Cell and Molecular Biology
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Week 12
Summary and Conclusions
Lecture - 44
Gene Therapy

Hello everyone, this is Dr. Vishal Tevedi from the Department of Biosciences and Bioengineering, IIT Guwahati. So what we are discussing in the course is Cell and Molecular Biology. So far, we have discussed the organism's origin of life, evolution, cell growth and regulation, apoptosis, necrosis, and we also discuss autophagy. In a couple of previous lectures and previous modules, we have also discussed the central dogma of molecular biology and immunology. So in this particular module, I have taken a few selected topics on which we are going to discuss.

So today we are going to discuss gene therapy. So, what is gene therapy? Gene therapy is the use of any collection of approaches for the treatment of human disease that depends on the introduction of DNA-based genetic material into an individual. It may be a gene that is going to be introduced, it may be removed, or there will be a change in the genetic material. So, the use of genetic manipulations by humans for the therapy of human diseases is a new and rapidly evolving field, and the purpose of gene therapy is that it should actually be able to correct the disease at the genetic level, right? So, this is being proposed or utilized for the treatment of cancers, hemophilia, and cystic fibrosis.

Spinal muscular atrophy, ADASID, and many more, right? The global gene therapy market, which was valued at 7.2 billion in 2003, has now increased to 8.85 billion in 2004, and it has been projected that this value could reach as high as 36.65 billion by the year 2032. Now, how does the disease explain why we need to do gene therapy? So if you get a disease, you actually have three different approaches to take care of it.

You have the option of taking medicine. So you can reduce the progression of that particular disease. You can actually use the surgical procedure to remove the diseased organs or tissue. But the gene therapy actually takes care of the disease at the root cause, right? So, it actually cures the cells and the genetic problem that is associated with a particular cell, and that is how it does not require supplementation with any kind of medicine or surgical procedure. Now this is very advantageous compared to taking medicine or undergoing a surgical procedure because gene therapy targets the root cause of the genetic problem associated with a particular disease.

So, as I said, you know the gene therapy is going to address the root cause, which is associated with the genetic alterations. It is going to be treated even for the parents' rights, and it can actually bring about changes in the offspring; it can also treat the parents for their own diseases as well. So, how has the field of gene therapy evolved? So, it started with the years 1960 to 1970 when the concept of gene therapy emerged, with a key paper in 1972 outlining its potential and the ethical considerations. So people propose that, you know, if there are possibilities, if there is advancement in genetic manipulation, why don't we actually alter the genes so that it can cure genetic diseases. Then, in the year 1980, it was decided to focus on fundamental research, particularly on using viral vectors to deliver the gene, leading to the first trial proposal in 1989.

Then in the year 1990, the first successful gene therapy trial was conducted on De Silva, who had SCID, making a major breakthrough. But, in the year 1999, the field trials suffered a setback with the death of Jesse, which actually failed because of the anaphylactic reactions, or I would say the site reactions to the viral vectors. In the year 2010, the first commercially available gene therapies were introduced, including Glybera in Europe and Luxturna for inherited retinal diseases, and these types, you know, the CAR T cell therapies, are being tested until 2027. Since 2012, the development of CRISPR-Cas9 mediated genetic modifications and manipulations has revolutionized the field of gene therapy, enabling the precise editing of the genome. And then in 2020, the first CRISPR-Cas based gene therapy was approved to treat sickle cell anemia and beta thalassemia, offering functional cures for these diseases.

Now, talking about gene therapy, the basic principle is that it actually takes healthy genes and introduces them into a patient, right? So imagine that a patient is suffering from a particular disease, and in that disease, one of the genes is non-functional or is not able to produce a particular enzyme. And then what we will do is take the healthy gene from a healthy individual and introduce that into a patient. And once you replace the defective gene with the healthy gene, you are actually going to produce the particular protein and that is how you can be able to cure a particular disease. Now talking about the approaches that you can use in gene therapy. So you can actually use the multiple approaches.

So, as far as the gene therapy type is concerned, we can have two different types of gene therapies. We can have somatic gene therapy; we can have germline gene therapies. So somatic gene therapies are where the somatic cells are actually going to be treated, and they are not going to go into the offspring. So it is actually only going to treat the parents, or it is only going to treat the particular individual. Whereas when you do germline gene therapies, the germline therapies are actually going to be done for the germ cells like the sperm and the ovum, and then they are actually going to go into the offspring.

As a result of that, it is actually going to cure the genetic disease. Apart from that, you can also make the genetic modifications. So, you can actually do gene replacement, gene corrections, or gene augmentations. And as far as the transfer method is concerned, you can actually use viral vectors or non-viral transfers such as the physical method, liposome-mediated methods, and the chemical method. Now, let's talk about the first therapy, which is somatic gene therapy.

So, somatic cells are components of all cells except the germ cells. So, you know that the germ cells mean the cells that are responsible for the production of offspring such as sperm and ova. So apart from these cells, whatever cells are present in an individual actually fall into the category of somatic cells, such as those in the liver, lungs, brain, and all other types of endothelial cells. In this particular somatic gene therapy, you are targeting the modification of the gene in the somatic cells. So you are actually going to cure the individual with different types of defects.

It is non-inheritable, so it will not get into the offspring, and the gene transfer occurs via the viral vector, the liposome-mediated vector, or direct insertions. The examples are cystic fibrosis, adenosine deaminase, or cancer. So, you can actually do the virus escape, or the disruption of the important gene is a drawback of this particular gene therapy. So, in this particular gene therapy, there is a problem of virus escape that occurs when you inject the virus for dental delivery; the immune system is actually going to act against the viruses, and that is how you are not going to get the desirable results. Taking an example, what are the target sites for gene therapy? You are actually going to have target sites in all the somatic cells, such as endothelial cells, liver cells, muscle cells, and lung cells.

So this is all given here, right? You can actually do the somatic gene therapy in endothelial cells, brain, liver, muscles, lungs, and the skin, right? So, remember that these are not inheritable to the other offspring. So, it is not going to only cure that particular individual. Talking about the examples of how gene therapy works, we have taken the example of cancer. So cancer is the second largest leading cause of death in humans, and it occurs as a consequence of the accumulation of genetic modifications, which lead to uncontrolled cell growth. So, you can have uncontrolled cell division, you can have loss of apoptosis, and you are going to have angiogenesis; because of that, the cancer is going to spread from one side to the other.

Then you can also have metastasis, which actually helps the cancer cells move from one side to the other side. Then you are also going to have rapid glycolysis, and you are also going to have the loss of contact inhibition, and because of that, the cancer cells are actually going to grow in large numbers. Now, when you want to treat cancers, you are

actually going to take care of the different types of mutations that cancer cells are acquiring, and because of that, they are actually going to acquire uncontrolled cell division. So, what are the approaches that you can use? So, you can actually treat the cells that are associated with the cancer. So, you can actually use oncogenes or proto-oncogenes such as EGF, KRAS, ARC1, CF, CMIC, and HER2.

You can also use some of the genes that are, you know, tumor suppressor genes, so the function of these genes is that they actually suppress the proliferation of cancer cells or suppress the induction of cancer cells, so you can actually use the RB proteins or the p53 proteins. And you will also use the BRC 1, 2, and 3. And then you can also use some of the genes that are important for the DNA repair genes. So, some of these genes can also be used for gene therapy in the case of cancer cells. What approach can you use? So, you can actually use multiple strategies.

So, in strategy number 1, what you can do is something that will actually inhibit the progression of cancer cells. And the aim of this is to actually bring the cell cycle control within the cancer cells, and you know that cancer cells do not follow the cell cycle control; as a result, they actually proliferate for an indefinite period of time. So, what if you bring the cell cycle control? What they will do is, after a certain period of divisions, they will actually get into the phase where they will not divide, and then ultimately they are going to die. And then you can also use therapeutic genes such as tumor suppressor genes like p53, RB proteins, or BRCA1. And then you can also use antisense, ribozymes, or siRNA.

So basically, you have strategy number one, where you are actually going to make the cancer cells susceptible to normal physiology. So because of that they will actually go through with the process of apoptosis, they will go through with the process of death. Then in statue number 2, what you can do is actually deliver a suicidal gene into cancer cells, right? So, what you can do is actually induce cytotoxicity in the cancer cells with the help of suicidal gene-expressing cells, right? And this is a very novel strategy where you are actually inducing death in the cancer cell. So, strategy number one is taking care of that cell; the cancer cell should not be able to survive, or should not be able to survive for multiple divisions. In strategy number two, you are actually expressing the particular enzyme that will induce death.

And you can actually have the gene activating the cytotoxic products. One example is where you can actually use, for example, the cytosine deaminase, and that will actually convert 5-fluorocytosine into 5-fluorouracil, and 5-fluorouracil is actually going to be the active drug. It is actually going to disrupt the DNA or RNA synthesis, and as a result, it is going to induce cell death. And then we also have other approaches, such as using

recombinant viruses or oncolytic viruses for tumor lysis. You can also have the immunomodulators.

Remember that the immune system is a very important factor in managing cancer progression. It is an immunosurveillance, which is actually responsible for the production of cancer cells. So when the cancer cells are produced, they are identified by the immune system, and that's how they are removed from the circulation. But if the immuno-surveillance system is weak, it is actually going to allow the proliferation of cancer cells. So, if you are going to work with the immunomodulations that are also going to take care of cancer development or the elimination of cancer, then you can also have the anti-angiogenic and the anti-proteolytic gene therapy, where you are actually going to disrupt.

The ability of the cancer cells to form new blood vessels results in a lack of nutrition and prevents them from spreading to this site number 2. So this is all about cancer. We have taken another example of SCID. So SCID means severe combined immunodeficiency. So it is actually an immune system-related disorder where it is a rare monogenetic disorder characterized by defects in the development and function of T lymphocytes.

So it is actually going to weaken this person's ability to fight against the different types of infectious organisms. So indirectly it affects the B cell lymphocytes and NK cells, as they are dependent on the T cells. So it is basically a defect of the T cell, but indirectly it is also going to affect the development of the B cell as well as the NK cell. So overall, the SCID is going to induce immunocompromised states or immunodeficiency in a particular individual, and as a result, it will be more susceptible to different types of diseases. So, for example, the children with SCID are known as bubble boys because some of them were actually able to survive for several years in plastic bubbles in which the air coming inside was filtered and food and other objects were sterilized before introduction.

So, the person with SCID is going to be extremely susceptible to the different types of diseases. And these are the symptoms of SCID. So it can generally be seen in the early days of life, right? The immune system is impaired and not capable of protecting the child. Therefore, children affected with SCID are prone to a series of life-threatening infections, including pneumonia, respiratory diseases, meningitis, ear infections, sinus infections, skin rashes, chronic cough, and sepsis. Now, what are the different types of SCID? So you can actually have the adenosine deaminase deficiency SCID, you can have X-linked SCID, you can have the RAG1 and RAG2 deficiency SCID, and you can also have the IL-7R deficient SCID.

And what is the strategy so you can actually harvest and do the gene corrections, do the conditioning, and also do the infusion of engraft, which will actually cure the SEID in a

particular person? So this is another approach; right, this is another approach where you are actually going to do the germline gene therapy. So far, what we have discussed includes somatic gene therapy, and we have taken two examples: cancer and SCID. Now we will take another example of germline gene therapy. So in germline gene therapy, you are actually going to modify the germ cells, right? So, all the cells are considered somatic cells except for the cells that are responsible for the production of new babies, right? So, the germ cell means you are actually talking about the sperm and the ovum in the case of humans, right? So, any kind of modification to the germ cells will actually proliferate and get into the offspring, like the new babies, right? So, the genetic modification of germ cells such as sperm, eggs, or early embryos would be a target site for germline gene therapy. So, in this case, what you do is, for example, if you have parents who have the genetic disease.

So, what you can do is actually be able to do the manipulation. So, you can actually produce the baby, right? Which is actually going to have a zygote, and this zygote is actually going to have the defective gene. Now, the embryo is also going to have a defective gene. So, what you do is you actually bring the embryo into the Petri dish, right? And then you actually do the manipulations and because of that this defective gene can be replaced with a new set of healthy genes, right. So, you are actually going to do the enucleation of the X cells, right? So, you are actually going to remove the nucleus? which has been formed from the parents, and then you will introduce, you know, the new gene, or you will actually introduce the diaptic gene into the system, and as a result of that, you are actually going to produce the improved zygote.

And then you will put this improved zygote into the female, and you will allow it to grow, and ultimately you are actually going to have the healthy baby, right? So embryos that have the modified gene will actually give rise to a healthy baby, and this healthy baby will actually get rid of the particular disease for which you are going to make the modifications. So, a simple example is cystic fibrosis. So, cystic fibrosis is an autosomal recessive disorder. It occurs due to the mutation of the CFTR, or cystic fibrosis transmembrane conductance regulator, gene, which codes for a 250-kilodalton transmembrane protein. The CFTR protein is activated by cyclic AMP and acts as a chloride ion channel mediating the secretion of these ions outside the cell.

And the CF is caused by the deletion of nucleotide triplets coding for the phenylalanine at the position of I08. Absence or malfunctioning of CFTR mostly affects the respiratory and gastrointestinal tracts by producing very thick mucus that is rapidly colonized by pathogenic organisms, causing chronic airway inflammation. Cystic fibrosis occurs because there is a mutation of the CFTR protein, which is actually not allowing the proliferation of pathogenic organisms, and this causes the infection of the airways. CFTR

protein is a chloride ion channel involved in creating mucus, while digestive juices and sweat belong to the ABC superfamily and transport the chloride ion across the cell membrane, regulating the movement of water and sodium that keeps mucus thick and slippery, especially in the lungs, pancreas, sweat glands, intestine, and reproductive tracts. Now, in cystic fibrosis, the lungs are mainly affected as thick mucus is produced, which can also block the enzymes released in the pancreas and other organs.

That is how it can cause sinus infections or salty-tasting skin. And what is the treatment? So there is no permanent solution for curing cystic fibrosis in a like manner. So, keeping the airway patent and suppressing infection with antibiotics, you can take mucolytic agents, and you can also use CFTR modulators, such as these drugs. So this is what it's shown here, right? If there will be a CFTR that is closed and open, right? And it is actually going to allow the release of the chloride. And remember that when we were talking about the transport of the material, we discussed in detail how these kinds of transporters are working, right? So they will actually be forming a closed circle and an open circle like that.

With the help of the ATP, all those kinds of loops are binding to the inner side, right? So, what is the gene therapy for cystic fibrosis? So what you can do is take the healthy copy of the gene into the adenovirus vector and into airway epithelial cells. Then the gene is delivered using the adenovirus associated with fewer side effects, but it is also less effective. And then you can use non-viral gene therapy with liposomes and polymeric nanoparticles, or you can use antisense oligonucleotide therapies. Recently, you can also use the Talon or the CRISPR-Cas, which actually altered the gene sequences, and as a result, it is going to help in the cystic fibrosis gene therapy. Therefore, viral vector-mediated aerosol-mediated AAV2 CFTR gene transfer into the lungs of patients with cystic fibrosis was conducted by Moss et al.

And then you can also have a non-variolated method. So in a clinical trial where the compacted DNA nanoparticles were administered to the nasal mucosa of the cystic fibrosis subject, a check for the reconstitution of CFTR protein was conducted. Then the other disease is called the dystrophin gene. So, muscular dystrophy is the X-linked disease caused by the mutation of the dystrophin gene, which is located on the short arm of the X chromosome. Dystrophin is a very long filamentous protein located in the cytoplasmic side of the plasma membrane.

Protein is composed of the four domains: the N-terminal domain, central rod domain, cysteine-rich domain, and the C-terminal domain. Dystrophin mutation leads to the premature protein truncation, which usually leads to severe muscular dystrophy. If there is deletion in the central rot system, but the N and C terminal regions are intact, then the

phenotype is the milder form of Becker muscular dystrophy. What is gene therapy for muscular dystrophy? So, dystrophin protein acts like shock absorbers, protecting the muscle fibers from damage during contractions. Without functional dystrophin, muscle cells become fragile and are easily damaged.

leading to chronic inflammation and the replacement of muscle tissue with scars and fat tissue. It includes the delay of motor neurons, which independently affect sitting and standing abilities. So, muscular dystrophy is diagnosed by the level of creatine phosphokinase, which is involved in muscle degeneration. Muscular tissue biopsy and electromyography can also be used for the confirmations. Although there is no cure for muscular dystrophy, a few strategies are being used to delay the process, such as the use of medicines like prednisone and its derivatives, along with cyclosporine, which delay muscle regeneration.

Additionally, monoclonal antibodies against myostatin can also improve muscle function. What is gene therapy in DMD? So, the probable gene for the GMD can be introduced into the dystopian cDNA while the adenovirus or truncated cDNA is used for the amino dystopian production in the myofiber. We can introduce the whole cDNA of the dystopian gene into the plasmid injected into the bloodstream. We can suppress the premature termination of the dystrophin, or you can use the exon-skipping approach of ASO pairing, which marks the normal splicing signal on the dystrophin P of mRNA, determining the exclusion of the pathogenic exon from the mature messenger RNA.

All the processes are ongoing in the clinical trials. So, what are the steps in gene therapy? So, you take the adenovirus, adenovirus-associated virus, right? Carrying the micro dystrophin gene is infused into the patient's bloodstream. Once inside the muscle cell, the virus releases the micro dystrophin gene; the cell's machinery then uses this new genetic print to initiate production. producing the micro dystopian proteins. The newly produced micro dystopian protein integrates into the muscle cell membrane, restoring the production and slowing the cycle of muscle degeneration. Then we also have an example of hemophilia, one of the classical genetic diseases where blood clotting is a problem, right? And we discussed that in detail when we talked about the genetic diseases, right? And we said that hemophilia is an X-linked recessive disorder in which the body fails to form a blood clot, a process necessary to stop the bleeding.

So these are the cascades of reactions that are responsible for blood clotting, and there are many factors that are responsible for the formation of blood clots. And the different types of hemophilia are hemophilia A, hemophilia B, and hemophilia C, and they all have a similar phenotype in that they have defects in blood clotting. So in hemophilia A, there will be a deficiency of factor VIII. In Haemophilia B, you have a deficiency of factor IX,

whereas in Haemophilia C, there will be a lack of factor XI, but it also has an autosomal recessive nature. Now, what is the treatment for Haemophilia? So the treatment is the replacement of the clotting factor produced via infusion.

So it may be prophylactic or episodic, or you can use a monoclonal antibody like this one, which mimics the function of factor VIII, and it is highly effective for Haemophilia A. And this all provides temporary control of the symptoms of the disease, not a permanent cure. What is gene therapy? What can you do in hemophilia? So gene therapy provides a permanent solution. Here, an effective copy of the gene is inserted into the liver cell, which cascades to produce the essential clotting factor to prevent hemophilia. So, you can actually produce a gene, right, and you can actually introduce that into the system, right? And that is how it is actually going to start producing that particular factor.

Then we also have the different types of gene-transfer methods. So we have the viral vectors; we have a non-viral vector. So within the viral vector, you can choose to use retroviruses, adenoviruses, or adeno-associated viruses. You can use the herpes simplex virus, the vaccinia virus, or the baculovirus. Whereas in the non-viral vectors, you can use either the physical method or the chemical method. So physical methods you can use are electroporations, microinjections, or gene therapy.

These methods you are going to use depend upon the type of organisms, type of target tissue, and type of target site. And within the chemical method, you can use the liposome methods, the calcium phosphate method, or the polymers. And then we also have the ex vivo approaches. So, cells are genetically modified outside the body and then reintroduced into the patient. So, one of the classical examples is the ah cortisols, right? So, you can actually isolate the defective genes from the patients, then you do the genetic modifications, and then you are going to do the cell expansion and quality control.

So, you will know that these cells are good, they will be in high numbers, and then you are going to put these cells back into the patient, and that is how you are actually going to get the cure for that particular disease. So what you are going to do is isolate the cells from the patient, and you are going to do the in vitro culturing. And while you are doing the culturing, you are actually going to replace the defective gene with the correct gene or the healthy genes and then you are going to do the proliferation and then you will put them back into the system, right. For example, if there is a problem with insulin production, you can actually take out the beta cells, correct the beta cells, and put the beta cells back, right? So, for example, this is an example of CAR T-cell therapy, right? So, what you can do is actually correct the T cells; you can collect them, so this is a patient, right? And you can actually collect the T cells from the patients, then you are going to do the genetic alteration of these T cells; you will actually train them so that they can cure or

identify the cancer cells, and they will be able to destroy the cancer cells. And then you are actually going to produce the CAR T cells, and they will express different types of receptors and antigens for identifying the cancer cells.

And then you are actually doing the conditioning chemotherapy. You will perform the CAR T cell infusion on the patient. And then once you infuse the CAR T cells into the patients, these CAR T cells, since they have already been expressing the recombinant receptor on their cell surface, will actually attack the cancer cells, and that is how they will eliminate the cancer cells. This is another example of, you know, where you are actually going to take a therapeutic gene, and then you insert that therapeutic gene into a suitable carrier. So in this case, for example, you can use the adenovirus, retrovirus, or lentivirus, and then you will enter that into a patient, and the patient is going to integrate that particular gene into its genome. And that is how the patient is going to start producing that defective gene, or they will start producing that particular protein, and that is how it is actually going to help overcome the particular disease.

And then we also have gene modification. So you can actually have gene silencing or gene knockout. You can have the epigenetic modifications or the gene knock-in. So in gene silencing, what you do is use siRNA or miRNA, and that will inhibit gene expression. So basically, it is actually going to suppress the production of that particular gene or the production of that particular protein so that it will actually not cause any harmful effects. Gene silencing is the regulation of gene expression without altering the DNA sequences.

So it is actually going to be a temporary solution. So it is only going to be until that particular transcript is available in that cell. And then we also have RNA. So RNA is another way of doing gene silencing only. It works by introducing a small double-stranded piece of RNA that is called siRNA.

And the siRNA enters the cell and is loaded onto the protein called rSAC. And rSAC unwinds the siRNA and uses one strand as a guide. And the rSAC guide is complex, patrols the cell, finds the target messenger RNA, and perfectly matches the guide, making it for the destruction. So basically, whether you are using the siRNA, gene alterations, or knockouts, it is actually going to only suppress the production of the particular protein from the particular RNA. Then we also have the antisense RNA techniques that are also going to the same; it is actually going to reduce the production of that particular protein in that particular cell. As a result, it is actually not going to alter the genome of that particular cell; it is only going to change the transcriptome, and it is going to reduce the amount of RNA available for protein production.

And what is the advantage of gene silencing? So it is reversible, it is highly specific, and it avoids the DNA risk, right? You know, when you do gene therapy, when you do genetic manipulations, when you do genetic mutations, right? You are actually changing a particular organism at a very gross level, aren't you? That sometimes may have the adverse effects that different types of effects, which you may not be able to know at this moment, but may be available after three or four generations. You probably know that the target site has been modified, but the off-target site could also be modified, which you probably will not be able to know at that point. As a result, many people think that if you cure the disease in a safer way, the safer way is to just reduce the production of that particular protein through gene silencing. And that is because these processes are reversible, they are very specific and do not allow the alteration of the genome; they will probably be much safer compared to the processes that are responsible for genetic alterations. So, the types of gene modification you can actually have are either the addition of a gene or gene editing, and so on.

So, in gene editing, you can use the zinc finger nuclease, you can actually use the TALENs, and you can use CRISPR-Cas. Conclusions about gene therapy are no longer a question of if, when, or how; its ability to precisely rewrite the code holds the ultimate promise of not just treating but truly curing the most challenging human diseases. So gene therapy is not only about the development of more efficient delivery systems and personalized medicine for rare diseases. So in this particular lecture, we discuss gene therapy, the different aspects of gene therapy, and that gene therapy could be of two types.

It could be somatic gene therapy or germline gene therapy. Somatic gene therapy is where you treat a particular individual for a specific type of disease, and it is not inheritable, so it will not be passed on to the offspring. And apart from that, you can also have germline gene therapy, where you are actually going to do the treatment in either the germ cells, such as sperm or the ovum, or you will actually do the alteration in the embryos. And as a result of that it is actually going to cure that particular genetic defect in that particular family and as a result of that the subsequent generations are also not going to face the similar kind of diseases. Apart from that, we have also taken examples of somatic gene therapy, and we have taken an example of the diseases from germline gene therapy as well. So, we have taken an example; we discussed the example of hemophilia, we discussed the example of SCID, and we also discussed muscular dystrophy.

So, with this brief discussion about gene therapy, we would like to conclude our lecture here. In our subsequent lecture, we will discuss more aspects related to this particular course. Thank you.