Module 6 Gene Therapy and diseases-II

Lecture 35

Severe combined immunodeficiency syndrome (SCID)(part-I)

Severe combined immunodeficiency syndrome (SCID) is a group of rare inherited disorders related to the immune system which is characterized by a substantial reduction or absence of T-lymphocytes function. Reduction or absence of T-lymphocytes functions causes deficits of both cell-mediated and humoral immune responses. The immune system normally fights off attacks from invading pathogenic bacteria and viruses, but as people with SCID have a defect in their immune system they are vulnerable to potentially deadly infections.

With the advancement in transplantation technology the cure rate of SCID by bone marrow transplant using matched sibling donors is more than 95%.

Generally there are several types of SCID.

- X-linked severe combined immunodeficiency
- Adenosine deaminase and purine nucleoside phosphorylase deficiencies
- Other autosomal recessive SCIDs

<table>
<thead>
<tr>
<th>Type of SCID based on gene mutation</th>
<th>Affected Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-Linked SCID gamma chain gene mutation</td>
<td>IL2RG</td>
</tr>
<tr>
<td>Types of Autosomal Recessive SCID based on gene mutation</td>
<td>Affected gene</td>
</tr>
<tr>
<td>CD45 gene mutation</td>
<td>CD45</td>
</tr>
<tr>
<td>Artemis gene mutation</td>
<td>ARTEMIS</td>
</tr>
<tr>
<td>Jak3 gene mutation</td>
<td>JAK3</td>
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</table>
Several mutations as depicted in the table above are responsible for the development of SCID. The most common form of SCID is caused due to mutation in the SCIDX1 gene that is found on the X chromosome. SCIDX1 gene encodes for a protein which responsible for the formation of a receptor called IL2RG (interleukin-2 receptor). IL2RG receptors are found generally in the plasma membrane of immune cells. Their major function is to allow the interaction and communication of the T cells and B cells. When the SCIDX1 gene gets mutated then the receptor formation is impaired and receptors are not present on immune cells. This absence of receptors causes non interaction between the immune cells. So the immune cells cannot mount an immune response against the invading pathogens. Thus the body cannot fight back; the individual is highly susceptible to infections and are called immune-compromised individuals.

IL2RG receptor is involved in the activation of an important signaling molecule called the Janus Kinase-3(JAK3). JAK3 gene is found on chromosome 19 and a mutation in this gene can also result in SCID.

Another form of SCID occurs due to the deficiency of the enzyme adenosine deaminase. This form of SCID is due to an autosomal recessive mutation of the ADA gene found on chromosome 20. The accumulation of dATP takes place in the absence of the ADA gene. Elevated dATP level inhibits the enzyme ribonucleotide reductase. Ribonucleotide reductase is responsible for the reduction of ribonucleotides to generate deoxyribonucleotides (dNTP). In the absence of ribonucleotides reductase dNTP synthesis is impaired. Usually dNTPs are required for lymphocyte proliferation and in the absence of dNTPs the effectiveness of the immune system is compromised and suppressed. The bottomline is that without functional ribonucleotide reductase, lymphocyte proliferation is inhibited and the immune system is compromised.

<table>
<thead>
<tr>
<th>ADA gene mutation</th>
<th>ADA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-7R alpha chain mutation</td>
<td>IL-7Rα</td>
</tr>
<tr>
<td>RAG1 and RAG2 mutation</td>
<td>RAG1/RAG2</td>
</tr>
<tr>
<td>CD3-δ/CD3-ε mutation</td>
<td>CD3-δ/CD3-ε</td>
</tr>
</tbody>
</table>
35.1 Purine nucleoside phosphorylase deficiency

Purine nucleoside phosphorylase (PNP) is an important enzyme involved in the purine salvage pathway. An autosomal recessive disorder involving mutations of the purine nucleoside phosphorylase (PNP) gene results in the absence of PNP enzyme, thus hampering the conversion of dGTP to riboflavin. As a result of impairment of PNP, dGTP accumulation takes place. An elevated level of dGTP is toxic to T-cells and causes its deficiency. T-cell deficiency inhibits the functioning of the immune system and is a cause for the development of SCID.

Figure 35.1 Schematic representation of purine pathway:
35.2 SCID Symptoms

Symptoms of SCID can be seen generally in the early days of life (first two to three months). Since, the immune system is impaired and not capable of protecting the child therefore children afflicted with SCID are prone to a series of life threatening infections including pneumonia (lung infection), respiratory diseases, meningitis (brain infection), ear infection, sinus infection, skin rashes, chronic cough, and sepsis (blood infection). SCID patients do not show any recovery even on administration of antibiotics (used to treat bacterial infections). Early diagnosis of SCID is very important, because without quick and early treatment, children afflicted with the disease will not survive more than two years age.

35.3 SCID Mouse

SCID mice are model organisms extensively used for conducting research related to the immune biology. Immune response studies based on cell transplantation methods, and the effects of disease on mammalian systems can be addressed by using SCID mice. All kinds of tissue transplant studies like malignant tissue, normal tissues etc. are routinely conducted in SCID mice. In addition to conducting immune response study these mice are superb organisms for testing the efficacy and the level of safety of new vaccines or therapeutic agents which would be used later to treat immune-compromised individuals.

SCID mice are incapable of

- making T and B lymphocytes
- activate complement system
- fighting infections
- rejecting tumors
- rejecting transplants

These incapacibilities of the SCID mice arise due to a rare recessive mutation on Chromosome 16. This rare mutation causes deficiency in the activity of an enzyme(Prkdc or "protein kinase, DNA activated, catalytic polypeptide") that plays a critical role in DNA repair. In this case V(D)J recombination fails to take place as a result the humoral and cellular immune systems fail to mature.
Even more efficient immune-compromised SCID mice can be generated by crossing SCID mice with mice carrying other mutations in related genes like IL2RG. Thus we can vary the amount of mutation and immune suppression and design strains of SCID mice according to the experiment or research requirement.
Lecture 36

Severe combined immunodeficiency syndrome (SCID) (part II)

36.1 Nude mouse

A nude mouse lacks the presence of body hair, which gives it the phenotypic "nude" nickname. It is a valuable model animal for research related to immunological aspects. It is developed in the research laboratory by disrupting the FOXN1 gene. This mouse was developed from a strain with a genetic mutation and because of this mutation thymus is absent, thereby causing a suppressed immune system. The inhibited immune system results in the reduction of number of T cells.

36.2 SCID pathogenesis

Most frequently the SCID presents an X-linked recessive pattern of inheritance, and is therefore referred to as X-linked SCID. In case of X-linked recessive disease male are the real sufferers (since males carry only one copy of the X chromosome and only need to inherit one defective copy of the gene to display the symptoms of the disorder), female on the other hand are generally the carriers and rarely afflicted with the disease (only in homozygous condition they display the symptoms of the disease but in heterozygous condition they inherit one defective X chromosome, they still have one healthy allele hence in heterozygous condition they do not display the symptoms of the disease). Thus females are associated with carrying the faulty gene and passing it on to their children.

Gene therapy strategy is being tried as an alternative treatment for SCID rather than the bone marrow transplant. Viral vectors like AAV, lentivirus, retrovirus are being explored for conducting transduction of the missing gene to hematopoietic stem cells and many trials are underway for ADA SCID and X-linked SCID.

In the year 1990 the first patient to experience successful gene therapy was a Sri Lankan named Ashanthi DeSilva (4 years old). Blood was collected from this patient and white blood cells were isolated. Using a virus vector a healthy adenosine deaminase (ADA) gene was transduced into those cells which were then injected back
into the patient. As a result normal enzyme expression was achieved. Enhanced expression was obtained by weekly injections of ADA which boosted the expression and corrected her deficiency. In case the non transduced cells survive in the presence of injected ADA then the transduced cells will have no selective advantage to proliferate and thus gene therapy approach may face a setback.

In the year 2000 gene therapy clinical trials using retrovirus vectors were successfully used to enhance the immune system functionalities in SCID patients. These trials were later stopped when it was found that by the insertion of the genecarrying retrovirus near an oncogenetwo of ten patients in one trial had developed leukemia. These serious adverse effects of gene therapy clinical trial were revisited in the year 2007 whenfourof the ten patients registered for the gene therapy treatment developed leukemia. Thus the major focus of gene therapy strategy is to modify the viral vector in such a way that such instances of oncogenesis do not reoccurr. Precise targeting of gene insertion using zinc-finger nucleases is being optimized. Gamma c gene is oncogenic when expressed by a retrovirus; cases of leukemia have not been reported in trials of ADA-SCID where the gamma c gene is not used. Clinical trials of gene therapy have been successful in restoring the immune system functionalities in the last decade where almost 20 patients have benefited and shown signs of recovery from the ADA-SCID and X-SCID disorders.

36.3 Gene therapy clinical trials for SCID

1. In the year 2009,Aiuti A .et al., conducted gene therapy clinical trial for immunodeficiency caused as a result of deficiency of the adenosine deaminase enzyme. The authorssenquired the extended or long term result of gene therapy for severe combined immunodeficiency (SCID) in the absence of adenosine deaminase (ADA), a fatal disorder of purine metabolism and immunodeficiency. The introduction of CD34+ cellstransduced with a retroviral vector containing the ADA gene into 10 children with SCID due to ADA deficiency.All patients are alive after a median follow-up of 4.0 years. Transduced CD34+ hematopoietic stem cells have stably engrafted and differentiated into myeloid cells containing ADA and lymphoid cells. Eight patients did not require enzyme-replacement therapy because ofcontinuousexpression of ADA gene in blood cells. Nine patients had immune reconstitution with increases in T-cell counts and normal functioning of T-cell.
Serious adverse events included prolonged neutropenia, hypertension, central-venous-catheter-related infections, Epstein-Barr virus reactivation, and autoimmune hepatitis. In conclusion the study showed effective treatment for SCID in patients with ADA deficiency.

2. In 2012, Sauer AV et al., enquired the defective B cell tolerance in ADA deficiency by using gene therapy. ADA gene defects are among the most common causes of SCID. Purine metabolism and disorder in the immune functions can be rectified by enzyme replacement therapy, or more effectively by bone marrow transplant. Autoimmune complications such as autoantibody production frequently occur in ADA-SCID patients following treatment. The authors tested the reactivity of recombinant antibodies isolated from B cells of SCID patients. The authors identified that before gene therapy by hematopoietic stem cells; more auto-reactive antibodies were present in SCID patients, indicative of defective central and peripheral B cell function. They also identified that patients have altered B cell receptor and T cell receptor function. In conclusion the ADA defect leads to altered T and B cell functions.
Lecture 37

Gene therapy of non-heritable disorders (part I)

Non heritable genetic disorders are those disorders which do not pass from one generation to the next. Some disorders like the X-linked SCID, Hemophilia are examples of heritable disorders. The disorders like cancer, some neurological disorders are both heritable as well as acquired. We have already discussed many disorders like Hemophilia, Cancer, DMD, Crigler Najjar syndrome, Tyrosinemia, Cystic fibrosis etc. In this section we shall look into the gene therapy aspects of disease like the cardiovascular disease, Neurological disease mainly stroke and acquired epilepsy.

37.1 Gene therapy for treatment of neurological diseases

Neurological diseases are of various kinds. Based on the region or cells or the metabolites involved the fatality of the disease can be assessed. In this section eight such neurological diseases which are suitable candidates for treatment by gene therapy have been tabulated. The alzheimer’s disease is caused due to environmental as well as genetic factors. The cause of epilepsy is unknown. The expansion of polyglutamine in Huntington gene locus (Htt) is the cause of huntington’s chorea. The lysosomal storage disease (LSD) occurs due to the deletion or mutation of the lysosomal enzyme gene and defective cofactor/transport protein gene. The Motor neuron disorders (group of disorders like amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), familial spastic paraplegia (FSP), spinobulbar muscular atrophy (SBMA)) occur due to missense mutations, abberative gene splicing and gene deletion. The parkinson’s disease occurs due to defect in α-synuclein, parkin, ubiquitin and hydrolase gene. The environmental factors like pesticides, neurotoxicants and fungicides are also responsible for inducing parkinson’s disease. The stroke in the brain is caused due to the oxygen deprivation or hypoxic condition leading to cell necrosis and apoptosis.
### Table 37.1 Neurodegenerative disorders which are suitable candidates for treatment by gene therapy:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mechanism of disease</th>
<th>Age at which the disease starts</th>
<th>Cause of the disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease</td>
<td>Accumulation of fibrillar peptide β-amyloid; Hyperphosphorylation of tau protein; Loss of synapse</td>
<td>Around 50-80 years</td>
<td>1. Environmental factors like viral infection and less nutritive or compromised diet.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Rarely genetic: involved genes are Amyloid precursor protein (APP), presenilin (PS), Apolipoprotein E4.</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Faulty aberrative local or global neural network hypersynchronization</td>
<td>Childhood</td>
<td>Unknown</td>
</tr>
<tr>
<td>Huntington’s chorea</td>
<td>Loss of striatal spiny neurons</td>
<td>Around 20-50 years</td>
<td>Genetic: expansion of polyglutamine in Huntington gene locus (Htt).</td>
</tr>
<tr>
<td>Lysosomal storage</td>
<td>Deficiency of lysosomal enzyme which results in the accumulation of lysosomal protein and leading to gradual degeneration of peripheral nervous</td>
<td>Infancy</td>
<td>Genetic: deletion or mutation of the lysosomal enzyme gene and defective cofactor/transport protein gene.</td>
</tr>
<tr>
<td>Disease</td>
<td>System and Condition</td>
<td>Age</td>
<td>Causes</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td>--------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Motor neuron disorders (group of disorders like amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), familial spastic paraplegia (FSP), spinobulbar muscular atrophy (SBMA))</td>
<td>Loss of spinal motor neuron</td>
<td>Around 40-60 years</td>
<td>Genetic: Missense mutations cause ALS, Abberative gene splicing and gene deletion causes SMA, Trinucleotide repeat expansion causes (SBMA).</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>Loss of pigmented DA neurons of midbrain</td>
<td>Around 50-70 years</td>
<td>(Rarely) Genetic: defect in α-synuclein, parkin, ubiquitin, hydrolase gene. Environmental: pesticides, neurotoxicants, fungicides.</td>
</tr>
<tr>
<td>Stroke</td>
<td>Oxygen deprivation or hypoxic condition leading to cell necrosis and apoptosis</td>
<td>At different ages no fixed age group.</td>
<td>Due to both genetic and environmental conditions.</td>
</tr>
</tbody>
</table>
For designing the gene therapy strategy for neurodegenerative diseases various viral vectors like adenovirus (Ad), Adeno-associated virus (AAV), recombinant Herpes simplex virus (rHSV), Herpes simplex virus amplicon, HSV-AAV hybrid amplicon as well as lentivirus are being explored.

<table>
<thead>
<tr>
<th>Viral vector</th>
<th>Tropism</th>
<th>Capacity</th>
<th>Expression period</th>
<th>Associated properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Neuronal and glial cells</td>
<td>Around 30Kb for gutless adenovirus and 7-8Kb for first and second generation adenovirus</td>
<td>Gutless adenovirus shows expression for many years whereas first and second generation adenovirus expresses for few months.</td>
<td>Gutless adenoviruses are less immunogenic as compared to first and second generation adenovirus. Episomal persistence is seen in both.</td>
</tr>
<tr>
<td>Adeno-associated virus (AAV)</td>
<td>Neuronal cells</td>
<td>Around 4.5Kb</td>
<td>Expresses for many years.</td>
<td>Causes minimum immune response.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Both episomal and integrative.</td>
</tr>
<tr>
<td>Recombinant Herpes simplex virus (rHSV)</td>
<td>Tropism in neuronal cells is more than in glial</td>
<td>Around 10Kb</td>
<td>Expresses for months</td>
<td>Causes some type of cytotoxicity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Episomal persistence is seen.</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Tropism in</td>
<td>Around 130Kb</td>
<td>Expresses for months to one</td>
<td>Since all viral genes are deleted thus</td>
</tr>
<tr>
<td>amplicon</td>
<td>neuronal cells is more than in glial cells</td>
<td>year</td>
<td>very little immune response is evoked. Episomal persistence.</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------------------</td>
<td>--------</td>
<td>-------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>HSV/AAV hybrid amplicon</td>
<td>Neuronal</td>
<td>&gt;20Kb</td>
<td>Expresses for months to one year</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>All viral genes are deleted therefore very little immune response evoked.</td>
<td></td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Neuronal</td>
<td>About 8 to 10 Kb</td>
<td>Expresses for many years</td>
<td>Integrative</td>
</tr>
</tbody>
</table>
Lecture 38

Gene therapy of non-heritable disorders (part II)

38.1 Gene therapy for stroke:

The apoptotic pathway for ischaemic stroke is depicted in the schematic representation below. The commitment phase of apoptosis is due to the transition of the permeability of mitochondria. Thus, this anomaly can be overcome by invoking an over-expression of the Bcl-2 protein family members as well as the Neuronal apoptosis inhibitory protein (NAIP). Experiments have been conducted that involve using Herpes simplex virus (HSV) based vector system wherein Bcl-2 gene was introduced and an over-expression of this protein was obtained. Over-expression of Bcl-2 reduced apoptosis and also improved neuronal survival both in vitro and in vivo. AAV has also been used to carry the NAIP and has shown to reduce the caspases levels thus preventing the degeneration of neurons. The expression of interleukin-1 receptor antagonist using virus as delivery vehicle has been shown to reduce inflammation (inflammatory response is a pre-stroke phenomena) within the brain and thus helping in suppression of other factors responsible for mounting the potential stroke. Over-expression of various genes like GLUT-1, D28K, SOD-1, GPX, using HSV vector has shown improvement of neurons affected by stroke in animal models. Sendai virus and recombinant adenovirus has also been used in a gene therapy strategy for CNS disorders. These have been used to carry neurotrophic and angiogenic factors like GDNF, BDNF and NDF.
Figure 38.1 Adaptive and pathological response in neurons:

Adaptive and pathologic response in the neuronal cells caused by ischemia:
This schematic representation demonstrates the process of programmed death pathway of neuronal cells based on the three phases which develop as a result of extrinsic as well as the intrinsic factors. Specific gene products are involved in blocking the three phases separately and preventing apoptosis. Blocking of Initiation phase occurs by the expression of Hsp 72, glut-1 etc. The blocking of commitment phase takes place by the Bcl-2, Bcl-XL whereas apoptosome machinery deactivation is done by the ZVADfmk, IAPs and the CrmA.
38.2 Gene therapy for treating epilepsy:

Epilepsy is a neurological disease which comprises of a group of neurological maladies characterized by hyperactivation or excitation of cells causing fits or seizures. The causes or etiology of acquired epilepsy is unknown but has been linked to some events in life that trigger the hyperactivation. In epilepsy due to hyperexcitation of nerve cells for a prolonged period of time the neural substrate is exposed to the neurotransmitters causing chronic conditions like the mesial temporal sclerosis. The full pathway of this condition has not been elucidated. Yet, literature survey provides with the information that the accumulation of N-acetyl-L-aspartate (NAA), which is the marker of injury to neurons, accumulation in the brain causes seizures. Tremor rats act as a model of human petit mal epilepsy and lack the aspartoacylaseenzyme (ASPA) gene. The ASPAis responsible for NAA metabolism to aspartate and acetatewithin the glial cells. This lack of ASPA gene is due to deletion mutation.

Gene therapy treatment for seizures restricted to certain areas have been attempted by using AAV mediated antisense therapies that focus on lowering the level of N-methyl-D-aspartic acid receptor subunit. This therapy elicits antagonistic effects on seizure sensitivity in vivo. Spontaneously Epileptic Rat (SER), is an indispensable animal model for epilepsy derived from the tremor rat, consists of double mutation and it demonstrates tonic convulsions along with absence like seizures. Administration of adenovirus mediated ASPA gene in SER has resulted in decrement of tonic convulsions. The limitation of the adenovirus mediated ASPA gene is that its expression is transient and for very less time duration.

A protein-L-isoaspartyl methyltransferase (PIMT) deficient mouse also acts as a perfect model for epilepsy. PIMT is involved in repairing of defective proteins (defect due to isomerized aspartate residue and deamidated asparagine residues). Isoaspartate (IsoAsp) accumulation takes place inside the brain of PIMT mouse that results in development of fatal epileptic seizures. Many neuroactive peptides have been identified recently and their antiseizure, anticonvulsion or antiepileptogenic role has been determined.
Recombinant Adeno-associated virus (AAV) vector containing galanin (GAL) and fibronectin (FIB) when administered to rat model at the inferior collicular cortex shows remarkable recovery with the weakening of seizures. Various degree of protection has been observed when AAV-FIB-GAL is administered to the hippocampus region of the rat model.

Identification of a lot of antiepileptogenic gene targets has opened up new possibilities that can be explored and used to design better gene therapy strategy for epilepsy.

38.3 Gene therapy for treatment of cardiovascular disease:

Cardiovascular disease comprises of coronary heart disease, hypertension, cardiomyopathy, myocardial infarction and valve dysfunction. When the heart is under huge stress due to these maladies two compensatory mechanisms are activated to aid the ailing heart. These mechanisms are rennin angiotensin system (RAS) and the cardiac hypertrophy.

Cardiac hypertrophy: Generally all cells divide by the process called hyperplasia but the cardiac cells do not divide by this process, instead they grow in size by the process called hypertrophy. The embryo or foetal myocytes show hyperplastic growth but after birth hyperplastic growth stops and hypertrophic growth starts. Under controlled condition as during the development phase this growth is good but uncontrolled growth is dangerous. With the growth in size the functionalities of the heart muscle to effectively contract and relax, gets compromised. Angiotensin-converting enzyme (ACE) inhibitors are involved in inhibiting this condition and they may also revert back the hypertrophic condition.

Renin Angiotensin System (RAS): Steps involved in the activation of RAS are as follows:

- Decrement in the renal perfusion pressure stimulates activation of RAS system
- Renin is released by the kidneys
- Renin converts Angiotensinogen released by liver to angiotensin-I (A-I)
- Lungs release ACE that causes the conversion of A-I to A-II
• ACE causes: cardiac hypertrophy, peripheral blood vessel constriction and retention of sodium and water

**Figure 38.2 Schematic representation of renin angiotensin system:**

![Diagram of the renin angiotensin system]

Schematic representation of cascade of physiological events leading to the synthesis of Angiotensin II causing the heart failure due to hypertrophy

All these responses add burden to the ailing heart eventually leading to myocardium failure. Adenovirus, AAV, First generation Adenovirus (FGAd), Retrovirus, Foamy virus, Helper dependent virus (HDAd) and the lentiviruses are the mostly used vectors in the gene therapy strategy of cardiovascular diseases.
Morpholino oligonucleotides are being used in gene therapy strategy development for the treatment of cardiovascular disease. Success to some extent has been obtained in various studies carried on model organisms like rabbits and pigs. The therapeutic mechanism used for treating a coronary artery disease in a pig model utilises the c-myc antisense morpholino oligonucleotide and helps in the decrement of hyperplasia (increment in number of cells) related to intima (inner lining of artery, vein or lymphatic vessel). The morpholino oligonucleotides administered using catheters or stents implanted in the heart vessels have been shown to decrease the size of coronary lesion remarkably.
Lecture 39

Recent advancement in gene therapy (part I)

The concept of gene therapy is being differentially perceived by various researchers, the modification and advent of new strategies are being anticipated based on the understanding of the disease. As already discussed in previous lectures we should clearly understand that the success of gene therapy relies on the understanding of the disease and making a simple and novel strategy to tackle the complications associated and provide a cure for the disease. Gene therapy involves the following strategies

- Delivery system strategy
- Therapeutic strategy
- Gene expression, detection and regulation strategy
- Disease targeting strategy
- Disease associated clinical trials
- Regulation of gene therapy

39.1 Delivery system strategy

This strategy involves various vectors viral as well as non-viral. A clever selection of the delivery vector increases the success rate of the gene therapy strategy. The delivery systems being extensively explored include the following

- Adenovirus vectors
- Modified adenovirus
- Adeno-associated virus
- Helper dependent adenovirus
- Lentiviral vector
- Herpes simplex viral vectors incapable of replication
- Vaccinia virus vector
- Retrovirus vector
- Bacterial vectors
- Baculovirus vector
- Receptor targeted polyplexes
- Redox responsive polymers and dendrimers
- Electroporation
- Magnetofection
- Photochemical internalization
- Macromolecular complexes
- Needle free delivery systems

39.2 Various other therapeutic strategies

These include the application of antisense oligonucleotide based strategy, in vivo methods which employ morpholino oligonucleotides, RNAi therapeutics, use of selectable markers, suicide gene therapy, use of polymeric nanoparticles as well as microparticles as delivery agents of the antisense oligonucleotides and siRNA etc. It is important to note that these strategies incorporate the fundamental idea of simplifying the method and finding the shortest route with least or no side effects for curing the disease. Embryonic stem cells are being explored for their application in regenerative medicine and tissue engineering.

39.3 The Gene expression and detection

Gene expression is one of the most important parts of gene therapy strategy. The transgene that is introduced must be regulated by optimal utilization of multiple gene switches or tissue specific or tissue restricted promoters. In recent years many strategies have been employed to restrict the expression of the transgene at the desired or specific site. Different promoters are being used to drive target specific expression in order to enhance the efficacy of the strategy for example: the use of active promoters from proliferating cells to restrict the transgene activity to tumor tissues, use of glucose sensitive promoters to control insulin expression etc. To attain control over the time for which the transgene expresses small molecule dependent gene switches are being employed. Some of these switches are gene switches involving bacterial derived transactivator, ecdysone receptor based gene switches, progesterone receptor based gene switches, fully humanized gene switch based on estrogen receptor, gene switches activated by dimerizers and gene switches comprising of tetracycline repressor derived transactivator.
**Figure 39.1** Schematic representation below depicts the working mechanism of the tetracycline dependent regulatory system:

1) Regulatory switch cassette produces Transactivators

- **Promoter**
- **Transactivator**
- **Poly A**

**NO GENE ACTIVATION**
Due to non binding of Transactivator-Dox complex

- **Poly A**
- **Transgene**
- **hminCMV**
- **Tet O**

**TRE**
- **Dox**

**GENE ACTIVATION**
Due to binding of Transactivator

- **Poly A**
- **Transgene**
- **hminCMV**
- **Tet O**

2) Regulatory switch cassette produces Transactivators

- **Promoter**
- **Transactivator**
- **Poly A**

**NO GENE ACTIVATION**
Due to non binding of Transactivator

- **Poly A**
- **Transgene**
- **hminCMV**
- **Tet O**

**TRE**
- **Dox**

**GENE ACTIVATION**
Due to binding of Transactivator-Dox complex

- **Poly A**
- **Transgene**
- **hminCMV**
- **Tet O**

**Schematic representation of Tetracycline dependent regulatory systems**

1) Tet-OFF regulatory switch
2) Tet-ON regulatory switch

Dox=Doxycycline, TRE=tetracycline response element, Tet O=tetracycline operator
39.4 Various disease targeting strategy

In the last decade many disease targeting strategies have been developed based on the history or knowledge of the disease and its pathogenesis. Most of the disease that are being clinically investigated for treatment using gene therapy includes hematopoietic disorders, cardiovascular diseases, all types of cancer, type 1 and type 2 diabetes, cystic fibrosis, neurological diseases, Inborn error of metabolisms like tyrosinemia, Crigler Najjar syndrome, duchenne muscular dystrophy, severe combined immunodeficiency syndrome, HIV infection, skin and systemic disorders, childhood blindness and so on. Here let us understand the RNAi based therapy for the treatment of HIV infection.

Recently Rous sarcoma virus, polio virus, hepatitis B and hepatitis C virus have been targeted using synthetic siRNA. Amongst the various infectious diseases HIV was the first disease which was targeted by RNAi. The life cycle of the HIV and its genetic expression has been fully explored. HIV contains env, gag, pol, nef, rev, tat, vif, vpr protein encoding RNA which has been targeted by using siRNAs and shRNA. The mutation rate increases if the virus is directly targeted and the mutated virus may escape. This problem can be evaded by targeting the cellular mRNAs of the host cells which encode for proteins that are essential for the HIV entry and its replication. Downregulation of CCR5, CXCR4, CD4, NFκ and NFβ has been found to block virus entry as well as replication. It is interesting to note that a 32 bp homozygous deletion in the CCR5 gene provides resistance against HIV infection whereas the heterozygous deletion provides a delayed development and progress of HIV disease. Targeting CXCR4 and CD4 is not a good choice due to their role in other intercellular interactions. The HIV may change its tropism from CCR5 to CXCR4 and become even more virulent. Thus, it is important to target both the HIV as well as the host cell co receptor transcripts using siRNA. Employing siRNA as a microbicide to stop viral transmission is the future prospect against HIV. The highly conserved sequences of the virus must be targeted in order to efficiently block all strains of the virus and prevent the development or emergence of siRNA resistant mutant strain. The efficacy to inhibit the virus and mutant strains by multiple combinatorial targeting using siRNA/shRNA should be carefully explored and their toxicity should be determined. Presumably, RNAi is inhibited by the HIV Tat
(transactivator) and TAR (transactivating response element). Tat inhibits the dicer and TAR binds to the TRBP (transactivating response RNA-binding protein) a component of the RISC (RNA induced silencing complex). The amount of TRBP synthesized in the cell is limited thus binding of TAR RNA to TRBP prevents TRBP from interacting with RISC and limits the efficacy of the RNAi based therapy. One of the strategy makes use of a combinatorial approach where the vector is loaded with the anti tat/rev shRNA, an anti CCR5 ribozyme and a nucleolar localizing TAR decoy. The co-expression of this vector has been successfully used to demonstrate the potency of RNAi as an anti-HIV regulatory mechanism.

39.5 Magnetofection:

Christian Plank and Christian Bergemann invented the technique magnetofection. It is a simple, easy and novel way of transfection into cell culture system. The principle of this method is that it uses magnetic force which is exerted on gene vectors or nucleic acids with magnetic particles to draw them towards the target cells. These magnetic particles are coated with cationic molecules. The magnetic nanoparticles are made of iron oxide, which is fully biodegradable coated with cationic particles and varies with applications. This method ensures 100% dose of the vector into the cells. Clubbing of magnetic nanoparticles with the gene vectors is accomplished by electrostatic interaction and salt-induced colloidal collection. Magnetic particles then get condensed on the target cells by the pressure of an external magnetic field. Further the cellular uptake of the genetic material is achieved either by endocytosis or by pinocytosis. The application of magnetofection is adapted to all types of nucleic acids and also in primary and secondary cell lines. The whole procedure takes very less processing time, shows high transfection efficiency as compared to standard transfection procedure because it’s been seen that within a short incubation period of cells with gene vectors the transfection efficiency is pretty high. The magnetic nanobeads are completely non toxic at recommended dose and even at higher dose. An appropriate magnetic field supports for the uptake of nanoparticles by the cells. In this case membrane architecture remains intact. Depending on the formulation used, the nucleic acids or other vectors are released into the cytoplasm.
Lecture 40

Recent advancement in gene therapy (part II)

40.1 Disease associated clinical trials

Recently full-fledged clinical trials are being performed for cardiovascular diseases, cancer, and hematopoietic disorders. With the knowledge of tumor suppressor genes, p53 is being explored for treating cancer. Here we shall try to understand the efficacy of the recombinant adenoviral p53 agent for treating cancer.

Numerous clinical trials were conducted to carefully estimate the efficacy of the recombinant adenovirus containing the p53 tumor suppressor gene (rAd-p53). After many trials it was concluded that rAd-p53 was endured by patients without any serious side effects apart from development of fever, localized pain at the injection site and fatigue. rAd-p53 injection with the trademark Gendicine is being produced by Shenzhen SiBiono Gene Tech in China. On October 16th, 2003 Gendicine was approved by the State Food and Drug Administration (SFDA) of China. Thus, Gendicine is the first gene therapy product of the world that was approved by a governing body. The anti-tumor effect of Gendicine is due to substitution of E1 region of the human serotype 5 adenovirus with the human wild-type p53 expression cassette driven by rous sarcoma virus (RSV) promoter. Gendicine administration to the tumor cells causes expression of p53 which triggers apoptotic pathways that gets amplified by the “bystander effect”. It also causes decrement in glucose uptake by cancer cells and inhibits multidrug resistance genes. As a result of downregulation of multidrug resistance genes the tumor cells become susceptible and lose resistance to chemotherapy and radiotherapy. Apart from the p53 gene the adenoviral vector is also believed to be a key player in mounting the immune response which limits the tumor growth. Side effects of radiotherapy have been found to be suppressed on administration of Gendicine with radiotherapy (GTRT). One death was reported when a high systemic dose of a wild type Ad-5 was injected to a patient for the treatment of ornithine transcarbamylase (OTC) due to the patient’s strong immune response. Apart from this tragedy no other fatality has been recorded and Ad5 is believed to demonstrate the weakest pathogenicity level among the adenovirus family. The
prescribed dose based on the various clinical trial is 1 vial for every injection (this 1 vial contains 1×10^{12} VP).

### 40.2 Regulation of gene therapy

Two federal agencies, the Food and Drug Administration (FDA) and the National Institute of Health, Office of Biotechnology Activities (NIH, OBA) belonging to the Department of Health and Human Services (DHHS) regulate the gene therapy clinical trials. FDA is involved in the determination of the quality, safety, purity potency and identity of the gene therapy product so that it can be safely administered to patients whereas the NIH is responsible to oversee the quality of science or research used in the development of the gene therapy. NIH provides funds to institutes and scientists involved in gene therapy research work. It also precisely evaluates and reviews the composition of Institutional Bio safety Committees (IBC). Public reviews are conducted and full awareness program of the various novel clinical trials is communicated and discussed through the quarterly meetings of the Recombinant DNA Advisory Committee (RAC). Confidentiality of the review of Investigational New Drugs (INDs) is maintained by the FDA reviewers. The pros and cons of the preclinical and clinical investigations are carefully determined by the FDA and the NIH whereas the ethical, legal and social issues are carefully considered by the RAC. FDA and the NIH have to be informed with proper document submission prior to initiation of human gene therapy clinical trials. IND application has to be submitted by the sponsors of a gene therapy clinical trial in a prescribed format to the Centre for Biologics Evaluation and Research (CBER). The approval of the Institutional review Board (IRB) and the IBC is essential for getting a clearance to conduct the clinical trials. The complete information and the protocol have to be submitted by the investigators to the NIH, OBA in accordance with the prescribed NIH guidelines. The protocol is also submitted to the RAC which after careful review and public discussion gives comments to the investigator and sends recommendations to the IRB, IBC and the FDA. By conducting public reviews the ethical, societal issues are addressed and the acceptance and progress of gene therapy is determined.
Various phases are involved in the development of gene therapy product and its approval. The first stage for the development of the product is known as the pre-IND stage followed by the three investigational phases (phase-I-III) during which the data produced helps in getting a license for the product. After getting the license, in the post licensing phase (phaseIV), post marketing studies are conducted. The FDA is involved in reviewing all the phases of IND.

Phase-I is conducted to monitor and assess the product safety in a given population of patients. It enables assessment of the mechanism of action, structure-activity relationship and metabolic as well as the pharmacologic behaviour of the drugs in human. Effectivity of the drug and the associated side effects can also be established with the change in dose. Generally 10-40 study subjects are administered with the drug in the phase-I gene therapy study. The scientific design of phase-II
studies depend on the amount of information accumulated with respect to the pharmacokinetics as well as the pharmacological effects of the drug.

**Figure 40.2 The flow chart for the gene therapy clinical trial:**

Phase-II study involves as few as 100 number of study subjects. This phase is designed to closely study and evaluate the efficacy of the gene therapy product for specific ailments and associated side effects in a well controlled manner. The phase-III study follows after the establishment of drug efficacy and involves several hundreds to thousand patients. After successful scrutiny and establishment of the drug as a good candidate license for drug production is issued. Phase-IV is the post marketing stage where the drug is further developed and comparatively analysed.

Gene therapy has evolved in the past decade and a range of new products are being developed to treat disease like cancer, hemophilia and other rare diseases. CBER along with NIH and FDA stringently controls the quality of drug development and the scientific research involved in the gene therapy strategy. The field of gene therapy has given hope for treatment of otherwise incurable diseases and has
completely changed the face of medicine. In the coming years let us hope that this field develops to an even greater extent with proper developed specific gene therapy treatments for all heritable, non heritable, congenital, and other incurable diseases.