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Nucleic Acid Therapies for Genetic Modifications inTherapeutics

Kamal Kishore and Saurabh Mishra*
Department of Pharmacy, M.J.P. Rohilkhand University
Bareilly-243006 (U.P.) INDIA
Corresponding author E-mail: saurabhmcm2002@gmail.com

Abstract:
Nucleic acid therapies work by modulating gene expression of either endogenous or invading genes. The replacement of defective genes with normal or suppressing the function of genes is a novel approach to treat genetic disorders. The production of therapeutic proteins in cell of an organism by transferring genes or cDNA for such protein into cells is referred to a gene therapy. Total suppression or reduction of the expression of undesirable proteins using defined antisense oligonucleotides. The inhibition of transcription at the level of DNA requires the inactivation of the transcription from only one or two active copies of a gene present in the genome; this is the rational for triplex inhibition of gene expression. The inhibition of protein activities and interference with their functions by specific, high-affinity binding of oligonucleotides to the proteins, this approach to therapeutic development is called Aptamer therapy. Ribozyme therapy means when an RNA precursor found to be self-splicing; because this RNA is enzymatically active, subsequently, itcleaves other RNA targets in a sequence-specific manner. The present review is focused on applications, processes and mechanism of nucleic acid therapies. This will help in understanding of nucleic acid therapies and also improve the knowledge about their applications in the field ofmedical sciences.

Key Words:
Vectors, Nucleic acids, DNA, RNA, Genes, Oligonucleotides, Antisense, Triplex, Aptamer, Ribozyme

Introduction:

The concept of using nucleic acid either DNA or RNA, polynucleotides, and oligonucleotides as therapeutic agent is a unique consequence of the development of rDNA technology (Glick, B.R. and Pasternak, J.J., 1994). Early development of nucleic acid which were responsible to replacement therapy, or correction by administration of the protein products of the defective genes. These therapies work by modulating gene expression of either endogenous or invading genes (Millroy and Khati, 2011). As currently defined, nucleic acid therapy approaches fall into five basic categories:

(A) GENE THERAPY
(B) ANTISENSE THERAPY
(C) TRIPLEX THERAPY
(D) APTAMER THERAPY
(E) RIBOZYME THERAPY

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(A) GENE THERAPY

Gene therapy can be broadly defined as the transfer of defined genetic material to specific target cells of a patient for the ultimate purpose of preventing or altering a particular disease site. Expression of entire genes producing therapeutic proteins, foreign to a cell, increasing the level of a normally expressed protein, or replacing a defective protein. Gene therapy is the correction of inborn error of metabolism by the insertion into the affected organism of a normal gene. Ideally, the inserted gene will be correctly targeted and regulated. The replacement of defective genes with normal or suppressing the function of genesis a novel approach to treat genetic disorders like AIDS. The production of therapeutic proteins in cell of an organism by transferring genes or cDNA for such protein into cells is referred to a gene therapy (Glick, B.R. and Pasternak, J.J., 1994).

Aim of gene therapy

Gene therapy can be used to correct genetic deficiencies through protein replacement or for expression of therapeutically beneficial proteins in selected groups of cells. Because the expression of a functional protein is the goal of gene therapy, the introduction of the entire coding sequence of a protein, with appropriate regulatory signals for the transcription of its gene, and the translation of the resulting mRNA are both required(Glick, B.R. and Pasternak, J.J., 1994).

Types of gene therapy

(I) Somatic gene therapy: Deals with the insertion of normal gene into the target somatic cells.

(II) Germ cell gene therapy: Involves the insertion of a normal gene into the cell (sperm, egg, or one cellembryo). This is not permitted legally.

Somatic gene therapy is further of two types (Haritha et al, 2012)

(i) **In-vivo:** It means direct introduction of genetic material into target cells of a particular tissue of patient. The tissues used are liver, muscle, skin, spleen, lungs, brain and blood cells, by using non-viral vectors.

(ii) **Ex-vivo:** It involves the transfer of genes into the cultured cells like bone marrow cells or prior to implanting these into the tissues of the living body. This method has been frequently used in clinical trials. Usually, it can be applied to only selected tissues that can be cultured in laboratory. This technique involves the following steps:

(1) Collect cells from an affected individual or patient.
(2) Correct the genetic defect by gene transfer.
(3) Select and grow the genetically corrected cells.
(4) Either infuse or transplant them back into the patient.

So both the in-vivo or ex-vivo techniques are used to insert a gene into a cell (transduction process)as follow:

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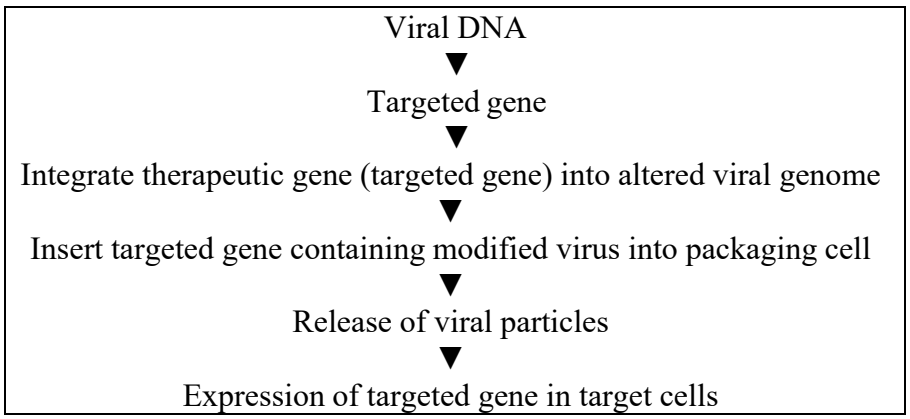

Methods for gene therapy (Glick, B.R. and Pasternak, J.J., 1994): **Vector system:**
Several methods have been used to introduce genetic material into recipient cells. These methods result in either transient (gene being expressed for a few days) or stable (gene) expressed for weeks or year expression of the therapeutic gene. The most commonly used techniques are viral gene transfer system and non-viral gene transfer (Glick, B.R. and Pasternak, J.J., 1994).

Vectors are of two types
(I) Physical vectors
(II) Biological vectors

(I) Physical vectors (Non-viral gene transfer system) (Kamimura et al., 2011)
(i) $CaPo_4$ mediated DNA uptake and electroporation
(ii) Liposomes-Fusion of DNA-loaded membranous vesicles to target cells
(iii) Microinjection method, or biolistic method or particle gun method
(iv) Plasmids Mammalian artificial chromosomes (MAC)

(II) Biological vectors (Viral gene transfer system or virus vectors)
(i) Retroviruses
(ii) Adenoviruses
(iii) Adeno-associated virus
   a. vaccinia vector (pox virus)
   b. herpes simplex virus-1vector
(iv) Other viral vectors
   a. HIV
   b. Hepatitis-B virus
   c. Influenza virus

$CaPo_4$: In the $CaPo_4$ precipitation method the cDNA for the gene of interest is allowed to precipitate by mixing it with $CaPo_4$. The dried powdered precipitate is then sprinkled over the target cell under sterile conditions. This will bind to cell Surface → Phagocytosis → Integration of cDNA into the genome.

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Electroporation: An electric current is passed in a cuvette containing a suspension of cDNA and the precipitant cells. The electric current opens the pores of the cell membrane and diffuse on of cDNA into cell is followed by integration of the transfected gene into the genome.

Liposomes: Are membranous lipids vesicles enclosing an aqueous volume. Therapeutic DNA can be encapsulated in the liposome's which are known to form complexes with them and can be transferred to recipient cells.

Microinjection: Direct administration of DNA into the disease site which leads to up taken and expression of the gene of interest.

Plasmids: Plasmids presents in a number of species of bacteria and yeast, float freely in the cytoplasm. Plasmid DNA along with foreign, replicates and produces many copies of itself and combined foreign sequences in the host cells. E. coli plasmid frequently used in industry as vehicle for the production of proteins structures.

MAC: Is a long term approach for gene therapy. A MAC is constructed in the laboratories, carrying the therapeutic gene within a large place of natural human chromosomal DNA. The MAC carried all the necessary genetic information necessary for chromosomal replication and has segregates non-randomly in mammalian cells.

Viral vectors: The delivery of nucleic acid into mammalian cells is more efficient using viral vectors capable of infecting virtually every cell in a target population. The viral vector is genetically modified in such a way that upon replication, its structural gene is replaced by target gene. The viral vector is then introduced into a packing helper cell line into which structural genes which lack a packaging signal have been transfected. This structural gene allows the production of virus particles containing the target gene. This safe viral particle can now infect the most cells releasing the target gene which will integrate randomly into the host genome; finally, the host cell expresses the target gene without any replication of virus.

Advantages of vectors

Vectors offer many advantages:

- High frequency of gene transfers.
- Transfer into specific cell types.
- More control over the final copy, numbers of a transfer genes.
- However, different species and cell types require different types of vectors. The various vectors are employed for this purpose are:

Retroviral vector: Are suitable for clinical testing of gene transfer and gene therapy but the major drawbacks to the use of retroviral vector is their requirement for dividing cells which limits their use in conditions such as Alzheimer's disease and the other problem, they may cause cancer (Kaplan, N.P., 1980; Glick, B.R. and Pasternak, J.J., 1994) as follow:

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Infection of a target cell

▼

Production of a DNA copy of the RNA genome, using recombinant technology that carried withinthe vision

▼

Transport of viral DNA to the nucleus

▼

Integration of viral DNA into the host genome at one of a number of different chromosomal sites

▼

Transcription of mRNA from the viral DNA that is driven by strong promoter within the 5' LTRsequence

▼

Translation in the cytoplasm of gag, poll and envelope proteins

▼

Formation of capsid and filling of this capsid with two RNA strands and recombinant technologymolecules

▼

Shedding of single membrane enveloped visions to the exterior of the cell

Adenoviral vectors: Are of interest because of their potential for in-vivo gene delivering. They are capable of efficiently infecting non-dividing cells and concentration. Preclinical studies with adenoviral vectors have been carried out involving the delivery of human genes α_1-antitrypsin and cystic fibrosis transmembrane regulator into the lungs tissues.

Adeno-associated virus (AAV): Integration of AAV genome into host DNA appears to be less efficient than retroviral integration. A number of other viral vectors designed to replace in the target cells are being developed as vector systems for application in gene therapy are:

- Herpes virus
- Vaccinia virus
- Poliovirus
- Other serial RNA viruses

Receptor-mediated endocytic pathway: For the uptake of DNA:

(i) DNA protein asialo - ovomucoidpolylysine complex which is also to target in-vivo with the samespecificity to liver cells.

(ii) Transferring DNA complex can target DNA to cells bearing the transferring receptor.

(iii) Gene transfer in specific cell lines:

- Germline gene therapy

(iv) Gene modification:

- **Replacement gene therapy:** A gene inserted in the genome so that its product could replace that of adefective gene.
- **Corrective gene therapy:** A mutant gene or a part of it replaced with a normal sequence.

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Therapeutic application of gene therapy:

- In the treatment of severe combined immuno deficiency syndrome.
- In cancer treatment.
- Familial hypercholesterolemia-by inserting low density lipoprotein receptor gene into hepatocytes.
- Severe neuropychiatric disorder, LeschNyhan Syndrome by inserting gene for the expression ofenzyme like hypoxanthine phosphoribosy transferase.
- Percutaneous transluminal coronary angioplasty.
- Restenosis-by gene inhibits growth of intimal cells.
- Malaria-by multicomponent nacked malaria DNA as a vaccine.
- Influenza-by nacked influenza virus DNA as a vaccine.
- Sickle cell anemia.
- Thalassemia.
- HIV-by fibroblasts expressing HIV envelope glycoproteins gene.
- Cancer-by implanting-
  - ❖ TNFα, IL-2&4 genes, interferon-γ, GM-CSF
  - ❖ Antisense genes
  - ❖ Multidrug resistance genes into the bone marrow cells to reduce toxicity of anticancer therapy.
  - ❖ Viral thymidine kinase into tumor cells that can convert a prodrug into toxic metabolite, that can killtumor cells selectively.
- Cystic fibrosis-may treated by regulation of expression of chloride channels. This can be achieved byinsertion of CFTR gene into respiratory epithelial cells.
- Gaucher disease.
- Amyotrophic multiple laberal psychosis
- Neurodegenerative like Alzheimer's disease, Huntington's chorea, familial amyltropic lateral sclerosis, Gaucher's disease and infectious diseases may be treated by supplementing the defective genes.
- Growth hormone deficiency may be removed by implanting cultured myoblasts transfected bygrowth gene.
- Thrombolysis-a mutant form of tPA may be inserted by adenovirus to specific clot sites and quicklylyse a clot.

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S. No.	Disease	Defective gene	References
1	Thalassemia	Hemoglobin	Herbert, L., et al., 2009
2	Sickle cell anemia	β-globin	Abbott, A., 1992; Wilson, J.F. and Jennifer, 200s
3	Severe Combined Immune Deficiency or bubble boy disease (SCID)	Adenosine deaminase	Hoggatt, J., 2016
4	Hemophilia	Factor VIII and IX	Morfini, M., et al., 2013
5	Gaucher's disease	Glucocerebrosidase	Whittington, R., Goad K.L., 1992; Stirnemann, J., et al., 2017
6	Inherited emphysema	α_1-antitrypsin	Abboud et al., 2005
7	Familial hypercholesterolemia	LDL-receptors	Soutar, A. and Naoumova, R., 2007
8	Cystic fibrosis	C.F. Transmembrane regulators	Meng, X., et al., 2017
9	Duchenne's muscular dystrophy	Dystrophin	Nowak, K.J and Davies, K.E., 2004
10	IDDM	Insulin-1	Wasserman, D.H and Zinman, B., 1994
11	Parkinsonism	Tyrosine hydroxylase	Haavik, J. andToska, K., 1998
12	Stroke, head injury, multiple sclerosis	Nerve Growth factor	Houlton, J., et al., 2019
13	Hypertension	Human tissue kallikerin	Zhang, J.J., et al., 1999
14	Anemia	Human erythropoietin	Tong, E.M. and Nissenson, A.R., 2001

Table No.: 1 : Some current and future targets for gene therapy

Rules and Regulation for rDNA technology and human gene therapy

Molecular biotechnology can potentially affect many aspects of modern society-including agricultural production and medical treatment. There are significant ethical, legal, economical and social issues that to be considered. In 1976, the united states of America (National institute of health) set some rules and regulations regarding the rDNA technology as :

(a) Used only those micro-organisms that least proliferates outside of the laboratory.
(b) Negative pressure inside the laboratory.
(c) Self-contained rooms.
(d) High quality filters system.

Human gene therapy

Aim of correcting an inherited defect in an affected person.

(a) Somatic (body) cells

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(b) Germ line cells (sperm or egg) or a fertilized egg (zygote)-it may inherited.
Through gene therapy enhancement of physical or mental attributes. Like human unable to synthesize vitamin-C but amphibians, reptiles, birds and non-primate is able to do so. So for these take genes of vitamin-C, made constrict vector, insert in human cells. Preimplantation embryo, make human germ line gene therapy feasible. Germ line gene therapy legally not permitted.

(B) ANTISENSE THERAPY

Total suppression or reduction of the expression of undesirable proteins using defined antisense oligonucleotides or nucleic acids which interact with the mRNA or the gene encoding the proteins by hybridization. Antisense RNAs or antisense oligonucleotides are used normally in nature to regulate gene expression, notably in prokaryotes and probably in eukaryotic cells, supports the use of exogenous antisense nucleic acids in regulating gene expression (UKEssays, 2018).

Inserting methods of nucleic acids into cells

A number of methods are used to insert of nucleic acids into cells:

(I) Physical methods

DNA Microinjection (Micro-injection pipette)

Microprojectile method (Particle gun, Biolistic method)

(II) Chemical methods

DNA uptake (CaSo4 method)

Cell fusion (MAC method)

(III) Electrical methods

DNA uptake (Electrophoresis)

(IV) Biological methods

Vector mediated

Liposomes

Cell fusion

(V) Receptor mediated transfer

DNA protein asialo-ovomucoid

Transferrin receptor

Functions of oligonucleotides

The suppression of gene expression can be achieved with small segments of DNA or RNA complementary to the mRNA for a protein:

- Single-stranded oligonucleotides of the order of 5-14 bases in length are capable of binding to the RNA transcript of a gene which great specificity by complementary base-paring. This binding interferes with maturation of the primary RNA transcript of the gene into a translatable mRNA and /or which translation of the resulting mRNA.
- Protein production from the mRNA may also be decreased by making the small RNAs, known as ribozymes, combine the ability to bind specifically to selected RNA species with the ability to cleave the target RNA enzymatically. The resultant destruction of the mRNA leads to a loss of protein production. These approaches are called as “antisense technologies”.

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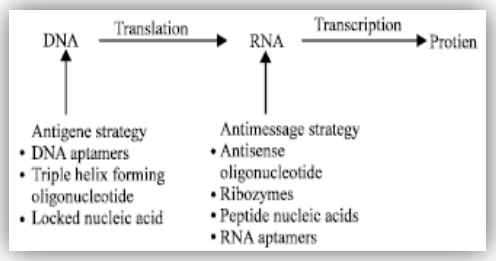
Mechanism of antisense inhibition

Anti-sense oligonucleotides may interfere with gene expression by several mechanisms-the mechanism of action is strongly dependent on the chemistry and length of the oligonucleotides.

- By administering antisense oligonucleotides exogenously.
- By producing antisense RNA by transcription of an antisense gene from within a cell.
- The antisense gene is constructed by placing a segment of the target gene, under the transcriptional control of a promoter, in the direction opposite to the normal transcriptional direction of the target gene.
- Antisense sequences hybridized to the mRNA interfere with its transcription by ribosomes.
- The bride region has also been suggested to make the RNA susceptible to nucleolytic degradation.



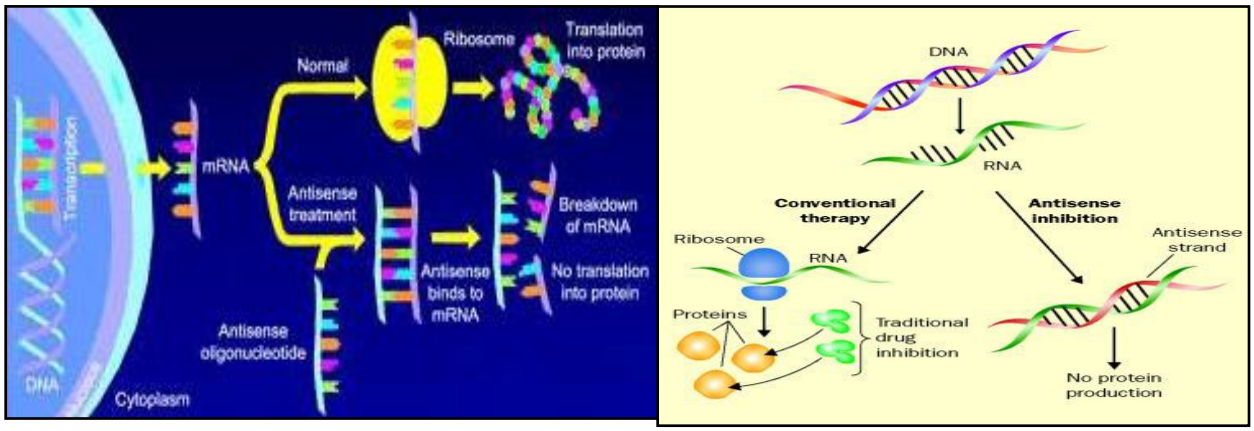


Figure No.: 1 Sites of action of antisense agents(Singh, J., et al., 2011; Gulam, M., et al., 2016)

Applications of antisense therapy (Singh, J., et al., 2011): It is useful in numerous disorders but causes fever and splenomegaly.

- Renal transplantation rejection reactions
- Cardiovascular disorders (Hypertension, Post angioplasty)
- Cancers
- Infections (viral, protozoal & fungal)
- CNS disorders (Alzheimer's disease, Cerebrovascular complications)
- Inflammatory and autoimmune disorders (asthma, Crohn's disease Psoriasis)
- Genetic disorders
- Acute myelogenous leukemia
- Chronic myelogenous leukemia
- HIV-infections

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- Genital warts caused by the human papilloma virus
- Viral infection

(C) TRIPLEX THERAPY

TRIPLEX INHIBITION OF GENE EXPRESSION

Antisense inhibition of RNA function requires the inhibition of thousands of copies of the target RNA present in the cell. However, inhibition of transcription at the level of DNA requires the inactivation of the transcription from only one or two active copies of a gene present in the genome; this is the rational for triplex inhibition of gene expression.

Some deoxyoligonucleotides are capable of complexing with double stranded DNA to form triplex helices. Resulting in transcriptional inhibition. Deoxyoligonucleotides of lengths over 15 bases can form triplex-stranded DNA by binding to the major groove of double stranded DNA. The bases in the two opposite strands of the target double stranded DNA remain hydrogen bonded to each other by crick-watson base pairing (A with T and G with C) as in the native state, but the oligonucleotide strands through a different type of base pairing. In this mode which in the oligonucleotide strand interacts with a G-C base pair of the double-stranded DNA and a T in the oligonucleotide strand interacts with an A-T pair. Further, interaction is only with one of the strands of double-stranded DNA, requiring that all the purines in the target region occur in the same strand of DNA. Thus in the most-studies current models, the binding oligonucleotides must be composed of pyrimidines C and T, and all of the purines in the target region must occurs in the same strand of the target DNA (Chan, P.P. and Glazer, P.M., 1997).

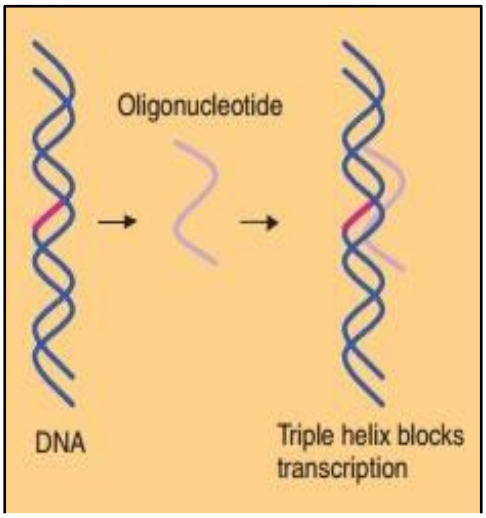

Figure No.: 2 Mechanism of Triplextherapy (Schiffelers R.M., et al.,2019)

Therapeutic potential

- To turn off the transcription of errant gene of viruses and those activated in cancers and otherdiseases.

(D) APTAMER THERAPY

Inhibition of protein activities and interference with their functions by specific, high-affinity binding of oligonucleotides to the proteins. It is now possible to screen random pools of oligonucleotides for binding to a protein and to isolate the ones that bind with high-affinity gene such oligonucleotides may themselves be useful as therapeutics or to leads for the development

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of other active molecules. This approach to therapeutic development is called aptamer therapy. Basically it is also an antisense therapy (Keefe, A., et al., 2010).

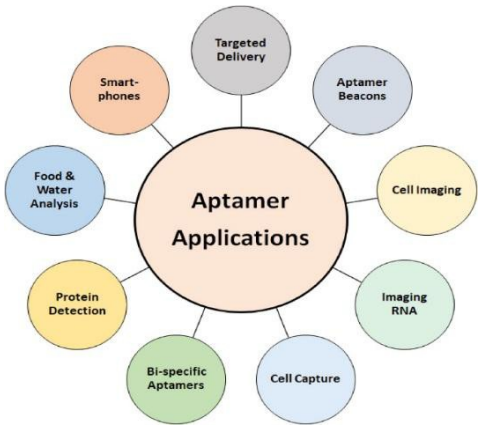

Figure No.: 3 Applications of aptamers oligonucleotide (Zhang, Y., et al., 2019)

Medical applications of aptamers oligonucleotides (Deisingh A., 2006).

Diagnostic potential

- Viruses
- Bacteria
- Parasites
- Cancers
- Inflammation
- Proteins

Therapeutic potential

- Kidney transplantation
- Skin drafting
- Aging-related complications
- Diabetes mellitus
- Cardiovascular disorders
- Blood & bone diseases
- Immunological problems
- Treatment of cancers

(E) RIBOZYME THERAPY

Ribozyme therapy was identified when an RNA precursor was found to be self-splicing; because this RNA was enzymatically active without the benefit of a protein component, it was termed a ribozyme.Ribozymes subsequently have been found not only to cleave themselves free from their RNA strand, but also to act in trans and cleave other RNA targets in a sequence-specific manner. This specificity, associated with Wastson-Crick base pairing, coupled with catalytic activity to cleave the substrate RNA, makes them potentially effective agents against mRNA of viral or oncogenic origin (Jose, A.M., 2002).

Types of ribozymes

I. Hairpin ribozyme
II. Hammerhead ribozyme

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III. Hepatitis data virus ribozyme
IV. Neurospors virus ribozyme
V. Tetrahyomenathermophila ribozyme

Construction of Ribozymes

The ability to designed ribozymes against selected RNA targets has expanded their therapeutic potential.

- Hammerhead ribozymes can be modified in their binding arms to be complementary to any nucleotide except guanosine. The optimum length of the binding arms appears to be 7/7 nucleotides on the 3' and 5' ends.
- The hairpin ribozyme, 4 nucleotides on the 5'-side and a variable number on the 3'-side of the cleavage side can be modified.
- In the HDV ribozyme only seven nucleotides at the position 5'-seide of the cleavage side need be changed to alter the specificity.

  These ribozymes are relatively small and they can be made either by chemical synthesis or by transcription from viral or non-viral vectors. Approximately 15 nucleotides, seven each in the 3'- and 5' arms and one at the catalytic core, is an optimal binding.

Methods of inserting Ribozymes

I. Receptors-mediated endocytosis.
II. By an oligonucleotide is incorporated into a vector.
  - Retroviruses
  - Adeno-associated viruses
  - Ribozymes vectors also delivered by aerosol to the lungs epithelium and target tissue ex-vivo (e.g.bone marrow).
III. Liposomes can sometimes facilitate cell-specific attachment and entry.
IV. An alternative method is the use of cationic lipids.

Mechanism of action : Ribozymes cleave their target either by transesterification or by hydrolysis (Figure: 4).The ribozyme has two domains; the catalytic domain acts as an enzyme, and the recognition domain binds to a specific target RNA. Once bound, the enzyme domain cleaves the target RNA (brainkart.com).

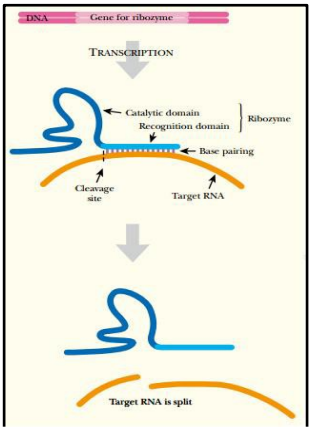

Figure No.: 4 Mechanism of Ribozyme Therapy

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Applications of Ribozyme Therapy (Lewin, A.S. and Hauswirth, W.W., 2001)

Therapeutic applications of the ribozymes are potentially broad and include use in viral infection and cancer. By causing changes in specific genes (H-ras, C-fos gene, bcr/abl gene, rex/tax gene) controlling proliferation. Most of the oncogenes were originally isolated as viruses containing genes of non-primates origin. These genes are mutations of proto-oncogenes, which are activated during embryogenesis, cell growth or specific tissue regeneration. Because oncogenes are over expressed and produce RNA that is distinguishable from the proto-oncogene, they are potentially excellent targets for ribozyme therapeutic activity.Ribozymes offers many therapeutic uses :

- The mRNA for any protein that is causative in a disease is a potential ribozyme target.
- Micro-organisms especially viruses, are targets for ribozyme therapy.
- Ribozymes to the arterial wall may be used to modify the restenotic process after angioplasty.
- A synthetic ribozyme could be injected into a joint space to suppress an inflammatory process byeliminating a particular cytokines or enzyme in the process.
- A viral infection in the lungs can be treated by aerosolization of ribozymes.
- Ribozyme delivered by a vector will be more uses systemic tissue (infection or non-infections) anddisease of a chronic nature (local or systemic).

Conclusion:

The recent development in nucleic acid therapies have provided an opportunity to modify or reduce genes which are responsible for causing disease. A perfect method to isolate specific gene sequence and insertions into desire targets is still require improvement. These therapies are really a firm dream in the development of novel methods to diagnose or treat various genetic disorders. The present review will help in understanding of nucleic acid therapies and also improve the knowledge about their applications.

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