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Review Article

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GENE THERAPY: A RECENT ADVANCES IN COVID – 19

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ABSTRACT

As gene therapy is one of the blistering themes of the latest century, it carries the enthusiasm of a cure to most of diseases, the dispute surrounding the altering of human defects, and the promise of a type of medical therapy most of us would never imagine possible. Gene therapy became feasible through the implementation of genetics and bioengineering that permit exploit vectors for delivery of extrachromosomal material to target cells. Safe techniques have been fabricated to do this, by means of several viral and non –viral vectors. Coronavirus disease(COVID-1 9) is a particular infectious respiratory disease caused by a newly discovered coronavirus, SARS-CoV-2.There are several challenges associated with the treatment of the COVID-19 during the contemporary outbreak. The virus is the positive strand RNA one with high homology to bat coronavirus. The pathogenic mechanism of the new coronavirus is still unclear, which is a significant obstacle to the development of drugs and patients rescue. The broadcasting and epidemic of the novel coronavirus highlighted the urgent need for new therapeutic technologies. Molecular biology tools that include gene editing approaches such as CRISPR-Cas12/13-based SHERLOCK, DETECTR,CARVER and PAC-MAN, antisence oligonucleotides, antisence peptide nucleic acids, ribozymes, aptamers and RNAi silencing approaches fabricated with cutting edge scientific advances equated to conventional diagnostic or treatment methods could be vital in COVID-19 and other future outbreaks. Thus in this review, we will explore potent of molecular biology approaches that can transfigure treatment of viral infections and therapies to fight COVID-19 in a highly specific, stable and efficient way.

KEYWORDS: Gene therapy, vectors, SARS-CoV-2, Novel CRISPR approaches.

INTRODUCTION

Defination: Gene therapy is the introduction of genes into the existing cells to prevent or treat a wide range of diseases. It is a procedure for correcting defective genes responsible for disease development. The first authorized gene therapy experiment occurred on September 14,1990 in US, when ASHANTI DESILVA was diagnosed for ADA-SCID. The major objective of gene therapy is to instigate a functional gene into a target cell and restore protein production that is absent or deficient due to genetic disorder. Gene delivery system is presently implemented in the treatment of diseases like cystic fibrosis, cardiovascular diseases, cancer and auto-immune disorders.

What Is Gene Delivery

- It is an experimental procedure to improve defective genes that are responsible for disease development
- The most common form of gene therapy involves introduction of a normal gene to replace an non-typical gene.
- A carrier molecule called vector transfers the therapeutic gene to patients target cells.
- Vectors generally used are viruses.
- Target cells such as the patients liver and lungs are injected with the viral or non viral vectors.
- The vector then discharges its genetic substance holding the therapeutic gene into the target cells.

Two Types of Gene Therapy

1) **Somatic Gene Therapy:** It includes launching a good gene into targeted cells with the end result of treating the patient - but not the patient future children because these genes do not get moved along to offspring's. In other words, even though some of the patients genes may be adapted to treat a disease, the possibility remains the same disease will overwhelm the patients children. This is the configuration of gene therapy that is used at most genetic laboratories throughout the world.
2) **Germline Gene Therapy:** It involves injecting foreign genes into fertilized eggs or in sperms fabricating cells, which will then pass any genetic modifications to future generations as well. However, although it has potential for obstructing inherited disease, this form of gene therapy is extremely disputable and presently very little

research is being done in this area, both for technical and ethical reasons.

ADVANTAGES

- A gene therapy has the capability to terminate and inhibit hereditary diseases such as cystic fibrosis, ADA-SCID etc
- It is feasible to treat for heart diseases, AIDS and cancers
- It gives individuals born with a genetic disease a chance to life
- It can be used to exterminate diseases from the future generations

Disadvantages

- Long lasting therapy is not attained by gene therapy, due to immediate dividing of cells, use-fullness of gene therapy is short lived
- Immune response to the inserted gene stimulates a potential endanger to gene therapy
- Disorders caused by deficiency in multiple gene cannot be diagnosed effectively using gene therapy
- Viruses used as vectors for gene transfer may be the reasons for toxicity, immune responses and inflammatory reactions in the host
- The cost is very high and the patient might need an insurance to cover the treatment
- The genetic testing, screening and research in finding the accessibility of certain gene is very controversy

Possible Applications or Potential Diseases Treated By Gene Therapy

A. Cancer Treatment: It includes the initiation of tumor suppressor genes, genes that produce apoptosis, genes that hinder tumor angiogenesis and genes coding for an enzyme to transform prodrugs to active drugs and immuno therapy. Some cancers such as malignant melanoma are exceptionally sensitive to immuno therapy, thus gene therapy and vaccines are being evolved against such cancers. Therapies used for certain cancers are immunotherapy, oncolytic virotherapy and gene therapy for different types of cancers such as lung cancer, metastatic cancer, prostate cancers and in solid tumors.EG: p53 is the defective genes progressed for cancer patients

B. Neurological Disorders: Disorders which can be treated by gene therapy are parkinsons disease, Alzheimer's disease and motor neuran disease. EG: prosavin is presently being trailed for parkinsons disease, which is produced by dopamine deficiency in the brain. Prosavin distributes into the brain via a lentivirus vector.

C. Inherited diseases

1) HAEMOPHILIA: Therapies are intended to transfer genes that express missing factors VIII and IX, persons would no longer need to administer exogenous clotting factors, research is going on with adeno-associated virus and lentivirus vectors

2) CYSTIC FIBROSIS: It is caused by mutations in the gene called "cystic fibrosis transmembrane conductance regulator(CFTR)".This mutations results in unusual ion transportation within lung and other cells.

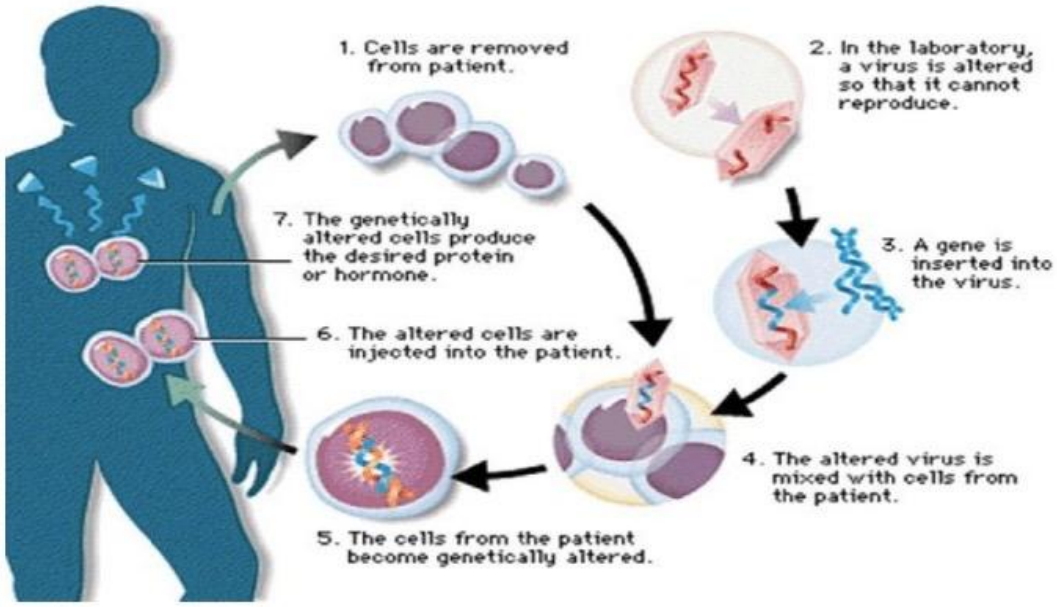
3) INHERITED RETINAL DEGENERATION: Occular gene therapies could provide a treatment strategy for people with inherited retinal degeneration.EG: Leber congenital amaurosis type 2, a type of blindness which can be treated by gene therapy

4) SEVERE COMBINED IMMUNO DEFICIENCIES(SCID): It is an inherited disorder that leaves affected children without fully functional immune systems. The most common types of SCID are SCID-ADA and SCID-XI.EG: retroviral vectors to deliver the ADA gene to patients with SCID-ADA

D. CARDIAC DISEASES: Gene therapy has been examined to target angiogenesis(the generation of new blood vessels) during cardiac surgery and to upgrade calcium-handling mechanisms in heart failures.

Target cells for gene transfer:

DISEASE	TARGET CELLS
Cancer	Tumor cells, antigen presenting cells(APCs),fibroblasts muscle cells
Inherited monogenic disease	Lung epithe;ial cells, macrophages,T cells
Infectious disease	Blood progenitor cells,muscle cells
Cardiovascular disease	Endothelial cells, muscle cells
Rheumatoid arthritis	Sinovial lining cells
Cubital tunnel syndrome	Nerve cells



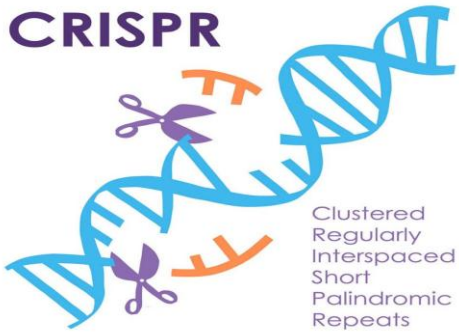
Steps involved in gene therapy.

Gene Editing And Irna Approaches For Covid-19 Diagnostics And Therapeutics

The commencing cases of novel coronavirus infected pneumonia(NCIP) seen in wuhan, Hubei province, china, on december 2019 in january 2020.COVID- 19 is a exceedingly contagious acute respiratory disease caused by severe acute respiratory syndrome related coronavirus (SARS-CoV-2). Firstly, the high number of cases overcomes diagnostic test proportions and proposes the need for a immediate solution for sample detections. Secondly, SARS-CoV-2 is nearly related to other significant coronavirus species and subspecies, so observation assays can give false-positive results if they are not effectively specific to SARS-CoV-2. Thirdly, patients with suspected SARS-CoV-2 infection sometimes have a divergent respiratory viral infection or co-infections with SARS-CoV-2 and other respiratory viruses.

- SARS-CoV-2 is an RNA virus that could be targeted and treated with the novel gene editing approaches called CRISPR approaches.
- Anti sense peptide nucleic acids, which have remarkable potential as antiviral agents, are crutial therapeutic candidates that can attack SARS-CoV-2 RNA with hybridiation affinity and stability.
- Although ribozyme and aptamer technologies are comparatively older than other gene-editing methods, that are still stronge candidates in the fight against the COVID-19 pandemic due to their high specificities and low toxicities, besides that aptamers are highly reproducible
- siRNAs that target structural and non-structural proteins of SARS-CoV-2, could hinder assembly and replication of SARS-CoV-2.

CRISPR-Cas13 IS ADAPTED AS AN ANTIVIRAL SYSTEM AGAINST SARS-CoV-2



CRISPR (Clustered regularly interspaced short palindromic repeats) systems have enhanced our ability to edit genes and regulate gene expression.The mechanism naturally safeguard bacteria against invading bacteriophages and other foreign nucleic acids.CRISPR effectors can suppress replication of double stranded DNA viruses or single stranded RNA viruses with DNA intermediates in mammalian cells.A technique called SHERLOCK was given by feng hangs group.This method allows multi-plexed, portable and ultra sensitive detection of RNA or DNA from clinically relevant samples.Open access SHERLOCK research protocols and design resources for SARS-CoV-2 are using CRISPR-Cas13.The Cas-13 enzymes can target and devastate the gene without editing the genome, which makes it a potential therapeutic for interfering gene expression without permanently modifying genome sequence.

Around two-thirds of the viruses that can infect humans, involving deadly Ebola, Zika and flu, have an ssRNA genome and there is no FDA- approved treatments for any of these type of viruses. Human viral pathogens are very complex and develop immediately, underlying both the developmental challenges and a great need for flexible antiviral therapeutic platforms.The technique harnesses the programmable RNA- targeting activity of Cas13 to build an end to end development platform called Cas13-assited viral expression and readout(CARVER), which integrates Cas13-mediated viral RNA cleavage with a fast, Cas13 based treatment readout using SHERLOCK platform.The development of CRISPR-Cas13 enzyme based technology named CARVER, which can be figured to kill or destroy RNA - based viruses in human cells.The researches designed and analyzed two RNA guides that acknowledged two sinatures of COVID-19 by using synthetic SARS-CoV-2 RNA fragments. By using CRISPR-Cas13 riboprotein complex that forms the SHERLOCK system, it is experienced to detect the presence of SARS-CoV-2 viral RNA in a COVID-19 patient specimen.

- The SHERLOCK COVID-19 virus identification method involves two major steps

1) The synthetic viral RNA amplification is employing recombinase polymerase amplification(RPA) method that is followed by invitro amplified DNA transcription back into RNA
2) Cas-13 RNA detection and crRNA targeting specific sequences.visual color readout utilizing a commercially accessible paper dipstick captures which cleaved reporter RNA with labrled ends on specific antibody bands.

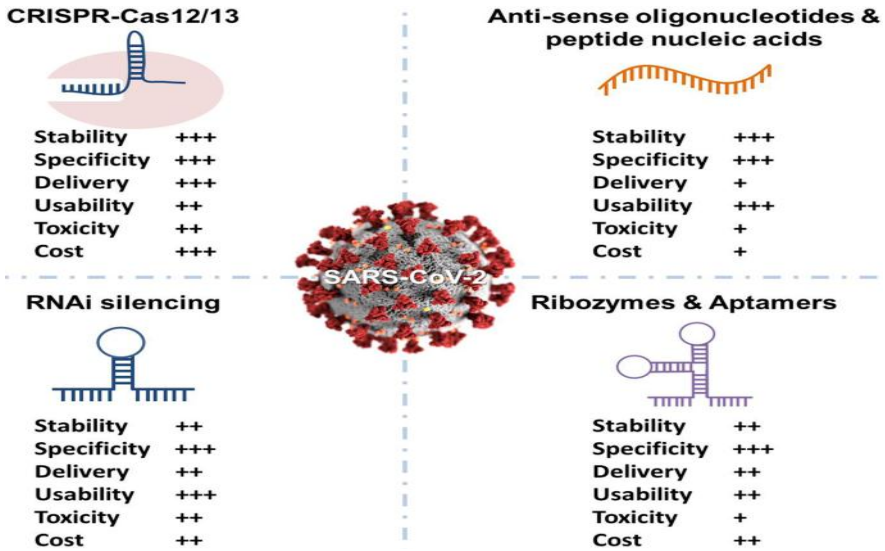
The investigation showed that SHERLOCK could identify coronavirus target RNA sequences with a sensitivity of 10-100 copies/µl of patient specimen. This recommends that RNA purified from patient nasal samples could be tested for the presence of COVID-19 in less than 1hr without special instrumentation.

rNAI Based Approaches For Covid-19 Therapy

RNA interference(RNAi) mechanism has been utilized for the silencing of genes that are linked to cancer, viral infections and autoimmune diseases. MicroRNAs, siRNAs and shRNAs are the essential molecules for the activation of RNAi mechanisma.Two different methods have been identified for siRNA mediated RNA interference. One is the RNA based method; synthetically produced siRNAs can be moved to target cells specific transporters. Another one method is the intracellular production of siRNA via DNA based strategies.

siRNA can be employed for the silencing of SARS-CoV-2 genes which encode structural and nsps. Genomes of SARS-CoV-2 encode four structural proteins, they are envelope(E), membrane(M), nucleocapsid(N) and spike(S) proteins which are significant for the assembly of the virion and inhibition of viral replication in humans. siRNAS have been shown to suppress SARS-CoV-2 gene expression. Along with gene suppressing expression, it also inhibits virus replication.As a different approach towards the COVID-19, instead of targeting SARS-CoV-2 genes by siRNA, the leader sequences of SARS-CoV-2 genes were targeted.

For the treatment of COVID-19, an appropriate invivo transport strategy should be selected when invitro analysis and characterization of siRNAs targeted to SARS-CoV-2 genes is complete.Naked siRNA molecules cannot effectively pass through the cell wall due to their large sie and high negative charges density. Acute lung injury has been entrenched because SARS-CoV-2 and SARS-CoV-2 viruses infect lung type-2 epithelial cells.For COVID-19, a respiratory disease, enlarged siRNAs, are given to patients via pulmonary route by inhalation, intratracheal aerosol delivery, intranasal delivery.



CONCLUSION

Coronavirus disease(COVID-19) is a peculiarly infectious respiratory disease produced by a newly identified coronavirus, SARS-CoV-2. The explosure and epidemic of the novel coronavirus highlighted the serious need for new therapeutic technologies that are fast, precise, stable, easy to manufacture and target specific for surveillance and treatment. Molecular biology tools that comprise gene editing approaches such as CRISPR-Cas12/13 based SHERLOCK, RNAi silencing therapies produced with cutting edge scientific advances compared to conventional daignostic or treatment techniques could be vital in COVID-19 and other future outbreaks. Thus, in this review, we outlined the robust molecular biology toolbox that is studied for treatment for SARS-CoV-2 and daignosis against the viral infections, which can be alternative biotechnological drugs in the future to conventional vaccines and drugs.

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