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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/11942
DOI URL: http://dx.doi.org/10.21474/IJAR01/11942

RESEARCH ARTICLE

SIRNA GENE THERAPY: A REVIEW

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Manuscript Info

Manuscript History
Received: 27 August 2020
Final Accepted: 30 September 2020
Published: October 2020

Key words:-
Mechanism, Design, Delivery Systems and Applications-Limitations of SiRNA

Abstract

RNA inference is new concept in gene therapy it acts by expression of specific genes to treat various diseases like cancer. It is also called as RNAi. In presence of dicer enzyme long double stranded RNA (dsRNA) is cut to small interfering RNAs called SiRNAs. SiRNAs are part of RNAi. It is mainly pursued for cancer therapy. It is the new promising agent which can produce specific gene expression and also post transcriptional gene regulation process for disease treatment. In this current review I gathered lots of basic information about SiRNA like it's structure, design, delivery system etc in short sweet manner.

Introduction:-

SiRNA is known as short interfering RNA or silencing RNA.It is mainly used for RNAi under class of double stranded RNA molecules.It is consisting of 20-25 base pairs in strength of length.It can produce expression of specific genes with complementary sequence of nucleotide and it makes degradation of mRNA after transcription process so degraded mRNA can't undergo translation process.They are also present in human bodies but in very less amount.They can be also introduced to eukaryotic cells artificially by making suitable delivery system.Entire process of gene silencing by SiRNA is called SiRNA knockdown.They arenon coding RNAs.In near future it will be possible that theywill be used for all types ofviral genome no matter if it's single stranded or double stranded genome.It can be also used for maintenance of proper and extended antiviral effect.Treatments of SiRNA are still under development and SiRNA have strong potential for cancer treatment .So SiRNA are new developing novel therapeutic strategy.A careful design is also required to use maximum safe potential of SiRNA.For the delivery of SiRNA to eukaryotic cells transfection process is mainly used in which there is use of chemical carriers to deliver SiRNA at target site and this is also a big challenge to deliver SiRNA at it's target site.It has lots of advantages over chemotherapy for cancer and also have some limitations in which delivery system, viruses escape and poor stability are major ones.(1)(2)

Structure:

Naturally occurring SiRNAs are double stranded, short and 20-25 nucleotide longer.It is consisting of two kind of strands.

1. Guide strand and
2. passenger strand
3. The guide strand will become part of RISC complex while passenger strand will be degraded.These strands are also know as sense and antisense strands.The sense strand has information that would be readable on RNA where anti sense is the non coding strand.Both of the strands have potential to guide post transcription gene regulation.SiRNA is double stranded because presence of double strands enhances their half life and also RNAi effect.This double stranded RNA have phosphorylated 5' end and 3' hydroxylated end and have overhanging two nucleotides.(3-4)

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Synthesis of SiRNA:
Currently there are five methods are available for synthesis of SiRNA
1. Digestion of long dsRNA by an enzyme dicer
2. Chemical synthesis
3. In vitro transcription
4. Expression in cells from an SiRNA expression plasmid or viral vectors
5. Expression in cells from pcr derived SiRNA expression cassettes.

1. Digestion of long dsRNA:In this method first long double stranded RNA are prepared by in vitro transcription using template that encodes 200-1000 nucleotides range of target mRNAs.Then it is digested in vitro with dicer enzyme (RNaselll enzyme) to produce SiRNA.This process is fast and inexpensive but not for long term studies.(5)
2. Chemical synthesis:In this method SiRNA are synthesized chemically.A protecting group AZMB[(azidomethyl) benzoyl] is introduced to ribonuclease 3'hydroxyl group to facilitating solution phase for synthesis of SiRNA.It is most expensive method and used for large amount of SiRNA in studies .It can not be used for long term studies.(6)(11)
3. In vitro transcription:Once template deoxynucleotide are obtained the process of synthesis takes about 24 hours.In this method the template deoxynucleotide undergoes to in vitro transcription in suitable cell and produces SiRNA.It is less expensive and not suitable for long term studies.(7)
4. SiRNA expression vectors:In this method SiRNA expression vectors are used and most commonly RNA pol. III is used as promoter to drive the expression of small hairpin SiRNA in mammalian cells.To use SiRNA expression vectors two oligodeoxynucleotides encoding the desired short hairpin RNA sequence are ordered, annealed, cloned to vector downstream of promoter.Suitable for long term studies but not for screening RNA sequences.(8)
5. SiRNA expression cassette:SiRNA expression cassette (SECs) are pcr derived SiRNA expression template Can be introduced to cells directly.SECs includes RNA polymerase III , a sequence encoding SiRNA hairpin, and termination site.Suitable for screeningSiRNA sequence are testing promoter but not for long term studies.(9)(10)

Design:
Designing of SiRNA is very difficult task.Designs are decided before their synthesis.Here's are the factors considered for design
1. Base contents (mainly contents of guanine and cytocin)
2. Thermodynamic properties
3. Structure
4. Accessibility to target site

There could be also use of some chemical modification in design for better stability and effects. (12)(13)(14)(15)(16)(17)(62)

Delivery system:
Because of the negative charge of SiRNA they are not suitable to penetrate the cell membrane solely and also it can undergo some interactions and produce unnecessary immune response so it requires delivery agent.(18)There are mainly 3 types of delivery methods are used for SiRNA,
1. Transfection
2. Electropermeabilization
3. Viral method.

Transfection:
1. In this method the prepared SiRNA is combined with the chemical delivery agents such as cationic liposomes, polymer nanoparticles or some positively charged ions like ca+2 etc.
2. This method is not suitable for all kinds of cells but still it is used for majority of cells.
3. Also this method has low in vitro efficiency.
4. This is the most used method.(19)(20)

Electropermeabilization:

1. In this method there is use of electroporators which generates electric pulses and it is used for that cells which are not suitable for transfection.
2. When this pulses are applied to cell membrane it causes temporary loss of semi permeability of cell membrane and produces hydrophilic pores through which uptake SiRNA is possible.(21)(22)

Viral method:

1. In this method there could be use of viruses as carriers.
2. In this method Viruses can deliver SiRNA through transduction process.
3. Some parts of viral genome are replaced by genes of SiRNA and they are delivered to target cells.
4. Sometimes viral vectors could be affected by immune response of host also some of them have poor target selection efficiency.(63)
5. Adenovirus, retrovirus etc could be used as viral vectors.(23)(24)

Mechanism:

Naturally occurring Long double stranded RNAs are cut by the enzyme dicer producing SiRNA in human bodies or artificial SiRNA is introduced to target site.

1. It gets incorporated to other proteins.
2. After that It produces RNA induced silencing complex(RISC) with the help of dicer.
3. After the production of RISC passenger strand of SiRNA is degraded and now SiRNA is single stranded with combination of RISC.
4. Then the single stranded SiRNA along with the RISC finds the mRNA for target and scans it.
5. It binds with this complementary mRNA and cuts this mRNA which causes cleavage in mRNA.
6. Due this cleavage in mRNA now it is unsuitable for translation so it is considered abnormal and it causes the silencing of gene which encodes for this mRNA.
7. Thus it produces the gene silencing and SiRNA is also able to target multiple genes of different pathways.
8. The degradation of target mRNA is caused by ATP hydrolysis process.
9. Efficiency of SiRNA is depends on its ability to produce RISC.(25)(26)(27)(28)(29)(30)

Applications and future prospects:

Note:

some of the applications including the therapeutic applications are still under the development the information in these applications are the proven potentials of SiRNA it shows what SiRNA is capable to do so please take a note of that.

1. Testing the hypotheses of gene function:thereare too many methods are available for identification of different expressed genes scientists and researchers Can make prediction for function of gene according to its expression.(31)(32)SiRNA are used to test the accuracy of these predictions.they could be also used for target validation.(33)(34)
2. Pathway analysis: It could be used for the analysis of various pathway.Works on the principle that reducing the expression of single gene has implication on expression of genes that are in same pathway.(35)(36)For example, reducing the levels of transcription factor like p53 will also reduce the expression of any genes which relies on p53.(37)(38)

3. Gene Redundancy: in some cases when major gene product from cell is eliminated some other genes helps the cell to survive these are called redundant genes and they can be identified by SiRNA.(39)(40)(41)(42)

4. In functional screening experiments but still there is no report on use of SiRNA for screening but screens in drosophila and C elegans are proven so it is opportunity to use it.(43)(44)(45)

Therapeutic applications:

Cancer: this disease is main target for use of SiRNA.it has advantages over the chemotherapy like it has higher degree of safety because it do not interact with the DNA also it has low cost.it is able to target multiple genes of different pathways so it will be effective approach to stop tumour profession.also development of personalized drugs for patients is possible so it will be more effective.co delivery of SiRNA with chemotherapeutic agents is also a big effective approach.due this abilities SiRNA will probably enter in class of anticancer therapeutic

agents.(46)(49)(50)(51)neurological disorders: there are proofs that SiRNA are effective against neurological diseases mainly for Huntington's disease.it was proven during preclinical trials on mice.(52)(53)(54)other diseases: topical microbicide treatments for herpes simplex viruse type 2 knockdown of host receptors and co receptors for HIV.silencing of hepatitis A&B gene silencing of influenza gene expression. all of these new approach can be lead to development of SiRNA in novel therapeutic agent.

Limitations and some tricks to overcome:

Virus escape:

virus escape means sometimes the virus can escape from SiRNA.

To overcome this there should be use of SiRNA targeting conserved viral or by factors involving in negative feedback regulation.(55)(56)

Insufficient cellular uptake:

1. Sometimes intake of SiRNA is not sufficient for gene silencing.
2. To overcome this and improvisation there should be use of synthetic nanoparticles composed of polymers,lipids, conjugate,also by incorporating cell specific targeting ligands in carrier.(57)(58)

Less stable:

Some chemical modification like 2'fluro and thioate linkage can improve it.(59)

Immunostimulation:

Sometimes SiRNA interacts with some agents present in the body and produces unnecessary immune response.

To overcome this there should detailed identification of cellular pathways involving immunorecognitionof RNA is required so according to that some methods could be developed to avoid this.(60)(61)(62)

Conclusion:-

SiRNA has proven strong therapeutical potential and it has also proven as useful tool for research as wellthey are able to target specific gene and silence them they are likely to be use for treatment of viral diseases as well as for cancer.For it's perfect performance it requires perfect design and delivery system so it can become stable.In coming years surely treatment of various diseases will be possible by SiRNA and it will high efficacy at low cost with less side effects so it is simply a future novel therapeutic strategy.

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