**Unit III : TOPIC: EMBRIYONIC   STEM CELL GENE TRANSFER**

**Gene Transfer to Human Embryonic Stem**

This method involves prior insertion of the desired DNA sequence by homologous recombination into an in vitro culture of embryonic stem (ES) cells. ... These cells are then incorporated into an embryo at the blastocyst stage of development. The result is a chimeric animal.

The delivery of genes to stem cells can advance cell-based therapies and the field of tissue engineering. Given the right conditions, pluripotent stem cells can potentially differentiate into any cell type of the body, allowing wide therapeutic utility including treatments for autoimmune diseases, spinal chord injury, Parkinson’s disease, and cardiac tissue engineering, among many others.

1.Gene delivery could allow for directed differentiation from a pluripotent stem cell into specific differentiated cell types of interest including hematopoietic cells, neurons, cardiomycytes, and osteoblasts, as well as reprogramming a differentiated cell back into a pluripotent state.2.Beyond controlled differentiation, ectopic expression of key growth and transcription factors could allow for elucidation of fundamental cell development pathways in vitro as well as regulation of growth in vivo once the cells are transferred to a patient. Gene delivery can also provide a mechanism for in vivo expression of secreted therapeutic proteins.

The use of recombinant DNA techniques to introduce new characters (ie. genes) into organisms (including humans) that were not present previously.

The term transgenic animal refers to an animal in which there has been a deliberate modification of the genome, in contrast to spontaneous mutation. Foreign DNA is introduced into the animal, using recombinant DNA technology, and then must be transmitted through the germ line so that every cell, including germ cells, of the animal contain the same modified genetic material.

If the germ cell line is altered, characters will be passed on to succeeding generations in normal reproduction.

If the somatic cell line alone is altered, only the organism itself will be affected, not its offspring.

Transgenesis may involve whole organisms, rather than individual cells, and there may be in vivo alteration of body function.

One use of transgenesis is gene therapy which is the alteration of the genetic make-up of of an individual organism in an attempt to correct an inborn error of metabolism, ie. cure inherited diseases. But this is generally only carried out with somatic cells and, therefore, will only affect one generation.

Do not confuse transgenesis with cloning which is the production of identical copies of molecules, cells or whole organisms. Cloning does not necessarily involve gene manipulation.

**Procedure for transgenesis**

The inserted DNA is known as the transgene.

Conventional recombinant DNA techniques are used to construct the transgene so that the desired gene product will be expressed in the desired location. Typical transgenes contain nucleotide sequences that correspond to the gene of interest, with all the components necessary for efficient expression of the gene, including a transcription-initiation site, the 5' untranslated region, a translation-initiation codon, the coding region, a stop codon, the 3' untranslated region, a polyadenylation site and a promoter. Different promoters can be used to cause gene expression in all tissues of the body (non-specific) or only in specific tissues:

eg. PROMOTER GENE EXPRESSION IN (beta)-actin promoter

many tissues of the transgenic animal simian virus 40 T antigen promoter

many tissues of the transgenic animal adipocyte P2 promoter fat cells myosin light-chain promoter

muscle amylase promoter acinar pancreas insulin promoter

islets of Langerhans beta cells beta-lactoglobin promoter

mammary glands .In pharming expression in the mammary glands is usually desired as this leads  to the appearance of the product in the milk of the animal - very convenient.

PROMOTER GENE EXPRESSION IN

(beta)-actin promoter many tissues of the transgenic animal

simian virus 40 T antigen promoter many tissues of the transgenic animal

adipocyte P2 promoter fat cells

myosin light-chain promoter muscle

amylase promoter acinar pancreas

insulin promoter islets of Langerhans beta cells

beta-lactoglobin promoter mammary glands

**In nut shell-:**

**Embryonic stem cell-mediated gene transfer**

This method involves prior insertion of the desired DNA sequence by homologous recombination into an in vitro culture of embryonic stem (ES) cells. Stem cells are undifferentiated cells that have the potential to differentiate into any type of cell (somatic and germ cells) and therefore to give rise to a complete organism. These cells are then incorporated into an embryo at the blastocyst stage of development. The result is a chimeric animal. ES cell-mediated gene transfer is the method of choice for gene inactivation, the so-called knock-out method.

This technique is of particular importance for the study of the genetic control of developmental processes. This technique works particularly well in mice. It has the advantage of allowing precise targeting of defined mutations in the gene via homologous recombination.

**Uses /Advantages of Gene transfer through ESC**

1 in toxicology: as responsive test animals (detection of toxicants);

2 in mammalian developmental genetics;

3 to introduce human genes into other organisms (particularly human) for the study of disease processes;

4 in molecular biology, the analysis of the regulation of gene expression;

5 in the pharmaceutical industry, the production of human pharmaceuticals in farm animals ("pharming"); targeted production of pharmaceutical proteins, drug production and product efficacy testing;

6 in biotechnology: as producers of specific proteins;

7 genetically engineered hormones to increase milk yield, meat production; genetic engineering of livestock in agriculture affecting modification of animal physiology and/or anatomy; cloning procedures to reproduce specific blood lines;

8 to speed up the introduction of existing characters into a strain/breed for improvement and modification;

9 developing animals specially created for use in xenografting, ie. modify the antigenic make-up of animals so that their tissues and organs can be used in transfusions and transplants.