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RNA interference and its therapeutic potential

Review Article

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Abstract: RNA interference is a technique that has become popular in the past few years. This is a biological method to detect the activity of a specific gene within a cell. RNAi is the introduction of homologous double stranded RNA to specifically target a gene's product resulting in null or hypomorphic phenotypes. This technique involves the degradation of specific mRNA by using small interfering RNA. Both microRNA (miRNA) and small interfering RNA (siRNA) are directly related to RNA interference. RNAi mechanism is being explored as a new technique for suppressing gene expression. It is an important issue in the treatment of various diseases. This review considers different aspects of RNAi technique including its history of discovery, molecular mechanism, gene expression study, advantages of this technique against previously used techniques, barrier associated with this technique, and its therapeutic application.

Keywords: *Cancer • MicroRNA • Interfering RNA • Disease*

1. Introduction

RNAi emerges as a new technique for gene regulation study at the present time. It shuts down the gene expression by degrading the mRNA at the post-transcriptional level with the help of a intricate network of the proteins (mainly dicer and RICS complex). Two types of small RNA molecules namely microRNA (miRNA) and small interfering RNA (siRNA) are central to RNA interference. MicroRNAs are post-transcriptional regulators (around 22 nucleotides) that bind to complementary sequences in the three prime untranslated regions of target messenger RNA (mRNA) and it usually results in gene silencing [5]. Small interfering RNA or short interfering RNA is a double-stranded RNA (dsRNA) molecule (20-25 nucleotides) that interferes with the expression of a specific gene. RNA interference is a cellular mechanism that naturally occurs in the cell, which plays an important role in development and maintenance of the genome. The siRNA is responsible for the successive degradation of the target mRNA in a homology dependent manner [74,22].

The discovery of the RNAi mechanism occurred in a successive way. The RNAi phenomenon was first reported by Napoli and Jorgensen in 1990 [59]. They found a surprising observation in petunias while trying to deepen the purple color of these flowers. They introduced a pigment-producing gene under the control of a powerful promoter and observed white color instead of deep purple. Jorgensen named the observed phenomenon "cosuppression", since the expression of both the introduced gene and the homologous endogenous gene was suppressed [53,59]. The suppression of the endogenous gene by the introduction of homologous RNA sequence was first noticed by Romano and Macino in *Neurospora crassa* in 1992 [58,59]. In animals, the first evidence that dsRNA could lead to gene silencing came from work in *Caenorhabditis elegans* [30]. They found gene silencing in their control set by using sense strand and it was quite an unusual result in comparison to the antisense technique [1,30,59]. In 1998, Fire and Mello recognized the fact that the dsRNA is responsible for endogenous gene silencing. According to their opinion the transgene was responsible for the production of the

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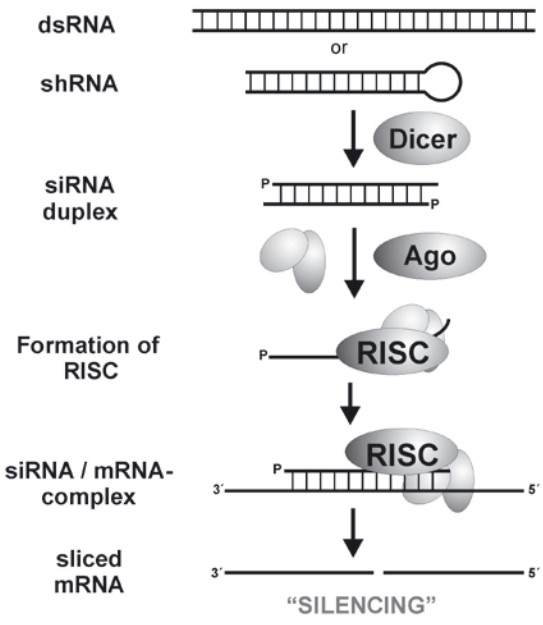
dsRNA in the case of plant and fungus whereas in worm there must be a contamination of the dsRNA with sense RNA [22,59]. Gene silencing has also been induced by shooting dsRNA into Drosophila embryos with a gene gun or by engineering flies to carry DNA containing an inverted repeat of the gene to be silenced [32]. At the end of 2005, it was reported that helicases played a major role in unwinding double stranded siRNAs and Ago2 has been proved to be responsible for cleaving the nonincorporated (passenger) strand of the siRNA duplex, allowing the other strand to be incorporated into RNA-induced silencing complex (RISC). Andrew Fire and Craig Mello got the Nobel Prize in 2006 in Physiology or Medicine for their discovery of RNA interference - gene silencing by double-stranded RNA [46].

There are some basic rules for designing an experiment of RNA interference. These include: length of siRNA targeted sequence should be 21 nucleotides, avoid intron, termination codon and regions within 50-100 bp of the start codon, GC content should be 30%-50%, avoid any nucleotide 4 or more in a row, avoid repeats and low complex sequence, avoid single nucleotide polymorphism (SNP) sites, avoid off-target effects on other sequence, designing negative control by scrambling targeted siRNA sequence, control and siRNA should be in same length and nucleotide composition. The siRNA transfection is a new method for introducing foreign genetic information by inducing RNA interference and it works by silencing key sequences on messenger RNA, which turns off specific genes by cleaving to them on the RNA strand. There are several methods for RNA transfection into the cell which includes chemical and biological reagents. The most widely used ways of siRNA preparation is chemical synthesis of siRNA, viral delivery, in vitro transcription, vectors, and expression cassettes. Low transfection efficiencies are the most frequent cause of unsuccessful knockdown. The optimal transfection condition on a given cell line is achieved by systematically testing each of several critical variables. There are many siRNA transfection reagents and protocols that are being supplied by different companies having different efficiency and variables. In general, the efficiency of siRNA transfection depends on two or more effective siRNA per target, efficient and reproducible delivery system, assay for evaluating siRNA effectiveness, and positive and negative siRNA controls. There are some ways of evaluating RNAi silencing efficiency in cells. One of the easiest methods is the cotransfection of two fluorescent probes and the efficiency of various shRNAs being directly monitored in vivo by fluorescence microscopy [82].

The siRNAs belong to a family of small RNA. They function as effector molecules in plant, fungi and animal

Figure 1. Mechanisms of RNA interference.



that triggers the sequence specific gene silencing or RNAi response in cells. Generally all eukaryotic organisms possess siRNA except budding yeast. The dsRNA is the precursor of the siRNA and it is formed when the RNA sequence is transcribed from a complementary DNA sequence. Sometimes viral infection is also responsible for the triggering of the RNAi response mediated by the cell as a part of their antiviral defense. During the replication of the viral genome, it produces RNA of both polarities that eventually enable the formation of dsRNA and triggers the RNAi response. The siRNA binds to its target (mRNA) in a sequence specific manner and cleaves the target mRNA in the middle of siRNA-mRNA molecule. The precursor double-stranded RNA for siRNA undergoes several rounds of processing before binding to the target mRNA. These include cutting of the dsRNA by dicer into small siRNA, binding of the siRNA with RISC complex, unwinding of the siRNA into a ssRNA, the attachment of the RICS complex associated antisense strand with the mRNA, and subsequent degradation of the target mRNA (Figure 1). After integration of the single ssRNA of the dsRNA into the RICS complex, argonaut-like proteins attach to it that in turn bind to the target sequence and lead to the destruction of the target mRNA. The function of all the argonaut proteins is yet to determine. But some of them are involved in the formation of the RICS complex. In Drosophila, Argonaute2 protein takes part in the formation of the RICS complex [33,57]. In plants, siRNA functions as an antiviral agent and it moves through the vascular system providing antiviral immunity to plants. The siRNA also

plays a vital role to keep the mobile genetic element (transposone) silent in plants and invertebrate [3,48,69]. RNAi technique has some advantages over previously known gene knockdown techniques since it is very specific in action. Moreover, very less amount of dsRNA is required to shut-off the effect of the targeted gene in this process and it has an effect far away from the introduction site [22].

The development of drugs by using siRNA has become a hot topic at the present time. The siRNA has enormous potentiality to make the particular gene silent that is responsible for the development of the disease though triggering the action of the siRNA at the particular site of the human body is the most problematic issue. Beside this, siRNA pinpoints a huge possibility to understand the underneath mechanism of disease development by silencing one or more target genes [6,31].

2. Barriers and advantages associated with siRNA therapies

Today, RNAi has become a promising technique in the field of life science as well as in the biomedical science to characterize and knock-down of gene or genes that are associated with the disease development. Cancer, AIDS, and hepatitis are diseases in which the effectiveness of RNAi has been already tested by scientists [15,74]. The use of RNAi against deadly diseases still remains in a state of trial. The main problem is associated with the delivery to the target. Gene therapy and antisense were unable to prove its effectiveness as a drug due to this problem [20].

In the beginning of the RNAi era, it was tested on invertebrate and plants, which revealed promising results but was found to be unsuccessful in human. The interferon activity was observed in the human cell after the insertion of the dsRNA, which leads to the global shout-down of genes and death of that cell down the track [20,49]. At that time scientists predicted that human had evolved interferon activity against the viral infection that was absent in lower animals. It then was discovered that dsRNA of shorter length (20-30 bp) was unable to induce interferon activity in the human cell though it leads to sequence specific degradation of the product (mRNA) of the targeted gene. As a result, RNAi remains as a back up system against viral diseases in humans [21]. There are generally two main techniques being followed regarding the delivery of the dsRNA into the human cell. Delivery of the dsRNA by using expression vector has an advantage over the introduction of dsRNA directly into the cell. This is because the effect of RNAi response is longer in the case of expression vector compared to the direct application of dsRNA into the cell [22,52,64,67,74]. Moreover, expression vector can infect both quiescent and actively growing and dividing cells. RNAi expression vectors are also ideal for transient, stable or regulated expression in cell lines that are easily transfected [13]. But this is not a good method for siRNA screening and it needs verification of insertion. Introducing synthetic siRNA direct into the cell can suppress target mRNA at the RNA-induced silencing complex (RISC) and effectiveness of the transfection reagent used is the key to success.

Therapies including interferon treatment, highly active antiretroviral treatment and chemotherapy have some unanticipated effects. No disturbance in cellular process has to be confirmed before using RNAi as a drug for human diseases. Generation of some residual interferon activity and off-target effect by degrading non-target mRNA are the main obstacles of RNAi approaches [20,38]. There is always a possibility of the non-specific effect after the use of RNAi mediated drug. Delivery of the siRNA into the body occurred in two different ways. All these events show two-fold non-specific effect on the cell system, which include interferon and immune response. There is still a conflict regarding the interferon activity in response to RNAi drug [35,40,45]. Different types of neurological disorders and cancer might occur due to the saturation of RNAi machinery [12,29]. The miRNA plays an important role in the regulation of many critical diseases though both over and less expression of miRNA leads to the development of cancerous cells. It is also evident that excess concentration of the external supply of siRNA influences the maturation path of the miRNA. It implies that control over the concentration of therapeutic dose of both siRNA and the siRNA should be crucial to obtain benefit from the siRNA therapy [36,37].

The use of RNAi as a screening tool opens a new horizon to identify gene or genes that are involved with disease development and help to find new therapeutic target. Two different types of screening methods are used in this issue. One is high throughput screening, where a specific gene is knocked-down at a particular time. This process is very laborious and time consuming. But in some cases, it has been used when two or more genes were involved in a particular process. In another method, large pools of RNAi viral vectors are introduced into the cell producing selective pressure isolation of the survival cell. It shows somewhat different behavior and survives in respect to the other tested cells [4,11,20,66]. Sequencing of those expression vectors reveals information of the desired genes. Genome scale wide screening is being used in case of many diseases like cancer, diabetes, and neurodegenerative disease [8].

Table 1. Inhibition of viral gene expression by RNAi-mediated technology.

Virus	Model system	Vehicle	Treatment type
Hepatitis C Virus	Reporter transcript	None	Co-transfection
Coxsakievirus B3	Mouse infection	None	Therapeutic
Respiratory syncytial virus	Mouse infection	None/TransIT	Prophylactic
Parainfluenza	Mouse infection	None/TransIT	Prophylactic
Influenza	Mouse infection	PEI-complex lentivirus	Prophylactic
	Mouse infection	Oligofectamine	Prophylactic
Hepatitis B virus	transient transfection	None	Transfecting artificial microRNA
	Hydrodynamic	None	Co-transfection
	Transgenic Mouse	Adenovirus	Therapeutic
	Hydrodynamic	Stabilized	Therapeutic
Monkeypox virus	Monkeypox virus		Therapeutic
Chikungunya virus		Mammalian cells	Therapeutic approach
HIV-1	Antiviral miRNAs or shRNAs		Targeting imperfect target sites
Marburg virus		VP30	Co-transfection
Semliki Forest Virus	Mammalian cell		Transfection

Sometimes siRNA-based therapy has been found to be inactive in some cancerous cells as well as against some viruses. These kind of cancerous cells alter the activity of miRNA and dicer and siRNAs do not exert its effect properly [34]. Some viruses show special kind of mutations in their gene by which they can escape themselves from termination by means of siRNA. On the other hand, some viral (HIV) gene product might interfere with the function of siRNA that lead ineptness of siRNA therapy against some viruses [61]. Complete cure of any disease is not achievable by using RNAi since it can only knock-down a particular gene, which is responsible for the development of a particular cancer or tumor. Complete relief is only possible after knocking out of a desired gene [73]. RNAi therapy can only prolong the survival rate of the patient by suppressing expression of the disease-causing gene or genes. The effectiveness of the siRNA against any disease is short and it is incapable of giving any life long relief from a disease. Before considering RNAi therapy as a medicine against a genetic disorder, stability of the siRNA inside the cell has to give priority. Some efforts have already been made by the scientists to make siRNA more stable inside the cell [16].

3. Benefits of RNAi therapy to cure complex diseases

RNAi is an advantageous process to cure diseases that happen to be due to the presence of single defective gene [10]. These types of diseases are generally categorized as cancer or neurobiological disorders. Use of RNAi as therapy sublimates these diseases leaving the option to know the structure of the gene product (protein). Many therapeutics or treatment has already been discovered for curing disease by RNAi technology (Table 1). The main issue is being focused on sequence that has to be targeted though there are some difficulties to use this technique. Despite that fact, some achievements have already been reported in cancer and neurobiological disorders by means of successful allele-specific silencing [51,68]. It is evident that RNAi is a more efficient technique as compared to the techniques like antisense and gene therapy though there is a difference in their experimental design. RNAi is a natural technique to manipulate gene expression since antiviral response is already present in the body of animal and plants [9]. RNAi also proved its effectiveness over antisense technology in case of combined therapy. This technique is more versatile for its target sequence identification and destruction for the presence of structural facilities (GC content and 3'overhang). A single dose of RNAi therapy can target more than one sequence of a single gene or a group of genes [7,55,60,72]. Nowadays, many commercial companies are being engaged to discover new products by using RNAi (Table 2).

3.1. Human immunodeficiency virus (HIV)

HIV is one of the most harmful disease that affect millions of people throughout the world. Nowadays, HAART is the only technique that is available to combat against the HIV. This technique extends the life span of a HIV infected patient but is unable to give any permanent solution [2]. Moreover, prolonged use of this technique may generate toxic effects inside the body. Due to

Table 2. Companies engaged with RNAi therapeutics development.

Company name	Diseases or disorders
Acuity Pharmaceuticals	Age-related macular degeneration; diabetic retinopathy
AGY Therapeutics	RNAi in neurons and glial cells
Alnylam Pharmaceuticals Inc.	Age-related macular degeneration; Parkinson's disease, respiratory syncytial virus; cystic fibrosis; influenza; spinal cord injury
Atugen AG	Metabolic disease; cancer ocular disease; skin disease
Benitec Australia Limited	Hepatitis C virus; HIV/AIDS; cancer; diabetes/obesity
Calando Pharmaceuticals	Nanoparticle technology
Cytrx Corporation	Diabetes/obesity; amyotrophic lateral sclerosis; cytomegalovirus retinitis
Devgen	Diabetes/obesity; arrhythmia
Genesis R&D	Allergy
Genta Incorporated	Cancer
International Therapeutics	HIV; Hepatitis B virus
Intradigm Corporation	Cancer; severe acute respiratory syndrome; arthritis
Nucleonics, Inc.	Hepatitis B Virus; Hepatitis C virus
Sirna Therapeutics, Inc.	Age-related macular degeneration; Hepatitis C virus; asthma; diabetes; cancer Huntington's disease; hearing loss
Archemix Corporation	Aptamer-DsiRNA therapeutics
GlaxoSmithKline	MicroRNA targeted therapeutics to treat inflammatory diseases
RXi Pharmaceuticals	Dermatology and ocular disease
Tekmira	Ebola virus infection
Access Pharma	Ovarian cancer

the insufficient solution of previously used techniques against HIV, RNAi has become a promising technique to find the alternative way. It has been reported that virus specific siRNA is able to eliminate the expression or replication of the virus very effectively either in-vivo or in-vitro. HIV has some kind of proteins that can interact with siRNA and make them resistance against the silencing. So it is better to target the cellular receptors and co-receptor that are responsible for the entry of HIV into the host cell. CD4, CCR5 and CXCR4 are the main receptors that facilitate the entry of HIV into the host. But before considering these possibilities, the effect of down regulation of these genes on other cellular processes should be well thought-out [54]. The antiviral activity of the siRNA becomes faded after its delivery into the cell due to degradation and dilution associated with cell division. It is observed that a lentiviral vector plays a desirable role to maintain its effectiveness. Lentiviral delivery of the siRNA against tat, tat-rev genes of HIV-1 in cell line, macrophages and primary T cells showed excellent result [43]. Application of siRNA has to be specified for either p24 gene of HIV-1 or CCR5 co-receptor before infecting in a non-dividing macrophage that can suppress infection up to 20 days and two targets siRNA for CCR5 co-receptor has been found to be worked well [62]. It is a challenge for scientists to make RNAi therapy at a clinical level but successful tests on preclinical model raised hope to make RNAi therapy against HIV infection. It has been recently found that delivery of anti-CCR5 and antiviral siRNA into viremia infected mice successfully reduce the population of the disease-associated CD4 T cells into the mice and provided resistance to the newly generated T cells by delivering antiviral siRNA into it [42]. Knockdown efficiency of miRNAs and shRNAs against wild-type and RNAi-escape HIV-1 variants has been reported too [44]. These findings reveal that imperfect sites by antiviral miRNAs or shRNAs may provide new RNAi approach for inhibiting of HIV-1 virus. Hopefully, RNAi–mediated knockdown of gene expression offers a novel treatment strategy for HIV infection. Humanized mice challenged with HIV after anti-CCR5 siRNA treatment has been found to enhance resistance to infection as assessed by the reduction in plasma viral load and disease-associated CD4 T-cell loss. This finding pinpoints the potential in vivo applicability of LFA-1-directed siRNA delivery as anti-HIV prophylaxis [41].

3.2. Cancer

Cancer is considered a life-threatening disease that has been put to the attention of most scientists working in the field of medical science. Before the application of therapy against any kind of cancers, it is very important to consider that it has to be non-destructive to normal healthy cells. The use of RNAi as a therapy against cancerous diseases becomes most popular for its

effective [25,35]. Nowadays, different experiments are performed by using RNAi therapy against cancer by exploiting every possibility like direct targeting of the oncogenes, stop progression and invasion of the tumor cells and sensitization of the tumor against drug [56]. It has been found that siRNA can prevent multiple oncogenic gene fusion and suppress disease development that is common in some special kind of cancer like lymphoma and leukemia [24]. In a preclinical model, siRNA is able to resist the development of tumor by targeting cellular p53 gene that is involved in the development of cancer [47]. It has been reported that difference in a single nucleotide between mutant and WTp53 gene in cells can express and thus able to restore both WT and protein function. This finding reveals the potential use of RNAi to suppress the expression of point mutated genes and antitumor therapy. RNAi technique also becomes successful against the spread of tumor growth and makes the tumor cell sensitized against the commonly used drug for treatment. Experiment on breast cancer reveals that siRNA is capable of blocking the further expansion of breast cancer by suppressing the function of the chemokine receptor CXCR4. Another experiment showed that tumor cells become sensitized to the common drug like chemotherapy agents once siRNA suppressed the function of the anti-apoptotic bcl-2 gene [26].

3.3. Neurobiological disease

To find the cause of many neurobiological diseases, scientists are often using invertebrate models (Drosophila and *C. elegans*) rather than vertebrate as a preclinical model. Different cellular processes are addressed that might be involved with the neurobiological diseases including membrane trafficking, formation of cellular junctions, intracellular signaling, neural plasticity, cell cycle, axon guidance and embryonic development [28]. Use of RNAi therapies to cure neurological diseases becomes very popular against the techniques like antisense, chemotherapy, etc. This preference indicates some positive sides, such as possibility of targeting multiple factors that are thought to be involved with the disease development process. At the same time, it can act without making any toxic effect to the cell. Some achievements have been made after applying siRNA in an in-vivo model. Its high degree of specificity to suppress the action of targeted gene or genes has been noticed [28,71]. This technique seems to be effective against retinal disorders, neurodegenerative disorders, CNS tumors, trinucleotide repeat diseases, etc. Modernization and achievement of this technique in the field of cancer and virology is showing a ray of hope in neurooncology and neurovirology.

3.4. Viral infections

Chikungunya has emerged as one of the most important arboviral infection and there is no effective antiviral or licensed vaccine available against Chikungunya infection so far. Symptoms of this disease include fever (104°F), petechial or maculopapular rash in trunk or limbs and arthritis affecting multiple joints [14]. Effectiveness of siRNAs against the conserved regions of nsP3 and E1 genes of Chikungunya virus were designed and up to 99% inhibition was observed [17]. Marburg virus is capable of causing haemorrhagic fever. RNA interference (RNAi) has been employed to destroy mRNA transcripts and disrupt replication [23]. Monkeypox virus causes the disease monkeypox in both humans and animals. Symptoms include swelling of lymph nodes, muscle pain, headache, rash and fever. Monkeypox or other orthopox viruses poses a serious threat to public health and there are no licensed drugs to treat these infections [18]. The potential role of RNAi as a therapeutic approach for monkeypox virus infections using MPV as a model has been reported [1].

3.5. Hepatitis

Hepatitis is an inflammation of the liver characterized by the presence of inflammatory cells in the tissue of the organ. Hepatitis may occur with limited or no symptoms, but often leads to jaundice, anorexia and malaise. A group of viruses (hepatisis A, herpes simplex, adenovirus, cytomegalovirus) are mostly responsible for this disease. Other reasons for this disease are toxin, leptospira, alcohol, etc. Hepatitis B virus (HBV) infection is a serious threat to health and increase the risk of chronic liver disease and hepatocellular carcinoma in human. RNAi has been applied to inhibit the production of HBV in mice transfected with a HBV plasmid. Immunohistochemical detection of HBV core antigen revealed 99% reduction in stained hepatocyres upon RNAi treatment [50]. It was also noticed that HVB replication and expression has been inhibited by artificial microRNA targeting the HBV S coding region in HepG2.2.15 cells [27]. This vector-based artificial microRNA could be a promising therapeutic approach for curing chronic HBV infection. Hepatitis C virus (HCV) is a small (55-65 nm in size), enveloped, positive-sense single-stranded RNA virus, which is a major cause of chronic liver disease and affects over 270 million individuals worldwide. The infection is often asymptomatic, but once establish, it can lead to the fibrosis and advanced cirrhosis. RNA interference activity has been used as a treatment by using of multiple siRNAs to reduce the devastating effects of HCV replication on the liver [70]. It was also reported that RNAi

has been specifically inhibit HCV RNA replication and protein expression in Huh-7 cells by using a selectable sub-genomic HCV replicon cell culture system [39].

3.6. Prion disease

Prion is a proteinaceous infectious particle, which consist of two or three parts: a helical molecule, protein coat and sometimes a viral wrapper [76]. All known prion diseases affect the structure of the brain or other neural tissue and all are currently untreatable and universally fatal. Symptoms include depression, unsteady gait, myoclonus, insomnia, memory problem, and inability to move. Prion disease refers to a group of fatal transmissible neurodegenerative diseases for which no pharmacological treatment is available. The cellular prion protein (PrPC) is required for both prion replication and pathogenesis.

RNAi has demonstrable therapeutic potential in animal models of several prion diseases, including Alzheimer disease, spinocerebellar ataxia, and Huntington disease. It has been reported that lentivector-mediated RNAi significantly reduced neuronal PrPC expression; effectively suppressed accumulation of the infectious protease-resistant form of PrP (PrPSc) in a persistently infected neuroblastoma cell line in mouse [77]. Lentivirally mediated RNAi of PrP has also been noticed to prevent the onset of behavioral deficits associated with early prion disease, reduced spongiform degeneration, and protected against neuronal loss [78]. This approach can now be used PrP knockdown for effective treatment of prion disease. It indicates that RNAi has therapeutic potential for prion disease treatment. It may also be possible to utilize RNAi to prevent or delay the occurrence of prion disease in subjects carrying pathogenic mutations in the human PrP gene.

3.7. Cardiovascular diseases

Cardiovascular diseases are involved with the heart or blood vessels (arteries and veins) that affect the cardiovascular system. It is the leading cause of death in the United States and many other industrialized countries. It most commonly results from the progressive occlusion of arteries in a process called atherosclerosis, which can ultimately culminate in a myocardial infarction or stroke [79]. High blood cholesterol, or hypercholesterolemia, is a major risk factor for atherosclerosis and heart disease. It has been reported that RNAi mediated silencing of PCSK9 gene lowered LDL cholesterol in cynomolgus monkeys and rodents without affecting HDL or triglyceride levels which indicates the potential role of RNAi therapies and the targeting of PCSK9 as a way to treat high cholesterol in human body [80]. It was also reported that mevastatin, an inhibitor of cholesterol synthesis, suppresses cell proliferation by inhibiting cyclin-dependent kinase-2 [81]. It is also possible in the near future to use the RNAi technology to intervene in the process of atherosclerosis or to reduce the damage to heart tissue and brain cells.

3.8. Other diseases

RNAi plays a significant role in other diseases including respiratory infection, ocular disease, inflammation and apoptosis. Respiratory syncytial virus (RSV) is a major causative agent of respiratory tract infection. No vaccine or drug has been developed to curtail the activity of RSV until now. The siRNA treatment on a RSV infected mice showed positive inhibition of RAV's NS1 gene whose product interferes with the host interferon response [75]. Age-related macular degeneration (AMD) is a kind of ocular disease that is characterized with uncontrolled growth, new blood vessels formation and invasion. Vascularization is a one of the major symptoms of ocular disease and it is thought that visualization is a cause of VEGF activity. It is revealed that VEGF specific siRNA effectively reduce its activity and prevent vascularization. It is even tested in human and showed successful results [63]. ALN-RSV01 siRNA has been probed to be directed against the mRNA of the respiratory syncytial virus nucleocapsi protein and has substantial antiviral activity in a murine model of respiratory syncytial virus infection [19]. In a certain type of diseases, it has been found to it activate innate immunity system. Moreover, siRNA seemded to be effective to control those cellular processes that activates innate immunity system. TNF α (a proinflammatory cytokine) plays a major role in rheumatoid arthritis and its suppression has been made through TNF α specific siRNA [68].

4. Conclusion

RNAi is a new technique that has been discovered in the past few years. It holds a great promise to investigate gene function and to make therapy of previously uncured diseases. In regards to therapeutic use, this technique is still in a developing stage and many problems are associated with its delivery and durability. But achievements in the preclinical model and some experiments on humans keep this technique in a top priority list in the field of molecular medicine, gene expression study and so on. A better understanding of the mechanism of the RNAi pathway, improved siRNA sequence selection, competent way of delivering RNA has to be understood

to achieve the future goal. Furthermore, development of efficient tissue-specific and differentiation-dependent expression of siRNA or shRNA (short hairpin RNA) is critical for transgenic and therapeutic approaches. Although there are many problems to face in this new field of gene therapy, successful in vitro and in vivo experiments raise hope for treating human disease with RNA interference.

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