BT501-HEALTH BIOTECHNOLOGY

1-What is biotechnology all about?

Biotechnology

- bio - life Technology - the application, or harnessing of science for a specific purpose
- technology, process or practice - modifies or harnesses any living organism or system to be useful to any human purpose

Classical Biotech vs Modern

- Classical Biotech –since ages ,Growing better crops, Breeding animals
- Cheese and yogurt production – use of microorganisms
- Mendel's work – Laying foundation of modern Biotech
- Major discoveries : DNA structure, Ligase, Restriction enzyme
- Birth of rDNA technology
- Cut Gene from one organism and paste into others

Applications of Biotech

- AgriBiotech –: Pest-resistant, Herbicide resistant, Abiotic stress tolerant crops
- Environmental Biotech: Bioremediation, Bacteria and Fishes as Biosensors

- Health Biotech: biotherapeutics (insulin, growth hormones) Diagnostics tools (PCR, FISH, microarray)

The field of biotechnology has been in use for ages in various forms, which include the growing of better crops (agricultural biotechnology) and animal breeding (animal biotechnology). Similarly, the use of biotechnology has been around for thousands of years, especially the application of microorganisms in the production of cheese and yogurt (food biotechnology). In addition, the tools of biotechnology have been implied in animal husbandry, to develop pest-resistant crops, bioremediation (environmental biotechnology), as well as in bioethanol production. But the most promising application of biotechnology is found to be in the medical field by generations of biotherapeutics (insulin, growth hormones) and diagnostics tools (PCR, FISH, micro-array technique). Before we discuss various applications of medical biotechnology, let us briefly go through the historical aspects of biotechnology and this information will make you to understand the field better.

It all began with the discovery made by Sir Alexander Fleming in the year 1918 where he observed that the mold *Penicillium* inhibited the growth of human skin disease-causing bacteria called *Staphylococcus aureus.* The discovery by Sir Alexander Fleming lead to the making of antibiotics that we use today. These antibiotics are highly recommended and extensively used medicinally for bacterial infections. These antibiotics are basically substances produced by microorganisms that normally inhibit the growth of other microorganisms. Later on, antibiotics became widely available as a drug for treating microbial infections in human beings, especially with the development of penicillin as the most used antibiotic. Currently, a variety of

microorganisms have been used to generate thousand liters of antibiotic drugs by using advanced biotechnology tools.

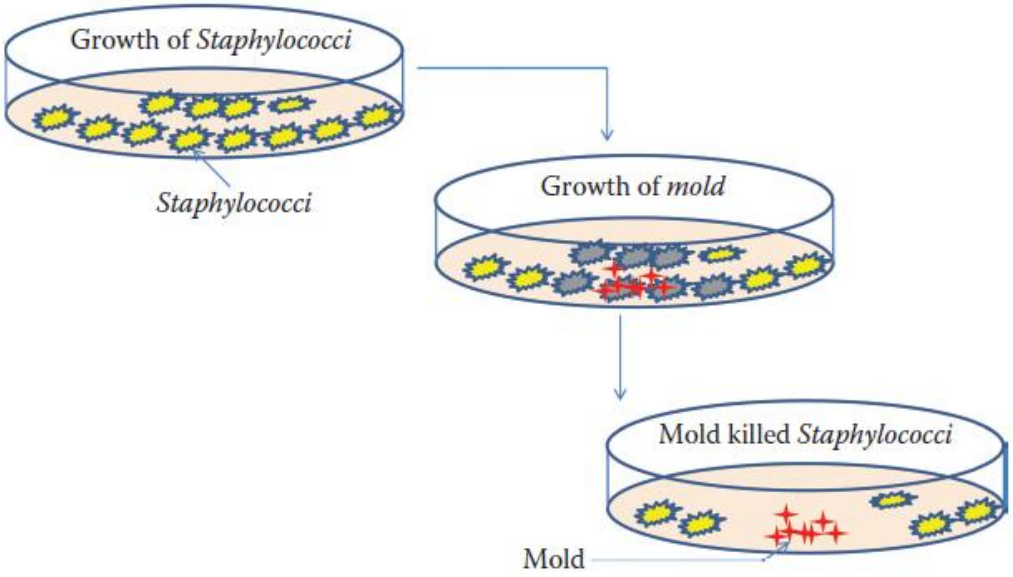
The field of biotechnology has taken a leap with the discovery of the double-helix structure of the deoxyribose nucleic acid (DNA) molecule and the credit goes to one research publication titled “This structure has novel features which are of considerable biological interest” authored by James Watson and Francis Crick in 1953, which claimed to discover the structure of the human DNA helix, the molecule that carries genetic information from one generation to the other. Nine years later, in the year 1962, they shared the Nobel Prize with Wilkins for cracking one of the most important of all biological puzzles. This discovery has led to the birth of genetic mapping, manipulation, and genetic engineering-type fields.

Surprisingly, with the help of genetic engineering, the gene of interest can be cut and inserted into the genome of other living organisms (microorganisms and viruses) and this process of gene insertion and manipulation is called recombinant DNA technology. Over the past few years recombinant DNA technology has been extensively employed to generate therapeutic products (insulin and growth hormones) for treating human diseases. With a rapid increase in the number of patients worldwide, there has been a tremendous scope to identify and create medicine for various human diseases. Thankfully with the availability of biotechnology tools, now it is possible to develop therapy for various diseases.

2-Medical products developed by using biotechnology tools:

Antibiotics

Penicillin is one of the earliest discovered antibiotics which is basically derived from molds such as *Penicillium*. Penicillin was discovered by Scottish scientist and Nobel laureate Sir Alexander Fleming in 1928. It all started with a basic experiment where Sir Fleming noticed a Petri dish containing *Staphylococcus* plate culture which he had mistakenly left open was contaminated by blue-green mold, which had formed a visible growth. But surprisingly he also found that there was a circle of inhibited bacterial growth around the mold. Later on, Sir Fleming hypothesized that the mold was releasing a substance that was preventing the growth and could contain a substance with antibiotic properties. In order to prove his hypothesis, he grew a pure culture and discovered the first antibiotic substance from the Penicillium mold, known as *Penicillium notatum*. Sir Alexander showed that *P. notatum* when grown in the appropriate substrate caused the release of chemical substances and these chemical substances were named as antibiotics. He later on named that chemical substance as penicillin. Soon after this discovery, penicillin was considered to be the most effective drug against bacteria (Gram-positive bacteria), and not at all effective against Gram-negative bacteria. The discovery of penicillin marks the beginning of the antibiotic production and so far more than 200 different types of antibiotics have been produced.



The pioneering experiment conducted by Dr. Alexander Fleming resulted in the discovery of the penicillin drug, an antibiotic drug.

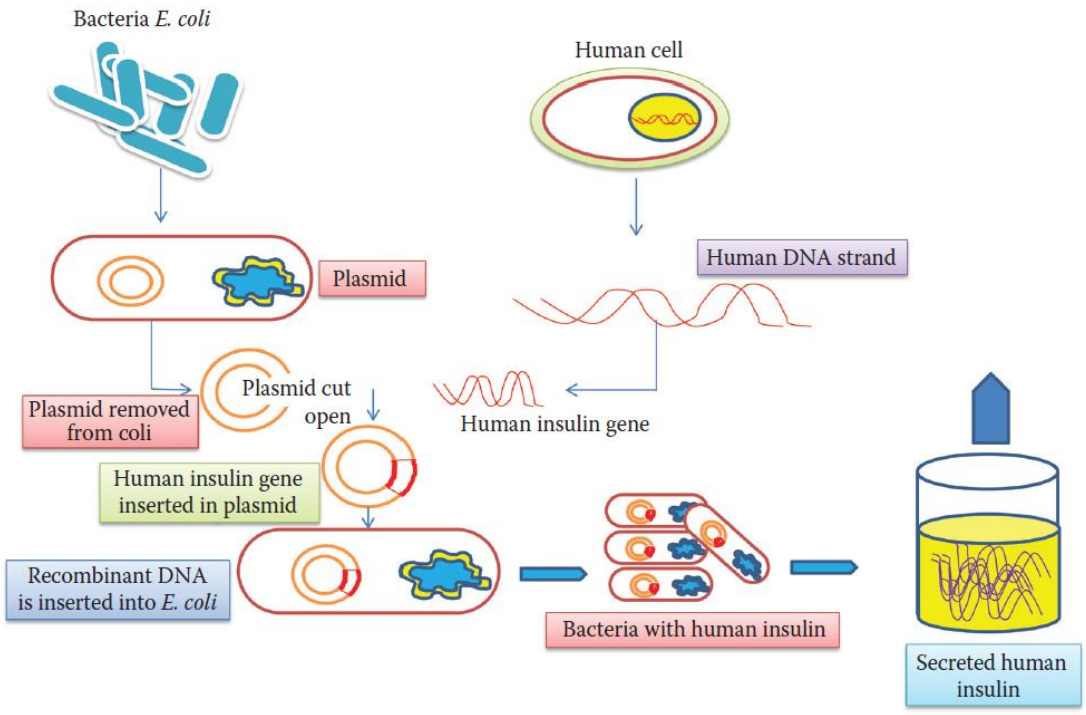
3-Recombinant insulin

Another discovery which was made in the medical field was the recombinant DNA technology, which has completely revolutionized disease treatments especially in the Type 1 diabetes mellitus, where insulin-producing cells become dysfunctional and do not produce sufficient amount of insulin to regulate blood-sugar level. In a healthy human being, insulin regulates glucose metabolism in the body. In diabetic conditions, the level of insulin decreases which causes the elevation of the blood sugar level and this clinical condition is known as diabetes mellitus. One of the best treatments of diabetes mellitus is insulin injections, where insulin is injected into the patient's body and the insulin regulates the blood-sugar level. The insulin is either synthesized chemically or produced by recombinant DNA technology. These synthesized

insulins are used medically to treat patients with Type 1 diabetes mellitus, whereas patients with Type 2 diabetes mellitus are insulin resistant.

Insulin Production -Advances

- Recently, human insulin gene into plants - safflower plant as bioreactors
- insulin analogues- chemically synthesized
- major hurdles - delivery of insulin - cannot be taken orally- improper absorption - biological activity lost.
- taken as subcutaneous injections - few companies oral form- trials are underway



Making of human insulin by recombinant DNA technology.

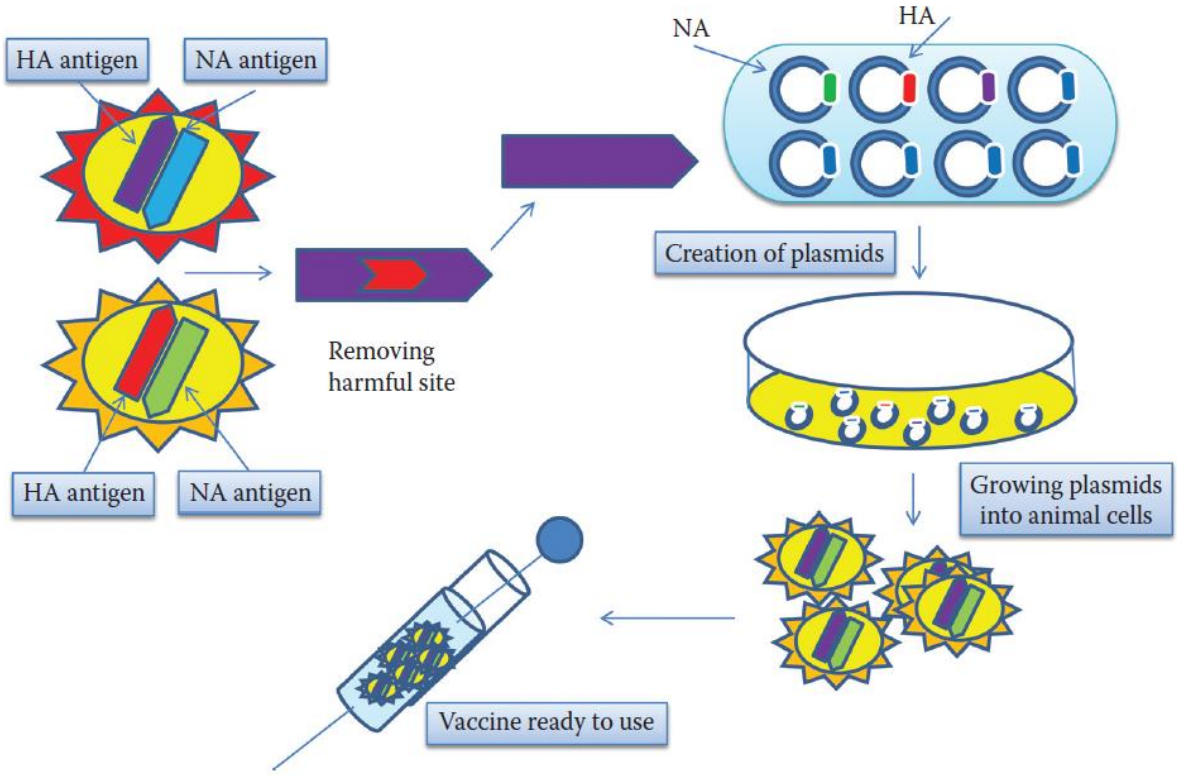
4-Vaccines

The vaccine is basically a biological preparation that improves the human immune defense system to fight diseases. The vaccines normally contain an agent that diligently resembles a disease-causing bacteria, and is frequently produced from debilitating or killed forms of the microorganisms. Upon injection into the human body, the vaccine can stimulate the body's immune system to recognize the agent as a foreign body, and recognize it, so that the immune system can recall and destroy or kill any of these microorganisms that later infect the human body. In a simpler way, the vaccine basically trains the human body to defend or fight or kill the microorganisms. The vaccines are classified based on its application; the vaccines that are used to prevent the effects of an upcoming infection by any natural or wild pathogen are called prophylactic, and the vaccines which are used against cancer are called therapeutic vaccines.

The story of vaccine development dates back to the seventeenth century when Edward Jenner found a milkmaid infected with smallpox, and a few days later he took pus from the hand of the milkmaid with cowpox and inoculated an eight-year-old boy with it. After six weeks he found that the boy did not contract smallpox. After a few years, Sir Louis Pasteur adapted Jenner's idea of developing a rabies vaccine and, subsequently, vaccine development progress started and became a matter of national concern. After that obligatory vaccination laws were passed in various countries around the globe. During the twentieth century, we have seen an introduction of several successful vaccines against diseases such as diphtheria, measles, mumps, and rubella. Interestingly, the major achievement was done in the 1950s when the polio vaccine was made and clinically used; also vaccines were synthesized to eradicate smallpox.

Successful vaccines

- Vaccines against diseases such as diphtheria, Anthrax, measles, mumps, and rubella, polio, smallpox, Hepatitis A, Hepatitis B, Flu etc



5-Monoclonal antibodies

Another milestone achieved in the beginning of the twentieth century was the making of monoclonal antibodies, which was proposed by Paul Ehrlich. He suggested that drug compounds can be accurately, precisely, and specifically delivered along with monoclonal antibodies. Monoclonal antibodies are mono-specific antibodies as they are made by identical cells that are all clones of a distinctive parent cell. Currently, it is possible to produce monoclonal antibodies

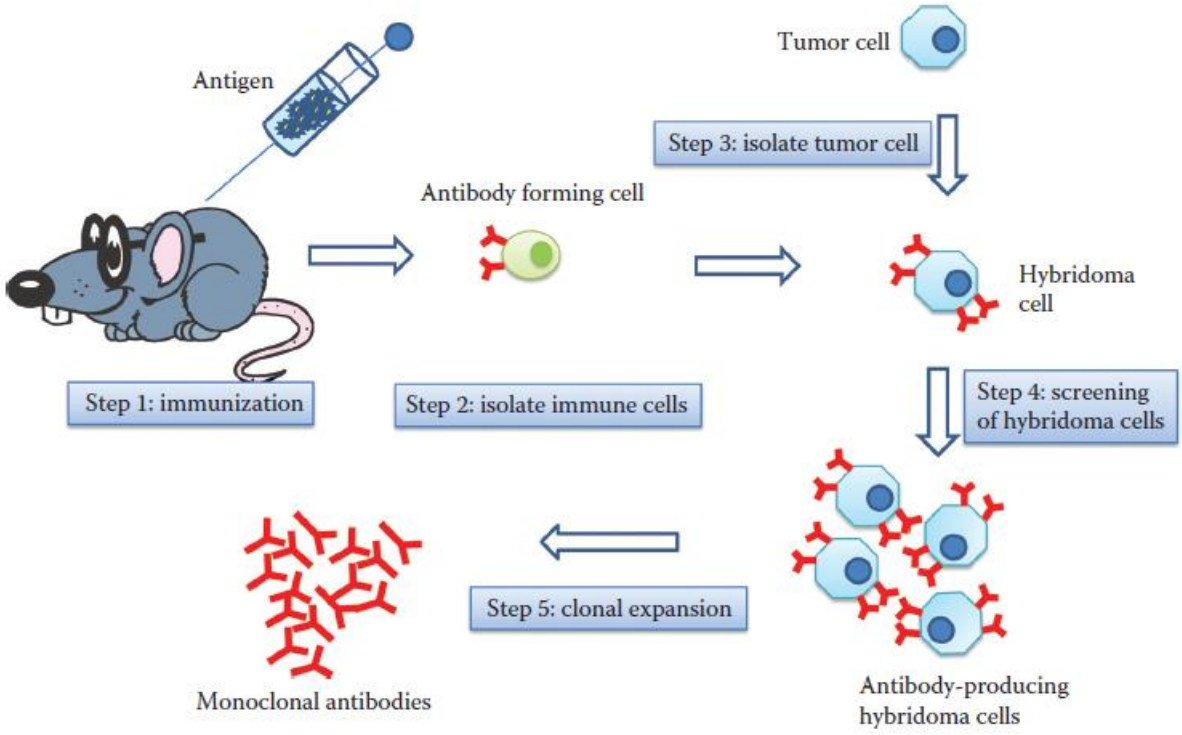
that specifically bind to that antigen and have a variety of applications, especially to detect or purify that substance (antigen), and has become an important tool in diagnostics and biomedical research.

In diagnostics and biomedical research, monoclonal antibodies are very useful tools. First, they are extremely specific; that is, each antibody binds to the specific site of the antigen. Second, some antibodies, once activated by the occurrence of a disease, continue to confer resistance against that disease; classic examples are the antibodies to the childhood diseases chickenpox and measles. Another important application of monoclonal antibodies is to develop vaccines against various diseases. As you are aware, a vaccine is made from bacteria or viruses either killed or inactivated. Upon introduction into the human body this vaccine stimulates the production of antibodies against the antigens to fight back the diseases. The production of monoclonal antibodies involves human and mouse hybrid cells and this technology is known as hybridoma technology. During monoclonal antibody production, tumor cells are merged with mammalian cells that produce an antibody against a particular antigen. The result of these merged tumors with mammalian cells is a called hybridoma, which can frequently produce antibodies. These antibodies are called monoclonal antibodies because they come from only one type of cell; whereas, the antibodies that are produced by conventional methods contain many kinds of cells and are called polyclonal antibodies.

Applications

- Diagnostic applications: e.g Quantification of blood hormones etc
- Therapeutic Applications: blocking the function of targeted molecule Protein Purification, Killing cancer cells

- Targeted Drug delivery
- Protein Purification



6-Bioengineered tissues

Over the last decade, tissue engineering became the most fascinating medical field, especially in body-parts reconstruction or cosmetic surgery areas. The basic concept about tissue engineering is to make human tissues through in vitro methods under controlled laboratory conditions and the making of such tissues is called bioengineered tissues. The definition of tissue engineering covers a broad range of applications in the healthcare field, however, in practice the term tissue engineering closely relates to repair or replacing portions of, or whole, human body tissues which include neurons, cardiomyocytes, bone, and cartilage. The bioengineered tissues are constructed by integrating certain mechanical and structural properties for proper functioning in

the human body, so one can say that bioengineering is basically the use of a combination of cells, engineering, and biocompatible materials, and is finally suitable to improve or replace biological functions. The bioengineered field was once categorized as a sub-field of biomaterials, but having grown in scope and application it is considered as a field in its own right. Tissue engineering is an emerging multidisciplinary field involving biology, medicine, and engineering that is likely to revolutionize the ways we improve the health of millions of people worldwide by repairing, maintaining, or enhancing tissue and organ function. The tissue engineering can also have diagnostic applications where the human cells or tissues are used to test drug metabolism and drug uptake, toxicity, and pathogenicity.

In vitro cell culture techniques have successfully cultured and constructed the human cells outside of the body. These outside body constructed cells are often referred to as bioengineered cells. There are various applications of bioengineered cells in clinical conditions.

The term regenerative medicine is frequently used synonymously with cell and tissue engineering, though in regenerative medicine, cell-based therapy is considered to be a main branch of regenerative medicine.

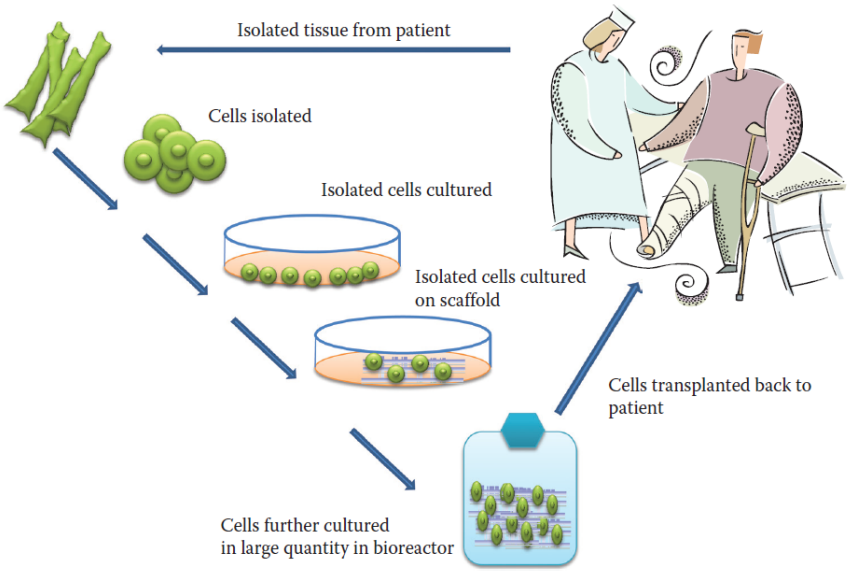
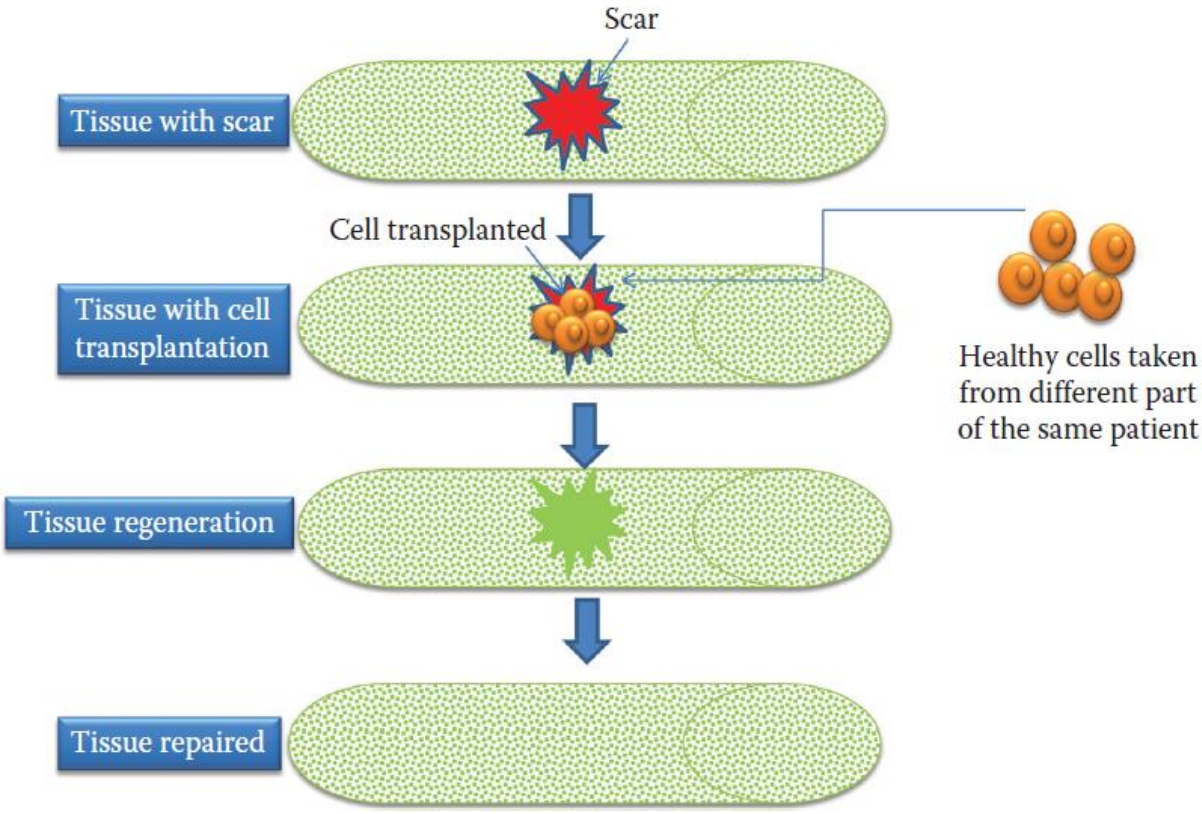


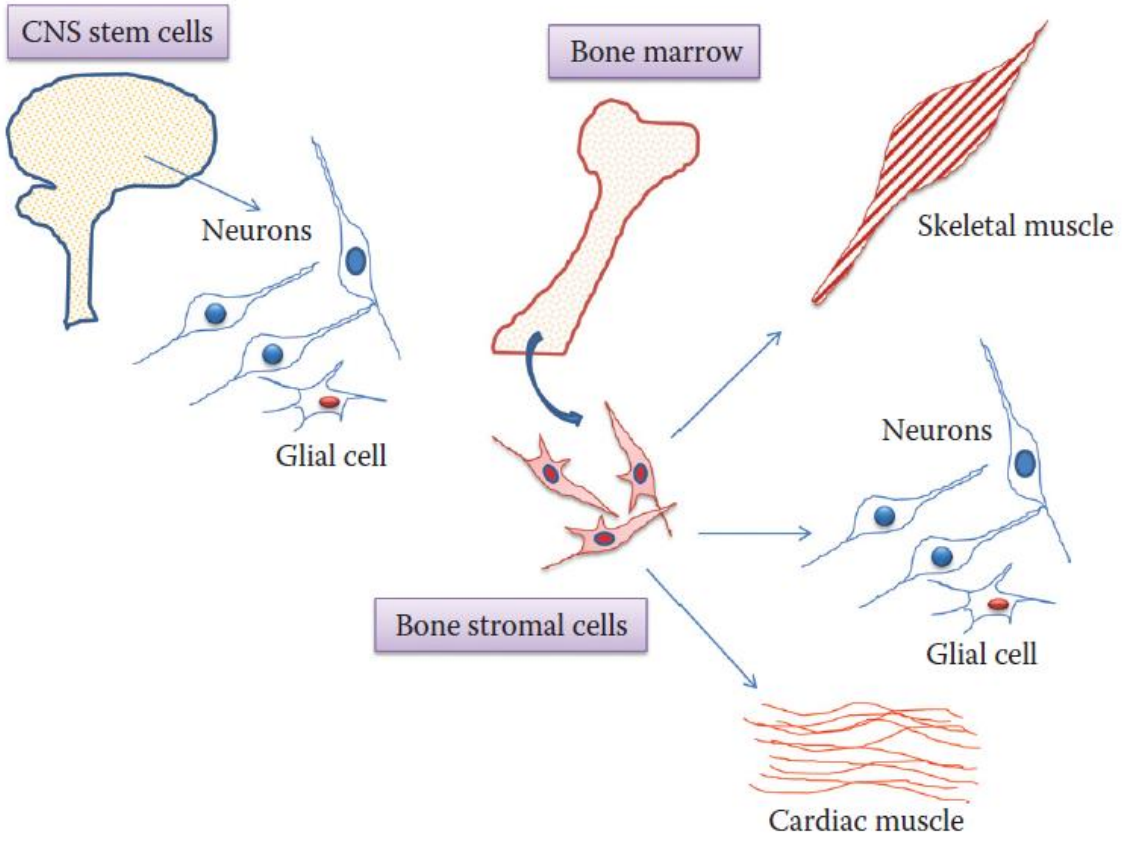
Figure. Cell and tissue engineering



7-Adult stem cell therapy

Over the last few years, there has been tremendous interest in adult stem cells for the autologous cell transplantation as these specialized cells have the potential ability to repair or restore the dysfunctional cells or tissues in the human body afflicted with various diseases that include blood cancer and neurological disorders. The stem cells are generally found in all multicellular organisms and they are characterized by the ability to renew themselves through cell division and differentiate into a varied range of specialized cell types. Moreover, mammalian stem cells may be broadly classified into two major types: adult stem cells and embryonic stem cells (ESCs) that are isolated from the inner cell mass of blastocysts.

One of the main roles of adult stem cells is that they remain in an undifferentiated state in the human body and multiply by cell division to replenish dying cells and restore damaged tissues and organs. The adult stem cells are also known as somatic stem cells; these stem cells can be found in both juvenile and adult ages. Moreover, adult stem cells are specialized cells that have the capability to divide and generate all cell types of the organ from which they originate, and can also possibly regenerate the entire organ. Unlike ESCs, the use of adult stem cells is not contentious as they are derived from adult source or tissue rather than by killing human embryos. Moreover, adult stem cells have been in use for many years predominantly in the treatment of cancer (such as leukemia and related bone and blood cancers) employing bone marrow transplants. Interestingly, the majority of the government funding especially in the United States is confined to adult stem cell-based research. Among adult stem cells, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) are found to be the most successful stem cells because they can be clinically used in patients. These stem cells can be directly injected or placed at the site of repair or injected through vascular delivery.

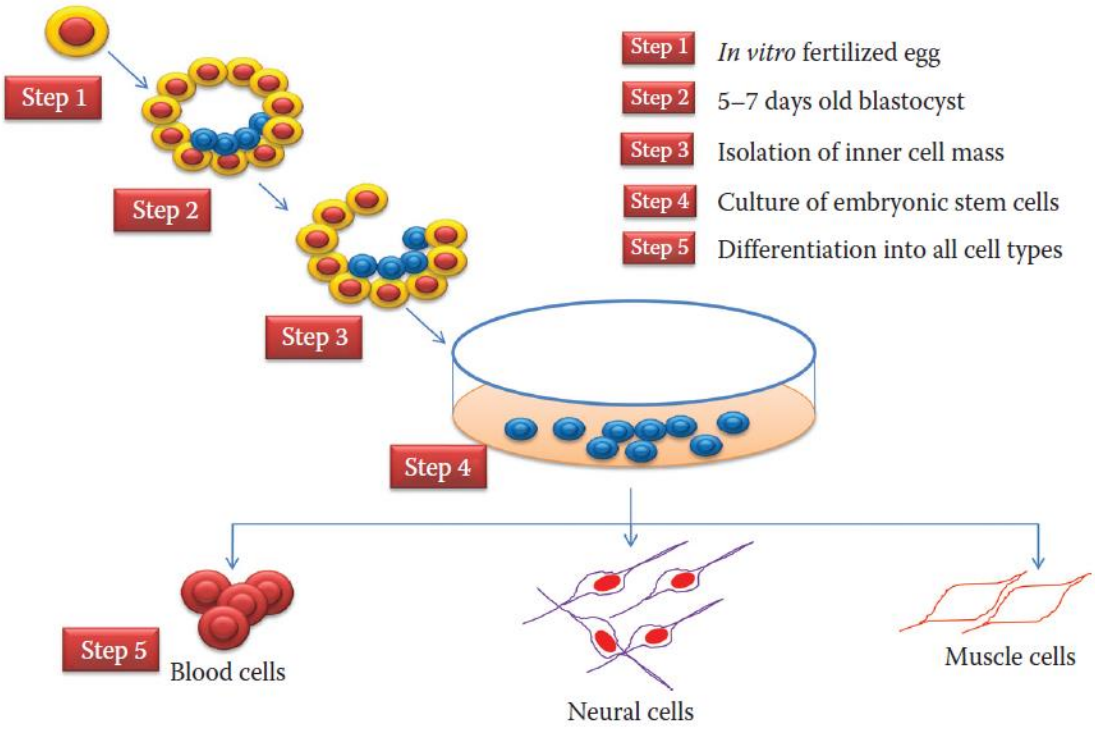


Adult stem cells

8-Emerging trends: Embryonic stem cells

Over the last few years ESCs have been a highly discussed and debated scientific topic at all levels which include public life, scientific forums, ethical, legal, and political platforms. Before we discuss the ethical or legal issues associated with ESCs, we will first learn what ESCs are all about. The ESCs are basically pluripotent stem cells isolated from the inner cell mass of the blastocyst, which is an early-stage embryo. Moreover, human embryos reach the blastocyst stage 4 to 5 days post fertilization, at which time they consist of about 50–150 cells. In addition to this, ESCs are adept at propagating themselves for an indefinite period under controlled culture situations. The culture and propagation of ESCs through in vitro methods is considered to be the

best technology to culture human cells in large quantities and to be engaged as valuable tools for both research and cell-based therapy.



Embryonic stem cells

One of the best applications of ESCs is to provide a large supply of required cells to be used as cell-based therapy and tissue replacement in patients who are suffering from degenerative diseases. Moreover, a number of diseases that could possibly be treated by ESCs are cancers, diabetes, Parkinson's disease, Alzheimer's disease, stroke, blindness, and spinal cord injuries. In contrast to its various therapeutic applications, the issues related to ESCs need to be resolved before making them useful for human use. These issues are the problem of immune rejection associated with allogeneic stem cell transplantation. These problems can be solved by using adult stem cells in autologous transplantation. Besides, cell-based therapy, the ESCs can be used as diagnostic tools to study an early human development and genetic disease, as well as in vitro

drug testing. Other issues related to ESCs transplantation is that there is a possibility that transplanted stem cells could form tumors and have the possibility of becoming cancerous if cell division continues uncontrollably. Contrary to this, supporters of ESCs research argue that such research should be continued because the resultant research findings will have significant medical potential. The recent development of induced pluripotent stems cells (iPSc) has created tremendous interest among scientists, as adult stem cells can be converted into pluripotent stem cells and there is no need to kill the human embryos.

In spite of all controversies over ethical use of human ESCs, on January 23, 2009, Geron Corporation, USA, has received an approval from US Food and Drug Administration (FDA) to test ESCs in humans. The Phase 1 clinical trial for transplantation of a human-ES-derived cell population into spinal cord-injured individuals became the first human ES cell human trial. The Phase 1 clinical study was conducted on eight to ten paraplegic patients who have had their injuries no longer than two weeks before the trial began, and stem cells were injected before the formation of scar in the tissue. Nevertheless, the researchers are stressing that the injections are not expected to fully cure the patients and restore all mobility. The success of such clinical trials goes back to the experiments conducted by University of California, USA, and which was supported by Geron Corporation of Menlo Park (Biotechnology Company), California. The results of an animal study showed that there was functional improvement in locomotive recovery in spinal cord-injured rats after stem cell transplantation. Interestingly, the first trial is mainly for testing the safety of cell-based transplantation. However, the trial had been put on hold in August 2009 due to apprehensions made by the FDA regarding a small number of microscopic tumors found in several treated rat models, but the hold has been lifted since.

9-Human genome project

The Human Genome Project (HGP) was an international collaborative research project with a primary aim to decode the human DNA genome. The main objective of the HGP was to map about 20–25 k genes of the human genome from both a physical and functional perspective. The initial task of the project was to identify the full set of genetic instructions enclosed inside human DNA. The project began with the end of several years of work supported by the United States Department of Energy (DoE). It has been reported that the $3-billion project was founded in 1990 by the DoE and the US National Institutes of Health. In addition to the United States, other countries also took part in the HGP, including the United Kingdom, France, Germany, Japan, China, and India. The team of scientists worked hard over the years and drafted a proposal for the genome project and the final sequencing of the human genome was done in 2006. Although the objective of the HGP is to understand the genetic organization of the human genome, the project has worked with other non-mammalian organisms.

Significance of the genome project

The reason for starting the HGP was to understand the role of genes in the development of diseases. There are reports that suggest more than 3000 genetic disorders known to be caused by genetic mutations. With the current treatment modalities, these genetic disorders cannot be cured without knowing the real cause. And to know the cause we have to know the role of genes in the diseases. Recent efforts have been made to find out the causes of cancer with genetic tools, but not much success was achieved. It has been recommended that information gained from the HGP would help to understand the genetic cause of the devastating illnesses, which include Parkinson’s disease, schizophrenia, or Alzheimer’s disease.

The genetic mutations play a role in many genetic diseases that include heart disease, diabetes, immune system disorders, and congenital defects. These diseases are thought to be the consequence from complex collaborations between genes and environmental factors. When genes for diseases have been identified, researchers can study how environmental factors, food, drugs, or pollutants can influence those genes. The location of the gene is important in identifying the type of protein which is produced by a particular gene. It has been reported that understanding the mechanism of a genetic disease is an important step in curing genetic diseases. It has been suggested that one day it may be possible to treat genetic diseases by gene therapy. Besides therapeutic applications, the information gained from the HGP can help to know the reason for the pluripotency nature of ESCs and how these ESCs can be differentiated into many different specialized cells such as muscle cell, neural cells, or hepatocytes.

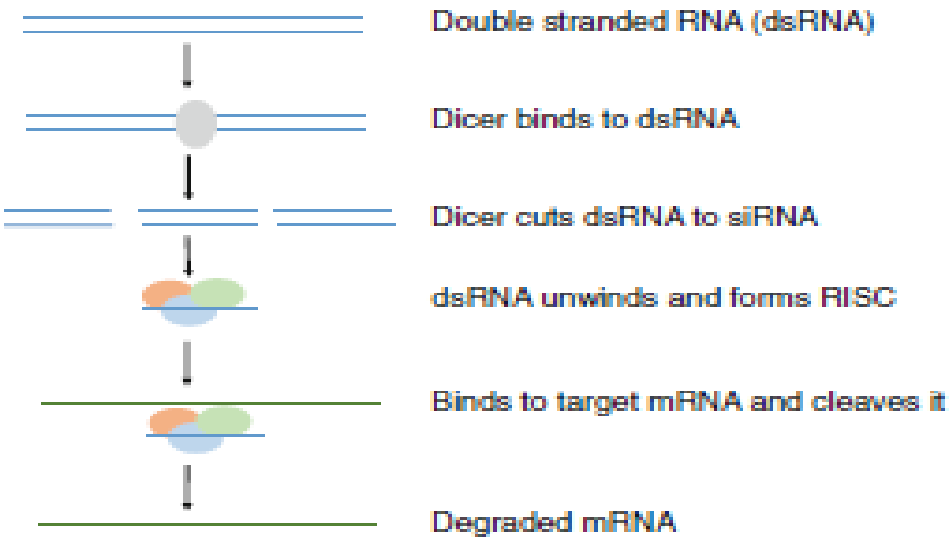
10-RNA interference technology

The RNA interference (RNAi) is a natural process that cells dictate to turn down or go to a silent state; such activity cell is regulated by a specific gene known as RNAi. The RNAi technology provides excellent tools to study the role specific gene in the development, causes, and progression of particular diseases, and has taken the biomedical community by storm. It has been reported that RNAi was first noticed in the plant *Petunia* when plant biologists tried to develop the purple color of the flower by introducing a pigment-producing gene. Surprisingly, the gene which was introduced to intensify color in the plants was found to suppress the color. In the end the resulting flowers developed white patches or became completely white. Surprisingly, a few years later another group of researchers observed a similar kind of gene-silencing effect in *Caenorhabditis elegans*. Later on, it has been reported that the gene-silencing effect is caused by

the presence of double-stranded RNA, and this double-stranded RNA is normally not found in healthy cells.

RNAi technology has been in use in various fields of biotechnology, particularly in the food plant engineering that produces lower levels of natural toxins. No plant that uses RNAi technology has yet passed the experimental stage; however, research work has been known to effectively reduce the allergen levels in tomato plants and also is known to cut the cancer-causing agents in tobacco plants. Also, other plant characters that have also been bioengineered include the production of nonnarcotic natural products by using the opium plant, development of resistance to common plant viruses, and protection of plants with dietary antioxidants. Interestingly, bioengineered plants such as *FlavrSavr* tomatoes and two cultivars of *ring-spot*-resistant papaya were originally developed by employing antisense technology. One of the pronounced applications of RNAi technology is its application in medicine. Over the past few years, efforts have been made to understand RNAi's role in normal and diseased cells, and also to use the RNAi technology for use in medical therapies. It has been suggested that human disease progression can be blocked by using RNAi-based therapies to turn down the activity of genes. Cancer, for example, is frequently caused by over-excited genes in the cells, and retarding their activities could stop the disease progression. Over the last few years, several pharmaceutical companies are using RNAi-based therapies to treat for various forms of cancer. In addition, viral infections can also be treated using RNAi-based therapies and this can be done by reducing the activity of key viral genes. It has been shown in the laboratory that human cells have successfully stopped the growth of HIV, polio, hepatitis C, and other viruses in human cell culture and RNAi-based therapies against HIV are under clinical trial stages. Moreover, the importance of RNAi technology has some beneficial effects in finding out the cause of the

disease. Using RNAi technology, the activity of a particular gene can be knocked down which will help us to understand its role in the disease development and progression. For many years, researchers have been studying how proteins regulate gene activity. Now with the help of RNAi technology, it would be possible to discover the role of proteins in gene regulation.

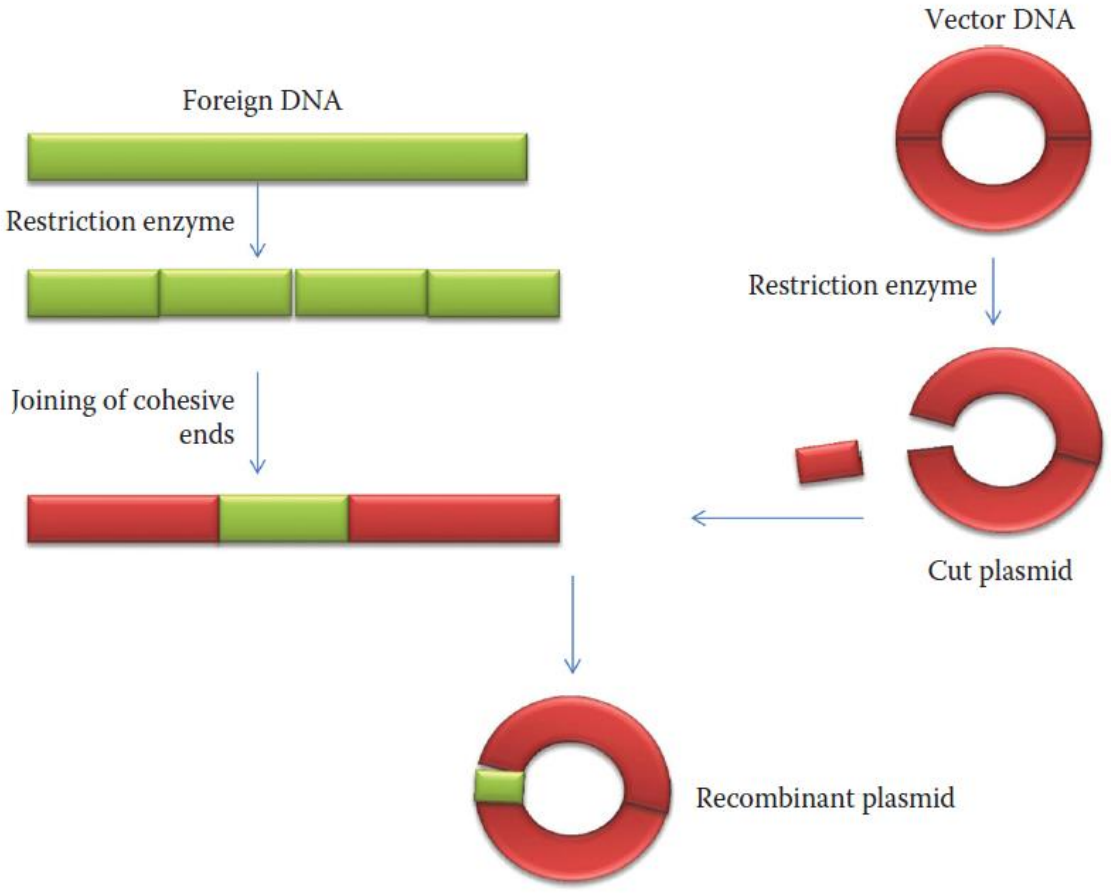


RNAi method for gene silencing. Dicer binds to dsRNA and cleaves it to small fragments. The fragments unwind and form RISC complex. The RISC complex binds to target mRNA and cleaves its sequence specifically.

11-Recombinant DNA technology

The process of r-DNA technology begins with the isolation of a gene of interest, which is then inserted into a vector; these vectors are further cloned into multiple copies. A vector is basically a piece of DNA that is capable of independent growth; bacterial plasmids and viral phages are the commonly used vectors. When the gene of interest (foreign DNA) is integrated into the plasmid or phage, this process is generally referred to as r-DNA. The next step in r-DNA technology is to introduce the vector containing the foreign DNA into host cells so that the cells

can express the desirable proteins. In order to get sufficient amounts of protein, the vectors must be cloned to produce large quantities of the DNA. Once the vector is isolated in large quantities, it can be introduced into the desired host cells, which include mammalian, yeast, or special bacterial cells. Finally, the host cells can synthesize the foreign protein from the r-DNA. When the cells are grown in vast quantities in the bioreactor or fermenter, the recombinant protein can be isolated in large amounts and the entire process is commonly referred as r-DNA technology. There are three different methods available through which r-DNA products are being developed: (1) transformation, (2) phage introduction, and (3) nonbacterial transformation.



Steps of constructing a recombinant DNA

The making of r-DNA has been briefly described below in a stepwise manner.

- Isolation of DNA
- Digestion of DNA
- Ligation of Two DNA Fragments
- Transferring Recombinant Vector into Host Cells (Transformation)
- Selection of the Transformed Cells

Goals of recombinant DNA technology

- To isolate and characterize a gene
- To make desired alterations in one or more isolated genes
- To return altered genes to living cells
- Artificially synthesize new gene
- Alternating the genome of an organism
- Understanding the hereditary diseases and their cure
- Improving human genome

Techniques used in rDNA technology

- Gel electrophoresis
- Cloning libraries
- Restriction enzyme mapping
- PCR
- Nucleic Acid Hybridization
- DNA Microarrays

Applications of rDNA technology

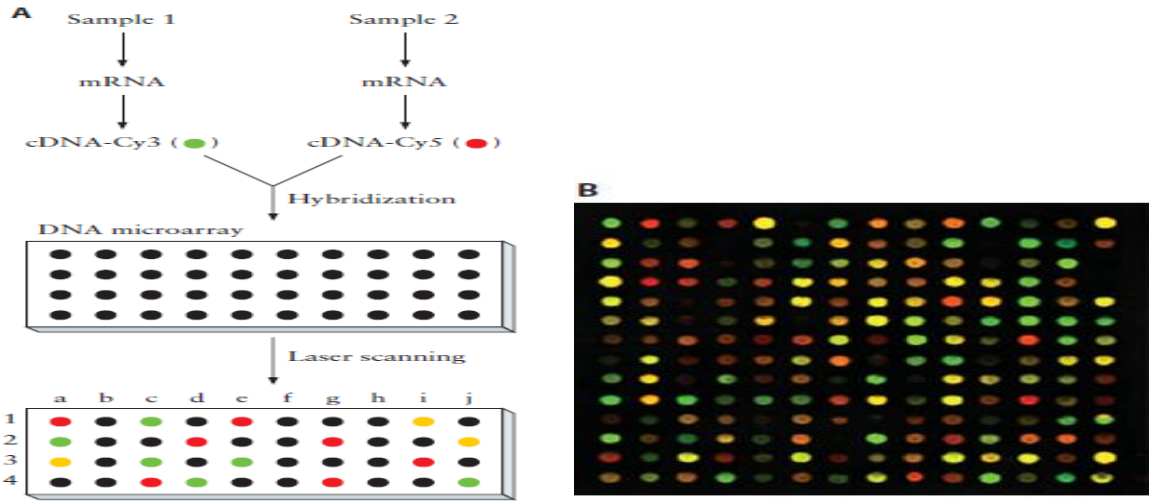
- Agriculture: growing crops of your choice (GM food), pesticide resistant crops, fruits with attractive colors, all being grown in artificial conditions

- Pharmacology: artificial insulin production, drug delivery to target sites
- Medicine: gene therapy, antiviral therapy, vaccination, synthesizing clotting factors
- Other uses: fluorescent fishes, glowing plants etc

12-Biochips

With the advent of information technology, it is possible to store biological information in a chip format which is called as a biochip. A microarray, also known as a DNA chip or biochip, is basically a collection of microscopic DNA spots attached to a solid surface (see picture). DNA microarray enables investigators to analyze expression of several genes in a single reaction. Typically it uses a solid surface such as a glass slide or a silicon film known as chip.

Gene expression profiling with a DNA microarray



(A) mRNA is extracted from two samples (sample 1 and sample 2), and during reverse transcription, the fi rst cDNA strands are labeled with the fl uorescent dyes Cy3 and Cy5,

respectively. The cDNA samples are mixed and hybridized to an ordered array of either gene sequences or gene-specifi c oligonucleotides. After the hybridization reaction, each probe cell is scanned for both fl uorescent dyes and the separate emissions are recorded. Probe cells that produce only a green or red emission represent genes that are transcribed only in sample 1 or 2, respectively; yellow emissions indicate genes that are active in both samples; and the absence of emissions (black) represents genes that are not transcribed in either sample. **(B)** Fluorescence image of a DNA microarray hybridized with Cy3- and Cy5-labeled cDNA

Applications

DNA microarray has increasing number of applications:

1-Expression analysis : In this method, the DNA of an organism is spotted on a chip and hybridized to cDNA obtained from mRNA of the same organism. The intensity signifies the abundance of a particular mRNA in the sample, signifying the expression level of the gene under a given condition. This is the most commonly used technique.

2-Mutation analysis : The genomic DNA is spotted on a chip and hybridized to DNA isolated from different individuals. Single nucleotide polymorphism can be identified by this method. It is important to analyze variation in a particular gene.

3- Comparative genomic hybridization: Increase or decrease of chromosomal fragments associated with a disease state. Prenatal chromosomal aberrations can be studied.

13-Gene therapy

To treat genetic disorders, the default or dysfunctional gene can either be replaced with a new gene or repaired with gene therapy. In brief, the gene therapy involves correcting defects in genes and in this process a normal/healthy gene is introduced to replace a defective gene. A person carrying the defect in a specifi c gene might develop disease, which may be inheritable. In gene therapy, nucleic acid is the therapeutic agent.

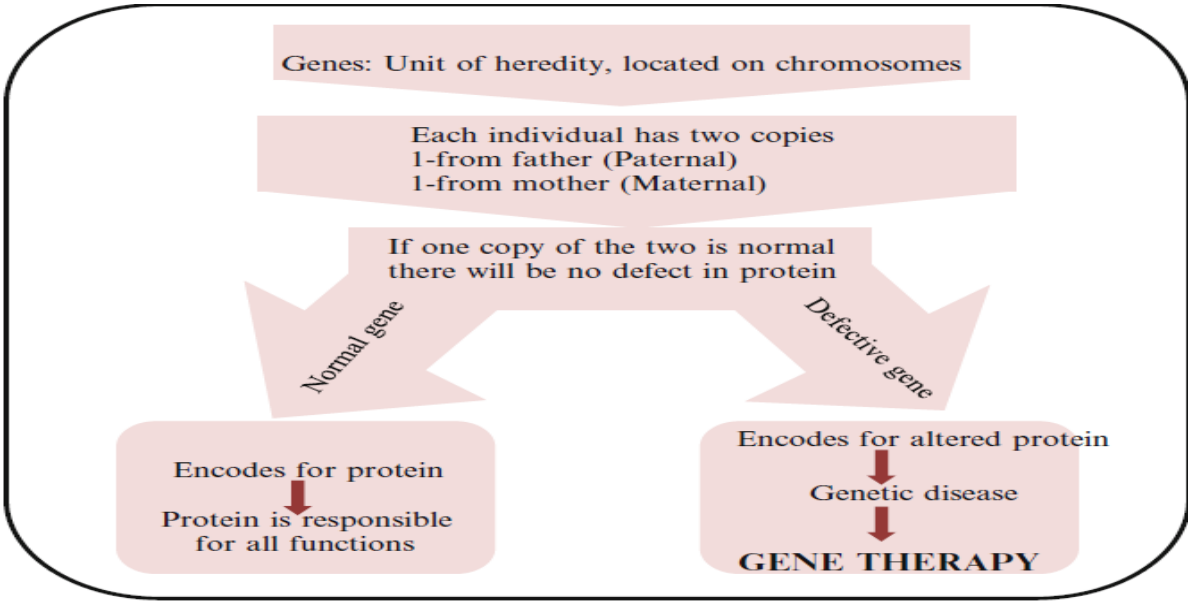
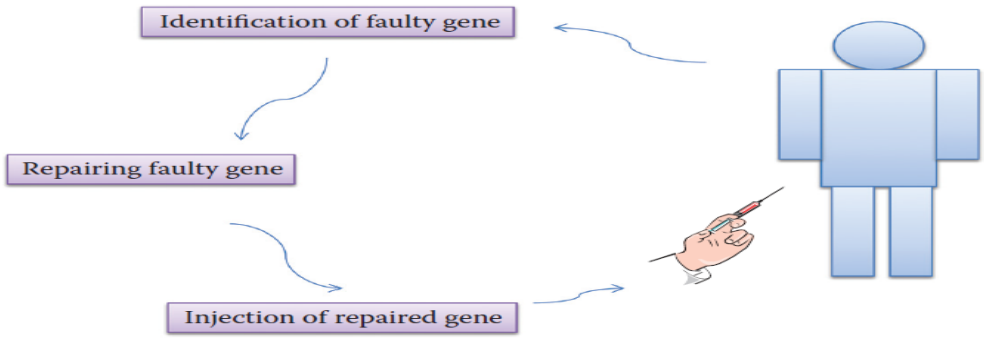


Fig.The function of normal gene and effect of defective gene, which causes disease. The individuals with defective gene are the candidate for gene therapy.

Gene therapy is still under development stages and various clinical trials are underway around the world. Its success will be based on the successful cure of genetic diseases by gene therapy.

The therapy is achieved by using a viral vector or gene delivery vehicles. The steps involved in the therapy in which the gene of interest enters the target cell via viral vector and begins expression are referred to as transduction.



The gene therapy can be classified into two major types: somatic gene therapy and germline gene therapy.

Somatic gene therapy

By using somatic gene therapy, somatic cells can be treated by inserting a vector loaded with the correct gene into a person's body. The somatic cells are cells that form in the body and cannot produce progenies. Gene therapy, in its present stage only treats somatic cells in humans.

There are two types of somatic gene therapy, *ex vivo* and *in vivo*.

Ex vivo somatic gene therapy: In *ex vivo* gene therapy genes or cells are modified outside the body and then transplanted back into the body. *In vivo* gene therapy, cells are modified or treated within the patient's body.

Germline Therapy

In the germline therapy, the modification is done in germ cells that are sperms or eggs, producing a permanent and genetically transferable modifycation. The functional gene is introduced into either of them, which then integrate in the selected germ cell, and subsequently the therapeutic gene is present in each cell of the body. The fertilized egg may be used to insert the therapeutic

gene and reimplanted into the mother. If successful, the functional gene is present and expressed in all cells of the resulting individual. The technique of making permanent changes might prove effective in the genetic diseases, but due to ethical and technical reasons, their use has been prohibited in humans.

Importance of vectors in gene therapy

Vectors should be undetected by the body's immune system to avoid immune rejection. Most viruses attack their hosts to insert their genetic material into the DNA of the host and this DNA contains instructions to produce viruses in large numbers. Considering these capabilities, scientist thought of using these viruses as a vehicle to deliver genetic materials to the host cells in order to treat genetic diseases. Currently the most common vector is used as a vehicle virus that has been genetically altered to carry normal human DNA. We have described various types of viral vectors which are being used in gene therapy and these viruses are found to be different in their mechanisms of action.

To make gene therapy effective, there are two classes of viruses—retroviruses and adenoviruses—found to be critical in gene delivery.

Recommendation to resolve the Legal and ethical issues

Nevertheless, there are some ethical concerns which have been raised in different parts of the world, and some ethical organizations have released recommendations to resolve the gene therapy issues and these recommendations include (a) establishing a national ethics body in each country to look at somatic gene therapy; (b) supporting somatic gene therapy research that follows the recommendations; (c) asking researchers, organizations, and governments to listen and respond to public concerns about gene therapy research; and (d) asking that research follows quality and safety controls.

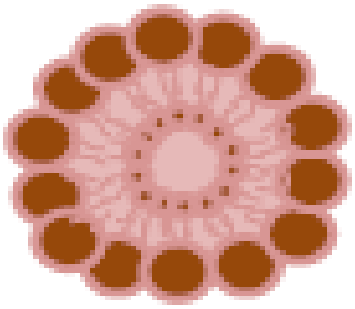
14-Liposome-Based Drug Delivery

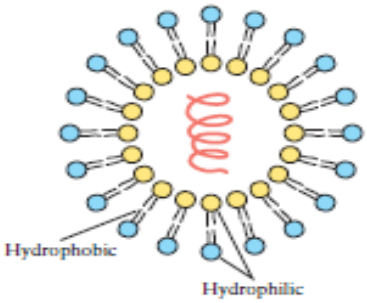
Liposomes are artificially prepared vesicles made of lipid bilayer. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases.

Liposomes have liquid core surrounded by phospholipid bilayer. Liposomes can be formed by sphingomyelins or lecithins and synthetic lipids as dimyristoyl, distearoyl, dipalmitoyl, and dioleoyl. They are 10 nm to several-micrometer spherical nanostructured molecules with defined shape and size. Liposomes are formed by an external phase having double phospholipid membranes and an aqueous internal phase with the capability to encapsulate both hydrophobic and hydrophilic compounds. Their advantages are biocompatibility, low toxicity, non-immunogenicity, and improved drug efficacy. They fuse to cell membrane and deliver the drug. They are used as carriers of antifungal, antibacterial, and cancer drugs:

- Liposome is used to deliver anticancer drug doxorubicin. Doxorubicin can induce myocardial toxicity. However when delivered in a liposomal preparation, the off-target toxicity is significantly reduced.

- Liposomes consisting of phosphatidylcholine, phosphatidylethanolamine, oleic acid, and cholesteryl hemisuccinate were developed by encapsulating a purified recombinant T4 endonuclease V . When delivered to the skin, they tend to improve DNA repair.





Structure of liposomes. They have liquid core surrounded by phospholipid layer. They are biocompatable, nontoxic, and non-immunogenic and used for packaging of drugs such as amphotericin B, ampicillin, polymyxin B, ciprofloxacin, anticancerous (daunorubicin and doxorubicin), insulin, and DNA. Used for drug delivery in fungal and bacterial infections, cancer cells, and diabetes.

15-Bionanotechnology

Bionanotechnology is a term that refers to the intersection of nanotechnology and biology. This discipline helps to indicate the merger of biological research in various fields of nanotechnology. Nanomaterial is the material used to make or construct biomaterials with nanomorphological features and these biomaterials can be used for various applications. These nanomaterials are basically smaller than 1/10th of a micrometer in at least one dimension; however, this term is occasionally used for materials smaller than 1 μm.

Application of nanomaterials in medicine

Therapeutic delivery: It has been reported that drug needs to be transported well in the body to easily reach the target site and there are times some drugs are not able to reach to target sites and get lost in the process. Recent efforts have been made to improve drug bioavailability, and nanomaterials play an important role in it. The word “bioavailability” denotes the presence of drug molecules where they are needed in the body and perform normal body function.

Nanoparticles can also be used as contrastagents, to take ultrasound and magnetic resonance imaging (MRI) pictures. Additionally, polymer-based nanoparticles can be designed to improve the pharmacological and therapeutical properties of drugs.

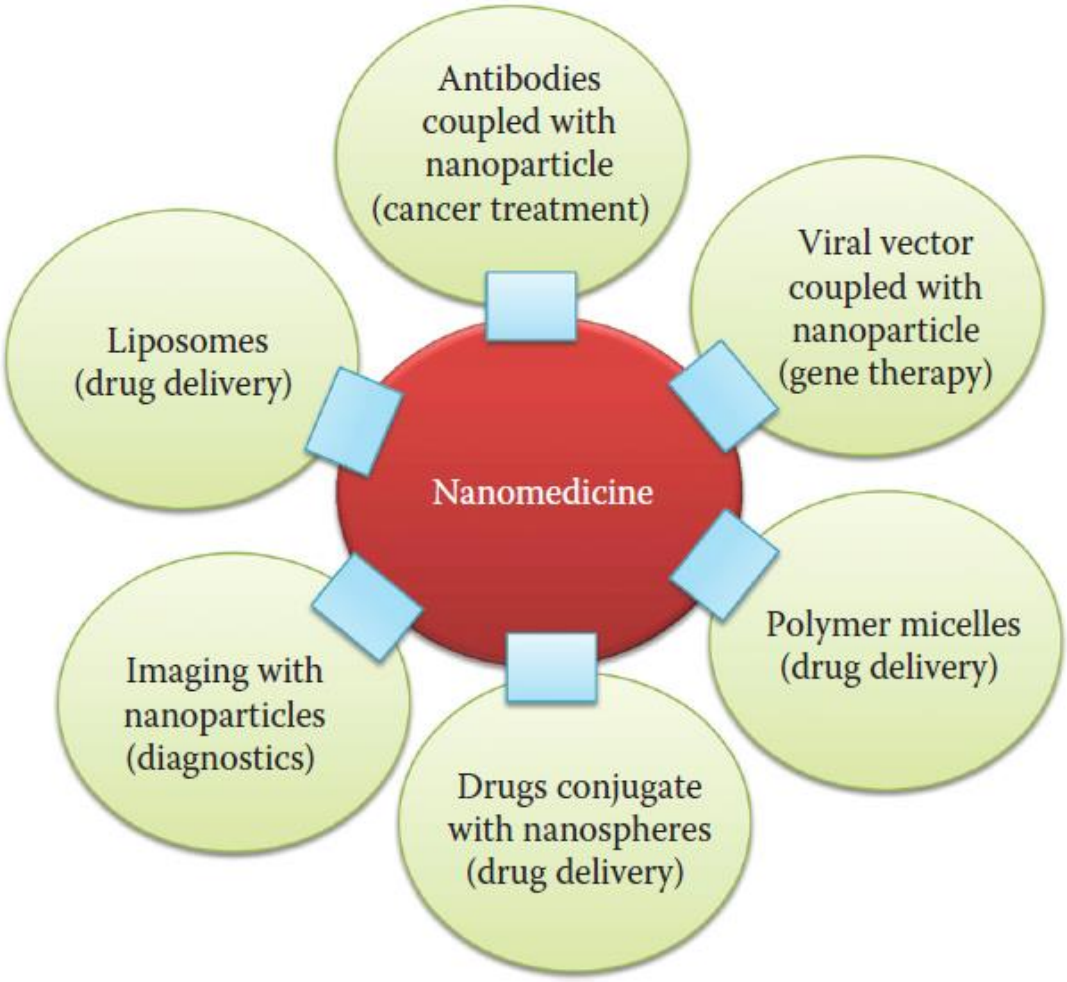


Figure- Applications of nanotechnology in medicine

The concepts that are enhanced through nanobiology include: nanodevices, nanoparticles, and nanoscale that occurs within the discipline of nanotechnology. Moreover, this technical method in biology allows scientists to imagine and create systems that can be used for biological research. Biologically inspired nanotechnology uses biological systems as the inspiration for technologies not yet created. The most important objectives that are frequently found in

nanobiology involve applying nanotools to relevant medical or biological problems and refining these applications. Moreover, developing new tools for the medical and biological fields is another primary objective in nanotechnology.

Despite the fact that biological systems are inherently nano in scale, nanoscience must merge with biology in order to deliver biomacromolecules and molecular machines that are similar to the human body or organ. In the twenty-first century, scientists have developed the technology to artificially tap into nanobiology. This process is best described as organic merging with synthetic. Interestingly, colonies of live neurons can live together on a biochip device. The self-assembling nanotubes have the ability to be used as a structural system as they would be composed together with rhodopsins, which would help the optical computing process and also help with the storage of biological materials. The most fascinating aspects would be of DNA as the software for all living things, which can be used as a structural proteomics system—a logical component of molecular computing.

16-Chromosomal Abnormalities

All nucleated cells contain chromosomes that consist of DNA and proteins (histones) in a compact structure. Chromosomes carry all of our genes and therefore all of our genetic information. DNA with its associated packaging proteins is referred to as **chromatin**. Some regions of chromosomes are tightly packed and are called heterochromatin, while other regions are less condensed and are called euchromatin. Less condensed packing of chromatin generally increases the transcription of genes in the region. Each species has a characteristic number and form of chromosomes, referred to as the **karyotype**. A **karyogram** is a photographic representation of stained chromosomes arranged in order of size, i.e., decreasing length (Fig. 1).

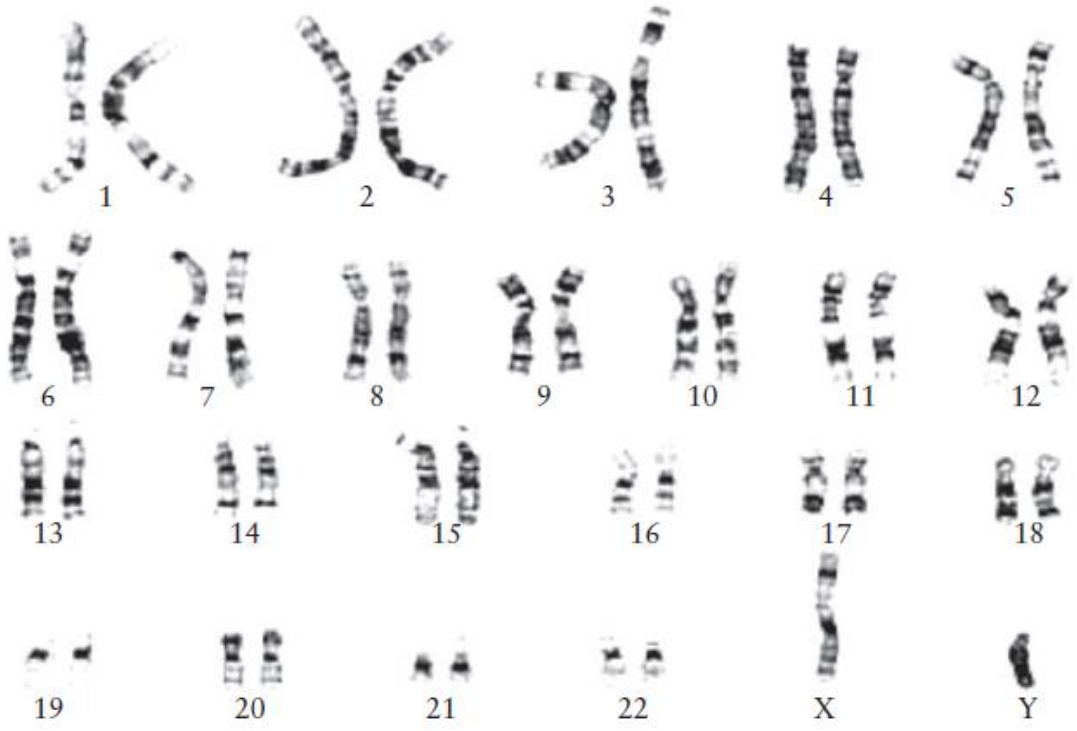


Fig. 1 Normal human male 46,XY karyogram. Humans have a total of 46 chromosomes that consist of two identical sets of 22 chromosomes (autosomal chromosomes) and 2 sex chromosomes (XX for female and XY for male). Because of the two identical sets of chromosomes, human cells are called diploid.

Each chromosome is paired with its matched or **homologous chromosome**. The matched chromosomes are identical in size and structure but may carry different versions (known as alleles) of the same gene. Humans have 46 chromosomes, or 23 chromosome pairs, to carry our approximately 25,000 genes. Cells in our body that contain 46 chromosomes are **diploid** ($n = 2$); 23 chromosomes are derived from the mother's egg cell, and the other 23 are from the father's sperm. Egg cells and spermatozoa each contain only 23 chromosomes (Fig. 2) and therefore are **haploid** ($n = 1$). In diploid cells, the 46 chromosomes appear as 22 homologous pairs of

autosomes (nonsex chromosomes) and one pair of sex chromosomes, XX in females and XY in males.

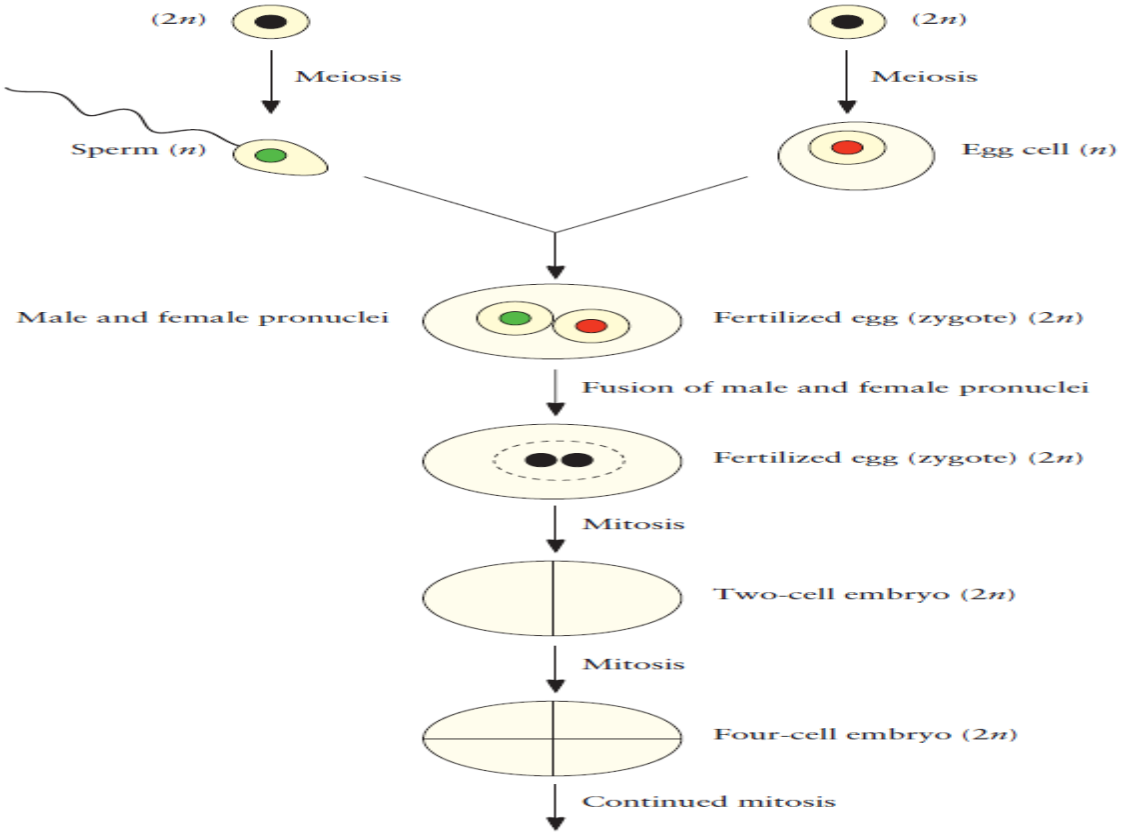


Fig.2 Generation of a diploid (2*n*) zygote. A diploid 2*n* fertilized egg (zygote) is produced by the fusion of a haploid (*n*) sperm and egg. Successive mitotic divisions generate many types of diploid cells in the body (somatic cells) during development and cell turnover (replacement of old cells with new ones) in an embryo.

Human chromosomes are usually studied in rapidly dividing cells, such as peripheral blood lymphocytes. Cell mitosis can be arrested in the metaphase stage of the cell cycle, and the chromosomes can be differentially stained to allow their identification microscopically. This microscopic analysis of chromosomes is known as **cytogenetics**. For routine karyotyping,

Giemsa staining is preferred, as this procedure produces alternating light and dark bands **(G banding)** that reflect differential chromosomal structures characteristic of each chromosomal pair (Fig. 1).These light and dark bands of chromosomes result from the specificity of binding of the Giemsa stain for the phosphate groups of DNA, as the stain attaches to regions of DNA where there are large amounts of A-T bonding. Thus, Giemsa staining can identify different types of changes in chromosomal structure as gene rearrangements.

Examination of a karyotype enables one to determine either if there is gain or loss of a chromosome(s) or if the structure of a given chromosome(s) is altered. The **centromere** of each chromosome separates the short arm (p) from the long arm (q). Most arms are divided into two or more regions by distinct bands, and each region is further subdivided into subbands (Fig. 3). For example, band Xp21.2 is found on the p arm of the X chromosome in region 2, band 1, subband 2. **Chromosome disorders** are caused by abnormalities in the number (increase or decrease of genes) or the structure of chromosomes. An individual's physical characteristics are called a **phenotype**, which is the combination of all of that individual's expressed traits, including morphology, development, behavior, and biochemical and physiological properties.
Phenotypes result from the expression of genes and environmental factors that interact with these genes. Thus, the phenotype of a person with a chromosomal disorder may vary with the type of chromosomal defect.

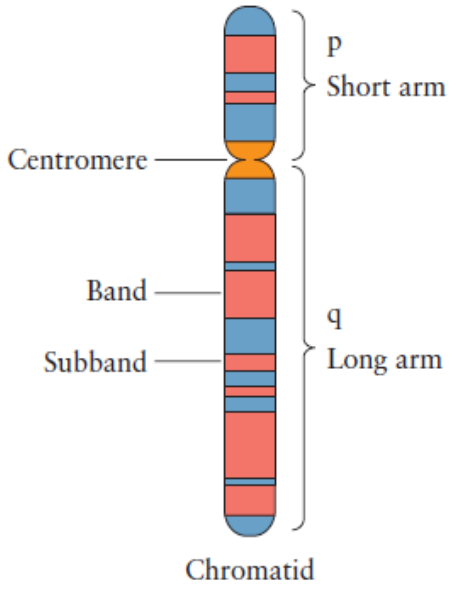


Fig.3Schematic diagram of the structures of a chromosome. The p and q arms, centromere, chromatid, bands, and subbands (described in the text) are shown. A chromatid is an individual chromosome that is paired with a replicated

copy of the identical chromosome. This pair of chromosomes is held together at the centromere for the process of cell division. The light and dark bands originate from the differential staining of the regions of the chromosome with the Giemsa stain, as explained in the text. The short arm of each chromosome is denoted with a "p" and the long arm with a "q." Thus, 7q refers to the long arm of chromosome 7. Each arm may be further divided into regions, depending on the size.

Risk factors for chromosomal abnormalities:

- Radiation
- Smoking
- Fertility drugs
- Alcohol consumption
- Oral contraceptive

17-Chromosome Structural Abnormalities

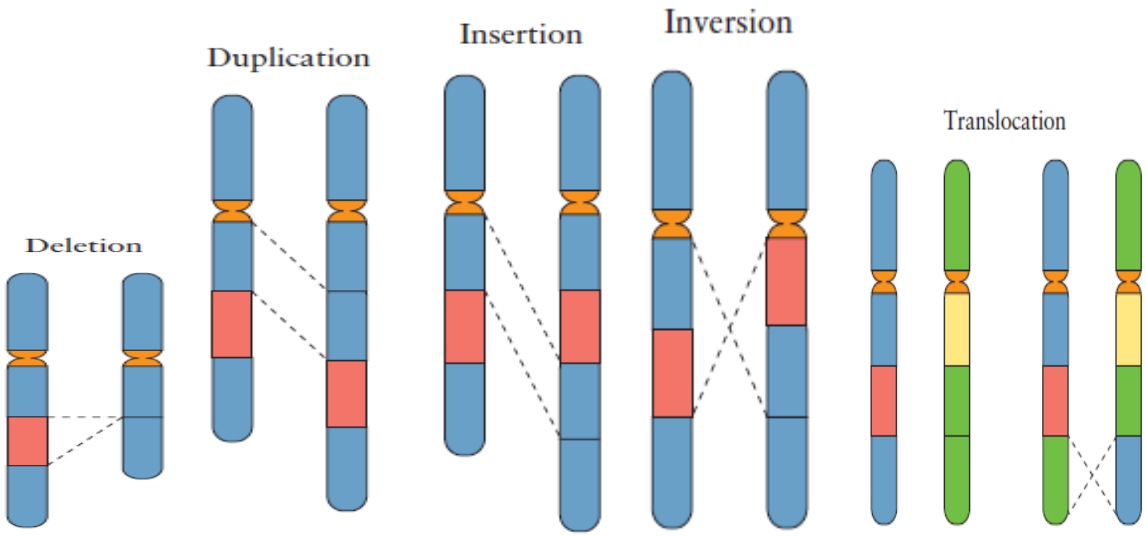
Genomic rearrangements may alter genome architecture and yield clinical consequences. Several genomic disorders caused by structural variation of chromosomes were discovered by cytogenetics. Recent advances in molecular cytogenetic techniques have enabled the rapid and precise detection of structural rearrangements on a whole-genome scale. This high resolution illustrates the role of **structural variants (**SVs) and **single-nucleotide polymorphisms (SNPs)** in normal genetic variation.

In analyzing the role of structural gene variants in cell function, it is important to consider the two types of such variants, i.e., **gain-of-function variants** and **loss-of-function variants**. A gain-of-function variant results from a mutation that confers new or enhanced activity on a protein. Most mutations of this type are not heritable (germ line) but, rather, are somatic mutations. A change in the structure of a gene that may arise during DNA replication and is not inherited from a parent, and also is not passed to offspring, is called a **somatic mutation**. Such a mutation that results in a single base substitution in DNA is known as a somatic point mutation.

A loss-of-function variant results from a point mutation that leads to reduced or abolished protein function. Most loss-of-function mutations are recessive, indicating that clinical signs are typically observed only when both chromosomal copies of a gene (one being inherited from each parent) carry such a mutation.

In a population, any two unrelated individuals are identical at about 99.5% of their DNA sequence. At a given chromosomal site, one individual may have the A nucleotide and the other individual may have the G nucleotide. This type of site in DNA is known as an SNP.

Each of the two different DNA sequences at this site (or gene) is called an **allele**. Molecular techniques such as array-based **comparative genomic hybridization (CGH)**, SNP arrays, array painting, and **next-generation sequencing** have facilitated and expedited the characterization of chromosome rearrangements in human genomes. These various genomic rearrangements can arise by several mechanisms, including deletions, amplifications, translocations, and inversions of DNA fragment. **Deletions** occur when a portion of a chromosome is missing or removed. **Duplications** result from the copying of a portion of a chromosome that results in extra genetic material. During a **translocation**, a portion of one chromosome is transferred to another chromosome. In a reciprocal translocation, DNA segments from two different chromosomes are exchanged. A DNA **inversion** results when a portion of a chromosome is broken off, turned upside down, and reattached. When a portion of a chromosome is disrupted, the chromosome may form a circle, or ring, without any loss of DNA.



18-Chromosome Numerical Abnormalities

An abnormality in which the chromosome number is an exact multiple of the haploid number (*n* = 23) and is larger than the diploid number (*n* = 46) is called **polyploidy**. Polyploidy arises from fertilization of an egg by two sperm (total number of chromosomes increases to 69) or the failure in one of the divisions of either the egg or the sperm so that a diploid gamete is produced. The survival of a fetus to full term of pregnancy is rare in the instance of polyploidy.

Aneuploidy occurs when the chromosome number is not an exact multiple of the haploid number and results from the failure of paired chromosomes (at first meiosis) or sister chromatids (at second meiosis) to separate at anaphase. Thus, two cells are produced, one with a missing copy of a chromosome and the other with an extra copy of that chromosome (Fig. 1). Examples of numerical chromosomal abnormalities are listed in Table 1.

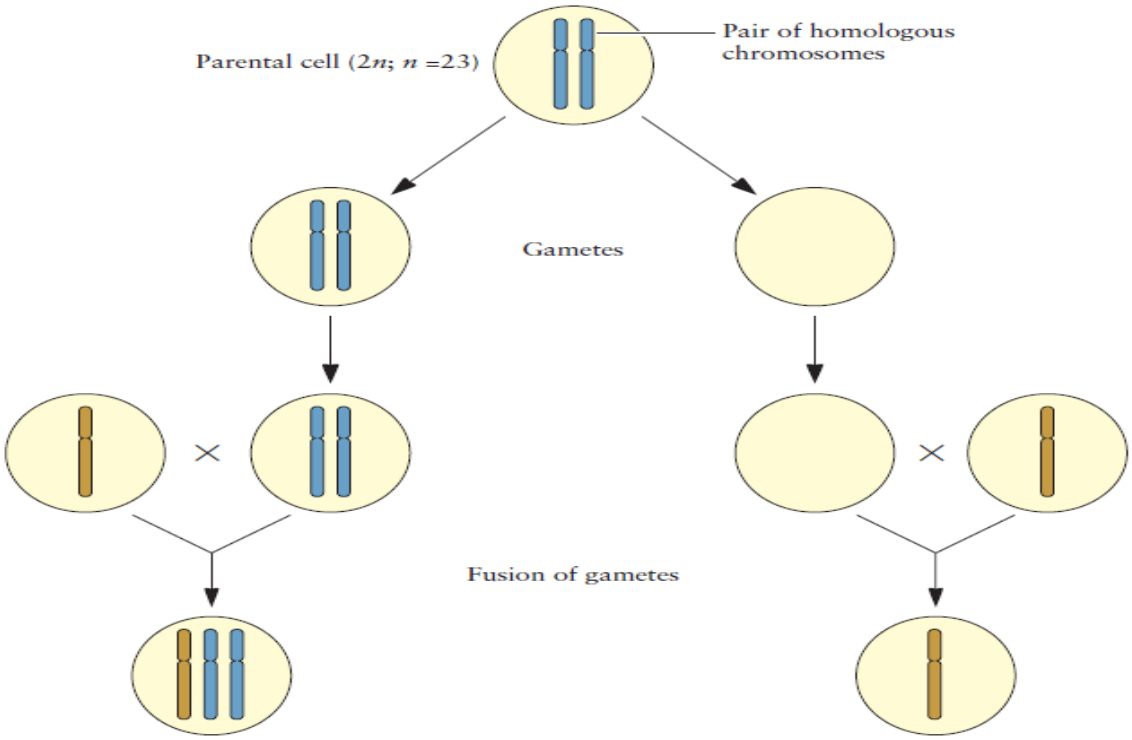


Fig.1 Nondisjunction in gamete cell formation and fusion of abnormal gametes with a normal haploid gamete. Aneuploidy occurs when paired chromosomes (at first meiosis) or sister chromatids (at second meiosis) do not separate from each other at anaphase, a stage of meiosis at which sister chromosomes move to opposite sides of the cell. This failure of paired chromosomes to separate, with the chromosomes instead moving to the same side of the cell, is termed nondisjunction. As a result, two cells are produced, one with a missing copy of a chromosome and one with an extra copy of that chromosome, as shown.

Table-1 Numerical chromosomal aberration syndromes

Aneuploidy condition	No. of chromosomes	Karyotype
Tetraploidy	92	XXYY
Triploidy	69	XXY
Trisomy 21 (Down syndrome)	47	XX+21
Trisomy 18 (Edward syndrome)	47	XY+18
Trisomy 13 (Patau syndrome)	47	XX+13
Klinefelter syndrome	47	XXY
Trisomy X	47	XXX
Turner syndrome	45	X

Trisomy 21, the first human chromosomal disorder discovered (in 1959), is an abnormality that displays an extra copy (total of 3 copies) of chromosome 21 (Fig. 2) and causes **Down syndrome**. The genes on all three copies of chromosome 21 are normal. However, not all individuals with Down syndrome show the same physical characteristics, indicating that their phenotypes can vary. People with Down syndrome have a typical facial appearance (the face is fl at and broad) that includes an abnormally small chin, skin folds on the inner corners of the eyes, poor muscle tone, a flat nasal bridge, a protruding tongue due to a small oral cavity, an enlarged

tongue near the tonsils, a short neck, and white spots on the iris. Growth parameters such as height, weight, and head circumference are smaller in children with Down syndrome than in typical individuals of the same age.

All Down syndrome patients have some degree of mental retardation, albeit moderate. Despite this condition, many persons with Down syndrome can be educated and live with minimal daily assistance, while others require much attention and care. There are several possible health concerns, including cardiac failure and hearing loss. Individuals with Down syndrome are now living longer than they used to and can survive into their 50s and 60s. However, those individuals who survive to that age are at very high risk of developing Alzheimer disease.

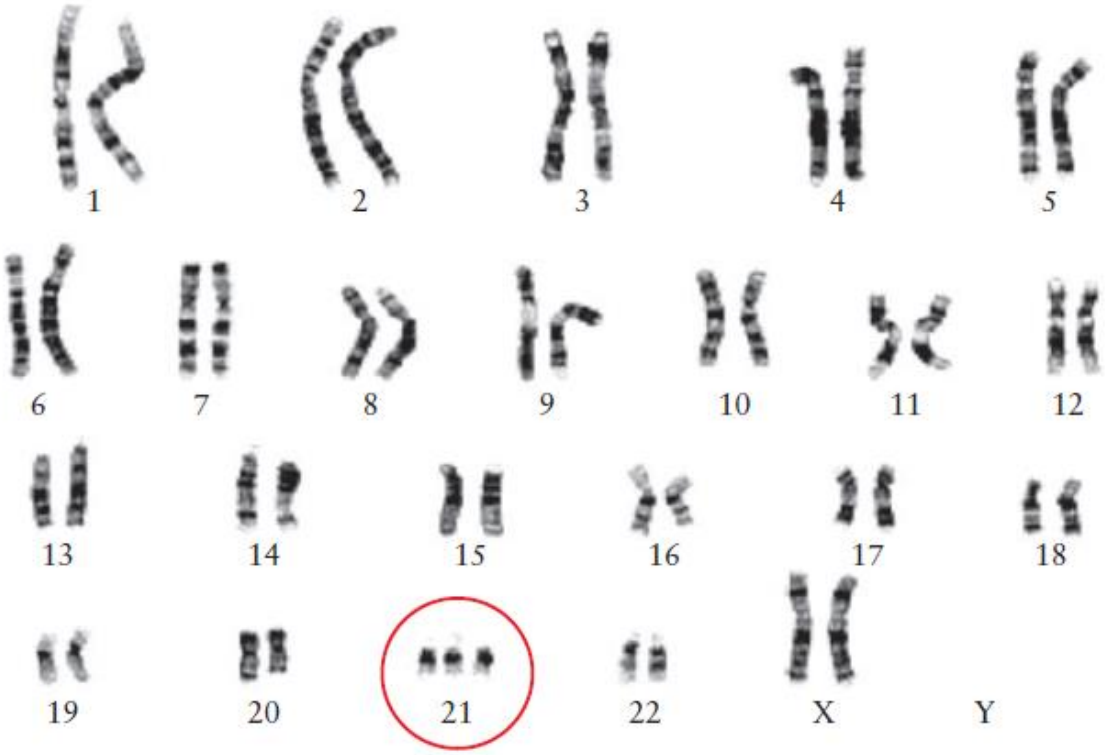


Fig-2 Trisomy 21. This syndrome appears when an individual inherits three copies of chromosome 21. The extra copy of chromosome 21 results in Down syndrome.

Trisomy 13, the presence of three copies of chromosome 13, causes **Patau syndrome**. Only about 5% of infants with this disorder survive past their first year, and most pregnancies involving trisomy 13 end in miscarriage. Children with trisomy 13 usually have a lot of trouble breathing, especially when they sleep, and many have seizures. All individuals with Patau syndome have severe mental retardation, and other common characteristics include a small head, extra fingers and/or toes, and a cleft lip or cleft palate.

Trisomy 18, the presence of 3 copies of chromosome 18, elicits **Edward syndrome**. Only about 10% of babies with this syndrome survive past their first year, and the majority of survivors are female, indicating a prenatal selection against males with trisomy 18 after the time of amniocentesis. Children with trisomy 18 usually have problems with breathing and eating, and many have seizures or serious heart conditions. All individuals with trisomy 18 have severe mental retardation. Most babies with trisomy 18 are very small and have certain recognizable facial features. They also tend to overlap their fingers in a very distinct pattern.

Prader–Willi syndrome results from the absence or nonexpression of a group of genes on chromosome 15. A specifi c form of blood cancer, **chronic myeloid leukemia**, may be caused by a chromosomal translocation, in which portions of two chromosomes (chromosomes 9 and 22) are exchanged. No chromosomal material is gained or lost, but a new, abnormal gene that leads to the development of cancer is formed.

19-Sex Chromosome Abnormalities

The phenotype of chromosomal disorders can vary depending on whether the chromosomal abnormality occurs on the maternally or paternally derived chromosomes (Table 1).

Turner syndrome (45,X) is an example of monosomy, in which a girl is born with only one sex chromosome, an X chromosome.

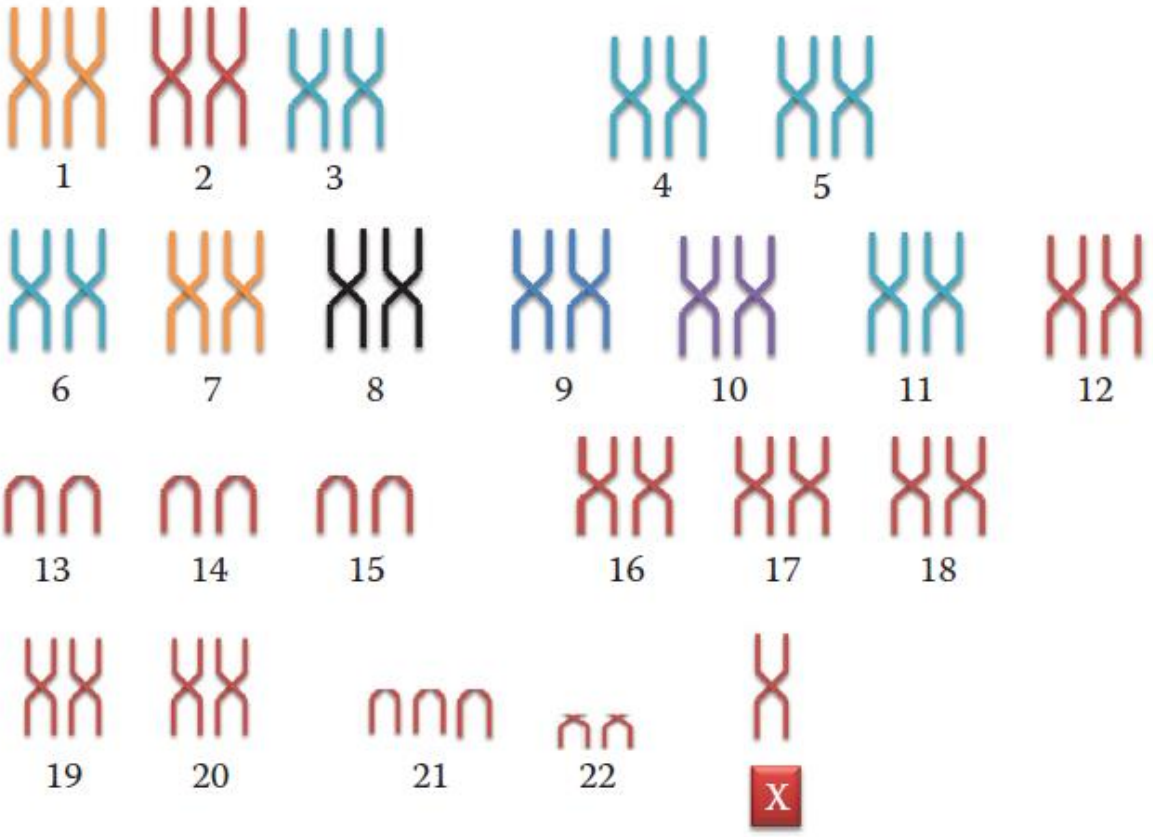


Fig- Turner syndrome (45 X).

Klinefelter syndrome occurs in males with the genotype 47,XXY. Such males have 47 chromosomes and are classified as having a sex chromosome trisomy (three sex chromosomes), since they carry two X chromosomes and one Y chromosome. This syndrome affects about 1 in 1,000 males. Most affected males are taller than average, and they may have more body fat in the hips or chest as well as little facial and body hair. Some Klinefelter males are mentally retarded, while many others have normal intelligence. The most common feature of this syndrome is infertility; about 2% of infertile men have Klinefelter syndrome.

20-Single Gene Disorders

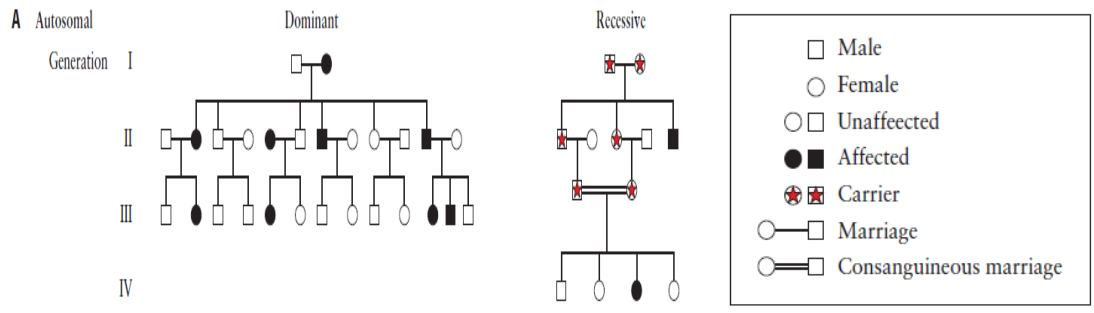
The genetic disorder in which a single gene gets mutated is called as a single-gene disorder. When a gene is mutated so that its protein product cannot carry out its normal body function, it leads to the development of a disorder.

Single-gene disorders, or monogenic diseases, result from a mutation(s) in a single gene occurring in all cells of the body (somatic cells). Inheritance of these types of disorders follows a Mendelian segregation pattern. Although very rare, such disorders affect millions of people globally. Currently, it is estimated that more than 10,000 human diseases are monogenic. These diseases can occur in about 1 out of every 100 births and thus can cause a significant loss of life.

So-called “pure genetic diseases” are caused by a single nucleotide change in a single gene in human DNA. The nature of disease depends on the functions performed by the modified gene.

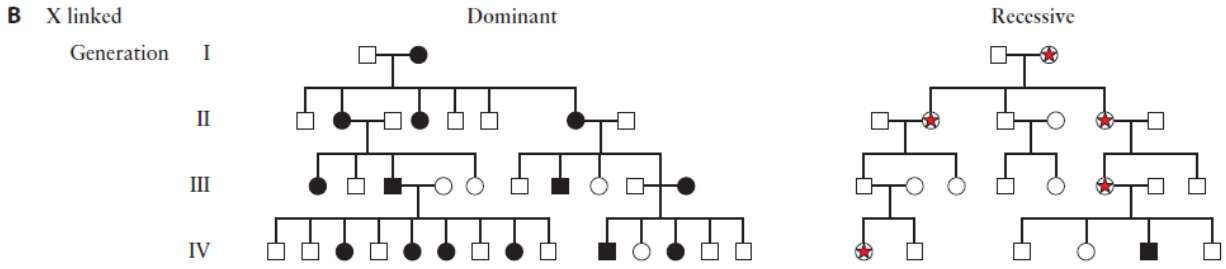
Monogenic diseases can be classified into three main categories: **autosomal dominant**, **autosomal recessive**, and **X linked** (Fig.).

All humans have two chromosomal copies of each gene or allele, one allele per each member of a chromosome pair. Dominant monogenic disorders involve a mutation in only one allele of a disease-related gene. In autosomal dominant inheritance, an affected person has at least one affected parent, and affected individuals have a 50% chance of passing the disorder on to their children. Huntington disease is an example of an autosomal dominant disorder.



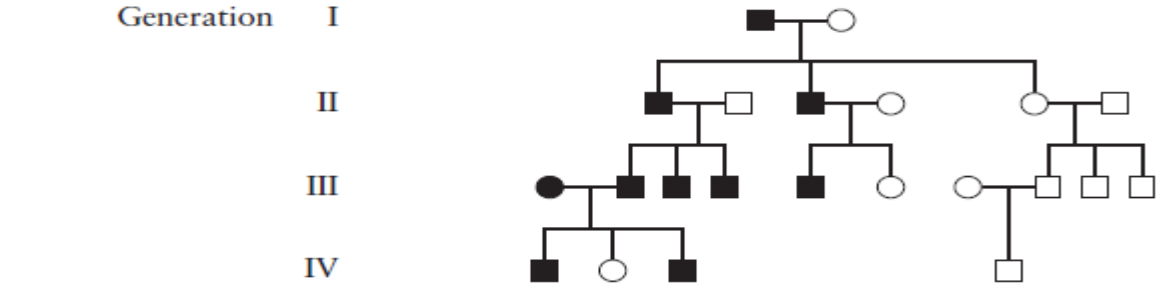
Recessive monogenic disorders occur due to a mutation in both alleles of a disease-related gene. In autosomal recessive inheritance, affected children are usually born to unaffected parents. Parents of affected children usually do not have disease symptoms but carry a single copy of the mutated gene. There is an increased incidence of autosomal recessive disorders in families in which parents are related. Children of parents who are both heterozygous for the mutated gene have a 25% chance of inheriting the disorder, and the disorder affects either sex. Cystic fibrosis and sickle-cell anemia are examples of autosomal recessive disorders.

X-linked monogenic disorders are linked to mutations in genes on the X chromosome. The X-linked alleles can also be dominant or recessive. These alleles are expressed in both men and women, more so in men, as they carry only one copy of the X chromosome (XY), whereas women carry two (XX).



Y-linked inheritance would affect only males, the affected males would always have an affected father, and all sons of an affected man would have the disease. However, no Y-linked diseases have ever been discovered. Apart from male infertility, mutations in Y-linked genes may not give rise to any disorders.

C Y linked



The prevalence of some monogenic inheritable disorders is shown in

Table

Disorder	Prevalence (approximate)
Autosomal dominant	
Familial hypercholesterolemia	1 in 500
Polycystic kidney disease	1 in 1,250
Neurofibromatosis type I	1 in 2,500
Hereditary spherocytosis	1 in 5,000
Marfan syndrome	1 in 4,000
Huntington disease	1 in 15,000
Autosomal recessive	
Sickle-cell anemia	1 in 625
Cystic fibrosis	1 in 2,000
Lysosomal acid lipase deficiency	1 in 40,000
Tay–Sachs disease	1 in 3,000
Phenylketonuria	1 in 12,000
Mucopolysaccharidoses	1 in 25,000
Glycogen storage diseases	1 in 50,000
Galactosemia	1 in 57,000
X linked	
Duchenne muscular dystrophy	1 in 7,000
Hemophilia	1 in 10,000

21-Cystic Fibrosis

Disorder and Genetics:

Cystic fibrosis is a genetic disorder that affects the respiratory, digestive, and reproductive systems. This disorder is mediated by the production of abnormally thick mucous linings in the lungs, can lead to fatal lung infections, and can give rise to obstruction of the pancreas and impair digestion. The severity of the condition varies from the mild forms (e.g., absence of the vas deferens in men) to the more common severe forms (epithelial gland dysfunction). Since an individual must inherit two defective cystic fibrosis genes, one from each parent, to get the disease, cystic fibrosis is classified as an autosomal recessive disorder.

In families in which both parents are carriers of the cystic fibrosis gene, there is a 25% chance that they will transmit cystic fibrosis to their child, a 50% chance that the child will carry the cystic fibrosis gene, and a 25% chance that the child will be a non carrier.

The cystic fibrosis gene encodes a protein known as the cystic fibrosis transmembrane regulator (CFTR), and the *CFTR* gene contains 27 exons within 250 kb of genomic DNA. The CFTR protein consists of 1,480 amino acids distributed in two membrane-spanning domains and two ATP-binding domains.

The membrane-spanning domains form a low-conductance cyclic adenosine monophosphate (cAMP)-dependent chloride channel. The ATP-binding domains control channel activity.

The most common mutation, a 3-bp deletion at codon 508 (ΔF508), results in the loss of a phenylalanine residue from the first ATP-binding domain and blocks the transport of the CFTR protein to the epithelial cell membrane.

The next most common mutation (≤5% of *CFTR* mutations) is also present in the first ATP-binding domain and G551D (glycine at residue 551 is replaced by aspartic acid) in exon 11. Both common mutations are associated with a severe form of the disease in the homozygous state.

Prevalence

Cystic fibrosis is a common cause of death in childhood and the most common inherited disease in white populations. The incidence of cystic fibrosis varies significantly in different countries: 1 in 2,500 births in the United Kingdom, 1 in 2,000 to 3,000 births in Europe, and 1 in 3,500 births in the United States. Cystic fibrosis can arise in nonwhite populations, but only very rarely (1 in 100,000 births in African-American and East Asian populations).

Diagnosis and Prognosis

Cystic fi brosis patients have many symptoms (salty skin; persistent coughing, wheezing, or shortness of breath; and excessive appetite but poor weight gain), and their symptoms may vary due to the >200 mutations of the *CFTR* gene.

A diagnostic sweat test measures the amount of salt in sweat of patients with cystic fi brosis (a high salt level indicates cystic fibrosis). This test is usually performed in babies older than 3 to 4 weeks and can also confirm the diagnosis in older children and adults.

If pancreatic enzyme levels are reduced, stool analyses may reveal decreased or absent levels of the digestive enzymes (trypsin and chymotrypsin) or high levels of fat. If insulin secretion is reduced, blood sugar levels are high. Lung function tests may show that breathing is compromised.

Genetic testing on a small blood sample can help determine whether an individual has a defective *CFTR* gene. During pregnancy, an accurate diagnosis of cystic fibrosis in the fetus is possible.

22-Fragile X Syndrome

Disorder and Genetics:

Fragile X syndrome is caused by a "fragile" site at the end of the long arm of the X chromosome. This syndrome is manifested by many changes in behavior and cognitive recognition that vary widely in severity among patients.

Fragile X syndrome is the most common cause of inherited mental retardation. Although it is an X-chromosome-linked recessive trait with variable expression and incomplete penetrance, 30% of all carrier women are affected.

Penetrance is the proportion of individuals carrying a particular variant of a gene (allele or genotype) who also express an associated trait or phenotype. Full penetrance occurs when all individuals carrying a gene express the phenotype. Incomplete penetrance occurs when some individuals fail to express the phenotype, even though they carry the variant allele.

Fragile X syndrome is caused by loss-of-function mutations in the fragile X mental retardation 1 (*FMR1*) gene. *FMR1* encodes the FMRP protein found in many tissues and at particularly high levels in the brain and testes. In the brain, it may play a role in the development of neuronal synapses and cell communication. The synapses can change and adapt over time in response to experience, a characteristic called **synaptic plasticity**. The FMRP protein may help regulate synaptic plasticity and there by control learning and memory.

Fragile X syndrome belongs to a growing class of **neurodegenerative disorders** known as **trinucleotide repeat disorders**. Among these disorders, 14 affect humans and elicit neurological dysfunction. Trinucleotide CGG repeat expansions (200 to more than 1,000 repeats) that inactivate the *FMR1* gene are the most common mutations observed at this locus.

Prevalence

Fragile X syndrome is the single most common inherited cause of mental impairment, affecting 1 in 3,600 males and 1 in 4,000 to 6,000 females worldwide. Approximately 1 in 259 women of all races carry the fragile X gene and may pass it to their children, whereas about 1 in 800 men of all races and ethnicities are carriers. Carrier females have a 30 to 40% chance of giving birth to a mentally retarded male child and a 15 to 20% chance of having a mentally retarded female child.

Diagnosis and Prognosis

The diagnosis of fragile X syndrome is made by the detection of mutations in the *FMR1* gene. Over 99% of individuals have an *FMR1* gene that expresses all of its known mutants. Tests used for diagnosis include chromosome analysis and various protein tests. Diagnosis is usually made when a child is young, and there is no current cure for this illness. Early diagnosis of the syndrome may allow for therapeutic interventions such as speech therapy, occupational therapy, psychotherapy, and special education, which can improve the quality of a patient's life considerably.