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RNA INTERFERENCE: APPLICATIONS AND CONFRONTATIONS

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ABSTRACT

Ribonucleic acid interference (RNAi) is one of modern techniques which involved in suppressing protein productions, this occurred via inserted of double stranded (ds) RNA into the cytoplasm in a cell which leads to particulate and effective degradation of complementary messenger (m RNA).

It discovered fortuitously during attempts to produce flowers with elevated color intensity. The possible of this technique to recognizing the physiological and pathological processes is very important, while its improvement as a therapeutic agent carries out much promise as targeted therapeutic agents.

This review article will discuss the basic biological processes that drive RNAi, indicate present and prediction future areas research, and some of applicable limitations.

Keywords: Ribonucleic acid, interference, (RNAi), m RNA

1. INTRODUCTION

RNA interference (RNAi) is a process of silencing gene function by inserting short sequences of ribonucleic acid (RNA) that coincide part of the target gene's sequence and therefore inhibition of protein production. RNAi has significantly obtained prominence as the technique of option for researchers sleuthing the structure and action of significance genes. RNAi has the ability to turn into a powerful therapeutic approach toward targeted and personalized medicine, the extent of disorders it could be treated is incredible; from cancer to cardiovascular diseases, neurodegenerative disorders and even HIV. Also it achieved a great improvement in agriculture field; the scientists have manufactured novel crops such as nicotine-free tobacco, non-allergenic peanuts, decaffeinated coffee, and nutrient fortified maize among many others.

The blocking of genes mechanism carried out at transcription, post-transcription and translation levels. In addition it might cause augmentation of gene expression because of immediate impactions in the translation process [1].

1.1 DNA-directed RNA interference

Utilize a DNA structure to stimulate the pathway of endogenous RNA interference in process known as gene silencing [1]. The DNA structure is designed to express self-complementary double stranded RNA especially the small or short hairpin [shRNA] which is more beneficial than RNAi in that it has approximately low rate of degradation and turnover, shRNA is normally expressed by delivery of plasmid [2]. This technique has an important role in the medical biotechnology field that concerns with possible treatment approaches by deactivated the pathogenic genes which demonstrated across a range of disease models. For example, HIV [3], hepatitis B [4], hepatitis C [5], lung cancer [6], retinitis pigmentosa [7], neuropathic pain [8], and advanced cancer [9]. Short hairpin RNA (shRNA) can be inserted in a certain delivery device to reach the target site after being encoded via DNA-directed RNA interference (ddRNAi). An Encoded RNA which is a transcription product when DNA carried into nucleus. Endogenous mechanisms are responsible of shRNA performance by controlling RNAi metabolic pathway and silencing genes which cause diseases [1] [Figure 1].

Figure 1: ddRNAi mechanism of action

1.2. Mechanism of RNA interference [10]

There are two phases for gene silencing mechanism that is occurred by RNAi.

1.2.1. The first phase is an initiation

The most important role in this stage is to produce siRNA that is developed by action of type III endonuclease Dicer that has two types: Dicer-1 or Dcr-1/Loquacious-PB (Loqs), and Dicer-2 or Dcr-2/R2D2, these two types have similar structures with different functions.

1.2.1.1.miRNA: Dicer-1 is an ATP-independent and applies some proteins in order to bind dsRNA. In a recent study on *Drosophila* was shown that Dicer-1 has a specific protein named Loqs used for dsRNA

binding, this result reached to the miRNA-regulated gene expression that Loqs proteins have an important affect in this process. There are two types of dsRBDs that result via encoding Loqs gene: PA and PB, PB plays critical role by promoting Dcr-1 affinity.

1.2.1.2.siRNA : Dicer-2 is an ATP-dependent, and it is need a specific protein for dsRNA binding which is called R2D2 that works with RNase enzyme, this protein will interact with long dsRNA but it does not have any regulating role for siRNA and Dicer-2 action.

1.2.2. The second phase: the effector stage

This phase is using a quid system involves single strand duplexes of the miRNA and

siRNA, which can incorporate into effector complexes as RNA-induced initiation of transcriptional gene silencing (RITS) or the RNA-induced silencing complex (RISC).

1.2.2.1. RISC

RISC contains mainly argonaute (Ago) proteins which have two types: Argo and

Piwi proteins, Argo binds to siRNAs and miRNAs, and the Piwi binds to piRNAs. A study was carried out on siRNA structure, and showed slight differences of siRNA structure that can change the determination of RISC complex guided strand.

Figure 2: A model for the mechanism of RNAi

1.3. Influence of overhang structure and sequence on Dicer specificity and efficiency

It is very important for Dicer recognition and specificity to determine which the characteristic of the overhang structure, Dicer synthesized and processed substrates

with different lengths and composition: 61mer MAPK14. In figure 3 that explained Dicer cleavage of all probable 2-nt overhang sequences (UU, UC, UG, UA, CU, CG, CA, CC, GU, GC, GG, GA, AU, AC, AG, AA) and illustrate which while cleavage product sizes are not changed via overhang

composition, nucleotide sequence does have an important role in determining Dicer efficiency [11]

The overhangs that hold a C and A in the penultimate and terminal positions (respectively) are processed efficiently, and those hold an A in the penultimate position and a U in the terminal position are less effective., classification of the Dicer cleavage efficiency of substrates reveals a hierarchy with $C > U = G > A$ will be the priority at the penultimate position and $A > G = U > C$ being the order of terminal nucleotides. Thus, RNA end structure, especially 30 overhang lengths, has an important role to determine the position and efficiency of Dicer cleavage. [11]. This knowledge has a deep effect for siRNA and shRNA design. shRNA expression via Pol III promoter results in shRNA termini with different overhang lengths (1–5 Us).

And this diversity drastically impacts the specificity of Dicer cleavage and therefore the cleaved siRNA function.

1.4. Effect of siRNA end structure on silencing efficiency

In recent researches it has been proposed that Dicer is involved in siRNA RISC loading [12]. By examination of Dicer cleavage in vitro, it elucidated that end structure impacts the effectiveness of Dicer.

This because of siRNA end structure influences overall silencing efficiency. Broadly, siRNAs with asymmetric overhangs on the antisense strand carried out preferable than siRNAs with asymmetric sense strand overhangs.

2. Designing siRNAs

Gene silencing will be controlled via making an expression vector for the siRNA. The siRNA sequence is processed to insert a short loop among the two strands. The output transcript is a short hairpin RNA (shRNA) that may be converted into a functional siRNA via Dicer [13]. The capability of gene silencing can be expressed by small interfering RNA (siRNA) which is determined by its sequence and it is varying among target sites. Cross hybridization and chemical modification can also change the efficacy of siRNA [14].

2.1. Location of siRNA

The target must be localized 50–100 nt downstream from the start codon (ATG). Thus, knowing the particular splice variants is important to define the target isoforms of the gene [14].

2.2. Modification

To achieve preferable siRNA activity, it should involve phosphate groups at the 50 end; therefore it is necessary not to block the 50 end of the antisense strand with other

modifications [14]. Transfected "infect (a cell) with free nucleic acid" non-modified 5'OH ends are speedily phosphorylated via cellular kinases, this appearing not to phosphorylate synthetic siRNAs [14]. Without paying attention of the aimed sequence, DNA 'TT' dinucleotide overhangs are sometimes added to the 3' ends.

2.3. Thermodynamic stability

The efficacy of siRNA is also impacted by its thermodynamic stability that is necessary for the function of siRNA and strand loading into RISC. The most efficient siRNA has a small T_m and duplex stability (less stable, more A/U rich) toward the 5' end of the guide strand and a relatively high T_m (more stable, more G/C rich) toward the 5' end of passenger strand [14].

2.4. Sequence characteristics and specificity

To achieve highest specificity, there are some sequence properties must be avoided in the guide strand such as homopolymeric runs and nine –base or greater segments of G/C bases. The site accessibility and the secondary structure of the target are important for activity of siRNA.

Moderate - low GC content (30–52%) is a feature of functional siRNA.[14].

3. Applications of RNA interference in various areas

RNAi technology is viable for gene silencing in different fields. RNAi has been used in *C. elegans* for genomics [15]. The promoters of RNA polymerase (pol) II and III (U6 and H1, alone or together) have been utilized for stable silencing approach. Retroviral-vector-based delivery of siRNAs has also been performed for more active silencing. There are two classes of retrovirus vectors have been used: (1) HIV-1-derived lentivirus vectors and (2) Oncoretrovirus-based vectors, such as Moloney murine leukemia virus (MoMuLV) and Murine stem cell virus (MSCV). Transgenic mice have been established with germline transmission of a shRNA expression cassette for silencing of genes not targeted by homologous recombination-based approaches

3.1. RNAi as a potential therapeutic for humans; genetic diseases

A genetic disease is an aberration or abnormalities of genes that formed in genome leads to pathogenic symptoms. The first recognized genetic disorder in a hominid was in the fossil species *Paranthropus robustus*, with over a third of individuals displaying Amelogenesis imperfect [16].

The Genetic disease could be hereditary, this significance which they are passed down from the offspring's genes. The second genetic disease, the disorder is happened via

DNA mutations. In these cases, the defect will only be passed down if it happens in the germline. There are certain forms of recessive gene diseases give an advantage in particular environments only if one gene copy is exist [17]. There is a big challenging due to absent of truth treatment, all the present treatment approaches is to control the symptoms, in these cases are happening only in the dominant negative genetic disease, that

its reason a mutant gene allele make an illness by existence of a second, normal copy, all of existed pharmacological or biological treatments are not suitable to target the selective abnormal genes which cause a disease, because of structural indifference of the normal variant of their targets from the disease-causing mutant ones [18]. Table 1

Table 1: the genes involved in applications of RNA interference (RNAi)

Genes	Fungi	Animals	Plants
Elf2c	QDE2	RDE1	AGO1
RdRp	QDE1	EGO1	SGS2/SDE1
RNA Helicase			MUT6
Rnase D		MUT7	
Coiled Coil			SGS3

Compiled from: <http://www.ejb.org> accessed on 3.08.2012

If the target is a single nucleotide polymorphism (SNP), in this case cannot utilize any structural or receptor protein in treatment, because of selectivity deficiency [18].

An auspicious progress is observed via utilizing RNAi in genetic disorders which provided by preliminary researches demonstrating how single nucleotide polymorphisms (SNPs) in mutant allele transcripts could be applied as selective targets for RNAi. But there is a challenging target in certain neurological disease which polyglutamine proteins encoded by CAG repeat containing transcripts, this is due to common of CAG repeats in different normal

transcripts so cannot be selectively targeted by siRNAs .the researchers observed in their systematic analyses of siRNAs that there is a high selective region in the complementary of polymorphic nucleotide with the middle region of the siRNA that gives an siRNA/SNP combination [19].

3.2. RNAi in gene regulation and antiviral responses

RNA interference (RNAi) has a significance role against virus and transposable elements (TEs) by degrading viral RNA, the hosts and viruses can utilize RNAi in order to manipulate each other's gene expression, and hosts will encode microRNAs which target the sequence of virus.

3.2.1 RNAi as innate antiviral immunity

The first experiment that demonstrated if RNAi has an antiviral effect was in plants when induced ‘gene silencing’ was aimed to achieve resistance to viruses that carrying an identical sequence [20], then it recognized as a normal innate antiviral immunity when find viruses which induce a similar response [21, 22]. The first antiviral RNAi recognized in animal was in *Drosophila* cell culture, which acting by the beetle Nodamura virus FHV (flock house virus) as an initiator and a viRNA pathway targeting [23], but the questionable information about the antiviral immunity in vertebrates are still under advanced research [24, 25, 26].

3.2.2 Viral suppression and evasion of the viRNA pathway

After detecting the naturalistic antiviral effect of RNAi was detected, the Hc-Pro protein of plant Potyviruses was revealed [27, 28, and 29] and the 2b protein of plant cucumoviruses [30] were recognized as VSRs. the B2 protein of the beetle virus FHV is The first VSR identified in an animal virus, it thought to be suppressed of RNAi in plants, before being shown to play the same role in insects. The RNAi effect can be minimized by viruses by forbidding dsRNA to be in contact with the RNAi pathway or by eschewing degradation once viRNAs formed.

Viruses have many modifications to diminish dsRNA production and protect the viral genome against degradation [31].

3.2.3 Viral evolution in response to host RNAi

In recent studies which observed that VSRs from different viral families have essentially no structural similarity, even where they have similar functions. This is particularly striking when the dsRNA-binding motifs are compared across different VSRs: the dsRNA-binding domains from P19 (tombusviruses), P21 (Beet Yellow Virus) and B2 (FHV) each appear to have evolved different and entirely novel dsRNA-binding motifs [32]. To check which VSRs evolve faster than other viral genes, the rate of protein divergence for all genes has been analyzed in pairs of isolates from 17 ssRNA plant viruses with experimentally proved VSRs. figure 4, they explained about the protein variation among pairs of isolates was significantly higher for VSRs than for other genes, which is proportionate with vigorous selection acting on VSRs [33].

3.3. Cancer

RNAi therapeutics for cancer treatment has a strong appeal due to the high specificity and potency to inhibit the process of gene expression. Growth factors, oncogenes and single nucleotide polymorphisms (SNPs) are

potential targets for different diseases. The ability to knocking out proteins with no harm to the cell unlike the chemotherapy that fight the tumors and killing both cancerous and healthy cells around them is what makes the RNAi interesting to many researchers. In vivo application of siRNA was operated on animal models targeting the colorectal cancer-associated gene beta-catenin. Following siRNA-mediated silencing of this gene in human colon cancer cells reduction in the proliferation and subsided invasiveness were observed. When the treated cancer cells were implanted in a nude mouse, prolonged survival was observed in comparison with mice received 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 [34]. Utilized transferrin containing non-lipid based nano-particles an example of RNAi therapeutics with targeted delivery to overcome the challenges of finding a good targeting and delivery with minimized side effects and were applied to target sarcoma cells in a mouse xenograph model [35]. This study provides a powerful proof of principle for systemic delivery of siRNAs to a metastatic cancer. RNAi may also have an interesting mechanism to aid in fighting the cancer by traditional cancer drugs. Including

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 [36]. Even if the trials are in their early phase, they increase the potential of using RNAi therapeutic in the future. The evolution over the past years in finding new technologies for delivery system and the advancing of RNAi therapeutics from pre-clinical into human trials with new trials of first substantial patient data from these RNAi based therapies on near future it is too early to dismiss RNAi therapeutics for cancer treatment.

4. RNAi technology

RNAi technology is serviceable in gene silencing as an approach treatment in different species. RNAi utilized widely in *C. elegans* for functional genomics. High-throughput investigation of most of the ~19,000 genes has been achieved. Ahringer and his fellow worker formed an RNAi library, illustrating ~86% of the genes of *C. elegans* [37]. This experiment achieved successfully chance in various model organisms, inclusive human [38]. Improvement of the 2'-O-acetoxyethyl (ACE) RNA chemistry and the combination of modified, particular cationic, nucleotides form the basis for the synthesis of highly

stable effective siRNAs. There is an obstacle regarding synthetic siRNAs is their low stability in serum. A latest research was carried out with much several chemistry at the 200 ribose position like aminoethyl and guanidinoethyl [39] and the siRNA half-life and efficacy can be promoted via inserting modifications at particular sites both in the passenger and the guide strand of the siRNA.

4.1. Technological applications of RNA interference in Gene knockdown

According to the organism and experimental system, the exogenous RNA could be a long strand designed to be cleaved by Dicer, or short RNAs designed to serve as siRNA substrates. Shorter RNAs are utilized in mammalian cells due to long double-stranded RNA molecules promote the mammalian interferon response, a form of innate immunity that interacts nonselective to foreign genetic material [40]. This reaction is approximately absent in mouse oocytes and cells from early mouse embryos and is therefore a common model system for studying gene-knockdown effects in mammals. Specific laboratory methods have been advanced to upgrade the using of RNAi in mammalian systems by obviate the direct introduction of siRNA, for instance, via stable transfection with a plasmid encoding the appropriate sequence from that siRNAs

may be transcribed [41], or via more accurate lent viral vector systems letting the inducible activation or deactivation of transcription, known as conditional RNAi [42].

5. Prospects of using RNAi techniques

An example which RNAi technology may be utilized to silence the gene(s) responsible for the production of b-oxalylaminoalanine-L-alanine (BOAA), a neurotoxin existed in a leafy vegetable known as *Lathyrus sativus*. Another case where RNAi may be strongly carried out is in the manufacturing of banana varieties resistant to the Banana Bract Mosaic Virus (BBRMV) which presently devastating the banana population in Southeast Asia and India [43]. The future researches of RNAi will attach for being inspected for other applications of human, animal and plant therapeutics.

6. Limitations of RNAi

The safety of RNA interference is controversial issue, exceptionally the probability for “off-target” effects, in which a gene with a coincidentally comparable sequence to the targeted gene is also suppresses. The rate of off-target reaction is determined by computational genomics study and its approximately equal 10%. This can explained the elevated death rate of animals in the experiments, could be related to be the result of “oversaturation” of the dsRNA

pathway [44]. In addition, while classical genetic screens can identify alleles that uncover regulatory mechanisms, RNAi is

purely a loss-of-function technique targeting the mature message [45] Figure 3.

Figure 3: Limitations of RNAi

7. CONCLUSION

The development of RNA interference application is continuously increased and achieving modern discoveries are being made on a daily basis, SiRNAs applications are presently highly used in gene function analysis, target identification and validation and as therapeutic agents. Despite of an applicable method for in-vitro experiments, obstacles with intracellular delivery of siRNA can affect gene silencing and therefore delay the successful techniques. At the end, the cost obstacle in improves of RNAi molecules is one of the most usage

limitation, especially for in vivo studies. Hopefully, cost must not be the barrier to use of this worthy technique. In conclusion, the presenting hype and excitement about posttranscriptional gene silencing technique has been justified, but experiments should converge on in vivo to achieve more applicable treatment approaches for most complicated genetic disorders.

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