MOLECULAR EMBRYOLOGY & GENE THERAPY

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Introduction

Most embryologists have been drawn to the field by the beauty of the developing embryo and the mystery of its structure emerging from a single fertilized egg. Human embryology is relatively new; but animal embryology predates human anatomy for it was founded by the Greek Philosopher Aristotle (384-322 BC). Aristotle studied extensively the chick embryo. However, it is amazing to note that human embryology may have been advanced in the days of the Jewish Pseudoepigrapha. For example the Apocalypse of Ezra 5:12-15 (Dated about 200 B.C) has this account on human embryology:

'Just as a farmer casts down the seed of corn into the earth, so a man casts down his seed into a woman's place. In the first (month) is a whole, in the second it is swollen, in the third it grows hair, in the fourth it grows nails, in the fifth it becomes milky, in the sixth it is ready and quickened, in the seventh it is prepared, [in the eighth..], in the ninth the bars of the gateways of the woman are opened and it is born healthy in the earth (Apocalypse of Ezra 5:12-15).

Until the second half of the 18th century, embryology was a matter of speculation rather than a matter of knowledge. One generally accepted theory was that of preformation (Knobil, et al., 1998). The preformation theory states that the complete animal with all its organs was believed to exist in the germ in a miniature form, needing only to unfold like a flower. It followed that each germ must contain within itself the germs of all its future descendants, one within another, as in a nest of boxes. Many naturalists believed the germ to be contained in the ovum, the female germ cell, but after the microscope had revealed spermatozoa, the male germ cells, in 1677, a school of so-called spermists (Parker, 1997; Nilsson and Lennart 1986) advanced the hypothesis that the germ was contained in the spermatozoon. Their drawings show the spermatozoon encasing a minute human figure, called the homunculus.

Little attention was given to the theory called the theory of epigenesis (Wolpert and Lewis, 1991), which the English physician and anatomist William Harvey had stated in 1651. This theory, which had been vaguely expressed much earlier by Aristotle, held that the specialized structures of the individual develop step by step from unspecialized antecedents in the egg. Proof of this theory was not forthcoming, however, until 1759 when the German anatomist Kaspar Friedrich Wolff reported on his study of the development of the chick in the egg and showed that the organs arise from undifferentiated material. The basic potential nature and organization of the structures of the organism are determined by the genetic constitution of the fertilized egg. Wolff is called the founder of modern embryology, a title also sometimes given to the Estonian naturalist Karl Ernst von Baer, who in the 19th century described the principal phases in the development of the chick and pioneered in comparative embryology (Vaughan and Christopher, 1996).

A firm basis for the new science was established by the cell theory formulated in 1838 by the German botanist Matthias Jakob Schleiden, who stated that all plants and animals are made up of cells. A year later his compatriot, the anatomist and physiologist Theodor Schwann, confirmed this theory. In later work these men demonstrated that tissues and organs develop by cell division.

Embryology today

Modern embryology began with the incorporation of experimental methods into what (embryology) had been basically a descriptive science. With the absorption of experimental methods into embryology, a new field known as experimental embryology or embryo manipulation emerged. Embryo manipulation or experimental embryology can be simply:

a) removing an embryo from the donor animal and immediately transferring it to a surrogate mother or
b) removing an embryo from donor animal and performing microsurgery such as pronuclear injection, nuclear transfer (cloning), embryo splitting or blastocyst injection on the embryo while maintaining the embryo in a special culture systems before transferring the embryo to the surrogate mother.

In order to understand the techniques of embryo manipulation, it is important to understand the early stages of reproduction. When the egg and sperm unite to form a zygote, each of the parents supply the zygote with half of the chromosomes necessary for a full set. The zygote, which is a single cell, then begins to reproduce itself by the cellular division process called mitosis, in which each chromosome is duplicated before separation so that each new cell has a full set of chromosomes. This is called the morula stage, and the new cells are called blastomeres. When enough cells have been produced (the number varies from species to species), cell differentiation begins to take place. The first differentiation appears to be when the blastocyst is formed, which is an almost hollow sphere with a cluster of cells inside; and the differentiation appears to be between the cells inside, which become the fetus, and the cells outside, which become the fetal membranes and placenta. However, the process is not entirely understood at the present time and there is some variation between species; so it is difficult to pinpoint the onset of differentiation, which some scientists believe occurs during blastomere division.

During the first stages of cell division, it is possible to separate the blastomeres with the result that each one develops into a separate embryo. Blastomeres with this capability are called totipotent. The purpose of this ability of a single blastomere to produce an entire embryo is probably to safeguard the process of embryo development against the destruction of any of the blastomeres. In theory, it should be possible to produce an entire embryo from each blastomere (and blastomeres are generally totipotent from the four to eight cell stage), but in practice it is usually only possible to produce two embryos. That is why this procedure is generally referred to as embryo splitting rather than cloning; although both terms refer to the same thing (cloning is the production of genetically identical embryos, which is a direct result of embryo splitting).

Interestingly enough, although the embryos produced from separated blastomeres usually have fewer cells than a normal embryo, the resulting offspring fall within the normal range of size for the species.

It is also possible to divide an embryo at other stages of development. For instance, the time at which embryo division is most successful is after the blastocyst has formed. Great care must be taken when dividing a blastocyst, since differentiation has already occurred to some extent and it is necessary to halve the blastocyst very precisely.

Another interesting embryonic manipulation is the creation of chimaeras. These are formed by uniting two different gametes, so that the embryo has two distinct cell lineages. Chimaeras do not combine the genetic information of both lineages in each cell. Instead, they are a patchwork of cells containing one lineage or the other. For this reason, the offspring of chimaeras are from one distinct genotype or the other, but not from both. Thus chimaeras are not useful for creating new animal populations beyond the first generation. However, they are extremely useful in other contexts. For instance, while embryo division as described above is limited in the number of viable embryos that can be produced, chimaeras can be used to increase the number. After the blastomeres are separated, they can be combined with blastomeres of a different genetic lineage. It has been found that with the additional tissue, the survival rate of the new embryos is more favorable. For some reason only a small percentage of the resulting embryos are chimaeric; this is thought to be because only one cell lineage develops into the cells inside the blastocyst, while the other lineage forms extra-embryonic tissue. It is believed that the more advanced cells are more likely to form the inner cells.

Another application of chimaeras could be for breeding endangered species. Because of the different biochemical environments in the uterus, and the different regulatory mechanisms

for fetal development, only very closely related species are able to bear each other's embryos to term. For example, when a goat is implanted with a sheep embryo or the other way around, the embryo is unable to develop properly. This problem can perhaps be surmounted by creating chimaeras in which the placenta stems from the cell lineage of the host species. The immune system of an animal attacks tissue it recognizes as "non-self," but it is possible that the mature chimaeras would be compatible with both the host species and the target species, so that it could bear either embryo to term. This has already proved to be true in studies with mice.

A further technique being developed to manipulate embryos involves the creation of uniparental embryos and same-sex mating. In the former case, the cell from a single gamete is made to go through mitosis, so that the resulting cell is completely homozygous. In the latter case, the DNA from two females (parthogenesis) or two males (androgenesis) is combined to form cells that have only female- or male-derived DNA. These zygotes cannot be developed into live animals, as genetic information from male and female derived DNA is necessary for embryonic development. However, these cells can be used to generate chimaeras. In the case of parthogenetic cells, these chimaeras produce viable gametes. The androgenetic cells do not become incorporated in the embryo; they are used to form extra-embryonic tissue, and so no gametes are recovered.

Aside from these more ambitious embryo manipulation endeavors, multiple ovulation and embryo transfer (MOET) could soon become a useful tool. MOET is the production of multiple embryos from a female with desirable traits, which are then implanted in the wombs of other females of the same species. This circumvents the disadvantages of breeding from a female line (which are that a female can only produce a limited number of offspring due to the time investment and physical rigors of pregnancy). At the present time, MOET is still too expensive for commercial application, but is being applied experimentally.

Embryonic stem cells are undifferentiated cells that can be isolated and cultured from early mammalian embryos. They (embryonic stem cells) readily proliferate in vitro and can incorporate foreign DNA and still retain their capability to differentiate into mammalian tissues. For example, a transgenic embryonic stem cell line can be created in vitro and the genetic modification be established in a living animal by combining the transgenic embryonic stem cells with a normally developing embryo. The resulting animal can be a chimera that contains cells including gametes, from both embryonic stem cells and the host embryo.

Hans Spemann envisioned the process of cloning in 1939, but it took scientist until 1997 to shock the world with the reality of cloning a mammal using an adult cell. That shock came in the form of a sheep and her name was Dolly. The world of science and agriculture has not been the same since.

Currently numerous species have been successfully cloned from adult cells worldwide. The process of nuclear transfer (cloning) involves removing the nucleus, or genetic material from an egg and replacing it with the genetic material of a different cell. The complex of enucleated egg and foreign genetic material is fused with a small charge of electricity and then allowed to develop in culture. Embryos that result are transferred to the reproductive tract of a surrogate mother for development. The process for cloning each species carries its own particular obstacles to overcome. Cloning processes work but with very low efficiency due in part to a high incidence of embryonic and fetal loss.

A major area of uncertainty for successful nuclear transfer is the type of donor cell used to contribute genetic material to the cloned embryo. Using a variety of cell types and different cattle breeds; several cloned calves have been produced in this laboratory. All the pregnancies, including currently ongoing pregnancies, are monitored for growth and development.

Cattle are the most commonly cloned livestock. Their importance to agriculture makes this species a natural area of interest for research. A project to scrutinize nuclear transfer bovine embryos at day-30 seeks to define placental development and the events of implantation of early cloned embryos. By describing gene expression, and the process of vascular growth

we hope to describe the mechanisms to improve nuclear transfer in cattle.
Large offspring born from manipulated mammalian embryos have been described for most species studied. The mouse provides a model to measure the contributions of fetal versus placental aberrations to abnormal development of cloned concepti. An exercise to combine cloned mouse embryos with in vivo-produced embryos subsequently transferred to surrogate mothers has been designed to show the respective influences of the embryo's trophectoderm and inner cell mass (ICM). Currently, numerous species have been successfully cloned from adult cells worldwide.

Among the most significant developments in experimental embryology are those of Hans Spermann (1869 – 1941) and his collogue Needleman. To uncover evidence about the ways in which numerous separate processes combine into the complete process of embryonic growth, Spemann experimented with newt embryos. Previous research had shown that the newt's eye develops from a structure known as the optic cup that grows from the developing brain. When the optic cup makes contact with skin cells, the skin cells begin development of a lens—the beginning of an eye structure. In his experiments, Spemann removed the skin cells where the eye would ordinarily form and transplanted tissue from a completely different region. He observed that the transplanted cells transformed, as expected, into the lens structure. This was proof that a signal from the optic cup determined what kind of tissue the cells would become. During the 1920s, Spemann developed a technique of splitting a newt egg into two halves by tying it with a single hair. If this splitting was performed early in the development of the egg, each half would develop into a complete, though small, embryo. Performed later in development, the process resulted in two half-formed embryos. Spemann concluded that at a fixed stage of embryonic development—a stage he called *determination*—the differentiation of cells is determined. In subsequent experiments, Spemann found that parts of the embryo, when transplanted to different regions, would not change their own development to fit the new region but would in fact influence the surrounding regions, sometimes causing the development of an entirely new embryo. Spemann referred to these regions of tissue as *organization centers*. He demonstrated that the interactions between these different *organizer* regions are an essential feature of embryonic development.
In performing his experiments in the tiny world of the developing newt, Spemann made many important advances in microsurgical equipment and technique. His findings on organizers propelled the field of developmental biology for the rest of the 20th century.

Embryology of the 21st century (molecular embryology)
Apart from experimental embryology, other fields that are presently gaining grounds world wide as a field of the 21st century are molecular embryology and sonographic embryology. Sonographic embryology is an emerging field of study which allows detailed examination of the fetal anatomy and measurement of the unborn in utero using real time ultrasound technology. Molecular embryology (defined as the use of knowledge of molecular biology in the elucidation of structure of the human embryo) is a field which takes into consideration genetic mechanisms or factors in the control of development. These genetic mechanisms may include genes (such as oncogens) which aid developmental processes. Analyses of the molecular processes taking place during embryonic development have shown (Carmona-Fontaine et al., 2007; Ogata et al., 2007; Bilic et al., 2007, Pinho and Niehrs C, 2007) that the principles guiding embryonic development in humans and in animals are very similar on a molecular level. In vertebrates, many genes that control development have been discovered through cell biological or embryological assays for function (Harland and Gerhart, 1997; Slack, 1998), through positional cloning in mice (Nusse et al., 1990; Herrmann et al., 1990), or through differential cloning (Davis et al., 1987). Through molecular studies on fate map of embryos and cell lineage of single precursor cells, scientists (Caneparo et al., 2007; Barreto et al., 2007; Niehrs, 2006) have able to garner a lot of information about growth and development *in utero* or *in vitro*.

The injection of genes into early embryo (Gene therapy) has also opened up a new exciting field of molecular embryology. That is why gene therapy is often referred to as the area of interest for the future of Molecular embryology

Fate maps/cell lineage of single precursor cells
Fate maps are depictions of what cells in various regions of the embryo of an embryo will become during development. Cell lineage studies can identify the range of phenotypes that arise from single cells. Construction of a fate map requires a means of following a cell from a defined region of the embryo and scoring the final phenotypes and and positions of their progeny. In some cases the molecular embryologist provides a unique cellular marker (in the form of cytoplasmic inclusions). In other cases a label is introduced to follow a cell lineage or construct a fate map (Fraser, 1992). Development involves groups of cells undergoing morphogenetic movements and phenotypic differentiation (Lewis, 2008; Horowitz and Simons, 2008).

Human embryo development
Human embryo refers to the developing human from the time of conception up to the end of the 8^{th} week of gestation. During this period it undergoes embryogenesis which can be crudely divided into early or late embryogenesis. Early embryogenesis is taken as development which occurs from zygote stage to the 14^{th} day of pregnancy when the primitive streak is formed and when there has been a completion of the initiation of pregnancy. The formation of the primitive streak heralds the conversion of the embryo from bilaminar to trilaminar embryo which is conversion from 2 germ layer to three. Early embryogenesis is characterised by zygote cleavage or mitosis. At the end of the 4^{th} mitosis the morula has been formed. Inmost mammals, by the time the early embryos enter the uterine cavity it has reached the blastocyst stage. In lower vertebrates his stage called the blastula is the stage of the embryo which allows for the formation of the 3^{rd} germ layer through the process of gastrulation.
In the amniote embryos the blastocyst is formed by a change in cell lineage (form one type which are all totipotent to a two type with the formation of two population of cells-trophectodermal cells and the cells of the inner cell mass. The trophectodermal cells take part in the formation of the fetal membrane and will not take part in the development of the fetus itself while the inner cell mass will form the fetus. Until recently the mechanism whereby these cells become differentiated into two distinct population of cell in the early embryos where not known; but they are gradually being mapped out now with the provision of data on inductive substance (Johnson 1988). Modern areas in protein identification technology have allowed for the characterisation of several factors which aid the separation and differentiation of cells in early and late embryo. Also immunological experiments have also been performed in which antibodies have been raised against specific proteins to determine the effect of antigen-antibody neutralisation of such proteins in early development. Thus certain stage specific protein antigens have been identified mainly by the work of Kapur and Johnson in California and recently implantation have been disrupted with the use of specific monoclonal antibodies against the implanting embryo. The formation of two population of cells in the early embryo have been identified to be under the control of teratocarcinoma antigen, epidermal growth factors and some other peptides. It was shown by Gaunt (1985) working in Cambridge that the ova of the mouse 30 minutes after fertilisation develops a teratocarcinoma antigen known as 2B5. This antigen is not present in *in vitro* fertilised egg until after 36 hours of incubation in growth medium. It was suggested therefore that the antigen may be elaborated in the oviductal epithelium. Teratocarcinomas are tumour cells of embryonic origin. Apart from the aforementioned, other stage specific antigens in the mouse early embryo had been previously characterised. They have also been found on teratocarcinoma (Teratocarcinomas are tumour cells of embryonic origin) cells and on certain mouse pluripotent cell types such as inner cell mass, embryonic ectoderm and primordial germ cells (Gaunt 1985). 2B5 antigen was described by Randle (Randle 1982) to be first expressed at the late 2 cell stage. SSEA-1 antigen was expressed initially at 8 cell mouse embryo (Solter and Knowles 1978) while M1/22.25 (Forssman) antigen was first expressed in the

late morula stage as first described by Willison and Stern (1978).

Gaunt while investigating the distribution of 2B5 antigen on mouse embryos found that the antigen disappeared from trophectodermal cells soon after blastocoels formation. He also found the antigen to be available in the epithelial lining of the oviduct of the adult mouse at the day of ovulation and also the endometrial lining of pregnant uterus. It was also detected on the adult male epithelial lining of discrete region of tubules in the caput epididymis. It was suggested by Bird and Kieber (1994) that such antigens as those described (e.g. SSEA-1) might be important in the mechanism of adhesion and compaction between embryonal cells in early embryogenesis. It is also possible that other teratocarcinoma antigens have such roles in early embryogenesis.

Embryonic growth factors

Embryonic growth factors can be broadly classified into three classes (Hill Strain and Milner 1987). These classes include:

a) Cell adhesion molecules (CAM)
b) Cell substrate adhesion molecules (CSAM) and
c) Peptide growth factors

Cell adhesion molecules (CAM) have been best studied in such phenomena as early embryogenesis, pattern formation, positional information all of which aid cell lineage formation and differentiation. CAMs have been shown to be homotypical, (they are all of similar types in all cells). They bind to membrane of two cells, which then adhere to each other as a result of recognition complementary sites on the two cell membranes with their contained cell adhesion molecules. CAMs are important in early and late embryogenesis, and even in fetal growth, morphogenesis and organogenesis. Migratory cells have been shown to loose their CAMs when they are migrating but once they are about to aggregate into tissues, they immediately gain them. These molecules thus allow cells to form compact tissues or glands after their migrations, especially in morphogenesis. CAMs are therefore important in differentiation of cells and also for the formation of large population of cells in organogenesis. CAMS can be further classified into three types

1) Liver type: These are found mainly in endodermal and liver tissues,
2) Neural type. Found mainly in neural tissues
3) Neural-glial type.

Cell substrate adhesion molecules (CSAMs) are important mainly in extracellular connective issue. They are of less importance in embryogenic growth and differentiation when compared to CAMs.

Peptide growth factors are the most important factors recently elucidated in embryonic and fetal growth. They have been found to be of considerable importance in various cells and tissues such as the nerve cells, especially in their differentiation into neuritis that form fasciculi or tracts. They are also important in fibroblasts, liver cells. Examples of peptide growth factor:

1. Somatomedin or insulin-like growth factors. These are substances that potentiate the effect of growth hormones. There are two types, IGF1 an IGFII. They are all under the control of hormones like insulin, growth hormone, and thyroxin in the fetus. They are potent mitogens for cells which are derived from all primitive germ layers. In postnatal life they are of importance in longitudinal skeletal growth through a stimulation of chondrocyte proliferation and also the maturation of epiphyseal growth plate. It has been shown that although the main source of IGF is the liver, in postnatal life, it is also found as tissue mitogen in most body tissues.
2. Insulin itself is also a growth factor. Supraphysiological concentration of the hormone is needed to promote cell replication in isolated postnatal connective tissue.
3. Epidermal growth factor (EGF). This is of considerable importance in the fetus and even in adult tissues. It is present both and fetus and adult tissues, mostly in the epidermis of skin, and bone. Recently, it has been shown to aid wound healing and therefore it is being

tried out as a therapeutic agent for the healing of wounds. In postnatal tissues it is found in the brain, pancreas, Brunner's glands of the duodenum, mammary gland, thyroid gland and lso the kidneys.

4. Neural growth factors (NGF). This is the only embryonic growth factor that does not aid mitosis and is therefore not a mitogenic. They are found in neural tissue and they probably aid the metabolism and non multiplicating growth of neural tissues. They are therefore non-mitogenic but they maintain the survival of certain populations of neurons and are chemotactic in property aiding regeneration of tissues. They also regulate the expression of catecolamine biosynthetic enzymes such as tyrosine hydroxylase, somatostatin and substance P.
5. Platelet derived growth factor (PGDF). Factor is found as a peptide stored in the a granule of platelets and released during clotting of blood. It is thought to stimulate the proliferation of cell types derived from mesoderm and they include fibroblasts, smooth muscle cells and also glial cells (Duel and Huang 1984). They are also released by platelets at sites of vascular injury. They are also released by platelets at sites of vascular injury. They are therefore also important in wound healing.
6. Fibroblast growth factor has been isolated from bovine pituitary gland ad brain. It has also been found to be in the adrenal cortex, corpus luteum, placenta, kidney and retina.
7. Transforming growth factors were first isolated in tumour cell products and they exist in two forms- TGFα and TGFβ has the ability to modulate the mitogenic activity of other peptide growth factors.
8. Embryonal carcinoma derived growth factor.

Oncogenes

An oncogene is a protein encoding gene, which when deregulated participates in the onset and development of cancer. Genetic mutations resulting in the activation of oncogenes increase the chance that a normal cell will develop into a tumor cell. Proto-oncogene on the other hand is a normal gene that can become an oncogene due to mutations or increased expression. Proto-oncogenes code for proteins that help to regulate cell growth and differentiation. They are often involved in signal transduction and execution of mitogenic signals, usually through their protein products. Upon activation, a proto-oncogene (or its product) becomes a tumor inducing agent or an oncogene (Todd and Wong, 1999). Examples of proto-oncogenes include RAS, WNT, MYC, ERK and TRK. Oncogenes are figuratively thought to be in a perpetual tug-of-war with tumor suppressor genes which act to prevent DNA damage and keep the cell's activities under control. There is much evidence to support the notion that loss of tumor suppressors or gain of oncogenes can lead to cancer.

Oncogenes are genes normally repressed in the cell of adult tissues but which due to certain retroviral stimulation allow for the expression of the gene; therefore the genes are expressed in abnormal adult cells usually virally infested causing cancer (Esquela-Kerscher and Slack, 2006). These genes are responsible for production of growth factors which allows for considerable mitosis which are responsible for stupendous division of cells that occur in cancerous cells. Many of the genes have been identified and sequenced. Oncogenes are also said to be stimulated or derepressed by certain carcinogens.

It was shown that certain viral oncogenes which have been introduced into cells by retroviruses and which are known to code for certain proteins that have served as cellular tumour markers are closely homologous to normal cellular genes (Bishop 1989). It is suggested that during the evolution of retroviruses these normal cellular genes became integrated into the viral genome. The viral oncogenes are designated as v-onc and the cellular genes as c-onc. It was the realisation that v-sis oncogene of the simian virus which encodes for the protein p28 structural similar to the β-chain of platelet derived growth factor that first allowed for the speculation that oncogenes

may encode for growth factors (Waterfield et al., 1983; Hill Strain and Milner 1987)

Homeobox genes

Several genes are now known to direct embryogenesis. Some of these include homeobox genes which have been the most studies (Kumar, 2008; Kawakami et al., 2000). A homeobox is a DNA sequence found within genes that are involved in the regulation of patterns of development (morphogenesis) in animals. It is about 180 base pairs long and also encodes a protein domain (the homeodomain) which can bind DNA (McGinnis et al., 1984)..

Genes that have a homeobox are called homeobox genes and form the homeobox gene family. The most studied and the most conserved group of homeodomain protein are the Hox genes, which control segmental patterning during development (Cavalli et al., 2008); although, not all homeodomain proteins are Hox proteins. They were discovered independently in 1983 by Walter Jakob Gehring and his colleagues at the University of Basel, Switzerland, and Matthew Scott and Amy Weiner, who were then working with Thomas Kaufman at Indiana University in Bloomington. Homeobox genes encode transcription factors which typically switch on cascades of other genes (McGinnis et al.,1984).. The homeodomain binds DNA in a specific manner.

However, the specificity of a single homeodomain protein is usually not enough to recognize only its desired target genes. Most of the time, homeodomain proteins act in the promoter region of their target genes as complexes with other transcription factors, often also homeodomain proteins. Such complexes have a much higher target specificity than a single homeodomain protein.

The homeobox genes were first found in the fruit fly *Drosophila melanogaster* and have subsequently been identified in many other species, from insects to reptiles and mammals.

On the Drosophila there are 8 homeobox genes which specify the structures which develop on each body segments. They are located on two regions on Drosophila chromosomes 3. There are a total of 38 genes which have homeoboxes in mammals. These have a higher degree of similarities with the Drosophila homeobox genes. These genes are called Hox in murine and HOX in human genome. They found in 4 clusters as follows (Ryan, 2007):

1. in mouse chromosome 11 and human chromosome 7
2. Mouse chromosome 11 and human chromosome 17
3. mouse chromosome 2 and human chromosome 2
4. mouse chromosome 2 and human chromosome 2

The Hox genes number from 1 to 13. Hox a-6, Hox b-6 and Hox c-6 are referred to as paralogous group. There are non homeobox genes which take part in development. They include Msx, Mox, Dix, Otx, Cax, Emx, Mox, DIc , Otx, Cax, Emx Goosecoid etc

Gene Therapy

Genes, which are carried on chromosomes, are the basic physical and functional units of heredity. Genes are specific sequences of bases that encode instructions on how to make proteins. Although genes get a lot of attention, it's the proteins that perform most life functions and even make up the majority of cellular structures. When genes are altered so that the encoded proteins are unable to carry out their normal functions, genetic disorders can result.

Gene therapy is a technique for correcting defective genes responsible for disease development. Researchers may use one of several approaches for correcting faulty genes:

1. A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common and can be done by use of microcell, calcium precipitation, direct intra-nuclear injection or via viral carriage (SV-40 retrovirus)
2. An abnormal gene could be swapped for a normal gene through homologous recombination.
3. The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.
4. The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

An organism whose genome has been altered by gene injection is said to be transgenic. The first most spectacular gene injection was that performed on the embryo of mouse using growth hormone gene of a rat by Palmiter and his associates (Palmiter et al., 1982). The mouse grew as big as a rat.

In most gene therapy studies, a "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene. A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes.

Target cells such as the patient's liver or lung cells are infected with the viral vector. The vector then unloads its genetic material containing the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state.

Some of the different types of viruses used as gene therapy vectors:

a. Retroviruses - A class of viruses that can create double-stranded DNA copies of their RNA genomes. These copies of its genome can be integrated into the chromosomes of host cells. Human immunodeficiency virus (HIV) is a retrovirus.
b. Adenoviruses - A class of viruses with double-stranded DNA genomes that cause respiratory, intestinal, and eye infections in humans. The virus that causes the common cold is an adenovirus.
c. Adeno-associated viruses - A class of small, single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome 19.
d. Herpes simplex viruses - A class of double-stranded DNA viruses that infect a particular cell type, neurons. Herpes simplex virus type 1 is a common human pathogen that causes cold sores.

Besides virus-mediated gene-delivery systems, there are several nonviral options for gene delivery. The simplest method is the direct introduction of therapeutic DNA into target cells. This approach is limited in its application because it can be used only with certain tissues and requires large amounts of DNA.

Another nonviral approach involves the creation of an artificial lipid sphere with an aqueous core. This liposome, which carries the therapeutic DNA, is capable of passing the DNA through the target cell's membrane.

Therapeutic DNA also can get inside target cells by chemically linking the DNA to a molecule that will bind to special cell receptors. Once bound to these receptors, the therapeutic DNA constructs are engulfed by the cell membrane and passed into the interior of the target cell. This delivery system tends to be less effective than other options.

Researchers also are experimenting with introducing a 47th (artificial human) chromosome into target cells. This chromosome would exist autonomously alongside the standard 46 --not affecting their workings or causing any mutations. It would be a large vector capable of carrying substantial amounts of genetic code, and scientists anticipate that, because of its construction and autonomy, the body's immune systems would not attack it. A problem with this potential method is the difficulty in delivering such a large molecule to the nucleus of a target cell.

As at today, there is no any human gene therapy product that has been approved for sale. Current gene therapy is experimental and has not proven very successful in clinical trials. Little progress has been made since the first gene therapy clinical trial began in 1990. In 1999, gene therapy suffered a major setback with the death of 18-year-old Jesse Gelsinger. Jesse was participating in a gene therapy trial for ornithine transcarboxylase deficiency (OTCD). He died from multiple organ failures 4 days after starting the treatment. His death is believed to have been triggered by a severe immune response to the adenovirus carrier.

Another major blow came in January 2003, when a temporary halt was placed on all gene therapy trials using retroviral vectors in blood

stem cells. This action was taken after it learned that a second child treated in a French gene therapy trial had developed a leukemia-like condition. Both this child and another who had developed a similar condition in August 2002 had been successfully treated by gene therapy for X-linked severe combined immunodeficiency disease (X-SCID), also known as "bubble baby syndrome."

Biological Response Modifiers Advisory Committee (BRMAC) met at the end of February 2003 to discuss possible measures that could allow a number of retroviral gene therapy trials for treatment of life-threatening diseases to proceed with appropriate safeguards. In April of 2003 the FDA eased the ban on gene therapy trials using retroviral vectors in blood stem cells.

The factors that have kept gene therapy from becoming an effective treatment for genetic diseases are:

i. Short-lived nature of gene therapy - Before gene therapy can become a permanent cure for any condition, the therapeutic DNA introduced into target cells must remain functional and the cells containing the therapeutic DNA must be long-lived and stable. Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits. Patients will have to undergo multiple rounds of gene therapy.
ii. Immune response - Anytime a foreign object is introduced into human tissues, the immune system is designed to attack the invader. The risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a potential risk. Furthermore, the immune system's enhanced response to invaders it has seen before makes it difficult for gene therapy to be repeated in patients.
iii. Problems with viral vectors - Viruses, while the carrier of choice in most gene therapy studies, present a variety of potential problems to the patient --toxicity, immune and inflammatory responses, and gene control and targeting issues. In addition, there is always the fear that the viral vector, once inside the patient, may recover its ability to cause disease.
iv. Multigene disorders - Conditions or disorders that arise from mutations in a single gene are the best candidates for gene therapy. Unfortunately, some the most commonly occurring disorders, such as heart disease, high blood pressure, Alzheimer's disease, arthritis, and diabetes, are caused by the combined effects of variations in many genes. Multigene or multifactorial disorders such as these would be especially difficult to treat effectively using gene therapy. For more information on different types of genetic disease, see Genetic Disease Information.

Some recent developments in gene therapy research include:

1. Results of world's first gene therapy for inherited blindness show sight improvement. 28 April 2008. UK researchers from the UCL Institute of Ophthalmology and Moorfields Eye Hospital NIHR Biomedical Research Centre have announced results from the world’s first clinical trial to test a revolutionary gene therapy treatment for a type of inherited blindness. The results, published today in the New England Journal of Medicine, show that the experimental treatment is safe and can improve sight. The findings are a landmark for gene therapy technology and could have a significant impact on future treatments for eye disease.

Previous information on this trial (May 1, 2007):

A team of British doctors from Moorfields Eye Hospital and University College in London conduct first human gene therapy trials to treat Leber's congenital amaurosis, a type of inherited childhood blindness caused by a single abnormal gene. The procedure has already been successful at restoring vision for dogs. This is the first trial to use gene therapy in an operation to treat blindness in humans.

2. A combination of two tumor suppressing genes delivered in lipid-based nanoparticles drastically reduces the number and size of human lung cancer tumors in mice during trials conducted by researchers from The University of Texas M. D. Anderson

Cancer Center and the University of Texas Southwestern Medical Center.

3. Researchers at the National Cancer Institute (NCI), part of the National Institutes of Health, successfully reengineer immune cells, called lymphocytes, to target and attack cancer cells in patients with advanced metastatic melanoma. This is the first time that gene therapy is used to successfully treat cancer in humans.
4. Gene therapy is effectively used to treat two adult patients for a disease affecting nonlymphocytic white blood cells called myeloid cells. Myeloid disorders are common and include a variety of bone marrow failure syndromes, such as acute myeloid leukemia. The study is the first to show that gene therapy can cure diseases of the myeloid system.
5. Gene Therapy cures deafness in guinea pigs. Each animal had been deafened by destruction of the hair cells in the cochlea that translate sound vibrations into nerve signals. A gene, called Atoh1, which stimulates the hair cells' growth, was delivered to the cochlea by an adenovirus. The genes triggered re-growth of the hair cells and many of the animals regained up to 80% of their original hearing thresholds. This study, which many pave the way to human trials of the gene, is the first to show that gene therapy can repair deafness in animals.
6. University of California, Los Angeles, research team gets genes into the brain using liposomes coated in a polymer call polyethylene glycol (PEG). The transfer of genes into the brain is a significant achievement because viral vectors are too big to get across the "blood-brain barrier." This method has potential for treating Parkinson's disease.
7. RNA interference or gene silencing may be a new way to treat Huntington's. Short pieces of double-stranded RNA (short, interfering RNAs or siRNAs) are used by cells to degrade RNA of a particular sequence. If a siRNA is designed to match the RNA copied from a faulty gene, then the abnormal protein product of that gene will not be produced.
8. New gene therapy approach repairs errors in messenger RNA derived from defective genes. Technique has potential to treat the blood disorder thalassaemia, cystic fibrosis, and some cancers.
9. Gene therapy for treating children with X-SCID (sever combined immunodeficiency) or the "bubble boy" disease is stopped in France when the treatment causes leukemia in one of the patients.
10. Researchers at Case Western Reserve University and Copernicus Therapeutics are able to create tiny liposomes 25 nanometers across that can carry therapeutic DNA through pores in the nuclear membrane.
11. Sickle cell is successfully treated in mice.

Just like any other technique used in humans, some of the ethical considerations for using gene therapy include:

1. What is normal and what is a disability or disorder, and who decides?
2. Are disabilities diseases? Do they need to be cured or prevented?
3. Does searching for a cure demean the lives of individuals presently affected by disabilities?
4. Is somatic gene therapy (which is done in the adult cells of persons known to have the disease) more or less ethical than germline gene therapy (which is done in egg and sperm cells and prevents the trait from being passed on to further generations)? In cases of somatic gene therapy, the procedure may have to be repeated in future generations.
5. Preliminary attempts at gene therapy are exorbitantly expensive. Who will have access to these therapies? Who will pay for their use?

Conclusion

As the tools of molecular biology and experimental embryology have been combined, the pace of progress in the field of developmental anatomy has exploded. It has become a field with sufficient breadth to fruitfully compare developmental mechanisms across all animal kingdom, and with sufficient

depth to attack patterning questions at the tissue, cellular and molecular levels.

I was told by my supervisor – since you are young go into sonographic embryology and today, I have no regrets. I say to the young people today the same thing: If you are young and an anatomist; go into molecular embryology. By the same token; if you want to be a gynaecologist, go into molecular embryology.

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