Transgenic and gene disruption techniques from a concept to a tool in studying the basic pathogenesesis of various human diseases

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Abstrak

Teknologi transgenik kini biasa digunakan dalam mengkaji dan menganalisis fungsi gen dan elemen regulatori yang berperan pada perkembangan dan diferensiasi jaringan. Gabungan teknik ini dengan teknik disrupsi gen, rekayasa genetik dan teknik lain seperti teknik biologi molekular, histologi dan lain-lain, banyak digunakan untuk mengkaji patogenesis dasar dari berbagai penyakit manusia. Artikel ini menyarikan teknik yang paling popular dalam modifikasi genom mamalia in vivo. Selain itu, artikel ini juga menerangkan tentang keuntungan teknik ini dalam mempelajari penyakit manusia, dan juga beberapa contoh patogenesis dasar penyakit manusia yang ditemukan menggunakan teknik ini, serta kemungkinan penggunaan teknik ini dalam mengobati penyakit manusia di masa depan.

Abstract

Transgenic technologies now have become commonly used in studying and analyzing the function of many genes and regulatory elements involved in the development and differentiation of tissues. By using the combination between these techniques and gene disruption techniques, including genetic geneering, and many other techniques, such as molecular biology techniques, histotechniques, etc, now many researchers have used it to study the basic pathogenesis of various human diseases. This article reviews the most popular technique for modification of the mammalian genome in vivo. This article also explains the advantages of these techniques in studying human diseases and some illustration of basic pathogenesis of human diseases that had been discovered by using these techniques and the future potential of these techniques in the treatment of human diseases.

By definition, transgenic organism is a life being whose germ line genome has been artificially modified by means of genetic engineering.

A foreign DNA can be randomly integrated into the genome. In mammals for example, linear DNA fragments introduced into cells are rapidly ligated end to end by intracellular enzymes to form long tandem arrays, which usually become integrated into a chromosome at an apparently random site.

A mouse egg injected with 200 copies of linear DNA molecules will often develop into a mouse, containing in many of its cells, tandem arrays of copies of the injected gene integrated at apparently random site in one site of one of its chromosomes. If the modified chromosome is present in the germ line cells (eggs or sperm), the mouse will pass these foreign gene on to its progeny. Animals that have been permanently altered by this way are called transgenic organisms and the foreign genes are called transgenes.

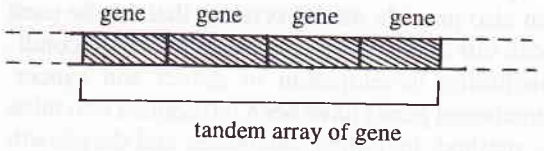

Figure 1. Tandemly arranged copies of the gene are inserted randomly into one chromosome in each cell

Transgenic mice are generated by microinjection of a cloned DNA fragment into the fertilized mouse egg, with subsequently embryogenesis, birth and post natal life. The successful incorporation of DNA into the

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germ line can be determined later by analysis of genomic DNA in the offsprings. In very early embryo the foreign DNA can be incorporated into the chromosome of some embryonic cells and maintained in them as they proliferate and differentiate into adult tissues. When the foreign DNA have been integrated into the genome of the recipipent embryo, the embryo or the adult animal can be analyzed to determine the pattern of expression and the phenotypic effect of the introduced gene.

Transgenic mice had been generated for the first time in 1981 by Palmitter and Brinster, while Spradling and Rubin produced transgenic fruit flies.[1]

This article reviews the most popular technique for modification of the mammalian genome in vivo, i.e. microinjection of fertilized eggs, targeted gene deletion through homologous recombination, and other techniques. This article also explains advantages of these techniques in studying human diseases and some illustration of basic pathogenesis of human diseases that had been discovered by using these techniques, and the future potential of these techniques in the treatment of human diseases.

THE USE OF TRANSGENIC MICE

In most cases the transgenic approach aims at specific modification of the genome, i.e. by introducing whole transcriptional units into the genome, or by inactivating pre-existing cellular genes.

Transgenic method allows the function of specific genes and regulatory elements to be examined in the context of the whole animal with its complex program of development and tissue differentiation. Transgenic mice can also provide model systems that can be used to broaden our understanding of human disease conditions, including development of defect and cancer. Many functional genes have been introduced into mice by these method, including oncogenes and the growth hormone gene. By adding the regulatory sequences along with the coding region of interest, expression of the gene may be limited to specific tissues or periods of development. A more complicated method has been developed to replace a functional gene with an inactive allele in transgenic mice.[2] By using this method, the function of many genes can be studied more easily and the basic mechanism of many human diseases can be explored and analyzed more extensively, increasing our understanding of the pathogenesis of various human diseases.

The generation of animal models for human diseases by means of transgenesis has promoted a leap forward in our understanding of the pathogenesis of various human diseases. However, translation of these concepts into experimental and clinical applications is proceeding slowly, because the development of efficient strategies for gene transfer in vivo is not easy.

Now gene transfer is already being tested as a therapeutic approach in animal models and in a growing number of human disorders. In addition, the time lag between advances in basic understanding and progress in applications has been steadily decreased in recent years. The potential of transgenic technologies for the advancement of medical science has encouraged molecular biologists to learn about the basic facts of medicine, while at the same time clinical researchers are increasingly making use of the tools provided by the new technologies.

Transgenic technology can be used as a "bridge" between genetic engineering, molecular biology, and other basic medical science's concept in one side and the "real" phenomena in in vivo biological study and clinical research. Finally transgenic technologies can act as communicator in the interaction of many disciplines of knowledges such as molecular genetics, histology, pathology, and clinical researches.

MANIPULATION OF THE MAMMALIAN GENOME IN VIVO

Compared to the transfer of genetic information to prokaryotic organism or cultured eukaryotic cells, genetic modification of a multicellular organism is a complex and inefficient process. In the last decade three approaches have evolved in manipulating the mammalian genome: (1) introduction of complete transcriptional unit or minigene into the germline of mice, rat and sheep, to generate a transgenic animal; (2) introduction of foreign genes into specific somatic cells; (3) ablation or subtle site directed modification of the endogenous genetic stock (colloquially termed the "knock out" approach).

INTRODUCTION OF TRANSGENES

Rudolf Janish and his colleagues in 1985 showed that infection of pre-implantation mouse embryos with an ectopic murine retrovirus can lead to proviral integration into the host genome. Subsequent transfer of such infected embryos to the reproductive tract of foster mothers and breeding of the offsprings enable germline transmission of the exogenous genetic

material to the filial generations in a Mendelian fashion. Shortly thereafter, Erwin Wagner, Tim Stewart and Beatrice Mintz introduced an alternative technique allowing for a higher flexibility in the generation of transgenicmice, i.e., pronuclear microinjection of zygotes, followed by transfer to the oviduct.[3] This techniques has been slightly modified in the subsequent years.[4]

SETTING UP A COLONY FOR THE PRODUCTION OF TRANSGENIC MICE

The animals maintained in a colony for the production and analysis of transgenic mice can be divided into 5 categories: (1) female mice for mating to produce fertilized eggs, (2) fertile stud male mice, (3) sterile male mice for the production of pseudopregnant female mice, (4) female mice to serve as pseudopregnant recipients and foster mothers, (5) transgenic mice lines derived from these founders.

The most important thing in preparing female for mating to produce fertilized eggs is the strain of mice. Mice used in generating transgenic mice must have good genetic background. Superovulated females, i.e., female mice induced by using hormons substituting/similar to FSH and LH. Such female mice are used almost exclusively over naturally ovulating females for the production of fertilized eggs in large numbers.

The stud male mice used in generating transgenic mice should be in good reproductive performance to fertilize the females' eggs in large number. Male mice reach sexual maturity between 6 to 8 weeks of age and can be used as stud for 1-2 years depending on the strain, but it is better to replace the stud at 8-10 months of age, as their reproductive performance tend to decrease after that age. Each superovulated female is placed individually with a stud and is checked for the copulation plug the next morning. If a stud fails to plug a superovulated female several times in a row, or if his plugging average is less than 60-80%, he should be replaced. Because the sperm count will be depressed for several days after mating, a stud should not be used for about a week after plugging a female.

Sterile male mice are required for mating to generate pseudopregnant resipient, and usually are produced by vasectomy. For vasectomy, males of at least 2 months of age from any strain with good breeding performance will be suitable. Before using a vasectomized male in an experiment, it should be tested for sterility by mating it with females to obtain at least three plugs. If the vasectomy was successful, most of the plugged females should not become pregnant.

Pseudopregnant mice are prepared by mating females in natural estrus with vasectomized males. The qualification of female as a foster mother are (a) at least 6 weeks of age, (b) more than 20 grams in body weight, (c) already succesfully reared a litter. Many investigators use coat color differences between the strain of the egg donor and the strain of the pseudopregnant recipient to be certain that any mice born actually derived from the donor eggs. Most mice will give birth on day 19 of pregnancy (counting the day of the plug as day 0), although this may vary between strains.

Potential transgenic mice are usually screened for the presence of the injected gene by Southern or Dot blot hybridization to DNA extracted from the tail. Mice that develop from injected eggs are often termed as "founder mice". As soon as it has been determined that a given "founder mice" is transgenic, it is usually mated to start establishing a transgenic line. If the founder is a female and we want to develop a transgenic line from her, it is necessary to wait until she has given birth and raised at least one litter before she can be sacrified. If the founder is a male, he can be placed with two females which are checked every day and will be replaced with a new female as soon as each one is plugged. In this way, the male can generate many litters within a few week. As soon as a male has plugged a sufficient number of females (6-8) he may be sacrified, if necessary, for the analysis of gene expression. However, if one wants to be sure of establishing a transgenic line, the founder should not be sacrified until positif transgenic progeny has been identified.

Although most transgenic founder will transmit the foreign gene to 50% of their offsprings, approximately 20% of transgenic founder are mosaic and transmit the gene at a low frequency (i.e. 5-10% instead of 50%). In addition, a proportion of females that are plugged will fail to get pregnant, for example if the male is sterile or semisterile or will not raise a litter succesfully. Homozygote transgenic mice are produced by setting up heterozygous intercrosses. Teoretically, one quarter of the progeny from such a cross should be homozygous with respect to the integrated foreign DNA; the rest, one half should be heterozygous, and one quarter non transgenic.

GENETRATING TRANSGENIC MICE

The generation of transgenic mice is a time consuming and difficult procedure. Many steps are required to

generate transgenic mice. Figure 2 shows the flow-chart of numerous steps required for transgenic mice generation.

The first step is to prepare the cloned DNA (DNA insert) for injection. The DNA insert should contain a promoter, polyadenylation site, complete protein coding region and at least one intron.[5] DNA fragment after restriction enzyme digestion of plasmid DNA was purified by agarose gel electrophoresis and electroelution. Although prokaryotic cloning vector sequences have no apparent effect on the integration frequency of microinjected genes, it is important to note that they can severely inhibit the expression of eukaryotic genes introduced into the mouse.[6] It is not known whether a specific sequence is responsible for this inhibition or whether it is a general property of prokaryotic DNA. So it is advisable to remove all vector sequences from a cloned gene before introducing it into the mouse gene line, if optimal expression of the introduced gene is desired.

The second step is to obtain the fertilized eggs (embryos) from a superovulated female mouse. This superovulated female mice are mated with the stud male mice good in reproductive performance to produce the fertilized eggs in large numbers. These fertilized eggs then are collected, washed, and prepared for injection by incubating them in culture medium.

The third step is to inject the male pronucleus of fertilized eggs (embryos) with DNA insert. For this procedure a special microscope/micromanipulator set up is required. Each fertilized eggs is individually immobilized by a holding micropipette using gentle suction, while DNA insert, are injected into the male pronucleus through a second injection pipette. The microinjection of DNA directly into the pronuclei of fertilized mouse eggs result in the stable chromosomal integration of foreign DNA in 10-40% of the resulting mice.[3,7-9] In most cases, integration appears to occur at the one-cell stage because the foreign DNA is present in every cell of the transgenic animal, including all primordial germ cells. Following injection, eggs (embryos) containing DNA insert are transfered into the infundibulum of pseudopregnant female mice by using transfer pipette. Then these foster mothers are bred for about 20 days until giving birth. After birth, the litters of these female mice and their mothers are bred together. At about 3 weeks after birth, 2 cm of their tails are clipped for tail DNA isolation, and finally the integration of the transgene within the genome are screened by using Southern blot analysis or PCR analysis. Then these transgenic mice can be used for further breeding or for analyzing the phenotypic expression.

INACTIVATING THE FUNCTIONAL GENES USING TRANSGENIC TECHNIC

A method had been developed to replace a functional gene with an inactivated allele in transgenic mice; this method is called the gene disruption technique.[2,9] If a DNA molecule carrying mutated mouse gene is transfered into a mouse cell, it usually inserted into the chromosomes at random sites and these nearly always include both ends of the engineered DNA fragment. However, about one in a thousand times, it will replace one of the two copies of the normal gene by homologous recombination. Homologous recombinant incorporates the center of the engineered DNA fragment without the ends. The rare colonies of cells in which a homologous recombination event has occured and caused a gene replacement are isolated by double drug selection. Then the correct colonies among these are identified by Southern blotting. This show that the correct colonies contain recombinant DNA sequence, i.e. the inserted fragment has replaced all or part of one copy of the normal gene. By exploiting this rare "gene targeting" event, any specific gene in a mouse cell can be inactivated by direct gene replacement.

In the first step of this procedure, a cloned DNA containing the gene to be mutated is modified by genetic engineering, so that it contains a bacterial gene within it, called Neo. The integration of Neo gene into a mouse chromosome renders resistancy to a drug (usually antibiotic) that otherwise kills the mouse cells. Then a viral gene called Tk (thymidine kinase) is added, attached to one end of the mouse DNA. Then the modified DNA fragment is introduced into a special line of mouse embryo derivated stem cell (called embryonic stem cell or ES cell). The integration of Tk into a mouse chromosome makes the cells sensitive to a different drug. The addition of these two drugs will give us the easy way to select the correct colonies of embryonic stem (ES) cells which contain the recombinant DNA sequences (mouse mutant gene), generated by homologous recombination (Figure 3).

In the second step, individual cells from the identified colony are inserted into an early mouse embryo, to produce the ES cell-embryo chimaeras. Then these chimaeric embryos are transfered to the foster mother mice.

Superovulating female mouse induced by using hormons similar to FSH and LH

Obtain fertilized eggs (embryo)

Collect, wash and incubate in culture medium

DNA Injection of fertilized eggs/ embryos (into male pronucleus)

Implant transgenic embryos into the infundibulum of a pseudo pregnant female mouse

Take care of foster mother mouse until giving birth

Birth of the litter

Clip 2 cm of tail for DNA analysis

Tail DNA analysis determines which offspring contain the injected DNA insert (by PCR or Southern blot analysis)

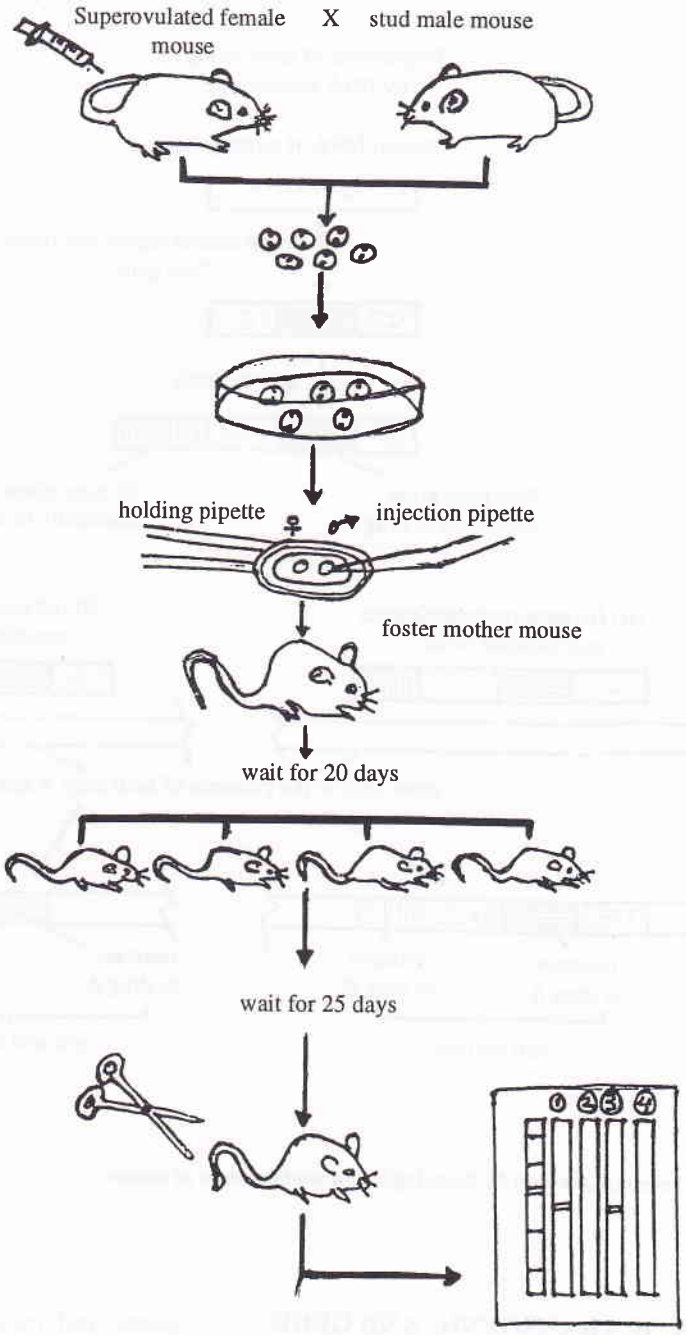

Figure 2. The flowchart of transgenic mice generation

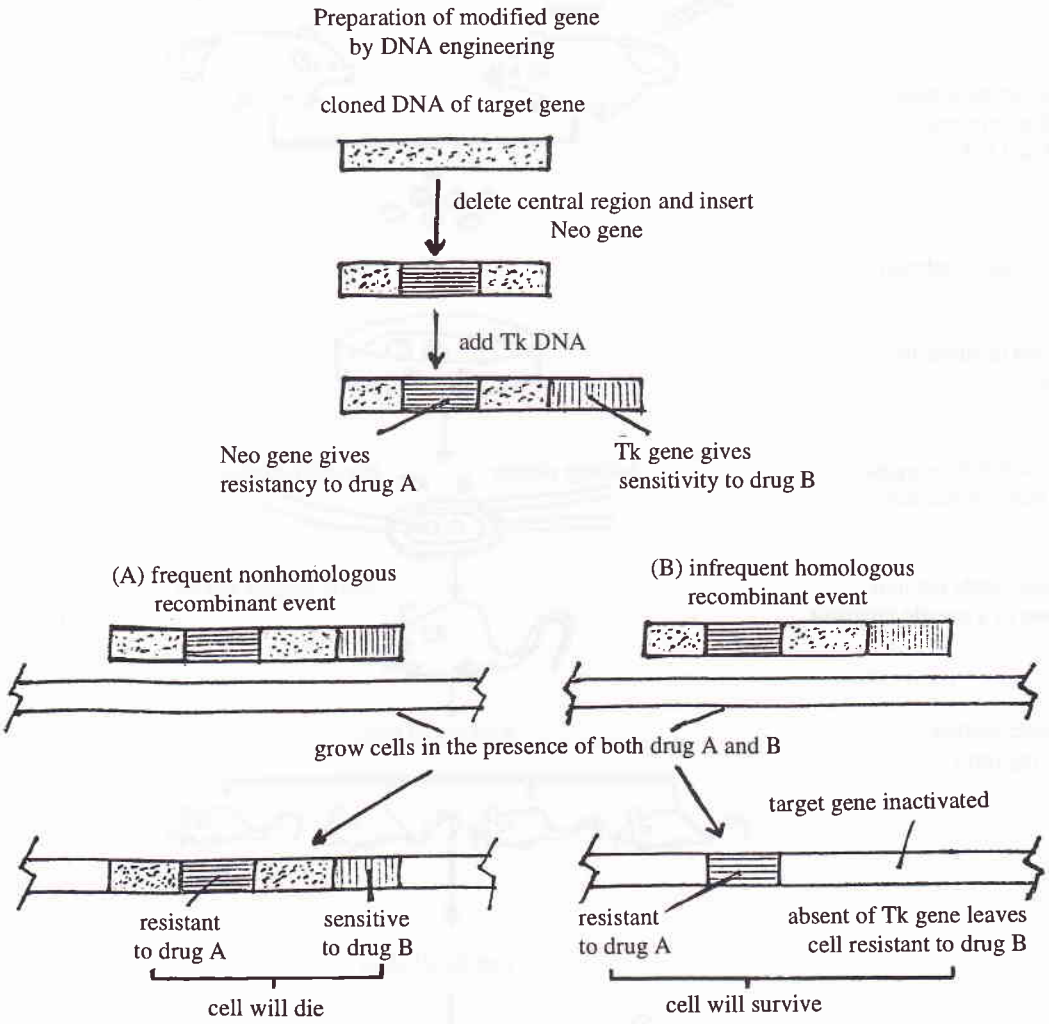

Figure 3. Selective gene replacement by homologous recombinantion in mouse.

APPLICATION OF TRANSGENIC AND GENE DISRUPTION TECHNIQUE

The ability to prepare transgenic mice lacking a known normal gene is a major advance, and the technique is now being widely used to dissect the functions of specific mammalian genes.

Since the generation of the first transgenic mice in 1981,[1] especially after the gene disruption technique was developed to replace a functional gene,[2,9] these techniques had been the most commonly used and useful techniques to analyze the function of specific genes and regulatory elements. Furthermore, these techniques were used in exploring the basic pathogenesis of many human diseases, including metabolic diseases, immune system disorder, cancer, degenerative diseases, defect in tissue development and differentiation, etc. Transgenic and gene disruption techniques are not only used to study the pathogenesis of human diseases, but also can be used to search an alternative method in the treatment of those diseases. Following description will explain the correlation of particular genes with some human diseases, which are studied by using transgenic and gene disruption techniques as a tool.

Correlation between Connexin 37, gap junction and infertility

At birth the ovary contains primordial follicles consisting of meiotically arrested oocytes surrounded by a single layer of granulosa cells. Periodically several primordial follicles undergo further development, and each cycle, one of it proceeds until ovulation. After ovulation, oocytes resume meiosis and the granulosa cells differentiate into steroidogenic cells, forming the corpus luteum. Until now little is known about the signals that control the follicle development, ovulation, differentiation of granulosa cells to become steroidogenic cells forming the corpus luteum and the relationship between oocyte and granulosa cells.

By using transgenic and gene disruption technique, it was shown that Connexin 37 played the important role in passing the signal that controlled the follicle growth, luteinization and oocyte maturation. Connexin 37 is a subunit of the proteins that form the gap junction between oocytes and granulosa cells. Loss of Conexin 37 in female mice causes infertility.[10]

Connexin is a family of the integral membrane proteins. Until now at least 12 members of connexin protein family were discovered. Each of them is encoded by its own gene, located at several different chromosomes. The expression of these genes on tissues are also different one from another. The structure of connexin consists of 4 transmembrane hydrophobic molecules (M1-M4), 2 extracellular loops (E1-E2), 1 cytoplasmic loop (CL), amino and carboxyl termini (figure 4). Extracellular, transmembrane, and N terminal cytoplasmic domains are well conserved among family members, while cytoplasmic loop and C-terminal cytoplasmic domain are highly variable in both sequence and size.

Connexin 37 is encoded by connexin 37 gene located at chromosome 4. This gene encodes a 1.5 kb mRNA and is expressed in several organs, such as heart, ovary, testis, lung, skin,[11] and blood vessel endothelial cells.[12,13]

All female mice lacking Connexin 37 (Cx 37) were infertile.[10] Although Cx 37 -/- mice showed no external abnormalities and appeared healthy, Cx 37 -/- females were found to be completely incapable of ovulation, even in gonadotropin stimulation. By using microinjection of neurobiotin into oocyte and electron microscopic analysis, it was found that gap junctions between oocytes and neighbouring granulosa cells were intact in wild type mice and completely absent in mutant mice. Further, it was discovered that abnormalities in follicular growth, control of luteinization and oocyte maturation underlay Cx 37 -/- female infertility. In Cx 37 -/- female mice follicular development consistenly arrested before full maturation, and the inappropriate formation of corpora lutea was occured. In normal animal, luteinization occured following ovulation caused by the lost of oocyte-granulosa cell communication. The premature luteinization observed in Cx 37 -/- females suggests a model in which junctional communication plays an important role in transfering the inhibitory signal from oocyte to granulosa cells and results in the prevention of corpus luteum formation.

Finally they concluded that cell-cell signalling through intercellular channels critically regulated the highly coordinated set of cellular interaction required for successful oogenesis and ovulation.

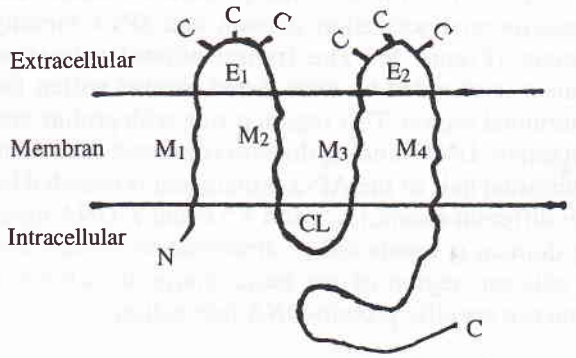

Figure 4. The structure of Connexin protein. The protein structure consists of 4 transmembrane hydrophobic molecules (M1-M4), 2 extracellular loops (E1-E2), cytoplasmic loop (CL), amino and carboxyl termini

Lack of transcription factor AP-2 causes defect in cranial closure and cranial development

Regulation of transcription is fundamental to dynamic process underlying cellular differentiation and development. The rate of gene transcription depends on its unique constellation of cis linked regulatory elements and on the combinatorial effect of transcription factors, both general and cellular type specific that binds to this element.

AP-2 is one of the transcription factors that may play a role in development. This nuclear protein was first identified and purified from Hela cells as 50 kDa in size that recognized sequences, common to enhancer

element of SV-40 and human metallothionein IIA gene. The binding sites for AP-2 was also found in a variety of gene promoters, including pro-enkephalin, collagenase, growth hormone, keratin K-14, murine major histocompatibility, C-myc and acetylcholin esterase. AP-2 amino acid sequence is highly conserved among frog, mice and human, indicating that this protein likely plays a fundamental role in development. During the closure of neural tube in mice, transcription factor AP-2 is expressed in ectoderm and neural crest cells migrating from the cranial neural fold.[14] Cranial neural crest cells provide patterning information for craniofacial morphogenesis, generate most of the skull bones, and together with placodal ectoderm, form the cranial ganglia.

The transcription factor AP-2 is encoded by a gene located on chromosome 6 near HLA locus. This gene consists of 7 exons and has a 18 kb genomic DNA. AP-2 protein structure has 2 main domains, the transcriptional activation domain and DNA binding domain (Figure 5). The transcriptional activation domain is encoded by exon 2 and located within the N-terminal region. This region is rich with proline and glutamine. DNA binding domain is located within the C-terminal half of the AP-2 protein and is encoded by four different exons, i.e., exon 4,5,6 and 7. DNA binding domain is composed of dimerization domain and an adjacent region of net basic charge to achieve a sequence specific protein-DNA interaction.

The function of AP-2 transcriptional protein in tissue development during embryogenesis was tudied by using disruption technique combined with transgenic method. Exon 6, which was important for dimerization and binding to DNA binding site in promoter area of the targeted gene was ommited. Mice lacking AP-2 gene died perinatally with cranio-abdominoschisis and severe dismorphogenesis of the face, skull, sensory organs and cranial ganglia. Failure of cranial closure between 9 and 9.5 days post-coitum coincided with increased apoptosis in the midbrain, anterior hindbrain and proximal mesenchyme of the first branchial arch, but did not involve the loss of expression of Twist or Pax-3, two other regulatory genes known to be required for cranial closure.

Mice lacking NCX/HOX 11 L-1 gene showed the megacolon phenomena, a novel pathogenesis for megacolon

Hirsprung's disease is a congenital disorder characterized by the congenital absence of the enteric nerve ganglia from the hindgut, resulting in intestinal obstruction, and causes the megacolon phenotype.

Enteric nerve ganglia were derived from neural crest cells. Neural crest cells that form a transient population of cells, emerged from the neural tube epithelium (neuroepithelium), located in the dorsal part of the neural tube, at the junction between neuroepithelium and surface ectoderm. These cells then migrate from its original sites at a define stage of development, disperse within the embryo and finally settle in many embryonic regions to yield ganglia and a large variety of connective tissues in the head. The migration and colonization of neural crest cells is mainly controlled by signals through the c-Ret protooncogene and the endothelin-B receptor gene. Most experimental models of Hirsprung's disease, including mutants with loss of function of the tyrosine kinase Ret receptor and its ligand, or the endothelin receptor and its ligand, also demonstrate loss of myenteric neurons.

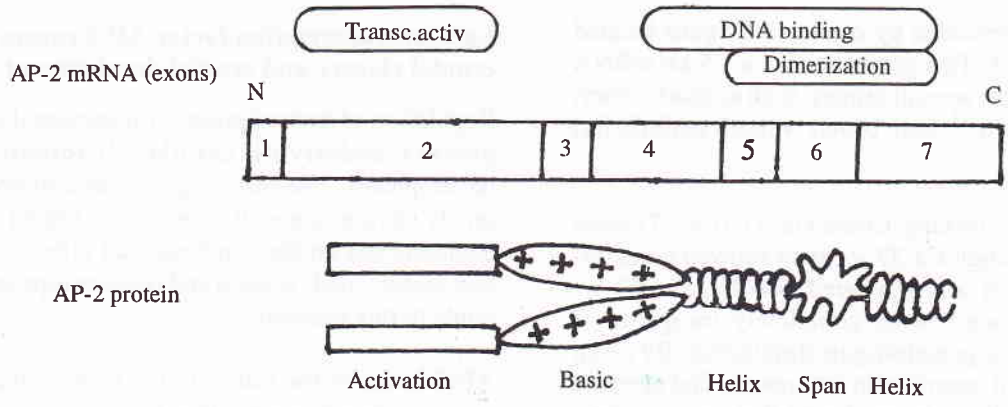

Figure 5. The correlation between the mRNA and protein of transcriptional factor AP-2

However, megacolon also results from neuronal intestinal dysplasia (NID), a human congenital disorder that often presents similarly to Hirsprung's disease i.e. a narrow rectum and distended bowel extending through hind gut into small bowel.

The neuronal intestine dysplasia is characterized by a normal number of ganglia or hyperplasia of enteric neuron. Experiments revealed that the human homologue of the NCX/Hox 11 L-1 gene can be a candidat gene causing NID in human. NCX/Hox 11 L-1 is a member of the Hox 11 homeobox gene family. NCX gene has 87% similarity in homeodomain sequences with Hox 11, and the helix 3 sequence that is important for determining the DNA binding spesificity is identical between them.[15] Homeobox gene products have a conserved domain (homeodomain) of 60 aminoacids, and are known to bind to specific DNA sequences. These genes are classified into several gene families depending on their homology of homeobox sequences and their locations on chromosomes in human and mice.

NCX/Hox 11 L-1 deficient mice developed myenteric neuronal dysplasia and megacolon after 3-5 weeks of age. Histochemical analysis revealed that the number of NADPH diaforese positive neurons in the proximal colon was higher in the NCX deficient mice than in the wild type mice. The number of substance-P positive neuronal cells and fibers also increased in the proximal colon of NCX deficient mice. NADPH diaforese is identical to nitric oxide synthase and nitric oxide functions as an inhibitory neurotransmitter to relax smooth muscle, while substance-P is an excitatory neurotransmitter to contract circular smooth muscles. Therefore, histochemical analysis revealed that the enteric neuron was hyperinnervated in the proximal part of the colon.

The pathogenesis of megacolon with the absence of enteric neurons can be explained by the abnormal movement of colon. In case of NID, the hyperinnervation may cause inappropriate action of enteric neurons and may result in functional abnormality of the colon.

The amelioration of the dystrophic phenotype of mdx mice using a truncated transgene

Duchene and Becker muscular dystrophies (DMD and BMD) are allelic X-linked recessive disorders which result in a distinctive pattern of progressive muscle wasting, and patient usually will die in early twenties, due to respiratory and or cardiac failure. The main symptoms and signs of this disease are muscle weakness, muscle pain and mental retardation; the value of serum creatinin kinase is elevated to 50-100 times of normal. Histological analysis shows progressive muscle fiber degeneration and regeneration. EMG analysis is characteristic with reduction in duration and amplitude of the wave. Immunohistochemical staining of dystrophin protein shows negative staining in DMD patient, faint in BMD disease, and mosaic pattern in DMD carrier.

The disease is caused by lack of dystrophin, a large membrane-associated protein expressed in muscle and brain that is localized to the inner face of the cell membrane. This cytoskeletal protein (427 kDa in molecular weight) is encoded by the largest known human gene with a 14-kB RNA transcripts containing 79 exons. Dystrophin has many isoforms generated through differential promoter usage and or alternative splicing at the 3'end of the gene (until now at least 5 distinct isoforms had been discovered).

Utrophin, another member of cytoskeletal protein (395 kDa in molecular weight) encoded by utrophin gene which is located at chromosome 6q24 in human and chromosome 10 in mouse, with a 13-kb RNA transcript, has an amino acid sequence that is 80% similar to the amino acid sequence of dystrophin. Like dystrophin, utrophin also consists of 4 domains, i.e. N and C terminal domain, rod domain and cysteine rich domain. The amino acid differences between dystrophin and utrophin is located only on the rod domain. Rod domain of dystrophin has 24 repeats of 109 amino acid sequence separated by 4 proline rich hinge regions, while utrophin only contains 22 repeats and 2 proline rich regions.

Utrophin was found in all tissues, particularly in the lung, bood vessel, and nerves. In normal adult muscle, utrophin is localized at the membrane of neuromuscular junctions. In fetal muscle development, utrophin is expressed before dystrophin. Like dystrophin, utrophin is localized at the sarcolemma and neuromuscular junctions, showing maximum expression at 17-18 weeks of gestation. However, when dystrophin is expressed, utrophin is gradually depleted from the sarcolemma, replaced by dystrophin and leaving the neuromuscular junction as the only site of utrophin localization. Because of this, many researcher suggest that overexpression of utrophin gene may decreased the dystrophic muscle phenotype of dystrophin disease.

Tinsley et al in 1996 had shown an alternative approach to treat DMD by upregulating the expression of closely

related protein, utrophin, which might be able to compensate for the dystrophic phenotype. For this purpose a truncated utrophin transgene has been expressed at high level in the muscles of the dystrophin deficient mdx mice. By this experiment, it had been shown that overexpression of utrophin gene could cause amelioration of the dystrophic phenotype of mdx mice.[16] Furthermore, because utrophin is normally expressed in all tissues including muscle, the use of this utrophin transgene rather than a dystrophin transgene in conventional gene therapy approaches using viruses or liposomes may avert any potential immunological respons against the transgene.

CONCLUSION

Transgenic technology now has developed rapidly from concept to a commonly used and useful technique in studying and analyzing the function of many genes and regulatory elements involved in the orchestra of development and differentiation of many cells and tissues. By combination with gene disruption and any other techniques, now transgenic technology is popular in studying the basic pathogenesis of various human diseases. Furthermore, overexpression of closely related gene might be able to compensate the impairment of particular gene in various human genetic based diseases. Finally, combination between transgenic technology, gene disruption and other methods can be used in the research for those purposes.

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