Antisense technology

Contents ;

Antisense technology ;

- Antisense technology is recently approches of specific modification or inhibition of gene.
- It is a tool used to study gene function and also utilize to manipulate the gene expression(to treat numbers of diseases).
- Sense strand/sequences – it is the coding strand within double- standard DNA that carries the translatable code in the 5’ to 3’ direction. It is complementary to the template strand the sense strand have sequence similar to mRNA.

- Antisense strand – it is the template strand of dsDNA, from which mRNA is transcribed(thus, the antisense strand is complementary to mRNA).
- In simple term “ sense” refers to original sequences(DNA or RNA) while “ Antisense “ refer to complementary copy.
- The antisense strand have base pair with its complementary mRNAstrand (sense RNA) and translated into a protein.

What is antisense technology??

- Controlling gene expression in a cell (by using synthesis antisense oligonucleotide).
- It target gene at the level of mRNA (rather than DNA).
- Preventing them to produce protein.

Using antisense oligonucleotide (AO)?

- Antisense oligonucleotides (AS ONs) are synthesize DNA oligomers that hybridize to a target RNA in a sequence-specific manner.

- They are successfully been employed to inhibit gene expression, modulate splicing of a precursor messenger RNA, or inactivate microRNAs.

- **Work ;** small piece of DNA or RNA that can bind to specific molecule of RNA. This blocks the ability of the RNA to make a protein or work in other ways (block the production of protein needed for cell growth)

- **How long (ASOs) work ;** chemistry and chemistry design, the effect of single- stranded phosphorothioate. ASOs on gene expression can last from 6 weeks to more than 6 month (single injection).

Mechanism of Antisense activity ;

- Rapid, specific and high throughout technique has enormous potential inresearch as well as treating a range of diseases including cancer, viral infection, cardiovascular, neurological and ocular diseases.

1. Antisemse oligonucleotides(AO) are synthetic, short, single- standard nucleotides that inhibit transcription of target gene.
2. By binding with high specificity to complementary mRNA sequence by watson and crick base pairing, forming a dimer.

3.Bound to the mRNA, antisense oligonucleotide(AO) advancing to protein synthesis by preventing access to the ribosome.

4.Occpying the mRNA protein, preventing translation and splicing;(by targeting the dimer for degradation by ribonuclease H1

5. Commonly utilized clinically by various antisense therapeutics.

Technical problem in Antisense technology

- Antisense oligonucleotide (AO) is had little therapeutic effect due to several difficulties in the mRNA inhibition.
- These issue includes inadequate tissue distribution and cell permeability, low specificity to their mRNA targets.
- High specificity is necessary to prevent toxicity by inhibition of undesired protein in fundamental cellular process (short half-life, not able to surppress gene expression for significance period).

- Antisense therapeutics is approved for clinical use nowadays.
- Chemical modification to the oligonucleotide have improved their stability and increased their resistance to ribonuclease digestion.

Applications ;

Antisense in viral infection :

- it has always been difficult to design and develop antiviral drug with low toxicity and improved specificity.
- Antisense technology can be easily applied to viruses.
- Antisense oligonucleotides are selective and highly specific and binds with targeted viral mRNA and down regulate the expression
- **VITRAVENE (Fomivirsen)** is the first antiviral antisense drugs used to treat cytomegalovirus retinitis (including human papillomavirus, HIV, hepatitis- B virus, influenza A virus and hrepes simplex virus.

- Antisense in cancer ;

1. Antisense drugs are less toxic then convertional drugs, many antisense oligonucleotides are currently investigation to treat various cancer in human or for the deactivation of oncogenes.

2. For the first time an antisense oligonucleotides in combination with **CISPLATIN** was approved to treat bladder cancer.

- Antisense in genetic research ;

1. The most widely used application of this technology is in gene therapy.
2. Antisense oligonucleotides are being used in genetic research to treat various genetic disorders.
3. In genetic disorders, some point mutations resulting in the formation of a defective mRNA and then defective protein.
4. The therapeutic objective of antisense technology is to block formation of defective protein form defective mRNA.
5. **B- THALESSEMIA** , genetic blood disorder can be completely treated using anisense technology in which o- alkly oligonucleotide or morpholino- oligonucleotides have been used.

- **Medical Applications of Antisense technology ;**

1. Antisense technology is used to investigate protein function in the living brain to study central nervous system(CNS) protein such as transmembrane receptors, ion channel, transport G- protein and growth factor
2. Antisense oligonuckeotides can be used to inhibit expression of a particular enzyme. For eg; inhibition of acetyl cholinesterase enzyme is the molecular target for the treatment of diseases like Alzheimer's diseases.

3. In inflammatory diseases, antisense oligonuckeotides is used to demonstrate the relative importance of various signalling components at the molecular level.

4.A novel respirable antisense drug called **RASONS** is used to treat various respiratory diseases including asthma, influenza, bronchitis, pulmonary fibrosis, pnemonia and lung cancer.

5. Antisense oligonucleotides can be used target specific membrane component that influences the pathophysiological mechanisms is renal and cardiovascular disorders.

Conclusion ;

- Antisense technology shows potential for diverse application to field of basic research & therapy.
- One of the most approved approches for inactivating a single specific gene.
- But it may sometime give undesirable.

- Generally, antisense RNA still lack effective design, biological activity, and efficient result of administration.

- Antisense technology form a very powerful weapon for studying gene function and for discovering more specific treatment of diseases.

References ;

- Life science 2019, Pranav Kumar and Usha Mina, Pathfinder Publication, New Delhi – India.

Ribozyme technology

Contents ;

What is Ribozymes ;

- Ribonucleic acid capable of catalyzing a chemical reaction.
- Vital for many biology process
- Natural ribozyme catalyze phosphodiester transfer or hydrolysis.
- A ribozyme(ribonucleic acid emzyme) is an RNA

That is capable of performing specific biochemical reactions, similar to the action of protein enzymes.

What is Ribozyme technology ??

- Ribozyme (ribonucleic acid enzyme) are molecules that have the ability to catalyze specific biochemical reactions, Including splicing in gene expression , similar to the action of protein enzyme.

- **Ribozyme ;** Ribozyme are catalytically active RNA molecule or RNA- protein complexes, in which RNA provide catalytic activity.

- **Example ;** small ribozyme include the hammerhead, the hairpin, the hepatitis delta ribozyme (made up 3000 nucleotides).

Kind of function ;

1. Transfer RNA maturation
2. Intron splicing
3. Replication of RNA virus or viroids
4. The regulation of mRNA stability
5. Protein synthesis

Catalytic Activities of Ribozymes ;

- Ribozyme are RNA molecules encoded with catalytic activity and capable of cleaving mRNA molecules in a sequence activity, catalytic manner.

- They contain sequences for selective ligation with target mRNAs which confers upon them high specificity.

- They also cantain sequences that perform cleavage reactions with the target mRNA.

- By modifying the substrate recognizing sequences ribozyme can be specifically tailored for the suppression of particular genes.

Types of ribozyme technology ;

1. Ribozyme may be classified into natural ribozymes and artificial ribozyme.

2. Natural ribozyme includes : peptidyl transferase 23S rRNA,RNase P, Group I & Group II intron , G1 R1 branching ribozyme leadzyme, hairpin ribozyme, Hammerhead ribozyme, HDV ribozyme, mammalian CPEB2 ribozyme, VS ribozyme, glms ribozyme, COTC ribozyme

- Artificial ribozyme are synthesised in the laboratory based on the dual nature of RNAs as a catalyst and an informational polymer.

- **23s rRNA** ; 23s rRNA has peptidyl transferase activity.

- Mutation in 23s rRNA, but not in any of the resistance to antibiotics that inhibit gene peptide bond formation

- Extraction of almost all the protein content of 50s subunit leaving <5 % of r – protein retain peptidyl transferase activity.

However, treatments that damage RNA abolish the catalytic activity.

- 23s rRNA prepared by invitro transcription can catalyze the formation of a peptide bond althrough with low efficiency.

- **RNase P ;** Ribonuclease P(RNase P), a ribonucleoprotein, is an essential tRNA processing enzyme found in all living organisms.

- **Hairpin Ribozyme ;** the hairpin ribozyme is a small section of RNA that can act as enzyme known as a ribozyme.
- first identified in the minus strand of the tabacco ring spot virus(TRSV).

- **Hammerhead Ribozyme ;** the hammerhead ribozyme is a RNA module that catalyze reversible cleavage and joining reaction at a specific site within an RNA molecule

- The minimal catalytic sequence active consists of three base- paired stems flanking a centre core of 15 conserved nucleotides.

- Hammerhead ribozyme play an important role as

1. Therapeutic agents
2. Biosensers, and
3. Its applications in functional genomics and gene discovery

- **Artificial Ribozyme :** the synthetic ribozyme made in the laboratory are known as **artifical ribozyme.**

- **Tang & Breaker** isolated self- cleaving RNA by invitro selection of RNAs originating from random – sequence RNAs.

- This approch take advantages of RNAs dual nature as both as catalyst and an informational polymer.

- The ribozyme are mutated by reverse transcribing them with **reverse transcription** into various **cDNA** and amplified with **mutagenic PCR.**

- **Lincoln and joyce** developed an RNA enzyme system capable of self replication by utilizing molecular competition (invitro evolution).

Application ;

A type of synthesis ribozyme durected agaisnt **HIV** shears has been developed and has entered clinical testing for HIV infection.

- **Ribozymes for Human therapy ;** the ability of ribozyme to recognize and cut specific RNA molecules makes them exciting candidate for human therapy.