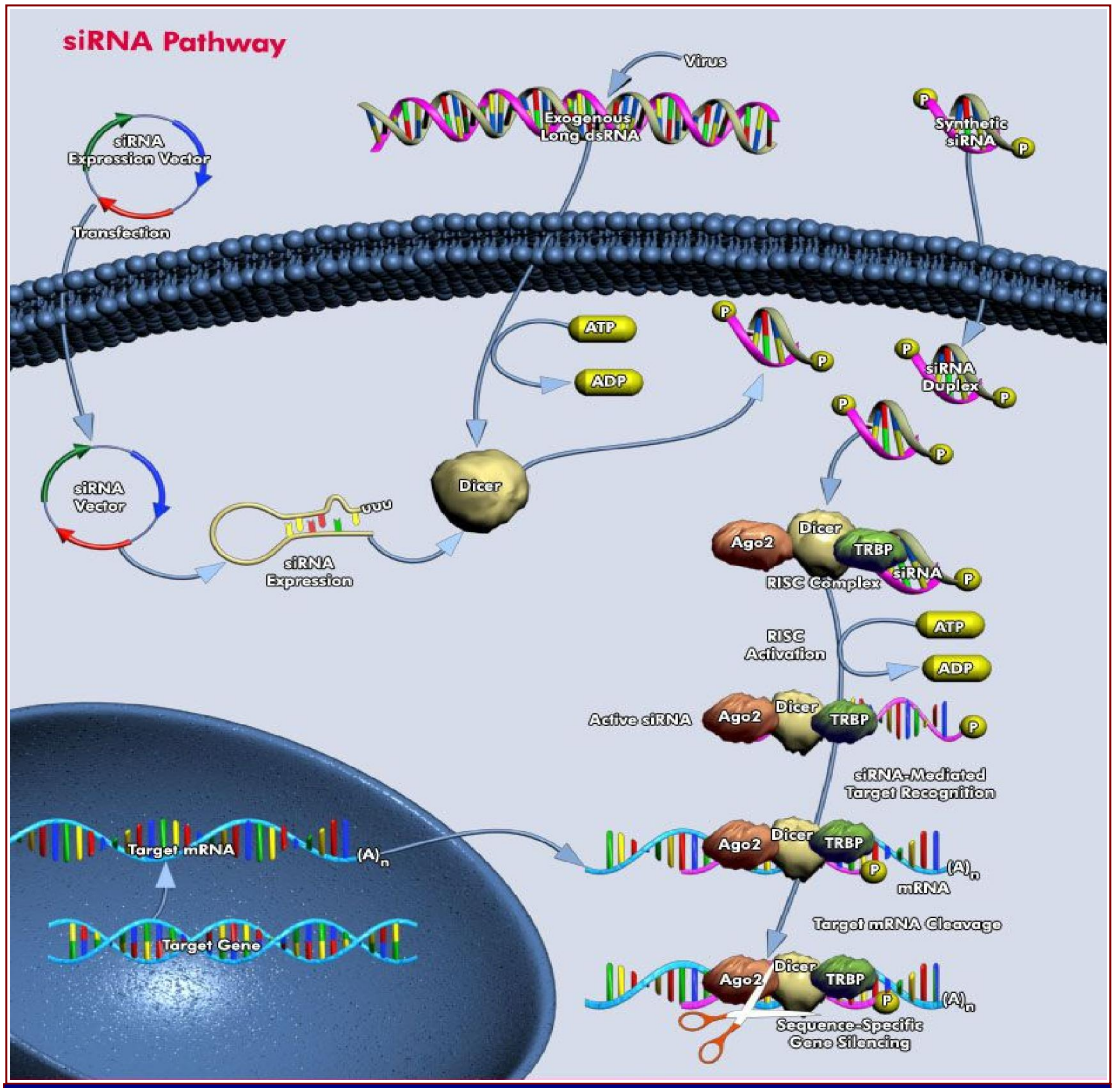


FIG. 4 SIRNA PATHWAY AND TRANSFECTION

si RNA administered by intranasal route successfully in experimental animal model like mouse and rats, also tested with various transfection reagents like Dharmafect 1,Fugene 6, Lipofectamin etc. along with different cell types as NCL HI299, A 549 etc. for treatment of many respiratory disorders [3]. The same experiment in primates also reported its therapeutic regimen against SARS coronavirus in Rhesus macaque [33].

After administration of complexed siRNAs, marked differences in the pharmacokinetics and biodistribution of the complexes are observed, the further absorption, distribution and elimination of various siRNA complexes being mainly dependent on the degree of uptake in liver, spleen, lung and kidney. The experimental research conducted for the administration in liver parenchymal cell (PC)-selective delivery of siRNA, siRNA was complexed with galactosylated cationic liposomes [34]. Galactosylated liposomes/siRNA complex exhibited a higher stability than naked siRNA in plasma. After intravenous administration of a galactosylated liposomes/siRNA complex, the siRNA did not undergo nuclease digestion and urinary excretion and was delivered efficiently to the liver and was detected in PC rather than liver non-parenchymal cells (NPC). Endogenous gene (Ubc13 gene) expression in the liver was inhibited by 80% when Ubc13-siRNA complexed with galactosylated liposomes was administered to mice at dose rate of 0.29 nmol/g. In contrast, the bare cationic liposomes did not induce any silencing effect on Ubc13 gene expression. These results indicated that galactosylated liposomes/siRNA complex could induce gene silencing of endogenous hepatic gene expression. The interferon responses by galactosylated liposomes/siRNA complex were controlled by optimization of the sequence of siRNA. Also no liver toxicity due to galactosylated liposomes/siRNA complex was observed under any of the conditions tested. In conclusion, they demonstrated the hepatocyte-selective gene silencing by galactosylated liposomes following intravenous administration.

In case of (Polyethylenimines)PEI(–PEG)-based siRNA complexes the induction of erythrocyte aggregation and hemorrhage is dependent on the degree and pattern of PEGylation as well as on the PEI/siRNA (N/P) ratio, and represents one important effect in the lung. Thereafter, siRNA uptake in liver and spleen, but not in lung or kidney, is

mediated by macrophage and is dependent on macrophage activity. In the kidney PEI (–PEG)/siRNA uptake is mostly passive and reflects the total stability of the complexes. Thus Liver, lung, spleen and kidney are the major players determining the in vivo biodistribution of PEI (–PEG)/siRNA complexes. Beyond their physicochemical and in vitro bioactivity characteristics, PEI (–PEG)/siRNA complexes show marked differences in vivo which can be explained by distinct effects in different tissues [35].

^{64}Cu Chloride Labeling used as the mixture of DOTA: ^{64Cu} chloride ratio of 250:1 to calculate the biodistribution kinetics of the si RNA molecules in mouse [12]. When the DOTA was conjugated to a siRNA molecule and labeled with ^{64}Cu (^{64}Cu-DOTA-siRNA), the observed biodistribution kinetics were characterized by rapid blood clearance through liver accumulation (≈23% ID/cm^3 at 60 min) and kidney filtration into the bladder (≈73% ID/cm^3 at 60 min). The total siRNA administered per mouse was 2.5 mg/kg, and ≈50% of this siRNA was ^{64}Cu-DOTA-siRNA (purified by ethanol precipitation). Micro-PET/CT was used to examine the kinetics of the biodistribution and tumor localization of siRNA nanoparticles after i.v. injection in mice in which they used a three-compartment model to investigate the impact of tumor-specific targeting on tumor accumulation and investigated the total dose of siRNA within the nanoparticles was the same as that used for naked siRNA (2.5 mg/kg), and again ≈50% of the total siRNA was DOTA-siRNA [12]. The biodistribution of the ^{64}Cu-DOTA-siRNA packaged into Tf-targeted nanoparticles appeared similar to that observed for naked ^{64}Cu-DOTA-siRNA, except that there was slightly higher liver accumulation (≈26% ID/cm^3 at 60 min) and a delayed peak in kidney accumulation (≈10 min after injection). Both naked siRNA and siRNA packaged into the carriers exhibited rapid blood clearance with tissue distribution mainly to the kidneys and liver within the first 15 min after injection. The plasma concentration ^{64}Cu-DOTA-siRNA of recorded an initial elimination half-life of 2.4 min and a terminal elimination half-life of 61.9 min. The rapid initial elimination half-life is expected for siRNA molecules whose small size (≈13 kDa) allows first-pass renal clearance in the mouse, these results, calculated [12, 36, 37, 38].

The pharmacokinetics of siRNA using ELISA-based quantification assay method and resulted that, the coefficient of variation (CV) of the ELISA quantification was 9.4%

for intra-assay and 12.1% for inter-assay. The assay was specific for double-stranded siRNAs. The intensity of the detected signal was reduced to background levels in the presence of single-stranded RNA. The ELISA-based assay revealed that the levels of methylated forms of siRNAs after transfection into A549 and HeLa cells were significantly higher than those of unmethylated siRNA forms [39].

Applying this assay to a study of the pharmacokinetic profiles of intravenously administered siRNAs, it is found that the higher blood concentrations were achieved using the methylated form of siRNAs than unmethylated form. Moreover, methylated siRNAs complexed to DOTAP-based cationic liposomes showed significantly higher and prolonged blood concentration–time profile, with 2.2-fold lower clearance rate (0.11 ± 0.02 ml/min) as compared to the uncomplexed form.

Extraction of siRNA from cultured cells, mouse blood and organs

The siRNAs transfected in vitro to cells can be extracted from cells after 6, 12, 24, 48, 72 and 96 h as described [40]. The siRNAs administered in vivo to mice by intravenous injection were extracted from blood after 30 min and at 1, 3, 6, 12 and 24 h [29]. The siRNAs delivered to mouse organs can be extracted, in a form of total RNA, from excised and ground organs 30 min after the intravenous injection of siRNA complexes. Extraction was done by using phenol and chloroform, and the extracted total RNA was purified by using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer.

Detection of siRNA administered to Cell and Animals

Small interfering RNA (siRNA) has excellent pharmacological features and is expected to be used for therapeutic drug development. Therefore, identification of si RNA administered to animals and cells is an essential tool to investigate the therapeutic role of si RNA. However, new RNA technologies have been established so that extremely small amounts (less than 1 pmol) of siRNA can be detected in organs of experimental animals and in human blood to facilitate pharmacokinetics studies. The siRNA, administered to cells and animals by using a fluorescence intensity distribution analysis polarization system [30]. This method uses high-power confocal microscopic analysis of fluorescence polarization in DNA probes that are bound to one of the strands

of siRNA and directly quantitates the copy number of siRNA molecule after extraction from specimens. A pharmacokinetic study to examine the blood retention time of siRNA/cationic liposomes in mice showed that this straightforward method is consistent with the other reverse transcriptase polymerase chain reaction amplification-based method and found that the entire process is simple and applicable for a high-throughput analysis, which provides excellent technical support for fundamental research on RNA interference and development of siRNA drugs. Similarly, a new method invented to amplify siRNA of 21-mers containing 3′-TdT to DNA after 3′-terminal dG-tailing by using terminal deoxytransferase and reverse transcriptase polymerase chain reaction (RT–PCR) with a primer containing oligo-dC, referred to as the TdT/RT–PCR method [29]. However, it can quantitate only siRNA having a 3′-terminal deoxynucleotidyl TdT sequence. Another method fluorescence-labeled siRNA and polyacrylamide gel electrophoresis can also be measured the retention time of siRNA stabilized with cationic polymers in the circulating blood of mice by this method but the method has several drawbacks it can not measure non-fluorescent (or non-radioactive) siRNA, such as unmodified siRNA in its natural form and technically tedious to run the gel electrophoresis. It also fails to obtain accurate estimates of the amount of siRNA and cannot handle many samples [34].

ADVANTAGES OF SI RNA THERAPY

siRNA therapeutics offering new types of evolutionary medicines in treatment of various diseases. siRNA is as a drug promises and has several advantages over traditional drugs like,

1] It has very high target selectivity.

2] Extremely effective at a low dose (nanomolar or subnanomolar concentrations)

3] It has very low toxicity due to metabolism to natural nucleotide components.

4] Excellent specificity of inhibition.

5] Very good potency.

6] The small size of the molecules and the diminished risk of toxic effects, e.g., immune responses.

7] Also effective against many untreatable systemic diseases like cancer.

8] It has many antiviral applications.

9] It has effective immunomodulatory effects.

10] siRNA therapeutics shows excellent procedure for cellular gene knockout experiments. Etc

DISADVANTAGES OF SI RNA THERAPY

Development of siRNA therapeutics, however, has been affected by several issues as, poor intracellular uptake due to the intrinsic negatively charged ~13K molecular weight structure and limited stability in circulating blood. Absence of convenient methods, to detect small amounts of siRNA in its natural form to monitor the pharmacokinetics in cells, circulating blood and organs. A small amount of siRNA present in the circulating blood is difficult to quantitate by conventional column chromatographic procedures due to limited ultraviolet absorption of the administered siRNA.

APPLICATIONS

Small interfering RNAs (siRNAs) are expected to have an excellent therapeutic application in human as well as in animal's therapy as drugs with a high specificity for their molecular target mRNAs. This therapy has wide range of successful therapeutic applications while some are still under the clinical trials. Though the continuous ongoing researches and experiments, the efficacy of siRNA therapy has been already recorded by the leading scientists, among which following are the successful achievements.

Anticancer activity

The expression of a large number of genes is known to be altered in cancer, and modifying the expression of these genes is an attractive method of cancer treatment. Therefore, siRNA therapies are currently being studied as potential cancer treatments.

The anticancer activity of RecQL1 helicase siRNA studied in mouse xenograft models [29]. They show that RecQL1 DNA helicase in the human RecQ helicase family participates in DNA repair and recombination pathways in the cell cycle of replication. Silencing the RecQL1 expression by RecQL1-siRNA induces mitotic death in vitro specifically in growing cancer cells. By contrast, the same RecQL1 silencing does not affect the growth of normal cells and resulted that RecQL1-siRNA in a complex with a

cationic polymer is a very promising anticancer drug candidate, and that in particular, RecQL1-siRNA formulated with a cationic liposome has an enormous potential to be used by intravenous injection for therapy specific for liver cancers, including metastasized cancers from the colon and pancreas. The effect of siRNA mediated gene silencing of C-erbB-2 was investigated [17]. This gene is overexpressed in some cancers such as breast, ovarian and lung cancer. Its expression is related to enhanced malignancy and metastatic ability, intrinsic chemoresistance and poor prognosis of tumors. Specific siRNA knockdown of C-erbB-2 in Calu-3 cells was found to effectively inhibit C-erbB-2 expression and cell proliferation. It also enhanced Calu-3 cell apoptosis thereby reducing the overall number of transformed cells.

In a similar study, siRNA directed against mutant K-ras and determined the antitumor effects of decreasing the levels of this protein in lung cancer cell lines. Results revealed that adenovirus-mediated siRNA can specifically target ras and may be a potential therapeutic to treat human lung cancer [41]. Fibronectin induces cell proliferation and inhibits apoptosis in the human bronchial epithelial cells BEAS-2B and 16-HBE were determined [21]. These pro-oncogenic effects are mediated by PI3-kinase and NF-κB and can be blocked by the administration of an anti p65 siRNA leading to an antioncogenic effect.

STAT3 siRNA effectively inhibits STAT3 gene expression in Hep2 cells leading to growth suppression and induction of apoptosis in Hep2 cells in larynge was demonstrated [42]. The use of siRNA technique may provide a novel therapeutic approach to treat laryngeal cancer and other malignant tumors expressing constitutively activated STAT3.

Anti viral applications

The unmodified siRNAs specific to influenza viral proteins to protect mice from lethal infection with influenza virus was used. Influenza virus was coadministered with siRNA, complexed with the cationic lipid Oligofectamine, by direct intranasal infusion in mice. Results show that viral titers in lung tissue were reduced as much as 63-fold using anti-influenza siRNA, compared to controls, with an improved survival [43].

Detection of RNA molecules occurs during viral infection and triggers antiviral innate defense mechanisms including the induction of type I interferons (IFN-alpha, IFN-beta) and down regulation of gene expression [44]. In another study a cell-based assay was developed to screen small interference RNA (siRNA) to block the expression of two genes of the severe acute respiratory syndrome (SARS) virus. These siRNA molecules could be used to examine the function of these genes in SARS virus replication and assembly and could potentially be developed into therapeutic agents for the treatment of patients with SARS [45].

The topical antiviral siRNA as microbicide for preventing or treating sexually transmitted diseases was demonstrated [46]. There are many viral diseases in which potency and efficacy of siRNA are very high and still under the clinical trails and research.

Immunostimulatory applications

In addition to the gene-silencing activity of siRNA, a number of recent studies have pointed to immunological effects of siRNAs, including the induction of proinflammatory cytokines. Immunorecognition of RNA depends on certain molecular features such as length, double- versus single-strand configuration, sequence motifs, and nucleoside modifications such as triphosphate residues. RNA-sensing immunoreceptors include three members of the Toll-like receptor (TLR) family (TLR3, TLR7, TLR8) and cytosolic RNA-binding proteins like PKR and the helicases RIG-I and Mda5 [44].

siRNA for the treatment of liver diseases

Zamore and Aronin [47] found siRNAs knock down hepatitis, as shut down the specific gene activities on an animal model of hepatitis. Mice infused with an siRNA against a cell death receptor recover liver function after experimentally induced injury. The Potential applications of siRNA in hepatitis C virus therapy, the Hepatitis C virus (HCV) genome is a single-stranded RNA that functions both as a messenger RNA and as a viral replication template, destruction of HCV RNA could eliminate not only virally directed protein synthesis, but also viral replication. It has been demonstrated that siRNAs interfere with HCV gene expression and replication, and this review will describe the use of RNAi as a tool to inhibit HCV gene expression [48].

siRNA for the treatment of diseases of the nervous system

Viral as well as nonviral delivery methods have been effectively tested in vivo for silencing of molecular targets and have resulted in significant efficacy in animal models of Alzheimer's disease, amyotrophic lateral sclerosis (ALS), anxiety, depression, encephalitis, glioblastoma, Huntington's disease, neuropathic pain, and spinocerebellar ataxia [49]. But the challenges for the application of this technology to neurological disease will be to identify appropriate disease targets, and to optimize the function, and particularly delivery of these RNA-based therapeutic molecules within the complex environment of the nervous system. In addition to this another studies of different new applications are under the experimental and clinical trials.

CONCLUSION

Si RNA therapeutics opens up an exciting new approach for the treatment of many disease conditions. Molecular biologists also working in the area of molecular pharmaceutics, to ensure that this technology reaches its full potential. The excellent biological activity of siRNA has also been tested for therapeutic drugs. siRNA as a drug promises several advantages over traditional drugs, offering new types of medicines that have a very high target selectivity and that are effective at a low dose as nanomolar or subnanomolar concentrations, with low toxicity due to metabolism to natural nucleotide components. Therefore, it can be concluded that the treatment of incurable diseases by the natural nucleotide components is the next sight of the world of therapeutics.

REFERENCES

1. Novina C D and Sharp PA: The RNAi revolution. *Nature* 2004; 30(6996):161-4.
2. Gary D, Puri JN and Won YY: Polymer-based siRNA delivery: perspectives on the fundamental and phenomenological distinctions from polymer-based DNA delivery. *J. Controlled Release* 2007; 121 (1−2): 64 -73.
3. Durcan N, Charlotte M and Cryan SA: Inhalable siRNA: Potential as a Therapeutic Agent in the Lungs. *Mol. Pharmaceutics* 2008; 5 (4): 559–566.
4. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K and Tuschl T: Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001; 411: 494–498.

5. Katoh T, Susa M, Suzuki T, Umeda N, Watanabe K and Suzuki T: Simple and rapid synthesis of siRNA derived from in vitro transcribed shRNA, *Nucleic Acids Res Suppl* 2003; 0: 249-50.
6. Zhu X., Li T, Dang Y, Feng Y and Huang P: A novel in vitro transcription method for producing siRNAs without specific sequence requirements. *Molecular Biotechnology* 2005; 31(3): 187-192.
7. Daniela C and Rossi JJ: Construction and Transfection of PCR Products Expressing siRNAs or shRNAs in Mammalian Cells. *Methods in Molecular Biology* 2002; 252 (2): 509-514.
8. Sioud, M: RNA interference and innate immunity. *Adv. Drug Delivery Rev.* 2007; 59(2−3): 153 -63.
9. Overhoff M, Wunsche W and Sczakiel G: Quantitative detection of siRNA and single-stranded oligonucleotides: relationship between uptake and biological activity of siRNA. *Nucleic Acids Res.* 2004; 32: 170.
10. Lingor P, Michel U, Scholl U, Bahr M and Kugler S: Transfection of 'naked' siRNA results in endosomal uptake and metabolic impairment in cultured neurons. *Biochem. Biophys. Res. Commun*. 2004; 315: 1126–1133.
11. Hu-Lieskovan S, Heidel JD, Bartlett DW, Davis ME and Triche TJ: Sequence-specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of Ewing's sarcoma. *Cancer Res.* 2005; 65: 8984–8992.
12. Derek WB, Helen Su, Isabel J, Hildebrandt, Wolfgang A, Weber and Davis ME: Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging. *PNAS* 2007; 104 (39): 15549-15554.
13. Buttgereit PS, Weineck G, Ropke A, Marten K, Brand T, Heinicke WH, Caselmann D, Huhn and Schmidt-Wolf IG: Efficient gene transfer into lymphoma cells using adenoviral vectors combined with lipofection. *Cancer Gene Ther*. 2000; 7 (8):1145-1155.

14. Yin, JQ, Gao J, Shao R, Tian WN, Wang J and Wan Y: siRNA agents inhibit oncogene expression and attenuate human tumor cell growth. *J. Exp. Ther. Oncol.* 2003; 3 (4):194- 204.
15. Uematsu K, He B, You L, Xu Z, McCormick F and Jablons DM: Activation of the Wnt pathway in non small cell lung cancer: evidence of dishevelled overexpression. *Oncogene* 2003; 22(46): 7218-7221.
16. Shieh YS, Lai CY, Kao YR, Shiah SG, Chu YW, Lee HS and Wu CW: Expression of axl in lung adenocarcinoma and correlation with tumor progression. *Neoplasia 2005*; 7(12):1058-1064.
17. Ren SH, Wang JW and Zhang L: Effects of Her-2/neu siRNA-mediated gene silencing on cell cycle and apoptosis of lung adenocarcinoma cells. *Zhonghua Yi Xue Za Zhi* 2005; 85 (22): 1530-4.
18. Miyawaki-Shimizu KD, Predescu, Shimizu J, Broman M, Predescu S and Malik AB: siRNA-induced caveolin-1 knockdown in mice increases lung vascular permeability via the junctional pathway. *Am. J. Physiol.: Lung Cell. Mol. Physiol.* 2006; 290 **(**2): L405 –L413.
19. Aggarwal S, Kim SW, Cheon K, Tabassam FH, Yoon JH and Koo JS: Nonclassical action of retinoic acid on the activation of the cAMP response element-binding protein in normal human bronchial epithelial cells. *Mol. Biol. Cell* 2006; 17 (2): 566 -575.
20. Amaar YG, Minera MG, Hatran LK, Strong DD, Mohan S and Reeves ME: Ras association domain family 1C protein stimulates human lung cancer cell proliferation. *Am. J. Physiol.: Lung Cell. Mol. Physiol.* 2006; 291 (6): 1185 -1190.
21. Han SW and Roman J: Fibronectin induces cell proliferation and inhibits apoptosis in human bronchial epithelial cells: pro-oncogenic effects mediated by PI3-kinase and NF-kappa B, *Oncogene* 2006; 25 (31):4341-9.
22. Demeneix B and Behr JP: Polyethylenimine (PEI). *Adv. Genet.* 2005; 53: 215- 230.
23. Zentilin L and Giacca M: In vivo transfer and expression of genes coding for short interfering RNAs. *Curr. Pharm. Biotechnol,* 2004; **5** (4): 341- 7.

24. Shen C, Buck AK, Liu X, Winkler M and Reske SN: Gene silencing by adenovirus-delivered siRNA. *FEBS Lett.*, 2003;539 (1−3): 111- 4.
25. Narvaiza I, Aparicio O, Vera M, Razquin N, Bortolanza S, Prieto J and Fortes P: Effect of adenovirus-mediated RNA interference on endogenous microRNAs in a mouse model of multidrug resistance protein 2 gene silencing. *J. Virol.* 2006;80 (24): 12236 -47.
26. Morris KV and Rossi JJ: Lentiviral-mediated delivery of siRNAs for antiviral therapy. *Gene Ther***.** 2006;13(6): 553-8.
27. Teichler ZD: US gene therapy in crisis. *Trends Genet,* 2000; 16(6): 272 -275
28. Musiyenko A, Bitko V and Barik S: RNAi-dependent and -independent antiviral phenotypes of chromosomally integrated shRNA clones: role of VASP in respiratory syncytial virus growth, *J. Mol. Med.* 2007; 85 (7): 745- 52.
29. Futami K, Kumagai E, Makino H, Sato A, Takagi M, Shimamoto A and Furuichi Y: Anticancer activity of RecQL1 helicase siRNA in mouse xenograft models. *Cancer Sci*. 2008; 99:1227–1236.
30. Abe T, Goda K, Futami K and Furuichi Y: Detection of siRNA administered to cells and animals by using a fluorescence intensity distribution analysis polarization system. *Nucleic Acids Research* 2009; 37**(**7): 56.
31. Dassie JP, Liu X, Thomas GS, Whitaker RM, Thiel KW, Stockdale KR, Meyerholz DK, McCaffrey AP, McNamara JSO and Giangrande PH: Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors. *Nature Biotechnology* 2009; 27:839 - 846.
32. Yokotaa TI, Iijimab S, Kuboderaa T, Katakaie CKY, Ageyamab N, Chena Y, Leeb YJ, Unnoa T, Nishinaa K, Iwasakib Y, Makif N, Mizusawaa H, and Akarib H: Efficient regulation of viral replication by siRNA in a non-human primate surrogate model for hepatitis C. *Biochemical and Biophysical Research Communications* 2007; 361(2): 294-300.
33. Li B, Tang JQ, Cheng D, Qin C, Xie FY, Wei Q, Xu J, Liu Y, Zheng BJ, Woodle MC, Zhong N and Lu PY: Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. *Nat. Med.* 2005; 11 (9): 944- 51.

34. Sato A, Takagi M, Shimamoto A, Kawakami S and Hashida M: Small interfering RNA delivery to the liver by intravenous administration of galactosylated cationic liposomes in mice. *Biomaterials* 2007; 28(7):1434-42.
35. Malek A, Czubayko F and Aigner A: PEG grafting of polyethylenimine (PEI) exerts different effects on DNA transfection and siRNA-induced gene targeting efficacy, *Journal of Drug Targeting* 2008; 16 (2): 124-139.
36. Soutschek J, Akinc A, Bramlage B, Charisse K, Constien R, Donoghue M, Elbashir S, Geick A, Hadwiger P, Harborth J, John M and Venkitasamy: Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 2004; 432:173-178.
37. Braascha DA, Parooa Z, Constantinescub A, Renb G, Ozb OK, Masonb RP and Coreya DR: Biodistribution of phosphodiester and phosphorothioate siRNA. *Bioorganic and Medicinal Chemistry Letters* 2003; 14(5):1139-1143.
38. Liu N, Dingb H, Vanderheydena JL, Zhua Z and Zhang, Y: Radiolabeling small RNA with technetium-99m for visualizing cellular delivery and mouse biodistribution, *Nuclear Medicine and Biology* 2007; 34(4): 399-404.
39. Kim SH, Lee SH, Tian H, Chen X and Park TG: Prostate cancer cell–specific VEGF siRNA delivery system using cell targeting peptide conjugated polyplexes, *Journal of Drug Targeting* 2009; 17(4): 311-317.
40. Futami K, Kumagai E, Makino H, Goto H, Takagi M, Shimamoto A and Furuichi Y: Induction of mitotic cell death in cancer cells by small interference RNA suppressing the expression of RecQL1 helicase. *Cancer Science* 2007; 99(1): 71-80.
41. Zhang Z, Jiang G, Yang F and Wang J: Knockdown of Mutant K-ras Expression by Adenovirus-Mediated siRNA Inhibits the In Vitro and in Vivo Growth of Lung Cancer Cells. *Cancer Biol. Ther*. 2006; 5 (11): 1481 -6.
42. Gao LF, Xu DQ, Wen LJ, Zhang XY, Shao YT and Zhao XJ: Inhibition of STAT3 expression by siRNA suppresses growth and induces apoptosis in larynge. *Acta Pharmacol Sin.* 2005; 26: 377-83.

43. Tompkins SM, Lo CY, Tumpey TM and Epstein SL: Protection against lethal influenza virus challenge by RNA interference in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101 (23): 8682 -6.
44. Schlee M, Hornung V and Hartmann G: siRNA and isRNA: Two Edges of One Sword. *Molecular Therapy* 2006; 14: 463–470.
45. Meng BO, Lui YW, Meng S, Cao C and Hu Y: Identification of effective siRNA blocking the expression of SARS viral envelope E and RDRP genes, *Molecular Biotechnology* 2006; 33(2):141-148.
46. Rossi JJ: Topical antiviral siRNA: A practical siRNA microbicide? *Gene Therapy* 2006; 13:1493–1494.
47. Zamore PD and Aronin N: siRNAs knock down hepatitis. *Nature Medicine* 2003; 9: 266 - 267.
48. Smolic R, Volarevic M, Wu CH and Wu GY: Potential applications of siRNA in hepatitis C virus therapy. *Curr Opin Investig Drugs* 2006; 7(2):142-6.
49. Satya P, Malhotra M and Rengaswamy V: RNA Interference: From Biology to Clinical Applications. *Methods in Molecular Biology* 2010; 623: 211-229.

For Correspondence:
Nitesh Kumar
Email: niteshprof@gmail.com