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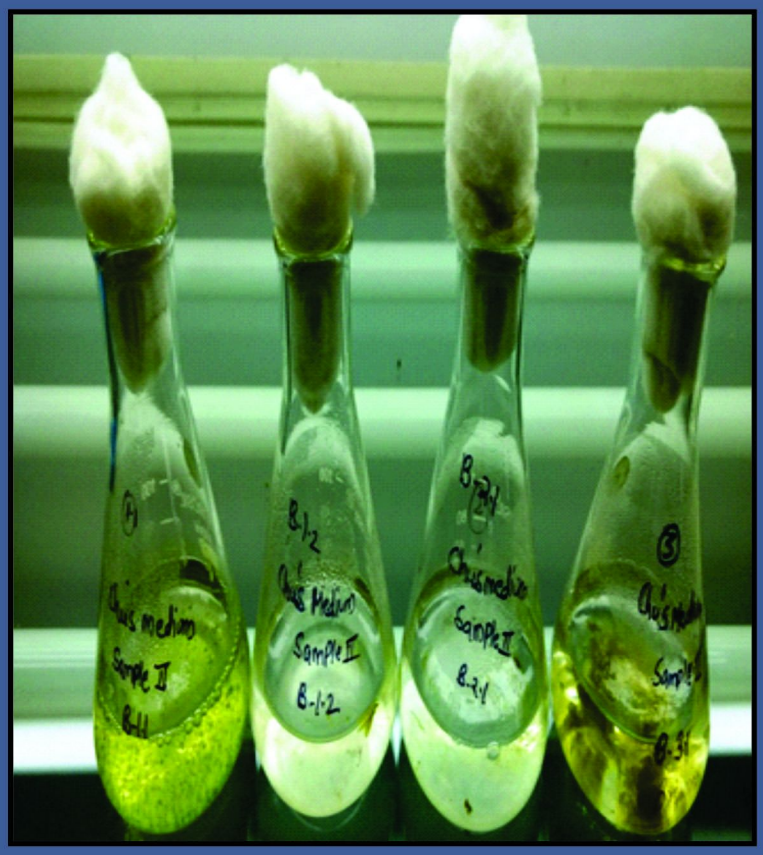
Basics of Molecular Therapeutics



JV'n Dr. Ritu Singh Rajput

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Basics of

Molecular Therapeutics

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Index

Chapter 1: Gene Therapy

Gene therapy is the introduction of genes into existing cells to prevent or cure a wide range of diseases.

Non-viral vectors in gene therapy:

Non-viral vectors are biocompatible moieties that are easy for cell or tissue targeting, easy to manipulate and can be produced on a large scale. The only drawback of a non-viral vector is its reduced cell or tissue transfection effectiveness. Different genetic materials such as antisense oligonucleotide (AON), plasmid DNA, small interfering RNA (siRNA), short hairpin RNA (shRNA), and micro RNA (miRNA) that acts on electrostatic interaction are used by non-viral delivery systems. On the other hand, cationic vectors contribute to the development of lipoplexes and polyplexes, such as lipids and polymers.

Liposomes in gene therapy:

The name liposome derives from two Greek words, "lipos" meaning fat and "soma" meaning body. A liposome is a tiny bubble (vehicle) formed from a cell membrane of the same content. Liposomes can be packed with DNA and can be used to treat cancer or other conditions. Phospholipids, which are molecules with a head group and a tail group, are typically made from membranes. The head is hydrophilic and the tail is hydrophobic and has a long hydrocarbon chain. Phospholipids are normally bilayer-stable membranes. There are a variety of structures formed during the packaging of the polynucleotide content in the liposome. Each shaped structure, based on characteristics of specific lipids used, is the most energetically favourable conformation. To show what form the amphiphile would take depending on the size variables, a dependent term known as the structure-packing parameter may be used.

Lipoplexes and polyplexes in gene therapy:

It must be shielded from damage to enhance the transmission of DNA into the cell, and its entrance into the cell must be encouraged. Due to their capacity to shield DNA from unwanted degradation during the transfection process, lipoplexes and polyplexes are used for this. In an ordered structure like a micelle or liposome, plasmid DNAs are coated by lipids. It is called lipoplex when the ordered structure is compounded with DNA. Three kinds of lipids

are available: cationic, anionic and neutral. For gene distribution, cationic lipids are more favoured because they are positively charged and thus well-complex with negative charged DNA and interact with the cell membrane, endocytose the cell and eventually release the DNA.

Naked DNA in gene therapy:

As a vector, the simplest non-viral gene distribution mechanism utilises naked DNA. Elevated levels of gene expression are created by direct injection of free DNA into certain tissues, especially muscles. In specific, it is applied to cancer tissues where the DNA may be either directly injected into the tumour or injected into the muscle to transmit tumour antigens that may act as a cancer vaccine. It may also be used to cure tissue genetic disorders, such as skin, and are available for direct injection. But the expression is typically non-uniform and the underlying histological and functional defects of the condition are not corrected. While it contributes to gene expression, there is a far lower degree of expression than in either viral or liposomal vectors. It is unsuitable due to the presence of serum nuclease for systematic administration. Its use is also limited to muscle cells and skin.

Immunotherapy:

Many of the gene therapy trials for cancer include immunotherapy. It has been well founded that cancer cells are weakly immunogenic because they are self-controlled and host immune responses are regulated in several ways. Many of the cancer gene therapy-related experiments include ex vivo tumour cell modulation to increase the development of interleukins, interferon gamma, and tumour necrosis factor. In comparison, in certain types of tumours, irradiated tumour cells should be used as vaccines. In order to strengthen the immune response against cancer, antigen presenting cells such as dendritic cells removed from a patient are currently modulated.

Gene therapy for infectious diseases:

Gene therapy may be an attractive tool for certain global infectious diseases, such as HIV, to be treated. More than 15 million people worldwide are infected by the disease, causing a drop in CD4+ cells in the body, leaving patients immunocompromised. Targeting siRNA to prevent virus replication, suicide gene therapy using adenoviral vectors, and transduction of HIV coat protein into patients to stimulate immune response are the latest methods. In addition, under tissue-specific promoter regulation, the cloning of the immunogenic gene into an effective plasmid expression vector can be used as a DNA vaccine to induce an immune response against many viral and bacterial diseases.

Virosome:

Virosomes are a delivery mechanism for medications or vaccines. It is a virus-like particle that serves as a carrier and adjuvant of the vaccine and therefore acts as an immune enhancement mechanism. Vaccines developed using virosome technology display high effectiveness with high purity and are thus a safe and efficient method of vaccinating babies and adults. Virosomes are viral envelopes reconstituted without the viral genome, comprising membrane lipids and viral spike glycoproteins. They are particularly effective as vaccine antigens and adjuvants because, because of the existence of viral glycoproteins, they induce humoral immune response. The primary benefit of virosomes over other drug delivery mechanisms, such as the liposomal and proteoliposomal carrier system, is that virosomes shield pharmaceutically active compounds from endosomal proteolytic degradation and low pH. This causes the substance to be intact until it enters the cytoplasm. The most widely used influenza virus is for the development of virosomes.

Thus, it would have hemagglutinin (HA) and neuraminidase (NA) glycoproteins of the influenza virus inside the phospholipid bilayer membrane in this case. It is possible to integrate different ligands, such as cytokines, peptides, monoclonal antibodies (MAbs), into virosomes and display them on the virosomal surface. HA protein virosomes have special fusion activity properties owing to the existence of influenza. By receptor mediated endocytosis, virosomes bind to the cell surface and enter the cell. The material of the virosome is released from the endosome into the cytoplasm of the target cell because of the presence of an acidic environment in the cell. Virosome technology is also an effective strategy for the transmission of particular immune stimulatory molecules encoding virus

antigens or DNA/RNA. It is unique to the antigen and can activate the immune response of all cells and antibodies to improve defence against the targeted disease. They are fully biodegradable, in addition to this.

Somatic cell gene therapy:

All the cells found in the body except the germ cells are somatic cells (oocytes and spermatocytes). Somatic gene therapy is not inheritable because, like liver cells, the new DNA is injected into a somatic cell of the body and thus the DNA does not reach the germ cells. In a person suffering from some genetic ailment, this transfer corrects the defect or disease. This procedure used for gene transfer can be either virus-mediated or liposome-mediated. In the nucleus, the DNA implanted is sometimes incorporated into the chromosome. Like bone marrow cells, the cells should be easy to separate and re-implant so they continue to differentiate for the whole life of the person producing blood cells. Therefore, illnesses that have a presence in the blood can be healed in this manner. The gene may also be inserted anywhere the target organ or tissue is found, such as the lungs, liver, muscle.

There are several concerns with somatic gene therapy, such as the possibility of mixing the viral vector with the cellular genome and thereby infecting the host. This is considered a viral escape. Activation of a proto-oncogene or disruption of an essential gene is another possibility. In somatic cell gene therapy, there are some obstacles to conquer. One is to target the cell or tissue needed to insert the gene. At times, viruses can be dangerous. For example, the right cell for cystic fibrosis should be located; the target should be the lungs and the stomach. Aerosol sprays should be used to deliver the gene to the lungs and the gene to be transmitted to the intestine can be packaged such that it swallows and prevents them from digestive enzymes before they operate.

The implantation of cells as an in vivo supply of some important biomolecules such as an enzyme, cytokine or coagulation factor, the transduction of activated lymphocytes, natural killer cells and cell populations such as hepatocytes or myocytes implicated in the execution of specific biological functions constitute somatic cell gene therapies. The original method to gene therapy was to insert the patient with the changed cell population, which is then altered by inserting a retroviral vector that expresses the corrected gene. The complexities of the treatment include the association of cells with other elements of the body, meaning that the

whole science should be evaluated with the greatest caution before being used for preclinical trials.

Target site for gene therapy

There are different target sites for the gene therapy

Endothelium: The advantage of using endothelium as a target site for gene therapy includes formation of new blood vessels and ease to get the gene of interest directly into the blood stream. Eg- Hemophilia

Muscle: It is easily accessible to blood stream and muscle specific promoter is well studied. Eg- Duchenne Muscular Dystrophy.

Liver: It has a capacity to regenerate and involve in a variety of functions. Thyroid binding promoter is well studied for liver specific gene transfer. Eg- Familial hypercholesterolemia.

Skin: Grafting of skin is possible and small piece can be grown to a large.

Brain: Important for nervous system related illnesses.

Lung: Airway epithelium is easily accessible organ. Eg- gene therapy for cystic fibrosis.

Transcriptional targeting approach

The specific cells or tissue can be transduced by a vector designed by using specific promoter that directs the expression of gene of interest. The gene expression of these vectors is only possible when the transcription factor proteins can bind to these promoters. Some of the promoter used for expressing the transgene is prostate specific antigen, cytomegalovirus promoter, β-actin promoter, and hypoxia inducible factors.

Germ line gene therapy

In germ-line gene therapy, the germ cells (sperm and egg) are manipulated. In germ cells or early embryos, foreign DNA is inserted. The value is that they are readily accessible outside the body and can thus be extracted and modified, and the implanted gene can be present in all

the patient's cells and can therefore be passed on to future generations. It would thus be inheritable. It is ideal for those who have inherited disabilities that are inheritable. But the issue is that the gene is targeted to the appropriate place when it may be inserted into the cell genome at any other location, inducing mutation or destroying a normal functioning gene.

Desirable improvements or modifications in the desired genes can be carried out in two ways in germline gene therapy. Until fertilisation, the genetic constitution of germ cells is changed in the first type and hence the changes in the genetic constitution move on in the form of mutation to the later generations. In today's world, though,

Changes in the genetic constitution was incorporated in the early stages of blastomere in the second form of germline gene therapy. In this form, the altered cells are able to differentiate and grow into an individual and the genetic modifications are obtained by just a few of these cells and the rest do not. Only if the cells acquiring the genetic modifications transform into germ cells of the body would the mutation be hereditary.

Germ line gene therapy has recently begun, while studies in poultry and some rodents have been performed. As no experiments have been carried out on humans to date, this treatment is also ethically unethical.

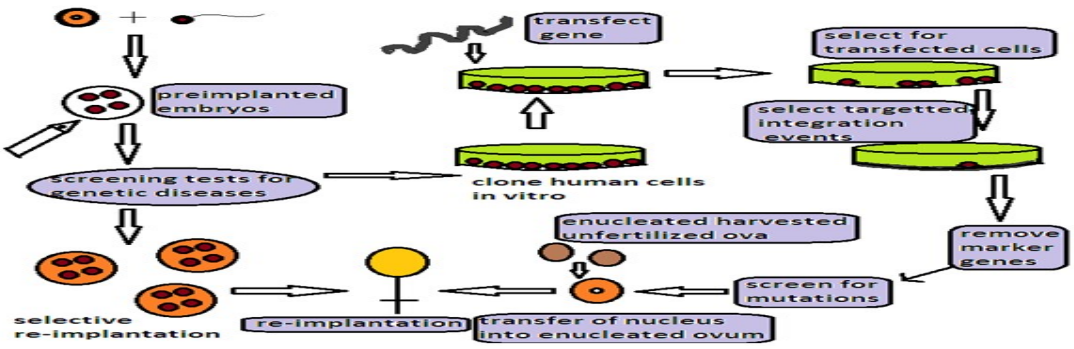


Diagram of Schematic representation of germline gene therapy

Steps of germ line gene therapy:

1. Isolation of totipotent embryonic cells at an undifferentiated stage
2. The determination of the genetic state of the embryo
3. Embryonic stem cell expansion in culture
4. Transfer of the genetic material into embryonic cell
5. Selection of stably transfected cell
6. Targeted gene replacement
7. Removal of marker
8. Confirmation of genomic integrity
9. Transfer of the nucleus
10. Re-implantation in the mother

Gene Replacement:

In understanding contemporary molecular cell biology, the techniques of genome mapping, mutation and protein expression have played a significant role. Nonetheless, a significant number of genes have been identified and analysed on the basis of their encoded protein's biochemical properties, the sequence resemblance of the encoded protein with proteins of a defined function, or their expression strategy during their progression. The in vivo function of such genes, if their mutant forms are not available, will be very difficult to understand. Gene replacement is a procedure in which a single gene is mutated in vitro and its natural copy is then replaced by its mutant version in order to explain its function in vivo. The substitution of genes is often often referred to as gene-targeted knockout or simply "knockout." In other words, gene replacement is a strategy for replacing or replacing an endogenous gene that requires homologous recombination. It may include point mutations, gene/exon elimination and even the introduction of a new gene.

For certain traditional animals, the procedure of gene replacement is a proven process and the method can differ among the broad variety of species used. Overall, in bacteria, a construct made from DNA is created. It normally consists of a targeted gene, a reporter gene, and a selectable marker that assists in the necessary selection process. Drosophila melanogaster is the species of choice for many genetic studies and has demonstrated its prompt role in gene therapy in species as well. In some cases, the use of engineered endonucleases such as zinc finger nucleases (ZFN) and homing endonucleases indicates a high occurrence of knockout studies. The above described construct is injected into the mouse embryonic stem cells in culture in knockout mice experiments. In order to use them to add to the tissue of a mouse by embryo injection, the cells with correct insertion are then chosen. Finally, only chimeric mice are taken and selected for breeding in which the reproductive organs are composed of modified cells.

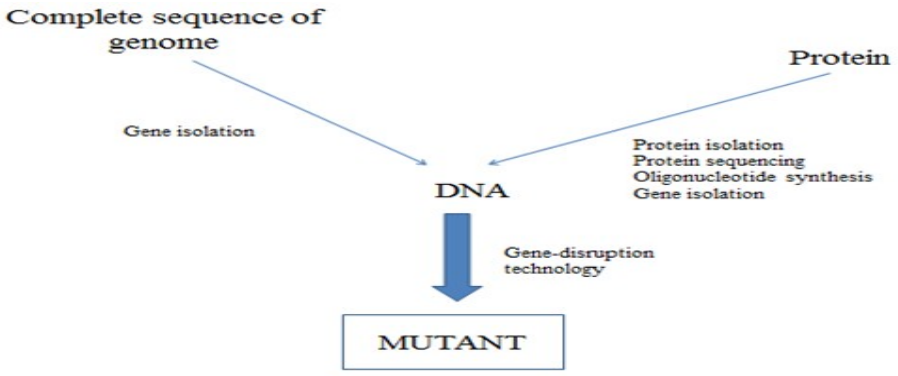


Diagram of Complete genome of an organism and its application

Gene addition:

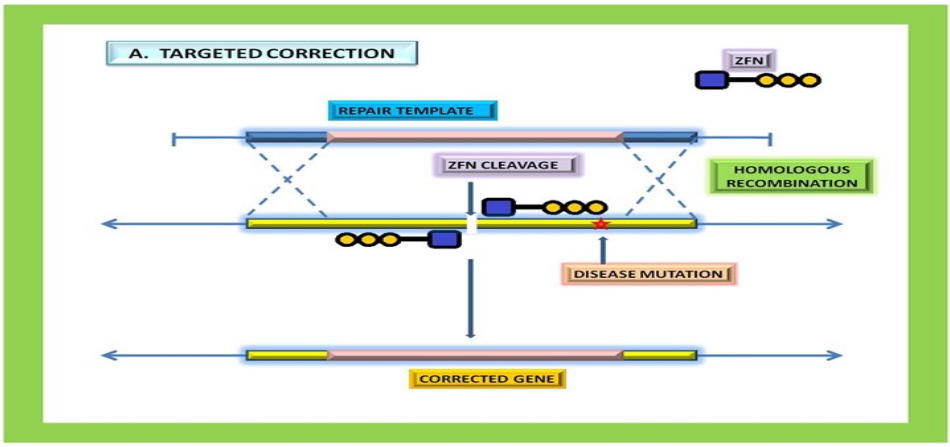
One of the most realistic alternatives to gene therapy in the modern situation is gene extension. The addition of an activated clone of a deficient innate gene is involved. Viral vector-based gene addition was successfully conducted to treat a patient in the U.S. named Thompson in the year 1990. Retroviruses were first used in the viral vector-based gene addition since they were believed and proved to be most desirable of all viruses for their adaptive nature to carry genes into cells. This is a good treatment because it has been found to be a substitute for gene replacement and has a very high efficacy in treating any disease. While gene addition therapy is reasonably adequate, it has its advantages and disadvantages

over gene replacement therapy. Its benefits are that it is possible to modify cells that do not usually express a single gene to express that gene.

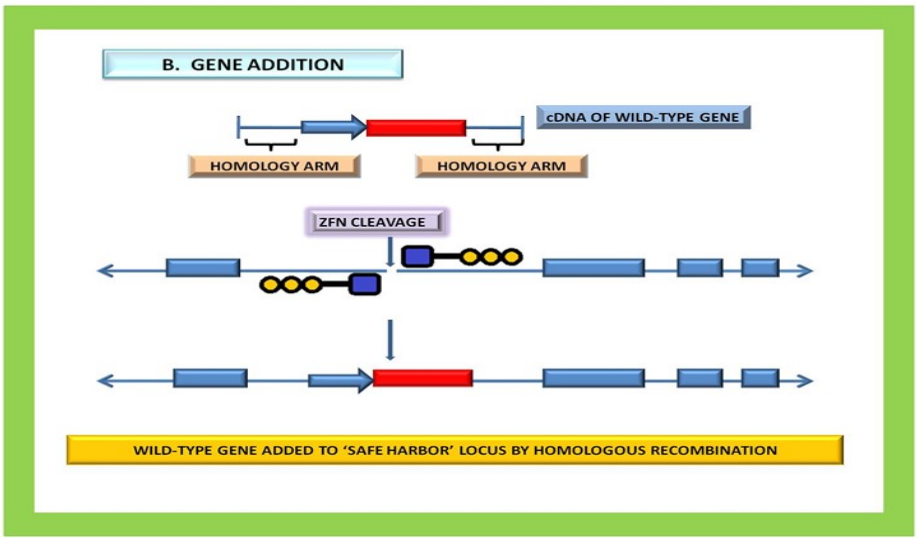
The spread of human immunodeficiency virus (HIV) could, for example, be limited by the transfer of genes that encode ribozymes capable of degrading HIV RNA. The disordered insertion of genes into the genome is a drawback of gene addition therapy. The injected genes may either be misrepresented or may induce abnormal gene expression immediately at the site of insertion.

The gene addition result relies on the successful incorporation of therapeutic genes inside the host cell at the required target location, preventing any disruption to cells, mutations or any form of adverse immune reaction. Zinc finger nucleases (ZFNs), including drosophila melanogaster, have become important reagents for engineering the genomes of many plants and animals. ZFNs, a specially designed laboratory, propose a common method of supplying to the genome a site-specific double strand break (DSB) and inducing a local homologous recombination by a large amount of magnitude. The plasmid-based approach to ZFN-encoding is successful in preventing all the problems associated with the viral distribution of therapeutic genes. By producing a site-specific DNA DSB at a fixed site in the genome, ZFNs drive extremely efficient genome editing. The consequent reconstruction of this DSB break by homology-directed repair (HDR) contributes to the addition of targeted genes.

In certain wet laboratory studies, the insertion of a split at a particular target site triggers gene addition to the use of CCR5-specific ZFNs as a model method using a homologous donor prototype. For certain therapeutic uses, such as the selective correction of human disease-causing mutations, the effectiveness of a single stranded break to direct repair pathway option could prove advantageous.



Diagrammatic illustration of targeted gene correction strategy



Diagrammatic illustration of targeted gene addition strategy

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converted into a beautiful deer? Hanuman is said to have mastered the great eight arts (Ashta Siddhis) of anima, mahima, laghima, garima etc where he could instruct his body to become as large as a mountain, or as tiny as an ant, or as heavy as a huge rock, or as light as a feather etc!

Competitive questions from today topic (2 questions Minimum)-

For site-directed mutagenesis, which one of the following restriction enzymes is used to digest methylated DNA?

A. KpnI B. DpnI
C. XhoI D. MluI
Exam Name: GATE2019

A mutation i the last three n a gene that codes for a polypeptide results in a variant polypeptide that lacks amino acids. What type of mutation is this?

A. Synonym ous mutation B. Synonymous mutat
C. Missense mutation D. Silent mutation
Exam Name: GATE2019

Questions to check understanding level of students-

- Gene-knockout mediated gene therapy is the knockout of the human ____ gene in T-cells to control HIV infection.
- The vectors which can accomplish _____are more preferred and desirable in case of insertional mutagenesis.

References:

Misra, S., 2013. Human gene therapy: a brief overview of the genetic revolution. *J Assoc Physicians India*, *61*(2), pp.127-33.

Chapter 2: Intracellular barriers to gene delivery

Vectors of non-viral gene therapy are highly suitable instruments for DNA integration into cells; they have stronger protection profiles than viral distribution approaches and are more likely to be provided repeatedly. Non-viral vectors include naked DNA, lipoplex (lipoplex) cationic lipid-DNA complexes, polymer-DNA complexes (polyplex), or lipid and polymer combinations. Effective distribution of genes depends on the vector of choice's ability to target a particular type of cell, enter the cell, and achieve appropriate levels of gene expression. This is not an easy mission, because both viral and non-viral vectors face many challenges that make this method challenging.

Next, the vector must have a means of attacking a single type of cell, while preventing extracellular insults, including nucleases and the immune system. Then, it must cross the plasma membrane and/or exit the endosome until the vector has reached its unique target, and travel through the dense cytoskeleton network en route to the nucleus. The nuclear envelope provides a final hurdle, because, in order to be transcribed, DNA must reach the nucleus. While viruses have developed pathways for accessing target cells, delivering their genetic material and continuing to replicate, these inherent mechanisms are absent in non-viral structures. Consequently, a great deal of work has been undertaken to characterise and resolve these obstacles in order to increase the effectiveness of these vectors.

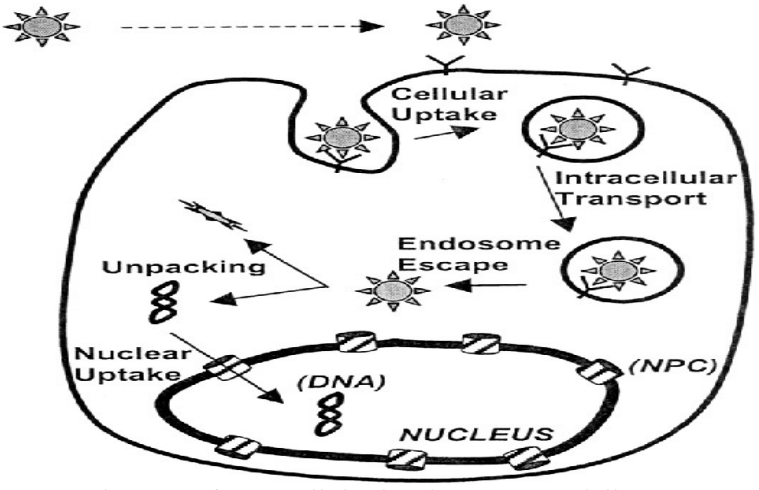

Diagram of Intracellular barriers to gene delivery

Methods

To deliver genes to host cells, there are a number of methods available. The mechanism is called transformation if genes are transferred to bacteria or plants, and it is called transfection whether it is used to deliver genes to animals. In regards to species, this is because transition

has a different definition, implying development to a cancerous state. There are no external methods for certain bacteria to add genes because they are naturally capable of processing foreign DNA. In order to make the cell membrane permeable to DNA and allow the DNA to be stably inserted into the host genome, most cells need some form of interference.

Chemical

Real or synthetic substances can be used to form particles that allow the transmission of genes into cells through chemical gene delivery methods. These synthetic vectors are able to bind DNA or RNA electrostatically and compress genetic information to handle larger genetic transfers. Chemical vectors typically enter cells by endocytosis and can shield genetic material from degradation.

Heat shock

One of the easiest methods is to change the cell's atmosphere and then stress it by giving it a heat shock. Usually, under cold conditions, the cells are incubated in a solution that includes divalent cations (often calcium chloride) before being exposed to a heat pulse. The cell membrane is partly destroyed by calcium chloride, which encourages the recombinant DNA to penetrate the host cell. It is proposed that, in cold conditions, exposing the cells to divalent cations can alter or weaken the structure of the cell surface, making it more permeable to DNA. Throughout the cell membrane, the heat-pulse is assumed to create a thermal mismatch, which forces the DNA to reach the cells through either cell pores or the weakened cell wall.

Calcium phosphate

Another simple solution involves binding the DNA with calcium phosphate and then exposing it to cultured cells. The cells encapsulate the solution, along with the DNA, and a small volume of DNA will be incorporated into the genome.

Liposomes and polymers

To introduce DNA into cells, liposomes and polymers may be used as vectors. Positively charged liposomes bind to DNA that has been negatively charged, whereas polymers that associate with DNA can be designed. They form lipoplexes and polyplexes, respectively, which are then taken up by the cells. It is also possible to merge the two structures. Polymer-based non-viral vectors interact with DNA and form polyplexes by using polymers.

Nanoparticles

The use of engineered inorganic and organic nanoparticles is another non-viral approach for gene delivery.

Physical

Artificial gene delivery can be mediated by physical methods which uses force to introduce genetic material through the cell membrane.

Electroporation

Electroporators should be used to make the cell membrane permeable to DNA. A form of competence promotion is electroporation. With an electric field of 10-20 kV/cm, cells are momentarily shocked, which is thought to create holes in the cell membrane from which the plasmid DNA can join. The holes are easily filled after the electric shock by the membrane-repair mechanisms of the cell.

Biolistics

A gene gun uses biolistics to inject DNA into cells. Biolistics, where gold or tungsten particles are filled with DNA and then fired into young plant cells or plant embryos, is another tool used to transform plant cells. Certain genetic material joins and changes the cells. This procedure can be used on plants not vulnerable to contamination with Agrobacterium and also helps plant plastids to be transformed. Using electroporation, which utilises an electric shock to make the cell membrane permeable to plasmid DNA, plant cells may also be converted. The transformation efficiency of biolistics and electroporation is smaller than agrobacterial transformation due to the damage caused to the cells and DNA.

Microinjection

Microinjection is where DNA is injected through the cell's nuclear envelope directly into the nucleus.

Sonoporation

Sonoporation uses sound waves create pores in a cell membrane to allow entry of genetic material.

Photoporation

Photoporation is when laser pulses are used to create pores in a cell membrane to allow entry of genetic material.

Magnetofection

Magnetofection uses magnetic particles complexed with DNA and an external magnetic field concentrate nucleic acid particles into target cells.

Hydroporation

A hydrodynamic capillary effect can be used to manipulate cell permeability.

Agrobacterium

In plants, DNA is often introduced using recombination induced by Agrobacterium, taking advantage of the sequence of Agrobacterium T-DNA that enables genetic material to be naturally incorporated into plant cells. Plant tissue is sliced into small pieces and immersed in a dissolved Agrobacterium-containing solution. Many of the plant cells revealed by the cuts would be bound to the bacteria. To move a DNA fragment called T-DNA from its plasmid into the plant, the bacteria uses conjugation. The transferred DNA is piloted to the nucleus of the plant cell and inserted into the genomic DNA of the host plants. The plasmid T-DNA is semi-randomly introduced into the genome of the host cell. By altering the plasmid to express the gene of interest, researchers will stably insert their desired gene into the genome of the plants. Its two small (25 base pair) boundary repeats are the only important parts of the T-DNA, at least one of which is necessary for plant transformation. The genes to be inserted into the plant are cloned into a plant.

Viral delivery

The transmission of virus-mediated genes exploits the ability of a virus to insert its DNA into a host cell and takes advantage of the ability of the virus to reproduce and implement its own genetic material. Viral gene delivery strategies are more likely to cause an immune response, but they are highly successful. Transduction is the mechanism that explains the insertion of DNA into the host cell mediated by viruses. Viruses are an especially successful method of gene delivery as the virus structure prevents degradation to the nucleus of the host cell through lysosomes of the DNA it delivers. In gene therapy, a gene that is meant for delivery is bundled to form a viral vector into a replication-deficient viral particle. Retrovirus, adenovirus, adeno-associated virus, and herpes simplex virus are the viruses used to date for

gene therapy. There are limitations of using viruses to carry genes into cells, though. Just very tiny bits of DNA can be delivered by viruses into the cells, it's labor-int.

Viral vector based gene delivery uses a viral vector to deliver genetic material to the host cell. This is done by using a virus that contains the desired gene and removing the part of the viruses genome that is infectious. Viruses are efficient at delivering genetic material to the host cell's nucleus, which is vital for replication.

RNA-based viral vectors

Because of the capacity to transcribe directly from infectious RNA transcripts, RNA-based viruses have been created. Since no processing is needed, RNA vectors are easily expressed and expressed in the targeted form. Integration of genes leads to long-term transgene expression, but delivery dependent on RNA is typically temporary and not lasting. Oncoretroviral, lentiviral, and human foamy viruses are found in retroviral vectors.

DNA-based viral vectors

DNA-based viral vectors are usually longer lasting with the possibility of integrating into the genome. DNA-based viral vectors include Adenoviridae, adeno-associated virus and herpes simplex virus.

Ancient Indian Literature Reference - https://www.hitxp.com/articles/science-technology/sanskrit-vedic-chants-and-the-secret-world-of-human-dna-wave-genetics-of-biological-internet-causing-hyper-communication/ Maricha of Ramayana who got himself converted into a beautiful deer. Hanuman is said to have mastered the great eight arts (Ashta Siddhis) of anima, mahima, laghima, garima etc where he could instruct his body to become as large as a mountain, or as tiny as an ant, or as heavy as a huge rock, or as light as a feather etc.

Competitive questions from today topic (2 questions Minimum)-

The p53 gene is responsible for:-

A. Initiating transcription of p21 which binds to cyclins
B. Over riding the G1 checkpoint
C. Damaging DNA X
D. vertical gene transfer

Exam Name: PU-2016

Which one of the following statements about alleles is NOT TRUE?

A. They may occupy different loci in the same chromosome

B. There may be several at one locus

C. One may be dominant over another

D. They may show co-dominance

Exam Name: DBT-2019

Questions to check understanding level of students-

What is an Intracellular barrier to gene delivery?

References:

Misra, S., 2013. Human gene therapy: a brief overview of the genetic revolution. *J Assoc Physicians India*, *61*(2), pp.127-33.

Chapter 3: Overview of inherited and acquired diseases for gene therapy

Gene therapy is the introduction of genes into existing cells to prevent or cure a wide range of diseases. It is a technique for correcting defective genes responsible for disease development. The first approved gene therapy experiment occurred on September 14, 1990 in US, when Ashanti DeSilva was treated for ADA-SCID.

Gene Therapy for Somatic Cells

Transferring therapeutic genes into somatic cells. Just ex. Eg. Gene introduction into the cells of the bone marrow, blood cells, skin cells etc. Later generations would not be generational. Currently, both experiments are aimed at repairing genetic mutations in somatic cells.

Germ Line Gene Therapy

Transferring medicinal genes into germ cells. Just ex. Eg. In eggs and sperm, genes are inserted. It is inherited and passed down to future generations. It is not being attempted at present for safety, ethical and technological reasons.

Ex vivo gene therapy:- gene transfer to and reinsertion of cultured cells. In vivo gene therapy: - guided gene delivery to the cells of a specific tissue in the body

Transplant the patient with the new cells. Pick, and evolve, genetically corrected cells. Introduce the genes for treatment . Growing cells in culture Separate cells from a patient with a genetic mutation. 1st gene therapy, Adenosine deaminase, to reverse enzyme deficiency (ADA). A 4-year-old girl, Ashanthi DeSilva, performed. Had SCID-Severe Mixed Immunodeficiency. Caused by ADA gene coding defects. T lymphocytes absorb and degrade deoxy adenosine. Disrupting immunity, suffering from infectious diseases and dying at a young age. Direct delivery of gene therapy. Carried out by viral or non viral vector systems. It can be the only possible option in patients where individual cells cannot be cultured in vitro in sufficient numbers (e.g. brain cells). In vivo gene transfer is necessary when cultured cells cannot be re-implanted in patients effectively.

Retrovirus Vector System

The recombinant retroviruses have the ability to integrate into the host genome in a stable fashion. Replication defective virus particles Target cell - dividing

Adeno Virus Vector System

Adeno virus with a DNA genome – good vectors. Target- non dividing human cell. Eg. Common cold adenovirus.

Gene Gun

Employs a high-pressure delivery system to shoot tissue with gold or tungsten particles that are coated with DNA Microinjection. Process of using a glass micropipette to insert microscopic substances into a single living cell. Normally performed under a specialized optical microscope setup called a micromanipulator.

Gene Inhibition Therapy

Long-lasting treatment is not accomplished by gene therapy; gene therapy effects are short-lived due to accelerated cell division. A possible concern for gene therapy is triggered by an antibody reaction to the transferred gene. Viruses used as gene transfer vectors can cause host toxicity, immune responses, and inflammatory reactions. Disorders caused by mutations in several genes using gene therapy will not be handled successfully.

Theoretically, for inherited disorders, gene therapy is the permanent cure. Yet there are many nuances of it. It is not available to most individuals at its current stage because of its tremendous expense. Any day, a breakthrough will arrive and a day will come when a gene therapy will be available for virtually any disease. Researchers found treatment for a widespread type of blindness (X-linked retinitis pigmentosa) that affects both young people and adults in a novel gene therapy technique developed by the University of Florida in January 2012. According to the findings of a Phase 2 analysis published in the journal Lancet Neurology in March 2011, a gene therapy called NLX-P101 significantly decreases mobility dysfunction in Parkinson's patients.

University Library Reference-

Dubey R.C, A textbook of biotechnology, 1st edition (2004)

Ancient Indian Literature Reference - https://www.hitxp.com/articles/science-technology/sanskrit-vedic-chants-and-the-secret-world-of-human-dna-wave-genetics-of biological-internet-causing-hyper-communication/ Maricha of Ramayana who got himself converted into a beautiful deer? Hanuman is said to have mastered the great eight arts (Ashta Siddhis) of anima, mahima, laghima, garima etc where he could instruct his body to become as large as a mountain, or as tiny as an ant, or as heavy as a huge rock, or as light as a feather etc.

Competitive questions from today topic (2 questions Minimum)-

The total magnification of a microscope is calculated by:-

A. Multiplication of the objective lens and condenser lens magnification powers
B. Square of objective lens power
C. Multiplication of the objective lens and ocular lens magnification powers
D. Addition of the objective lens and ocular lens magnification powers

Exam Name: PU-016

What is a genophore?

A. DNA in prokaryotes
B. DNA and RNA in prokaryotes
C. DNA and protein in prokaryotes
D. RNA in prokaryotes

Exam Name: CFTRI

Questions to check understanding level of students-

What is inherited and acquired diseases for gene therapy?

References:

Misra, S., 2013. Human gene therapy: a brief overview of the genetic revolution. *J Assoc Physicians India*, *61*(2), pp.127-33.

Chapter 4: Retro and Adeno virus mediated gene transfer

Retrovirus Vector System

The recombinant retroviruses have the potential to penetrate in a secure manner into the host genome.

A retrovirus is a type of virus that incorporates a copy of its RNA genome[a] into the DNA of a host cell infected by it, thus modifying the cell's genome. Once within the cytoplasm of the host cell, the virus uses its own reverse transcriptase enzyme to create DNA from its RNA genome, the reverse of the normal sequence (backwards). An integrase enzyme then integrates the new DNA into the genome of the host cell, at which stage the retroviral DNA is referred to as a provirus. As part of its own genome, the host cell then treats the viral DNA, transcribing and translating the viral genes along with the cell's own genes, creating the proteins needed to assemble new copies of the virus.

They have three basic classes, while retroviruses have various subfamilies. Oncoretroviruses (oncogenic retroviruses), lentiviruses (slow retroviruses) and spumaviruses (foamy viruses). Oncoretroviruses in certain mammals are capable of causing cancer, lentiviruses in humans and other animals are capable of causing extreme immunodeficiency and death, and spumaviruses in humans or animals are benign and not associated with any illness.

In humans, other mammals, and birds, certain retroviruses cause extreme diseases. HIV-1 and HIV-2, the cause of the disease AIDS, contain human retroviruses. The human T-lymphotropic virus (HTLV) also causes infection in humans. Murine leukaemia viruses (MLVs) cause cancer in mouse hosts. Retroviruses have been used extensively in gene delivery systems and are useful research resources in molecular biology.

Retrovirus virions consist of enveloped particles about 100 nm in diameter. Glycoprotein is made up of the outer lipid envelope. Two similar single-stranded RNA molecules, 7-10 kilobases in length, are also found in the virions. The two molecules are present as a dimer formed by base pairing of complementary sequences. Interaction sites were described as a "kissing -loop complex" between the two RNA molecules. While virions of multiple retroviruses do not have the same morphology or biology, all components of the virion are quite similar.

The main virion components are:

Envelope: consisting of lipids (obtained during the budding phase from the host plasma membrane) as well as glycoprotein encoded by the env gene. Three distinct roles serve the retroviral envelope: protection from the extracellular environment through the lipid bilayer, allowing the retrovirus via endosomal membrane trafficking to enter/exit host cells, and the ability to directly enter cells by fusing their membranes.

RNA: It consists of an RNA dimer. On the 5 'top, it has a cap and on the 3' end, a poly(A) tail. As a result of host RNA polymerase II (Pol II) action, genomic RNA (gRNA) is produced and processed as a host mRNA by inserting a 5' methyl cap and a 3 'poly-A tail. The RNA genome also has terminal noncoding regions that are critical in replication, and internal regions that encode virion proteins for gene expression. Four regions are found in the 5 'top, which are R, U5, PBS and L. At each end of the genome used during reverse transcription, the R region is a short repeated sequence to ensure proper end-to-end transition in the expanding chain. U5, on the other hand, is a short series between R and PBS that is special. PBS (primer binding site) is made up of 18 bases complementary to the 3' tRNA primer end. The L region is an untranslated leader region that provides the signal for the RNA genome to be packaged.

The 3' end includes 3 regions, which are PPT (polypurine tract), U3, and R. The PPT is a primer for plus-strand DNA synthesis during reverse transcription. U3 is a sequence between PPT and R, which serves as a signal that the provirus can use in transcription. R is the terminal repeated sequence at 3' end.

Proteins: made of gag proteins, protease (PR), pol proteins, and env proteins. The main components of the viral capsid are group-specific antigen (gag) proteins, which are about 2000-4000 copies per virion. Gag has two domains of nucleic acid binding, namely matrix (MA) and nucleocapsid domains (NC). One of the essential roles of Gag protein is precisely detecting, binding, and packing retroviral genomic RNA into assembling virions. Gag associations with cellular RNAs also control assembly components. The expression of gag alone gives rise to the assembly of plasma membrane-like immature virus particles. Of both retroviruses, the precursor to the inner structural protein is the Gag protein.

In multiple viruses, protease (pro) is distributed differently. It acts during virion maturation in proteolytic cleavages to create mature gag and pol proteins. The responsibility of Retroviral Gag proteins is to organise several facets of the assembly of virions.

Pol proteins are responsible for viral DNA synthesis and, after infection, incorporation into host DNA.

In the interaction and entrance of virions into the host cell, the Env proteins play a role. The ability of the retrovirus to bind to its target host cell using unique cell-surface receptors is provided by the surface component (SU) of the Env protein, whereas the ability of the retrovirus to reach the cell by membrane fusion is provided by the membrane-anchored trans-membrane component (SU) of the Env protein (TM). Therefore, the Env protein causes the retrovirus to be infectious.

The RNA in the retrovirus virus is associated with many protein species. The most abundant protein that covers RNA is the nucleocapsid (NC) protein, while other proteins are much smaller and have enzyme activity. RNA-dependent DNA polymerase (reverse transcriptase; RT), DNA-dependent DNA polymerase, Ribonuclease H (RNase H) Integrase and Protease are several enzyme activities found in the retrovirus virus. Three distinct cleavage modes have been shown to be retroviral RNases H encoded by all retroviruses, including HIV: internal, DNA 3' end-directed, and RNA 5' end-directed. In reverse transcription, all three cleavage modes form positions.

Therefore, in certain areas of reverse transcription, the activity of RNase H is important. The use of an RNase H operation during retroviral replication suggests a novel technique to copy a single-stranded RNA genome into a double-stranded DNA, as the minus-strand DNA is complementary and the base pairing to the retrovirus genome in the first stage of DNA synthesis.[18] The action of the RNase H ribonuclease is also important in the retroviral life cycle because it generates and extracts Retroviruses without RNase H activity are non-infectious.

Adeno Virus Vector System

Adeno virus with a healthy vector DNA genome. Target-Human Cell Nondividing. Just ex. Eg. Widespread cold adenovirus.

Medium-sized (90-100 nm), unenveloped (naked) icosahedral viruses consisting of a nucleocapsid and a genome of double-stranded linear DNA are adenoviruses. There are over 51 distinct serotypes in humans that are responsible for 5-10% of children's upper respiratory infections and also adult infections. Their DNA molecule is inserted into the host as these viruses infect a host cell. The genetic material of adenoviruses is not integrated into the genetic material of the host cell (transient). In the nucleus of the host cell, the DNA molecule is left free, and the instructions are transcribed much as every other gene in this extra DNA molecule. The only distinction is that when the cell is going to undergo cell division, these new genes are not repeated, meaning that the offspring of that cell will not get the extra chromosome.

As a consequence, adenovirus therapy will involve re-administration in a may population of cells, while the absence of penetration into the genome of the host cell may avoid the type of cancer seen in the SCID trials. This vector method has shown real potential in cancer research, and an adenovirus to cure cancer is also the first gene therapy product (Gendicine) to be approved.

The viruses of the Adenoviridae family infect numerous animal species, including humans. In human adenoids (tonsils), from which the name is derived, adenoviruses were first isolated. The largest non-enveloped viruses are adenoviruses, as they are the highest size that can be transported across the endosome (i.e. envelope fusion is not necessary). The virion also has a special "spike" or fibre connected with each capsid penton base (see image below) that helps to bind to the host cell on the surface of the host cell through the coxsackie-adenovirus receptor.

Adenoviruses are distributed mainly through respiratory droplets, but they can also be spread via faecal pathways. Many adenovirus infections result in upper respiratory tract infections. Infections of adenovirus sometimes present as conjunctivitis, tonsilitis, ear infections or croups. Gastroenteritis can also occur with adenoviruses (stomach flu). For adenovirus infections, a combination of conjunctivitis and tonsilitis is especially frequent. Any kids can contract bronchiolitis or pneumonia from adenovirus, all of which may be serious. Adenoviruses can also cause cough fits in babies that sound almost exactly like whooping cough.

Many individuals survive on their own from adenovirus infections, although individuals with immunodeficiency often die from adenovirus infections, and even previously stable individuals will rarely die from these infections.

Two sets of interactions between the virus and the host cell include the entrance of adenoviruses into the host cell. The knob domain of the fibre protein binding to the cell receptor initiates entry into the host cell. The two receptors currently in place are: CD46 for serotypes of human adenovirus group B and coxsackievirus adenovirus receptor (CAR) for all other serotypes. A secondary association accompanies this, where a specialised motif interacts with an integrin molecule in the penton base protein. It is the interaction of the co-receptor that activates adenovirus internalisation. Alpha-v integrin is this co-receptor molecule. Endocytosis of the virus particle via clathrin-coated pits results from binding to alpha integrin. Attachment to alpha integrin activates cell signalling and thereby causes polymerization of actin, resulting in the virion forming an endosome into the host cell.

The endosome acidifies, which changes virus topology by allowing capsid components to disassociate until the virus has successfully achieved entry into the host cell. These shifts, as well as the poisonous nature of the pentons, contribute to the release into the cytoplasm of the virion. The virus is transferred to the nuclear pore complex with the aid of cellular microtubules, from which the adenovirus particle disassembles. Viral DNA that can reach the nucleus through the nuclear pore is subsequently released. The DNA interacts with histone molecules after this. Viral gene expression can therefore occur and new particles of the virus can be produced.

By the DNA replication process, the adenovirus life cycle is divided into two phases: an early and a late phase. A primary transcript is produced in both processes, which is alternatively spliced to produce monocistronic mRNAs consistent with the ribosome of the host, facilitating translation of the goods. In order to express primarily non-structural, regulatory proteins, the early genes are responsible. The objectives of these proteins are threefold: to change the host protein expression needed for DNA synthesis; to enable other virus genes (such as virus-encoded DNA polymerase); and to prevent premature host-immune protection death of the infected cell (blockage of apoptosis, blockage of interferon activity, and blockage of MHC class I translocation and expression).

Any adenoviruses can turn cells using their early gene products under specialised conditions. In vitro immortalization of primary cells, E1a (binds Retinoblastoma tumour suppressor

protein) has been found to allow E1b (binds p53 tumour suppressor) to help and stably turn the cells. Nevertheless, to effectively convert the host cell and form tumours, they are dependent on each other. The early and late phases are distinguished by DNA replication. Once appropriate virus proteins, replication machinery and replication substrates have been released by the early genes, adenovirus genome replication can occur. A terminal protein that is covalently bound to the 5 'end of the genome of adenovirus serves as a replication primer. The viral DNA polymerase then uses a process of strand displacement to duplicate the genome, as opposed to the traditional Okazaki fragments used in mammalian DNA replication.

The late phase of the life cycle of adenovirus focuses on creating adequate amounts of structural protein to accommodate all the genetic material produced by replication of DNA. If the viral components have been successfully replicated, the virus is assembled and released from the cell into its protein shells as a result of virally mediated cell lysis.

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Ancient Indian Literature Reference - https://asia.nikkei.com/Business/Science/Ayurveda-the-ancient-Indian-medical-practice3. The science and practice of Ayurveda are narrated in ancient texts, including "Charaka Samhita," a treatise on general medicine. This refers to a large collection of Hindu sacred texts, the Vedas. In Vedic philosophy, human beings are part of nature, animals and plants are interdependent, and there is an inherent relationship between the universe and human beings. Unlike the animal kingdom, humans live in a more complex natural world where they are constantly exposed to environmental changes like weather, diet, work, society, the economy and lifestyle. It is believed that these forces can negatively influence people's state of mind, body and soul.

Competitive questions from today topic (2 questions Minimum)-

Rabies virus infects

A. Astrocytes B. Oligodendrocytes

C. Neurons D.Macrophages

Exam Namecusat-2015

A mutation i the last three n a gene that codes for a polypeptide results in a variant polypeptide that lacks amino acids. What type of mutation is this?

A. Synonym ous mutation B. Synonym ous mutation

C. Missense mutation D. D. Silent mutation

Exam NameGATE2019

Questions to check understanding level of students-

What is retrovirus?

References:

Lieber, A., Peeters, M.V., Meuse, L., Fausto, N., Perkins, J. and Kay, M.A., 1995. Adenovirus-mediated urokinase gene transfer induces liver regeneration and allows for efficient retrovirus transduction of hepatocytes in vivo. *Proceedings of the National Academy of Sciences*, *92*(13), pp.6210-6214.

Smythe, W.R., Kaiser, L.R., Hwang, H.C., Amin, K.M., Pilewski, J.M., Eck, S.J., Wilson, J.M. and Albelda, S.M., 1994. Successful adenovirus-mediated gene transfer in an in vivo model of human malignant mesothelioma. *The Annals of thoracic surgery*, *57*(6), pp.1395-1401.

Chapter 5: Liposome and Nanoparticles mediated gene delivery

Introduction

In both basic science and applied biology, the methodology of gene transfer is used very extensively. A basic and proven technique is the delivery of DNA into animal cells. It has been an invaluable instrument for gene cloning, the study of gene function and regulation, and the development for examination and verification of small quantities of recombinant proteins. The gene transfer experiment helps to express in the receiver cells the inserted genetic construct (or transgene) or disrupt or inactivate particular endogenous genes (resulting in a loss of function). There are multiple gene transfer uses such as large-scale industrial manufacturing of recombinant antibodies and vaccines and gene medicine or gene therapy. They range from the use of mammalian and gene therapy.

Every cell or a certain target cell population carries a different modification in the species or genetically engineered entire animals produced by Gene transfer. These animals are used to research the function and expression of genes, model human diseases, produce in their milk and other fluids recombinant proteins, and to enhance the efficiency of herds of livestock and other domestic organisms. Technology has helped to explain the roles of the multiple genes used in genome projects (functional genomics). Examples of such experiments include systematic DNA-mediated mutagenesis and gene trap systems in the mouse and in the fruit fly, Drosophila melanogaster, nematode genome-wide RNA interference experiments, Caenorhabditis elegans, and novel yeast-based protein interaction screens using mammalian cells.

The research that includes intentional alteration of the genetic material of plants or animals is genetic engineering of food. It is of great agricultural potential and medical importance to introduce DNA into plants. The effects of gene transfer may be temporary and stable transfection.

For therapeutic purposes, gene therapy can be defined as the intentional transfer of DNA. One of the key considerations of gene therapy is gene transfer, and it is one of the clone's key functions. Somatic (body) or germ (egg and sperm) cells may be targeted for gene transfer. In somatic gene transfer, the genome of the receiver is affected, but the alteration will not be

carried on to the next generation. In germline gene transfer, the egg and sperm cells of the parents are altered with the objective of passing on the changes to their offspring.

History

In bacteria, which are capable of at least four natural modes of genetic exchange, the principle of gene transfer between cells was first demonstrated. In 1928, Frederick Griffith discovered the first process called transformation in the Streptococcus pneumoniae bacterium. The essence of the transformative theory, however, he was unable to decide. Oswald Avery found in 1944 that the material exchanged between cells was DNA. Joshua Lederberg and Edward Tatum discovered a second method of gene transfer in Escherichia coli in 1946, called conjugation. The transmission of DNA via a direct connection between bacterial cells was involved in this process. E. In E. The shape of this conduit between cells was a protein tube known as a pilus. On a broad plasmid known as the F (for fertility) factor, the capacity of the cells to build the pilus and transfer DNA through it was coded.

The act of conjugation in most cases involved the transition of the plasmid alone, which was formed in the receiver cell, transforming it from an F- to a F+ phenotype. However, in some circumstances, the F plasmid could merge into the bacterial chromosome, and the transition of chromosomal genes could result from conjugation. Then, in 1951, Joshua Lederberg and Norton Zinder discovered a new form of bacteriophage-mediated gene transfer in Salmonella. They observed that often the newly developed phage could bundle some of the DNA of the host cell and then pass it in a subsequent infection to a second host cell.

Two modes of transduction, generalised and advanced transduction, were established. The phage head was erroneously stuffed entirely with host cell DNA during generalised transduction. The phage genome was inserted into the bacterial chromosome and bound to host DNA in advanced transduction. The fourth gene transfer pathway is mediated by full cell fusion in bacteria and occurs in many bacterial genera, including Bacillus and Streptomyces. The viral capsid is necessary under normal conditions to insert the oncogene and the rest of the genome into the animal host cell. To differentiate it from regular infection, the rare mechanism of gene transmission without the viral capsid was called transfection.

The word transformation continued to be used in bacterial genetics to characterise the absorption of naked plasmid or genomic DNA (essentially any DNA that had the potential to change the recipient cell phenotype), while transfection was used primarily to describe the absorption of naked phage DNA (or RNA), i.e. nucleic acid that had the potential to start a period of phage replication. In the absence of a biological vector, the term transfection has

commonly been agreed to mean the insertion of some type of DNA, phage, plasmid, genomic or otherwise, into an animal cell.

Different Gene Transfection Techniques

There are two intermittent and permanent modes of transfection. The transfected DNA is not incorporated into the host chromosome during transient transfection. In order to achieve a transient yet high degree of expression of the target gene, DNA is passed into a recipient cell. Permanent transfection is also called secure transfection. The transferred DNA is incorporated (inserted) into chromosomal DNA by a secure transfection, and the genetics of the recipient cells are permanently altered.

Gene transmission into animal cells must meet three distinct aims, independent of the delivery process. Next, it is important to transport exogenous genetics through the cell membrane. Transport through the membrane is accomplished by direct transfer in physical transfection processes, where the membrane is broken during transmission from which DNA and RNA can diffuse. In other delivery systems, before internalisation, the nucleic acid must form some kind of complex that bonds to the cell surface. The complex is formed between nucleic acid and a synthetic compound in chemical transfection methods, for instance, while the complex consists of nucleic acid wrapped within a viral capsid in transduction methods.

If the genetic material has been released into the cell via the cell membrane, it must be transferred to its site of expression or activity. Again, at this point, the nucleic acid is passive. DNA or RNA complexes are deposited in the cytoplasm in most transfection processes, following escape from the endosomal vesicle. DNA must be transferred to the nucleus, while in the cytoplasm, RNA can act directly. DNA may be transmitted directly into the nucleus in methods such as particle bombardment and microinjection, so intrinsic transport pathways are not necessary. As part of the infection cycle, many viruses often carry their nucleic acid cargo to the nucleus, often after contact with cell surface receptors and either internalisation within endosomes or direct plasma membrane fusion. There are some variations, however, such as poxviruses (e.g. Vaccinia virus) and alphaviruses (e.g. Sindbis virus) that replicate in the cytoplasm.

The exogenous genetic material needs to be triggered in the final step of gene transfer. It must be released for expression and/or association with the host genome from its complex and made competent. In the host, exogenous RNA occurs only temporarily, while exogenous DNA may occur temporarily or indefinitely.

The methods of gene transfer typically contain three categories: 1. Transfection by biochemical techniques; 2. Transfection by physical techniques; 3. Virus-instantaneous transduction. In order to form synthetic complexes, the first gene transfer protocols used naked DNA that was combined with unique chemicals. Such synthetic complexes either interact with the cell membrane and facilitate endocytosis uptake, or fuse with the membrane and directly deliver the DNA into the cytoplasm. These methods of chemical transfection have been used extensively. They are, however, generally inefficient for in vivo gene transfer. Physical transfection strategies, on the other hand, are useful for both in vitro and in vivo gene transfer.

Methods of physical transfection include breaking the cell membrane and physically inserting the nucleic acid into the cell or nucleus. While all sets of techniques have advantages and drawbacks, some of the most effective methods of transfection in use today include a mixture of chemical and physical processes. For the passage of uncovered, wild-type viral DNA into animal cells, chemical and physical transfection methods were first used. The introduction of plasmid vectors and recombinant viral genomes containing particular transgenes of concern is now more commonly employed of all strategies.

Chemical Transfection

A variety of limits must be resolved by chemical transfection techniques to deliver active DNA into the nucleus. They have to convince the cell to associate with exogenous DNA and filter it, and finally produce the nucleus with at least some intact DNA molecules. The first drawback of gene transfer is the hydrophobic and negatively charged cell membrane, whereas DNA is hydrophilic and negatively charged. Only by a synthetic complex can DNA interact with the cell membrane, in conjunction with DNA that bears a net positive charge, or whether it is either contained in a fusogenic capsule.

The synthetic complex's purpose is to form a complex that is positively charged. DNA transfer to the nucleus is the second obstacle to effective transfection, and it is the most complicated step in the methods of chemical transfection. Following membrane fusion, DNA encapsulated inside fusogenic particles is stored in the cytoplasm under the cell membrane, and is thought to find its way through an intrinsic transport pathway to the nucleus. But the mechanism is still little known. Complexes picked up by endocytosis, on the other hand, are transported into acidic endosomes and subsequently degraded into lysosomes. The DNA must find its way to the nucleus to attain a high degree of transfection quality.

By destroying endosomes and sabotaging the endosomal transport pathway, chloroquine is believed to enable DNA escape into the cytoplasm. For transfer to the nucleus, peptides with endosome-disrupting properties or the DNA sequence itself may also be essential. Vectors bearing the root of replication of the SV40 virus are transferred to the nucleus more successfully than identical vectors without this sequence. The nuclear import of exogenous DNA may also be facilitated by the incorporation of peptides with canonical nuclear localization sequences. Activation of the exogenous DNA by dissociation from the complex while inside the cell is the final barrier to effective transfection. Because only free DNA can associate with the genome of hosts, dissociation becomes essential.

In an experiment, naked DNA and DNA lipid complexes were injected with animal oocyte nuclei to search for the interaction with the genome of the hosts and the former were able to transmit transgenes. Dissociation is believed to occur either by easy diffusion or neutralisation of positively charged complexes with intracellular lipids and other molecules that are negatively charged.

Calcium phosphate Transfection

It was the first form of chemical transfection to be used for cells from animals. The most commonly used method of transfection is potentially calcium phosphate. This is a simple, accurate approach that applies to several lines of cultured cells, and the reagents are cheap. It can be used for both transient transformation and stable transformation. The theory of the procedure is that DNA is mixed softly with calcium chloride in a buffered phosphate solution, which allows a fine DNA-calcium phosphate coprecipitate to form. The precipitate collects on the cells, and endocytosis consumes some of the contaminants. In cells developing as a monolayer, since these cells are uniformly covered with the precipitate, the most successful transfection occurs. The technique was developed for the introduction of adenovirus DNA into rat cells by Graham and van der Erb in 1973.

In a study published in 1962 by Szybalska and Szybalski, the existence of calcium was shown to be responsible for the effective genomic DNA transformation of human cells. By calcium phosphate transfection, the first mammalian cell lines stably transfected with plasmid DNA were also developed in 1978.

Transfection with DEAE-dextran

The first transfection reagent to be produced was DEAE-dextran, and it was used very extensively until the introduction of lipofection reagents in the 1990s. It is a soluble

polycationic carbohydrate produced by electrostatic interactions to form aggregates with DNA. It gives a net positive charge to the whole complex, which allows it to interact with the cell membrane that is negatively charged and facilitates endocytosis uptake. Compared to the particles produced during calcium phosphate transfection, the complexes are very small. It is also possible to use much less DNA in each transfection experiment. The reagents are inexpensive, like the calcium phosphate process, and the technique is simple and effective. In order to generate stably transformed cell lines, DEAE-dextran-mediated transfection is not especially effective.

Lipofection

Liposome mediated gene transfer was first described in 1980 by Fengler. To form a fusogenic particle with DNA, liposomes are used. They are phospholipid hydrophobic, unilaminar vesicles. In culture, certain vesicles fuse with the cell membrane when combined with cells and carry DNA directly into the cytoplasm. Lipofection is the method of chemical gene transfer for gene therapy that is most commonly used. The downside is that the processing of liposomes containing DNA is difficult and labor-intensive. The ability to convert mouse cells in vivo by injecting liposomes into the tail vein was one particular advantage of the process. The first non-viral technique invented specifically for in vivo DNA transmission was liposome-mediated transfection.

By adding viral proteins that facilitate the successful fusion between viral envelopes and cell membranes, the efficiency of liposome-mediated gene transfer can be enhanced. This fusogenic particles have been known as virosomes. Lipofection, both transient and stable transfection, is highly reproducible and extremely efficient.

It makes transient transfection of up to 90 percent of culture cells. It also reveals stable transfection efficiencies that are up to 20 times better than conventional methods of chemical transfection. One downside to this technique is that the lipids in the laboratory are typically difficult to prepare. Therefore, they must be bought from a commercial supplier and they are very pricey. A positively charged head party, a linker, and a hydrophobic 'anchor' provide lipid-based transfection reagents. There are normally between one and four amine groups in the head group, and this is linked either by a glycerol bond to an aliphatic hydrocarbon chain anchor or by a variety of bonds to a cholesterol anchor.

Physical Transfection

In physical procedures, a form of physical force is used to deliver the DNA directly into either the cytoplasm or the nucleus. Interaction with the plasma membrane is not a prerequisite. This stops the endosomal system from being active and hence reduces the amount of damage caused by the exogenous DNA. The methods of physical transfection are typically costly if they use any kind of equipment that is needed to administer physical force. In solution, DNA can be added free of charge, although it can be helpful to protect it by creating chemical complexes (e.g. polyamines) to reduce the detrimental effects of shear forces during the transition process.

Electroporation

Neumann and colleagues first used this approach for animal cells in 1982. The transfection of cells after their exposure to a pulsed electric field is electroporation. This allows a number of nanometer-sized pores to expand for up to 30 minutes in the plasma membrane, allowing free DNA from the surrounding medium to be absorbed. Afterwards, with no apparent detrimental effects on the treated cells, the pores close spontaneously. For many existing cell lines, especially those recalcitrant to methods of chemical transfection, it is ideal. The standard method for electroporation is very clear. In an electroporation barrier, cells are suspended in or flooded and exposed to a short electrical pulse of high voltage.

The amplitude and length of the pulse determines the efficacy of the transfection, and for various cell lines, these parameters must be calculated empirically. It is reliable, highly reproducible, ideal for both stable and transient transfection, and has the added benefit of being able to at least partially regulate the number of transgene copies. The drawbacks of this approach include the need for advanced capacitor discharge devices capable of reliably regulating pulse duration and voltage, the need for greater cell numbers and higher DNA concentrations than those used in methods of chemical transfection, and the comparatively high degree of cell death that follows this phase. As a tool for in vivo gene transfer, electroporation has also been explored. The application of DNA into surface or near-surface tissues such as skin, muscle and melanoma and even into internal organs such as the liver has been used successfully.

Initially, electroporation was only applicable to cells cultivated in suspension, but it can also be used for monolayer cells by modifying the technique. A variation of the technique known

as nucleofection has most recently been identified. In order to facilitate direct electroporation-mediated gene transfer to the nucleus, this process blends precise electroporation conditions with multiple transfection reagents, resulting in the effective transfection of historically challenging targets, including primary cells. The pore forming, using laser therapy, is based on another in vitro transfection technology called laser poration. This involves a process of DNA uptake similar to electroporation, i.e. free DNA is picked up directly by transient pores formed by a precisely focused laser beam from the ambient medium. This method can only be extended to a limited number of cells at a time, like microinjection but with optimum DNA concentration, it can result in stable.

University Library Reference-

Dubey R.C, A textbook of biotechnology, 1st edition(2004)

Ancient Indian Literature Reference - Formulations containing mercury are only rarely mentioned in Charaka Samhita. The first reference pertaining to Parada and its utility in therapeutics mentioned in the classic is controversial.

Competitive questions from today topic (2 questions Minimum)-

Which one of the following statements about alleles is NOT TRUE?

a. They may occupy different loci in the same chromosome
b. There may be several at one locus
c. One may be dominant over another
d. They may show co-dominance
Exam NameDBT-2019

The total magnification of a microscope is calculated by:-

A. Multiplication of the objective lens and condenser lens magnification powers
B.Square of objective lens power
C.Multiplication of the objective lens and ocular lens magnification powers
D.Addition of the objective lens and ocular lens magnification powers
Exam NamePU-016

Questions to check understanding level of students-

What is Liposome?

References:

Zhou, M., Wang, L., Su, W., Tong, L., Liu, Y., Fan, Y., Luo, N., Zheng, Y., Zhao, H., Xiang, R. and Li, Z., 2012. Assessment of therapeutic efficacy of liposomal nanoparticles mediated gene delivery by molecular imaging for cancer therapy. *Journal of Biomedical Nanotechnology*, *8*(5), pp.742-750.

Chapter 6: Cellular therapy and stem cell

Stem cells are the raw materials of the body, cells from which all other cells are produced with special functions. Stem cells differentiate to form more cells called daughter cells under the proper conditions in the body or laboratory.

These daughter cells, such as blood cells, nerve cells, heart muscle cells or bone cells, either become new stem cells (self-renewal) or become differentiated cells (differentiation) with a more unique role. In the body, no other cell has the inherent capacity to produce new kinds of cells.

Increase knowledge about how illnesses emerge. Researchers and physicians may better understand how diseases and conditions grow by seeing stem cells mature into cells in bones, heart muscles, nerves, and other organs and tissues.

To substitute diseased cells, produce healthy cells (regenerative medicine). Stem cells can be directed to become unique cells that can be used in individuals to rebuild and heal sick or injured tissues.

Those of spinal cord injury, type 1 diabetes, Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, heart disease, stroke, fires, tumours, and osteoarthritis are individuals that can benefit from stem cell treatments.

Stem cells may have the ability to be grown for transplant and regenerative medicine to become new tissue. Researchers aim to advance the understanding and uses of stem cells in transplantation and regenerative medicine.

For protection and efficacy, research experimental medicines. Researchers can use certain types of stem cells to assess the medications for efficacy and consistency before using investigational drugs in persons. For cardiac toxicity testing, this method of testing would most likely first have a clear effect on drug production first.

The feasibility of using human stem cells that have been programmed into tissue-specific cells to research new drugs is a recent field of research. The cells must be engineered to possess the properties of the type of cells attacked by the drug in order for the development of

experimental drugs to be successful. Techniques continue to be researched to programme cells into particular cells.

For instance, to test a potential drug for a nerve disorder, nerve cells may be produced. Tests will see whether there was some influence of the new drug on the cells and whether the cells were affected.

Embryonic stem cells: These stem cells originate from three- to five-day-old embryos. An embryo is considered a blastocyst at this level and contains around 150 cells. These are stem cells that are pluripotent (ploo-RIP-uh-tunt), meaning they can break into more stem cells or becoming another sort of cell in the body. This versatility helps embryonic stem cells in most adult tissues, such as bone marrow or fat, to be used to rebuild or restore diseased tissue and organs. These stem cells are present in small numbers. Adult stem cells have a more restricted capacity to give birth to new body cells relative to embryonic stem cells. Until recently, researchers believed that only identical cell types could be formed by adult stem cells. Researchers, for example, assumed that only blood cells could give birth to stem cells located in the bone marrow. However, new research indicates that adult stem cells could be able to develop multiple cell types. Bone marrow stem cells, for example, could be able to produce bone or heart muscle cells.

Adult cells Altered to have embryonic stem cell properties (induced pluripotent stem cells). Using genetic reprogramming, scientists have successfully converted normal adult cells into stem cells. Researchers will reprogram the cells to behave similarly to embryonic stem cells by modifying the genes in the adult cells.

This new approach will allow researchers to use reprogrammed cells instead of embryonic stem cells and avoid the rejection of new stem cells by the immune system. Scientists do not yet know, however, whether the use of altered adult cells in human beings can cause harmful effects.

Researchers were able to take and reprogram normal connective tissue cells to become healthy heart cells. In research, animals that were injected with new heart cells with heart disease demonstrated increased heart function and survival time.

Stem cells of perinatal origin. Stem cells in amniotic fluid as well as umbilical cord blood were discovered by researchers. These stem cells have the capacity to develop into advanced cells as well. The sac which surrounds and protects a developing foetus in the uterus is filled with amniotic fluid. In samples of amniotic fluid taken from pregnant mothers, researchers have detected stem cells to screen for anomalies, a technique called amniocentesis.

Stem cells are the raw materials of the body, cells from which all other cells are produced with special functions. Stem cells differentiate to form more cells called daughter cells under the proper conditions in the body or laboratory.

These daughter cells, such as blood cells, nerve cells, heart muscle cells or bone cells, either become new stem cells (self-renewal) or become differentiated cells (differentiation) with a more unique role. In the body, no other cell has the inherent capacity to produce new kinds of cells.

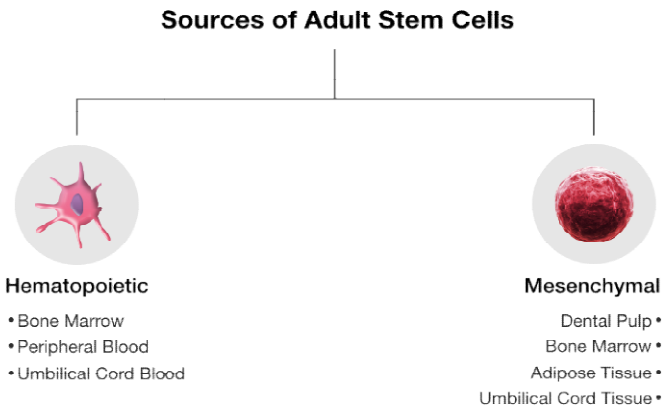

Diagram of source of stem cell

Stem embryonic cells. These stem cells originate from three- to five-day-old embryos. An embryo is considered a blastocyst at this level and it has around 150 cells.

There are stem cells that are pluripotent (ploo-RIP-uh-tunt), meaning that they can break into more stem cells or become another sort of cell in the body. This versatility makes it possible to use embryonic stem cells to rebuild or repair tissue and organs that are diseased.

Stem cells from adults. In most adult tissues, such as bone marrow or fat, these stem cells are located in limited numbers. Adult stem cells have a more limited ability to give birth to different cells of the body compared with embryonic stem cells.

Until recently, researchers assumed that only identical kinds of cells could be produced by adult stem cells. Researchers believed, for example, that stem cells found in the bone marrow could only give rise to blood cells.

Emerging data, however, shows that adult stem cells could be able to produce distinct cell types. Bone marrow stem cells, for instance, may be able to produce bone or heart muscle cells.

This study has contributed to clinical trials in the early stages to assess the efficacy and wellbeing of persons. Adult stem cells, for instance, are actually being studied in patients with neurological or heart disease.

Adult cells that are changed to have embryonic stem cell properties (induced pluripotent stem cells). Using genetic reprogramming, scientists have successfully converted normal adult cells into stem cells. Researchers will reprogram the cells to behave similarly to embryonic stem cells by modifying the genes in the adult cells.

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University Library Reference-

Dubey R.C, A textbook of biotechnology, 1st edition (2004)

Ancient Indian Literature Reference - "We had 100 Kauravas from one mother because of stem cell and test tube technology," said Rao, vice chancellor at Andhra University.

Competitive questions from today topic (2 questions Minimum)-

Error-free repair of double strand breaks in DNA is accomplished by

A. non-homologous end-joining B. non-homologous end-joining
C. homologous recombination D. mismatch repair.
Exam Name CSIR NET 2016

Hemophilia is X linked disorder. If a hemophilic male marries a normal female, what percentage of sons will have hemophilia?

A.25%
B.5%
C.0%
D.100%
Exam NameCFTRI-2012

Questions to check understanding level of students-

What is stem cell therapy?

References:

Chen, A.K.L., Reuveny, S. and Oh, S.K.W., 2013. Application of human mesenchymal and pluripotent stem cell microcarrier cultures in cellular therapy: achievements and future direction. *Biotechnology advances*, *31*(7), pp.1032-1046.

Chapter 7: Recombinant therapy and Clinical applications of recombinant technology

Genetic engineering or recombinant DNA (rDNA) technology is the umbrella term for a series of experimental techniques that allow the modification of individual genes and DNA sequences, resulting in genetically modified organisms (GMO) and products. In medicine, agriculture and manufacturing, many possible applications of rDNA have been added. Conventionally, proteins and other biological materials are mostly of poor purity, extracted from human or animal serum or tissue. There are many benefits to the manufacturing of therapeutic goods by means of rDNA technology, such as the supply of medicines that could not be made by traditional methods, the manufacture of adequate amounts of drugs and the provision for the manufacture of sterile drugs.

Nine drugs have been approved for marketing in India: insulin (diabetes medicine), alpha interferon (cancer medicine), hepatitis B vaccine, GMCSF, G-CSF, blood clotting factor 7, erythropoietin (kidney failure medicine), streptokinase (heart attack medicine) and human growth hormone. Both these drugs are imported, except for Hepatitis-B. The cumulative imports of these goods were 237 crores in 1996-97. Human insulin, alpha interferon, and erythropoietin are the four main recombinant drugs with elevated market potential in India.

Human Therapeutics from RDT:

The manufacture of human therapeutics such as hormones, growth factors and antibodies, which are not only barely available but still very expensive for human use, has become one of the main advantages of recombinant DNA technology. Proteins that aid the body combat infection or conduct particular roles, such as blood proteins, hormones, growth factors, interferons and interleukins, provide recombinant therapeutics. Recombinant biopharmaceuticals have increased tremendously in recent years, beginning with basic proteins such as insulin (the first recombinant medicinal product) and then growth hormone.

Due to their greater specificity and lack of side effects, therapeutic proteins are favoured over traditional medications. Therapeutic proteins are less harmful than chemical drugs and are not carcinogenic or teratogenic (capable of disrupting an embryo or fetus' growth and development).

Antisense Therapeutics:

Not only can the base-pairing rules be used to render artificial genes, but they can also be used to interrupt mRNA translation. This method is an example of scientists imitating nature, as is also the case. In normal cells, the development of an RNA molecule which is complementary to mRNA is an uncommon mechanism for regulating gene expression.

This complementary molecule is called antisense RNA because it binds to the mRNA bases that code for a protein by base pairing to the "sense" bases. The creation of a double-stranded RNA hybrid delays the translation of the mRNA, and the hybrid in the cytoplasm appears to rapidly break down.

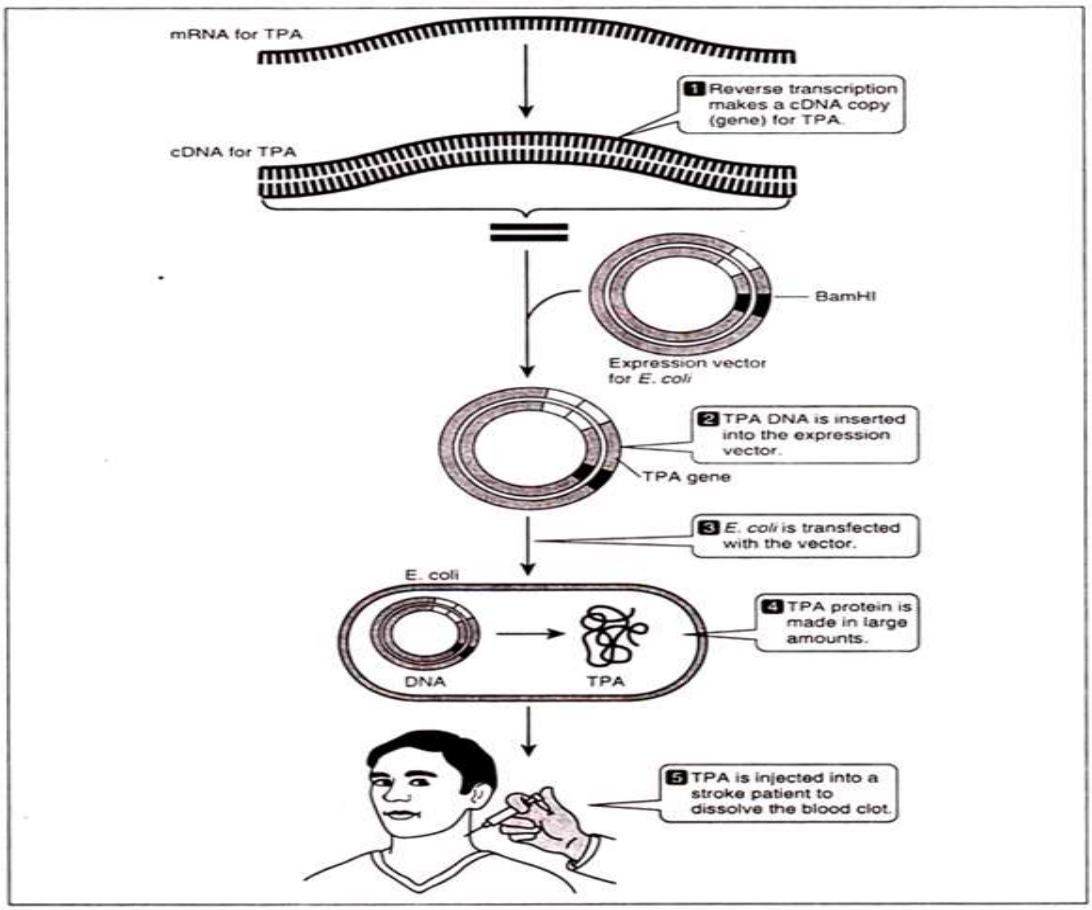

Fig. 2.3: Tissue Plasminogen Activator: From Protein to Gene to Drug TPA is a naturally occurring human protein involved in dissolving blood clots. Its isolation and use as a pharmaceutical agent for treating patients suffering from blood clotting in the heart or brain. Today RDT is helping us to produce TPA in large quantity.

Diagram of Recombinant therapy

Although the gene continues to be transcribed, translation does not take place. After determining the sequence of a gene and its mRNA in the laboratory, scientists can make and add specific antisense RNA to a cell to prevent translation of that gene's m RNA.

Genetically Engineered Proteins in Therapeutics:

Proteins that are obtained by the RDT process are genetically modified proteins. They are uniquely structured proteins that are tactfully formed by either avoiding or cheating the host to defend against it.

It is understood that geogenetically modified proteins block or imitate surface receptors present on the cell membranes. We can de-sign, for instance, a protein that can resemble a receptor protein to which HIV binds when entering WBC. Instead, HIV binds to this genetically modified pro-protein molecule and does not join the WBC.

Gene Therapy:

Biologists have now been permitted to attempt to cure genetic diseases in various ways by genetic modification. A procedure called gene therapy is one method. A genetic mutation is treated during gene therapy by inserting a gene into the cells of the patient. For diseases that result from the lack of a particular protein, gene therapy works well. The lung disease cystic fibrosis, for instance, arises from the absence of a functioning gene called the gene CFTR.

The gene encodes a protein when functional, which helps to transport ions in the respiratory passages into and out of cells. Bad ion exchange triggers the symptoms of cystic fibrosis without that gene, including the build-up of sticky mucus blocking the airways.

Clinical Application

Simply placed, in living cells, recombinant proteins are converted products of exogenous DNA. In general, the processing of recombinant proteins involves two main steps: molecular cloning and the expression of proteins. Today, one of the most potent methods utilised in life sciences is recombinant protein development. In medicine, science, and biotechnology, etc., recombinant proteins have wide applications.

1. Medicine

2. Research

3. Biotechnology

4. The Latest Researches of Recombinant Proteins

Recombinant proteins and diseases

Recombinant proteins and vaccines

Medicine

Many human disorders are due to disruption of specific proteins systemically or partly. For a number of disorders, such as diabetes, cancer, infectious diseases, haemophilia, and anaemia, therapeutic proteins provide essential therapies. Antibodies, FC fusion proteins, hormones, interleukins, enzymes, and anticoagulants are common therapeutic proteins.

For medicinal uses, there is a rising need for recombinant proteins. Human proteins acquired through genetic modification play a crucial role in the demand for medicinal medicines.

At present, most of the recombinant therapeutic proteins in mammalian cells are produced because mammalian cells are capable of producing high-quality proteins identical to those that exist naturally. Moreover, because of its well-characterized genetics, rapid development, and high-yield development, many permitted recombinant therapeutic proteins are produced in E.coli.

Therapeutic proteins will, essentially, be divided into four classes.

Class I: Enzymatic or regulatory function therapeutic proteins. These proteins replace a defective or dysfunctional protein, up-regulate or provide a new function or activity to an existing pathway.

Class II: Medicinal proteins with special activity targeting. Those proteins interact with or deliver other molecules to a molecule or organism.

Class III: Protein therapeutics as vaccines. These proteins help guard against tumours, autoimmune disorders and foreign agents.

Community IV: as diagnostics, medicinal proteins. Generally, these proteins are purified proteins and recombinant proteins.

Research

Recombinant proteins help to elucidate an organism's basic and fundamental values. These molecules may be used in various cellular activities, such as cell signalling, metabolism, development, replication and death, transcription, translation, and protein modification, to recognise and locate the location of the protein encoded by a particular gene and to reveal the role of other genes. Thus, in molecular biology, cell biology, biochemistry, structural and biophysical experiments, and many other scientific areas, recombinant proteins are widely used.

Recombinant proteins are useful tools in understanding protein-protein interactions. They have proven performance in several laboratory techniques, such as ELISA, Western Blot, and immunohistochemistry (IHC). To establish enzymatic assays, recombinant proteins may be used. Recombinant proteins may be used as standards, such as ELISA standards, when used in combination with a paired antibody pair. In comparison, in Western blots, recombinant proteins may be used as supportive controls.

Biotechnology

In industry, food packaging, agriculture, and bioengineering, recombinant proteins are also included. In the breeding industry, for example, enzymes may be applied to animal feed in order to maximise the nutritional value of feed products, minimise the cost of feed and waste disposal, encourage animal gut health, enhance the efficiency of livestock and improve the ecosystem. In addition, lactic acid bacteria (LAB) have been used for the processing of fermented foods for a long time, and lately, LAB has been engineered for the expression of recombinant proteins that will have wide applications such as optimising digestion and feeding for humans/animals.

A protein produced by the liver, alpha-1-antitrypsin is secreted into the bloodstream and then circulates through the body to protect the lungs. Patients who are unable to generate protein typically undergo an injection of alpha-1-antitrypsin protein, which is derived from the blood of the donor, on a daily and quantitative basis. Researchers from the Biosustainability Center

of the Novo Nordisk Foundation at the Technical University of Denmark have successfully developed alpha-1-antitrypsin in CHO scale cells. In addition, through genetic methods, the researchers have enhanced the proteins, thereby obtaining recombinant therapeutic proteins identical to human variants with a particular sugar structure. In the future, it is possible to eliminate the need for individual donors.

A research led by Georgetown University, released in Science Journals, found that, despite an inherited predisposition to feed at all hours, forced expression of the protein FGFBP3 (BP3 for short) in a laboratory strain of obese mice showed a surprising decrease in their fat mass. The findings of this study indicate that the FGFBP3 protein could provide novel therapy to reverse metabolic syndrome-related diseases, such as type 2 diabetes and fatty liver disease. But, since BP3 is a natural protein and not an experimental compound, after a final round of preclinical tests, clinical trials of recombinant human BP3 may begin.

The subject of the 2018 Nobel Prize for Physiology & Medicine is PD-L2, one of the main players in immune checkpoint therapy. This work, pioneered by US Professor James P Allison and Japanese Professor Tasuku Honjo, has contributed to therapies focused on immune checkpoint therapy for cancers such as melanoma, lung cancer and others. AMSBIO has recently added to its immunotherapy portfolio an important new product - a PD-L2 / TCR activator - CHO Recombinant Cell Line.

Recombinant proteins and vaccines

Human papillomavirus, referred to as HPV, has been linked to head and neck cancers in men and not only induces cervical cancer in women, certain strains. Currently, the vaccine against Gardasil-9 is used to prevent the infection. Of these individual vaccines, Gardasil 9 is nine combined into one, which can defend against numerous HPV variants. But it is fairly pricey, so few people can afford it. By adding the L2 protein to a pre-existing hepatitis B virus-like particle, researchers at the School of Life Sciences and the Biodesign Center for Immunotherapy, Vaccines and Virotherapy developed an HPV VLP (virus-like particle) and then they obtained a recombinant immune complex by fusing the L2 protein to an antibody. In plants, they scaled up the vaccine, getting a more affordable, plant-based vaccine that activates a robust immune response to the HPV virus that is widely defensive. This vaccine model also can be applied to other infectious agents. Next, the researchers would like to test safety and efficacy of the vaccine on humans or nonhuman primates.

As stated by researchers at the University of Edinburgh and the Pirbright Institute, a new vaccination technique, also known as the use of a specialist recombinant virus vaccine, could provide safety to millions of chickens at risk of extreme respiratory disease. To insert pathogens into cells in the body, these vaccines use harmless or weak forms of a virus or bacteria. In this case, to construct two copies of a harmless virus, experts used recombinant viruses with distinct spike proteins as vaccines.

A innovative vaccine, also referred to as LASSARAB, intended to shield people from both Lassa fever and rabies, has shown promise in preclinical studies, according to recent reports published in Nature Communications. A reduced rabies virus vector, or handler, is used by the inactivated recombinant vaccine candidate. In the rabies virus vector, the development team incorporated genetic material from the Lassa virus so that the vaccine expresses surface proteins from both the Lassa virus and the rabies virus.

The immune response to both Lassa and rabies viruses is triggered by these surface proteins. In order to "kill" the live rabies virus used to produce the driver, the recombinant vaccine was then inactivated.

Overall, biotechnology advances have improved and encouraged the development of recombinant proteins for diverse applications. Their functions in medicine, science, and biotechnology are irreplaceable, while recombinant proteins do have some disadvantages. We are still looking forward to making more success with recombinant proteins in the treatment of different diseases.

University Library Reference-

Dubey R.C, A textbook of biotechnology, 1st edition(2004)

Ancient Indian Literature Reference - “Studies using ancient DNA have been rewriting prehistory all over the world in the last few years and in India, there has been one fascinating discovery after another. The most recent study on this subject, led by geneticist David Reich of Harvard University, was published in March 2018 and co-authored by 92 scholars from all over the world - many of them leading names in disciplines as diverse as genetics, history, archaeology and anthropology.

Competitive questions from today topic (2 questions Minimum)-

Nucleotides are building blocks of nucleic acids. Each nucleotide is a composite molecule formed by

A. base-sugar-phosphate. B.base-sugar-OH.
C. (base-sugar-phosphate)n. D.sugar-phosphate
Exam NameAIIMS-2017

Human immuno deficiency virus (HIV) has a protein coat and a genetic material which is

A.Single stranded DNA. B.Single stranded RNA.
C.Double stranded RNA. D. Double stranded DNA.
Exam NameAIIMS-2017

Questions to check understanding level of students-

What is Recombinant therapy?

References:

Caplan, A.I. and Bruder, S.P., 2001. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends in molecular medicine*, *7*(6), pp.259-264.

Einhorn, T.A., 2003. Clinical applications of recombinant human BMPs: early experience and future development. *JBJS*, *85*(suppl_3), pp.82-88.

Chapter 8: Erythropoietin and Human growth hormone

Erythropoietin (EPO) is a kidney-generated hormone that stimulates the bone marrow to form red blood cells. The kidney cells that make erythropoietin in the blood that flows through the kidney are susceptible to low levels of oxygen. When the oxygen level is too low, these cells make and release erythropoietin. A low level of oxygen may mean a reduced number of red blood cells (anaemia) or molecules of haemoglobin that carry oxygen through the body.

The kidney cells that create EPO are specialised and are susceptible to the low amounts of oxygen entering the kidney in the blood. When the oxygen level is low in the kidney, these cells release erythropoietin. In order to generate more red blood cells, erythropoietin activates the bone marrow, which in turn enhances the oxygen carrying capacity of the blood.

The prime regulator of the development of red cells is the EPO. Its key functions are to facilitate the division and formation of red blood cells and to initiate the synthesis of oxygen transporting haemoglobin, the molecule inside red cells. Not only in the kidney but also, to a lesser extent, in the liver, EPO is made. On human chromosome 7, the EPO gene was found (in band 7q21). In order to regulate liver versus kidney development of EPO, separate DNA sequences flanking the EPO gene act. The blood EPO calculation can suggest bone marrow or kidney disease disorders. Natural EPO levels range from 0 to 19 mU/ml (milliunits per milliliter). In polycythemia rubra vera, a condition characterised by an accumulation of red blood cells, elevated amounts can be observed. In chronic renal disease, lower than average levels of EPO are seen.

EPO was synthetically developed using recombinant DNA technology for use in people with certain forms of anaemia: anaemia due to kidney disease, anaemia secondary to AIDS treatment with AZT, and cancer-associated anaemia.

In endurance athletes such as alleged cyclists (in the Tour de France), long-distance riders, speed skaters, and Nordic (cross-country) skiers, EPO has been highly misused as a performance-enhancing substance. EPO is known to be extremely dangerous when misused in such circumstances (perhaps because dehydration will further increase blood viscosity, raising the risk of heart attacks and strokes. The Tour, the Olympics, and other sporting organisations have prohibited EPO.

Causes: Inadequate or deficient development of red blood cells, a high rate of red blood cell loss, and heavy bleeding are the three primary causes of the disorder. One sort of deficient

red cell development is megaloblastic. Anemia can be moderate, easily treatable or extreme and requiring emergency intervention.

Growth hormone (GH) is a tiny protein produced and secreted into the bloodstream by the pituitary gland. The development of GH is mediated by a complex collection of hormones produced in the brain hypothalamus and in the intestinal and pancreatic tracts.

In pulses, the pituitary puts out GH; levels increase after exercise, pain, and sleep. There is more GH developed at night than during the day under normal conditions. This physiology is confusing, but it teaches us, at the very least, that intermittent blood testing to measure GH levels are useless because during the day, high and low levels alternate. But scientists who closely calculate the total development of GH report that it increases during infancy, peaks during adolescence, and decreases from middle age onwards.

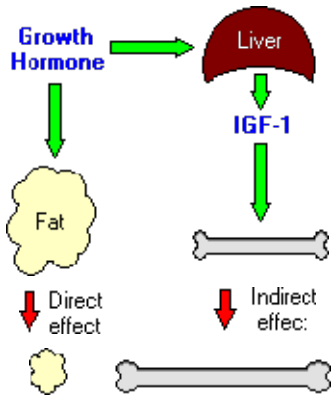

Diagram of growth hormone

In the human body, GH works on several tissues. It promotes bone and cartilage development in kids and teenagers. GH improves protein production in people of all ages, encourages the use of fat, interferes with the activity of insulin, and enhances blood sugar levels. GH also increases insulin-like growth factor-11 levels (IGF-1).

Human growth hormone benefits

As a prescription drug that is prescribed by injection, GH is available. For children with GH deficiency and those with very short stature, GH is suggested. Adult GH deficiency, a rare disorder that almost invariably occurs in combination with significant problems involving the hypothalamus, pituitary gland, or both, is now approved for care. Adult GH deficiency

diagnosis relies on special tests that promote the development of GH; basic blood tests are at best ineffective, at worst deceptive.

Injections of GH support adults with bona fide GH deficiencies. They appreciate fracture safety, expanded muscle density, enhanced ability and energy for exercise, and a decreased risk of potential heart disease. But the price to pay is there. Up to 30 percent of patients report side effects that include fluid loss, inflammation in the joints and muscles, carpal tunnel syndrome (hand pain and numbness triggering nerve pressure in the wrist), and elevated levels of blood sugar.

Human growth hormone and aging

A team of researchers evaluated 31 high-quality trials that were conducted after 1989 to assess the efficacy and effectiveness of GH in stable elderly people. Each of the experiments was limited, but 220 subjects who got GH and 227 control subjects who did not get the hormone were assessed together. Two thirds of the subjects were men; their average age was 69, and overweight but not obese was the standard volunteer.

The dosage of GH differed significantly, and the length of treatment varied between two and 52 weeks. Even, the various doses succeeded in increasing IGF-1 levels by 88 percent, which represents the amount of GH.

The treated people added an average of 4.6 pounds of lean body mass as opposed to the participants who did not get GH, and they lost a comparable amount of body fat. No significant changes were observed in the levels of LDL ('bad') cholesterol, HDL ('good') cholesterol, triglycerides, aerobic ability, bone density, or fasting blood sugar and insulin. But a high number of side effects, including fluid accumulation, knee pain, breast enlargement, and carpal tunnel syndrome, is encountered by GH recipients. In order to discern any improvement in the risk of cancer, the trials were too short, although other studies show an elevated risk of cancer in general and of prostate cancer in particular.

University Library Reference-

Dubey R.C, A textbook of biotechnology, 1st edition(2004)

Competitive questions from today topic (2 questions Minimum)-

Rolling circle model of replication in viruses

A .initiates with a nick on one strand

B. originates at oriC

C. results in only one copy of the genome

D. results in concatemers

Exam Name IIT-JAM 2016

The antibiotics have no effect on viruses because

A. viruses show metabolism of their own

B. viruses are too small in size for antibiotics to act upon them

C. viruses show no metabolism of their own

D. viruses produce a thick covering and encyst themselves as endospores

Exam NameAIIMS-2015

Questions to check understanding level of students-

What is Erythropoietin?

References:

Greenwell, P. and McCulley, M., 2008. *Molecular therapeutics: 21st century medicine*. John Wiley & Sons

Chapter 9: Antisense therapy and si RNA

The backbone in antisense medication is nucleic acids. Antisense oligonucleotide-based therapeutics require gene expression modulation downwards. RNA-based medicines that contain antisense oligonucleotides, by altering RNA and/or reducing, restoring, and changing protein expression by various molecular pathways, have significant therapeutic potential for the treatment of different diseases. Targeted antisense therapy pharmacology has provided the platform for translating its effectiveness to the clinic. Chemical modifications of antisense oligonucleotides have not only improved the specificity and potency over the years, but also decreased the side effects. This also altered the entire architecture of the clinical trial and propose newer treatment techniques.

Improvement in the science of antisense oligonucleotide therapy has permitted and taken studies from the bench to the clinic. In addition, both preclinical and clinical reactions have been seen with the use of small interfering RNAs, micro RNAs, ribozymes, and other antisense substances to combat dangerous diseases such as cancers. In addition, in the form of precision medicine, antisense therapy has tremendous potential to address particular genes of concern. In various disease contexts, optimization of improved distribution, precision, affinity and nuclease tolerance with decreased toxicity is ongoing.

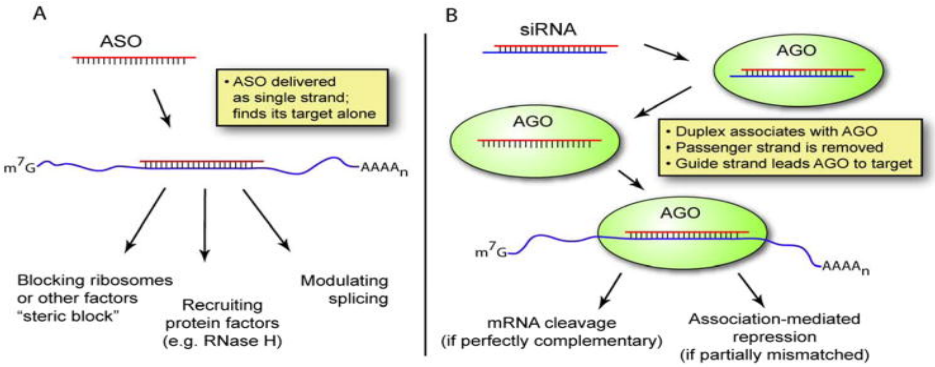

Diagram Antisense therapy

The success in the sequencing of the next generation helps us to understand the genetic history of many disorders, such as cancer, Parkinson's, rheumatoid arthritis, and Alzheimer's, which illustrates the rise of personalised medicine. This knowledge has been well adapted and embraced for diagnosis, but in order to remedy the genetic disorders causing illnesses, the research also lags towards pharmaceutical therapies. The two main groups of US Food

and Drug Administration (FDA)-approved medications are currently small molecules and proteins. Via competitive binding, small-molecule drugs inhibit target proteins, while protein-based drugs (such as antibodies) can bind to multiple targets with high specificity. Protein size and stability are the key drawbacks to their effectiveness for certain disease targets, and all disease-relevant proteins or genes may not be targeted by both protein and small-molecule drugs.

Thus, there is a current need for precision genomics to produce medicines. The medicines focused on mRNA and DNA are more effective therapeutically and have the tremendous potential to treat the genetic defect. As a potential candidate to cure diseases at the genetic (gene and RNA) level, RNA-based drugs have arisen. Due to a variety of reasons, such as nucleic acid architecture, delivery methods and materials for delivering RNA drugs to the site of interest, the delivery of therapeutic RNA has been limited. The recent progress in RNA and RNA-protein therapy has shown the great potential for RNA delivery production, and modulating gene/protein expression and gene editing has shown the therapeutic applications of RNA-based drugs.

Antisense oligonucleotides are regarded as a method to treat disease by using short DNA-like molecules. This is the most powerful and widely used technology for selective gene therapy to control gene expression and medications. Such antisense oligonucleotides bind to messenger RNA (mRNA) and affect the development of proteins and inhibit the expression of genes. In order to inhibit the action of the particular target gene of interest in the human genome, antisense molecules are synthetic replicas of specific mRNA sequences. Recently, antisense therapy has emerged as a potential method for the treatment of multiple diseases, and the FDA has licenced many antisense drugs for treatment. Chemically modified oligonucleotides complementing particular mRNA are injected into the cells that inhibit the translation of the specific protein for antisense gene therapy.

Similarly, the antisense drug contains the essential "noncoding mRNA" molecule, which prevents the translation of a particular protein. For the treatment of infectious infections, genetic/hereditary diseases, as well as cancers, antisense oligonucleotides may be very useful. The oligonucleotides that exist spontaneously carry poor stability and very low specificity and have a number of in vivo side effects. Therapeutic use of oligonucleotides can be accomplished by optimising molecular stability and specificity and reducing side effects by chemical alteration. Low interfering RNA, ribozyme, DNAzyme, anti-gene, CpG, decoy, and

aptamer are the most common therapeutic oligonucleotides. Antisense oligonucleotide chemical modification may increase its ability to enter the cells to bind the unique target gene sequences that further inhibit the activity of the targeted gene.

Several delivery mechanisms of antisense RNA and antisense oligonucleotides have been developed to transport antisense RNA or oligonucleotides across the cell membrane through the cytoplasm and nucleus, such as virus vectors (retrovirus, adenovirus, and adeno-associated virus) and liposomes. Ribonucleic acid (RNA) is the primary target of oligonucleotides, while small molecules and antibodies target proteins mainly because of their chemical properties and distinct molecular mechanisms of action. The protein mRNA codes for non-coding RNAs (such as microRNA, transfer RNA, small interfering RNAs, ribosomal RNA, and long noncoding RNAs). Transferring genetic material from DNA to protein is the key feature of noncoding RNAs. Due to their high affinity, selectivity, ease of chemical modifications, and less toxicity, the main therapeutic approach to target RNA-based therapy is antisense oligonucleotides. This chapter offers a detailed description of the treatment of antisense and the key therapeutic approaches.

The tremendous advancement in the area of gene therapy and antisense therapy is obvious from various clinical trials focused on gene therapy and antisense therapy that are currently ongoing worldwide.

The first gene therapy-based medication, Gendicine (Ad-p53), was approved in China for the treatment of squamous cell carcinoma of the head and neck in combination with radiotherapy. For the local treatment of cytomegalovirus-induced retinitis, one AON drug, Vitravene, has also been approved, and several others are in clinical trials, including siRNAs, miRNAs, and ribozymes that target different oncogenes and other cancer-promoting genes in mRNAs.

While in laboratory and clinical settings, the use of gene therapy and antisense therapy to mediate tumour regression is well illustrated, the impediment persists as this is converted into broad clinical application. The lack of delivery mechanisms that effectively administer an appropriate dose of a therapeutic gene(s) or antisense drug(s) to the desired tumour site are the key hurdles remaining in cancer gene therapy and antisense therapy. The distribution of targeted genes or antisense drugs for therapeutic approaches to distant tumours is a challenging job that urges the production of delivery vectors capable of overcoming several barriers. To transmit the therapeutic gene or antisense compound into the targeted tumour cells or tissues, many researchers have used viral and non-viral vectors.

While the reports of clinical trials focused on early gene therapy and antisense therapy using either viral or non-viral vectors have been promising, it is still difficult to find a single strategy that satisfies all the criteria for ideal gene transfer and vector expression.

Limitations of new vector technology have hampered the clinic's advancement in gene therapy and cancer antisense therapy. Therefore, one of the possible methods that must be further investigated in the future to increase gene therapy and antisense therapy against a wide variety of cancers is the development of suitable delivery mechanisms for targeting therapeutic genes and antisense agents into targeted tumour cells and tissues. It is hoped that a promising technology for systemic cancer gene therapy and antisense therapy will be the next generation of carriers.

Si RNA

A family of double-stranded RNA non-coding RNA molecules, 20-25 base pairs in length, similar to miRNA, and functioning within the RNA interference (RNAi) pathway is a small interfering RNA (siRNA), also known as short interfering RNA or silencing RNA. By degrading mRNA after transcription, it interferes with the expression of particular genes with complementary nucleotide sequences, resisting translation.

The mechanism by which siRNA induces gene silencing by transcription repression happens as follows.

siRNA Mechanism

An endo-ribonuclease called Dicer is cleaved by long dsRNA (which can come from hairpin, complementary RNAs, and RNA-dependent RNA polymerases). In order to form short interfering RNA or siRNA, Dicer removes the long dsRNA; this is what causes the molecules to form the RNA-Induced Silencing Complex (RISC). Once siRNA reaches the cell, the RISC is inserted into other proteins.

The siRNA is unwound to create one single stranded siRNA until the siRNA is part of the RISC complex. Because of its base pairing at the 5 'end, the strand that is thermodynamically less stable is selected to remain part of the RISC complex. The single stranded siRNA that is part of the RISC complex can now scan to locate a complementary mRNA. Once the single stranded siRNA (part of the RISC complex) binds to its target mRNA, mRNA cleavage is induced.

Now the mRNA is cut and recognised by the cell as irregular. This contributes to mRNA degradation and, in consequence, no mRNA translation into amino acids and then proteins. The gene that encodes the mRNA is therefore silenced.

SiRNA is also similar to miRNA, but miRNAs are derived from shorter stemloop RNA products, usually silence genes by translation repression, and have wider specificity of action, whereas siRNAs typically function before translation by cleaving the mRNA, and have 100% complementarity, hence very close specificity of the target.

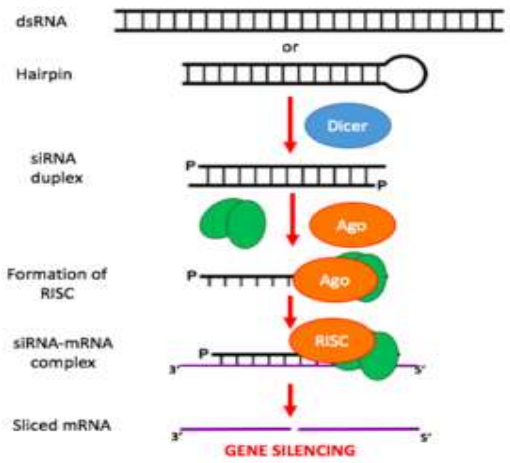

Diagram of Si RNA Mechanism

Library Reference book Dubey R.C, A textbook of biotechnology, 1st edition(2004)

Ancient Indian Literature Reference - https://www.hitxp.com/articles/science-technology/sanskrit-vedic-chants-and-the-secret-world-of-human-dna-wave-genetics-of-biological-internet-causing-hyper-communication/ Maricha of Ramayana who got himself converted into a beautiful deer? Hanuman is said to have mastered the great eight arts (Ashta Siddhis) of anima, mahima, laghima, garima etc where he could instruct his body to become as large as a mountain, or as tiny as an ant, or as heavy as a huge rock, or as light as a feather etc!

Competitive questions from today topic (2 questions Minimum)-

Nucleoside consists of

A. Sugar B. Sugar and phosphate

C. Sugar, phosphate and a nitrogenous base D. Phosphate

Exam NameCUSAT-2016

DNA strands run

A. Parallel B. Partly parallel, partly anti-parallel

C. Anti-parallel D. Horizontal

Exam NameCUSAT-2016

Questions to check understanding level of students-

What is si RNA?

References:

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Chapter 10: Transgenic animals

As models in biomedical science, transgenic animals are widely used in the laboratory. Genetically engineered rodents, mainly rats, are over 95 per cent of those used. In order to explain gene regulation in the form of disease tolerance, progression and to assess reactions to a therapeutic intervention, they are valuable instruments for studying human disease.

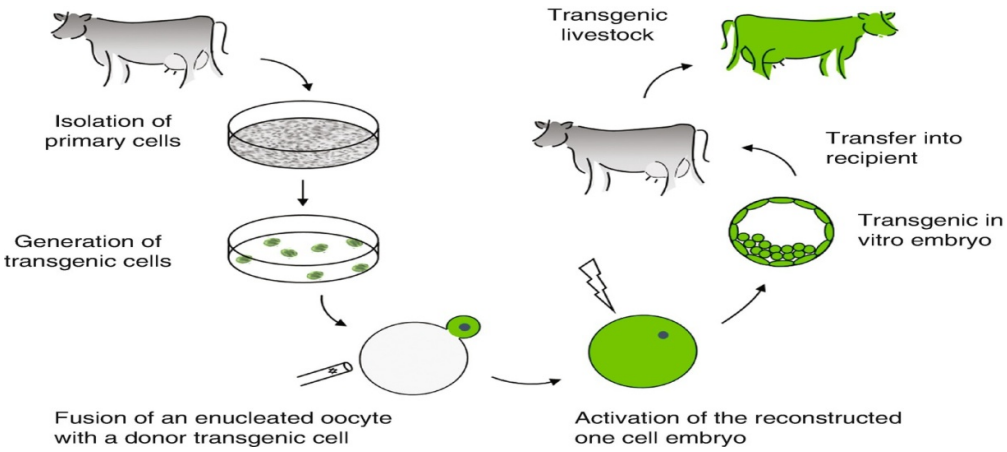

Diagram of transgenic animal

To spontaneously generate human antibodies for use as therapeutics, mice have now been genetically engineered. Transgenic mice were used to extract seven of the eleven monoclonal antibody drugs approved by the FDA between 2006 and 2011.

As a way to manufacture vast amounts of complex human proteins for the treatment of human illness, transgenic farm animals are also being explored. In mammalian cell-based reactors, such therapeutic proteins are currently being made, but this manufacturing process is costly. The construction of a new cell-based manufacturing facility for one therapeutic protein, for example, was expected to cost more than US$500 million in 2008.

A cheaper option would be to develop a means to produce recombinant proteins in the milk, blood or eggs of transgenic animals. Progress in this area, however, has been slow to-date. Only two biomedical products have so far received regulatory approval. The first is human antithrombin III, a therapeutic protein produced in the milk of transgenic goats, which is used

to prevent clots in patients with hereditary antithrombin deficiency receiving surgery or undergoing childbirth. A relatively small herd of goats (about 80) can supply enough human antithrombin III for all of Europe. The second product is a recombinant human C12 esterase inhibitior produced in the milk of transgenic rabbits. This is used to treat hereditary angiodema, a rare genetic disorder which causes blood vessels in the blood to expand and cause skin swellings.

Discovery

A variety of components depend on the ability to generate transgenic animals. The ability to transfer embryos is one of the first things required to produce transgenic animals. In 1891, in Angora rabbits, Walter Heape performed the first successful transfer of embryos. The ability to control the embryo is another essential aspect. Manipulation of embryos in mice in vitro was first recorded using a culture method in the 1940s. The capacity to manipulate eggs is also important. This was made possible by the efforts of Ralph Brinster, attached to the University of Pennsylvania, who devised a reliable culture egg system in 1963, and Teh Ping Lin, based at the California School of Medicine, who outlined a technique for micro-injecting fertilised mouse eggs in 1966 that allowed foreign DNA to be accurately inserted.

In 1974, the virologist Rudolph Jaenisch, then at the Salk Institute, and the mouse embryologist Beatrice Mintz at the Fox Chase Cancer Center announced the first genetic manipulation of animals. By injecting the SV40 virus into early-stage mouse embryos, they demonstrated the feasibility of altering genes in mice. In all of their tissues, the resulting mice carried the modified gene. In 1976, by infecting an embryo, Jaenisch announced that the Moloney Murine Leukemia Virus could also be passed on to offspring. Four years later, in 1980, together with Frank Ruddle, Jon Gordon and George Scango announced the birth of a mouse born with genetic material which they had injected into newly fertilised mouse eggs.

The successful implantation of foreign DNA into mice was documented by other scientists in 1981, thus altering the genetic makeup of the animals. These included Mintz at the Fox Chase Cancer Center in Philadelphia with Tim Stewart and Erwin Wagner; Brinster and Richard Palmiter at the University of Washington, Seattle; and Oxford University with Frank Costantini and Elizabeth Lacy.

Such work laid the groundwork for the development of genetically engineered transgenic mice to inherit unique types of cancer. As a laboratory tool to better understand the onset and

progression of cancer, these mice were generated. The benefit of such mice is that they provide the human body with a model that closely mimics it. Not only do mice offer a way to gain greater insight into cancer, but they also provide experimental drugs for research.

Application

Transgenic animals are animals (most commonly mice) that have been purposely inserted into their genome with a foreign gene. The microinjection of DNA into the pronuclei of a fertilized egg, which is subsequently inserted into the oviduct of a pseudopregnant surrogate mother, most usually generates such species. This results in the recipient animal giving birth to offspring that have been genetically engineered. To create a transgenic line, the offspring are then bred with other transgenic offspring. It is also possible to create transgenic animals by injecting DNA into embryonic stem cells, which are then micro-injected into an embryo that has grown for five or six days after fertilization, or by infecting an embryo with viruses that hold the DNA of interest. This final approach is widely used to modify a single gene, with the removal or 'knocking out' of a target gene in most cases. What's perceived as a 'knockout' animal is the end product.

Transgenic mice have been a primary model for disease investigation since the mid-1980s. Mice are not only the model of choice because their completed genome sequence is thoroughly studied, but their genome is identical to the human genome. In addition, physiological and behavioral experiments conducted on mice may be explicitly extrapolated to the human disease. For the generic manipulation of mouse cells and embryos, robust and advanced techniques are also readily available. The fact that they have a limited reproductive period is another advantage of mice. Other transgenic animals, such as pigs, sheep and rats, are also used, but due to technical restrictions, their use in pharmaceutical research has so far been limited. However, recent technical developments are setting the groundwork for the transgenic rat's broader acceptance.

In drug discovery and development, transgenic rodents play a variety of critical roles. Importantly, scientists are able to research the role of individual genes at the level of the entire organism, which has advanced the study of physiology and disease biology and enabled the discovery of new targets for drugs. Transgenic rodents may be designed to replicate human illness due to their similarities in physiology and gene function between humans and rodents. Indeed, a range of models of transgenic mice have been developed for this purpose. Mice are used to study obesity, heart disease, diabetes, arthritis, opioid

addiction, anxiety, ageing, Alzheimer's disease and Parkinson's disease as models, for example. They are also used for the study of various cancer forms. Furthermore, transgenic pigs are being studied as a source of transplant organs, which may solve some of the extreme donor organ shortages if proved clinically safe. The advent of the modern gene editing technology CRISPR, which significantly reduced the number of steps involved in the production of transgenic animals, has recently transformed the development of transgenic animals, making the whole process much faster and less expensive.

Library Reference book Dubey R.C, A textbook of biotechnology, 1st edition(2004)

Ancient Indian Literature Reference - Rubhus were three brothers (Rubhu, Vajra and Vibhu) created from the cell of their father. Their father was old but they were cloned young to bring back the youth of aging parents. Additionally, they also created a cloned horse and cow. Cloning of Rubhus is mentioned by seven different sages in seven different verses of Rigveda. All of these sages were from different generations thus depicting that such a technology existed over a long period of human life.

Competitive questions from today topic (2 questions Minimum)-

Which one of the following modifications occurs both on DNA and protein?

A. ADP-ribosylation B. Methylation
C.Sumoylation D. Ubiquitination
Exam NameIIT-JAM 2019

Which of the following statements about mature tRNA and/or mRNA are FALSE?

A. tRNAs end with CCA sequence at the 3 end
B Both form clover-leaf structures
C. Both are polyadenylated at their 3 ends
D. All tRNAs are devoid of introns
Exam Name IIT-JAM 2018

Questions to check understanding level of students-

What is transgenic animal?

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