# Post-Graduate Degree Programme (CBCS)
# in
# ZOOLOGY

## SEMESTER-IV

## SOFT CORE THEORY PAPER

## CANCER BIOLOGY AND MEDICAL GENETICS

## ZDSE(MN)T-408

## SELF LEARNING MATERIAL

**DIRECTORATE OFOPEN AND DISTANCE LEARNING**
**UNIVERSITY OF KALYANI**
**KALYANI, NADIA,**
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Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Amalendu Bhunia, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

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Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.



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Director
Directorate of Open and Distance Learning
University of Kalyani

# SOFT CORE THEORY PAPER (ZDSE(MN)T -408)

## CANCER BIOLOGY AND MEDICAL GENETICS

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# UNIT-I

# Multistep tumorogenesis; Cell immortalization; Differences of normal cells and cancer cells; cell transformation and factors for cell proliferation; DNA and RNA tumor viruses

**Objective:** In this unit we will learn about Cell immortalization; Differences of normal cells and cancer cells; cell transformation and factors for cell proliferation; DNA and RNA tumor viruses

## Introduction:

In multicellular organisms, cell division is a normal process. Cells divide for growth, for the development of organs, for healing of wounds and also for the replacement of older and damaged cells. Cell division is a very complex process which is controlled by a regulatory mechanism at both molecular and cellular level. Again, in higher multicellular organism, each and every cell belongs to a particular type of tissue like epithelial tissue, connective tissue muscular tissue etc.

Hence, when a cell of a specific tissue divides, it normally produces its own kinds of cell of the tissue to which it belongs. It never produces the cells of other tissues. Therefore, the process by which cells achieve this specification and specialization is known as cellular differentiation. Differentiation of cell begins during embryonic gastrulation stage and continues through tissue formation. Actually differentiation has a genetic basis and the process results from the interaction of the nucleus and the cytoplasm. After the cells become well- differentiated, they cannot go back normally to the undifferentiated stage unless disturbed internally or externally.

Therefore, in multicellular organism, the cell division, differentiation and survival of individual cells are carefully regulated to meet the needs of the organism as a whole. When this regulation is lost due to any reason, the cells behave unusually and defy their control mechanism. Then the cells grow and divide in an uncontrolled manner ultimately spreading throughout the body and interfering with the functions of normal tissues and organs. As a whole, this condition leads to cancer. Cancer develops from defects in fundamental regulatory mechanisms of the cell.

## What is Cancer?

Cancer is an abnormal and uncontrolled division of cells, known as cancer cells that invade and destroy the surrounding tissues. Generally cancer is defined as uncontrolled proliferation of cells without any differentiation. Cancer cells are different from normal cells in some aspects. They do not remain confined to one part of the body.

They penetrate and infiltrate into the adjoining tissues and dislocate their functions. Some of the cancer cells get detached from the main site of origin and travel by blood and lymph to sites distant from the original tumour and form fresh colonies, called metastasis or secondary growth.

## How Cancer Cells Differ from Normal Cells?

Normal cells have a limited life span. They are usually replaced by new cells through cell division and cell differentiation. Their production is regulated in such a manner that the number of a given cell type remains nearly constant. Normal cells show a property called contact inhibition.

Due to this property they contact with other cells, inhibit their uncontrolled growth. Cancer cells seem to have lost this property. But cancer cells do not respond to normal growth control mechanism. These cells proliferate in an unregulated manner and form clones of cells which can expand irregularly. This uncontrolled growth is called tumour or neoplasm.

## Meaning of Cancer:

Cancer is a non-infectious disease. It starts at the molecular level of the cell and, ultimately affects the cellular behaviour. Generally, it can be defined as uncontrolled proliferation of cells without any differentiation.

## Types of Cancer:

Cancer is a large class of diverse disease. All types of cancer can result from uncontrolled cell growth and division of any of the different kinds of cells in the body. So there are more than a hundred distinct typesof cancer which vary in their behaviour and response to treatment. The uncontrolled cell growth producesa mass of cells which are called tumours or neoplasm tumours may be benign or malignant. A benign tumor remains confined to its original location. They do not invade the surrounding normal tissues. They do not spread to distant body sites.

The most common example of tumour is the skin wart. A benign tumour consists of closely resembles normal cells and may function like normal cells. Generally benign tumours are harmless and can usually be removed surgically. However, these tumours may sometimes become quite harmful if they are located in organs like brain and liver. A malignant tumour does not remain confined to its original location. They

are capable of both invading surrounding normal tissue and spreading throughout the body via the circulatory or lymphatic systems. Malignant tumours become life-threatening if, they spread throughout the body. nly malignant tumours are properly designated as cancers. The cells of malignant tumour are derived from single cell, thus they are monoclonal in character. Malignant tumour is composed of aberrant cells. They behave like embryonic type, undifferentiated, having irregular, large nucleus, and deficient of cytoplasm. Malignant tumours are generally classified into four main types on the basis of cell type from which they arise.

### (i) Carcinomas:

It includes approximately 90% of human cancer. This type is principally derived from epithelial cells of ectoderm and endoderm. The solid tumours in nerve tissue and in tissues of body surfaces or their attached glands are example of carcinomas. Cervical, breast, skin and brain carcinomas are developed from malignant tumour.

### (ii) Sarcomas:

Sarcomas are solid tumours of connective tissues such as muscle, bone, cartilage and fibrous tissue. This type of malignant tumours are rare in human (about 2% of human cancer).

### (iii) Lymphomas:

It is a type of malignancy in which there is excessive production of lymphocytes by the lymph nodes and spleen. It accounts for approximately 8% of human cancers. Hodgkin's disease is an example of human lymphoma.

### (iv) Leukemia's:

This type of malignancy arises from the blood forming cell. Leukemia's are commonly known as blood cancer. Leukemia's are neoplastic growth (uncontrolled cell growth at the cost of remaining cells) of leucocytes or WBC. They are characterised by excessive production of WBC of the blood. The name leukemia is derived from Greek leukos (white) + haima (blood) the massive proliferation of leukemia cells can cause a patient's blood to appear milky.

In addition to the types of cancer mentioned above, cancers are further classified

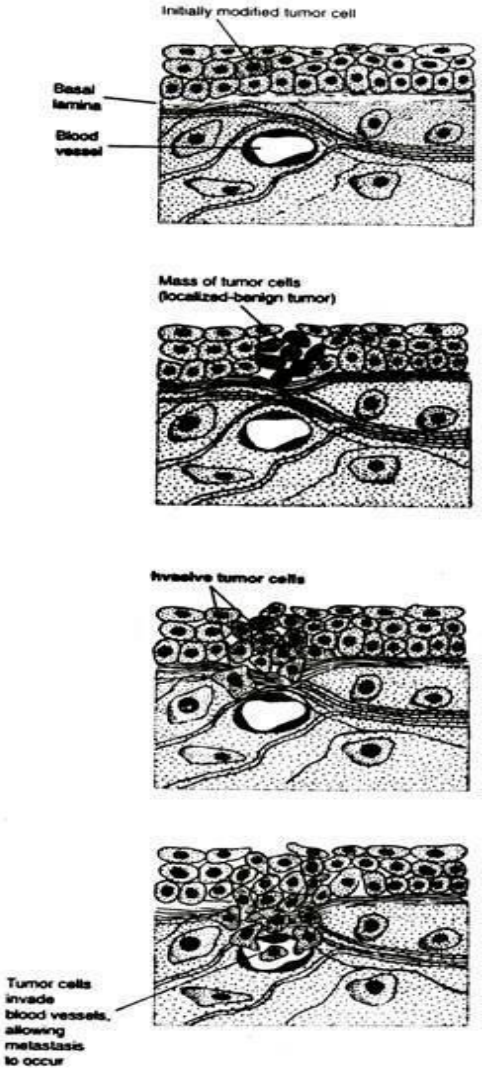


Fig. 23.1: Stages in tumour growth and metastasis.

according to tissue of origin, for example lung cancer, breast cancer, and the type of cells involved, for example fibro sarcoma arises from fibroblasts, erythromoid leukemia's from precursor of erythrocytes. Although there are many kinds of cancer, the four most common cancers are those of prostrate, breast, lung and colon/rectum.

## Development of Cancer:

The development of cancer is a multistep process in which cells gradually become malignant through a progressive series of alternations. This process involves mutation and selection for cells with progressively increasing capacity for cell division, survival, invasion and metastasis (spread of cancer cells through the blood or lymphatic system to other organ sites).

The first step in the process is when a single cell within a tissue of the organ concerned is genetically modified. The modified cell divides rapidly, although

surrounding cells do not— and a mass of tumour cells forms. These cells constitute a clone where cells are identical in terms of structure, characteristics and function. Rapid cell proliferation leads to the tumorous outgrowth or adenoma or polyp. This tumour is still benign. Tumour progression continues as additional mutation occur within cells of tumour population. Some of these mutations give a selective advantage to the cell such as rapid growth and the descendants of a cell bearing such a mutation will consequently become dominant within the tumour population. This process is known as clonal selection. Clonal selection continues throughout tumour development and, consequently, tumour become more and more rapid, growing and increasingly malignant. The tumour cells, by their rapid proliferation, invades the basal lamina that surrounds the tissue. Then tumour cells spread into blood vessels that will distribute them to other sites in the body. This is known as metastasis. If the tumour cells can exit from the blood vessels and grow at distant site, they are considered malignant (Fig. 23.1).

## Characteristics of Cancer Cells:

The uncontrolled growth of cancer cells results from accumulated abnormalities affecting many of the cell regulatory mechanisms. The process of cell change in which a normal cell loses its ability to control its rate of division and thus becomes a tumour cell is called cell transformation.

Cancer cells shows some typical characteristic properties that are absent in normal cells. Sometimes cancer cell properties are just opposite to the properties of normal cell. Cancer cells in vivo differ from their normal counterparts in several respects. Some characteristic properties of cancer cells can also be demonstrated by cell culture in vitro.

### (i) Immortalization:

Normal cell culture do not survive indefinitely For example, human cell culture die after about 50 generations. On the other hand, transformed cell cultures can go on indefinitely and remain immortal if the nutrition is provided and overcrowding avoided.

### (ii) Loss of Contact Inhibition:

Normal cells growing in tissue culture tend to make cell contacts by adhesion to neighbouring cells. At the points of adhesion some kind of electron-dense plaque is formed in both contacting cells. At the same time there is a slowing down of the amoeboid process which results in contact inhibition of movement. In contrast, cancer cells are unable to form adhesive junctions and do not show this type of contact inhibition. Experimentally, it has been observed that when normal cells have

become completely surrounded by other cells, their mobility stops and they form a monolayer. At the same time there is inhibition of growth and the number of cells in the petridish remains practically constant.

On the other hand, cancer cells continue to multiply and pile up forming irregular masses several layers deep. Cancerous cells undergo a change in property of their cell membranes and cell coat such as disappearance of gap junction, loss of coupling changes in glycolipid and glycoprotein and a reduction in gangliosides. In the cell coat fibronectin, a large glycoprotein found in footprints of moving cultured cells is reduced in cancerous cells. These changes enable the cells to dissociate from neighbouring cells and show loss of contact inhibition.

## (iii) Reduced Cellular Adhesion:

Most cancer cells are less adhesive than the normal cells due to reduced expression of cell surface adhesive molecules. When normal cells are transformed into cancer cells then a change of stickiness of their cell membrane results. Normal cells show stickiness or adhesiveness. If normal cells are grown in a liquid nutrient medium kept in a glass vessel, the cells stick to glass wall rather than float in the medium. But when cancer cells are allowed to grow in nutrient medium, they stick to each other less than do normalcells.

Adhesiveness shows considerable specificity. For example, a liver cell tends to stick with another liver cell and not to other types of cell such as kidney cell. Cancerous cells do not show this property. They are able to mix and stick to any type of normal cell. For example, a malignant liver cell can mix and stick to normal kidney cell. Hence this unusual behaviour of cancer cell explains that cancer cells can invade several normal organs.

## (iv) Invasiveness:

One of the most important characteristics of cancer cells is their invasiveness. It is the ability to invade other tissues. Malignant cells generally secrete proteases that digest extracellular matrix components, allowing the cancer cells to invade adjacent normal tissues. For example, secretion of collagenase by the cancer cells helps to digest and penetrate through basal laminae to invade the underlying connective tissue.

Cancer cells also secrete growth factors that promote the formation of new blood vessels. This is known as angiogenesis. Angiogenesis is necessary to support the growth of tumour beyond the size of about a million cells at which point new blood vessels are needed to supply oxygen and nutrients to the multiplying tumour cells. Actually the growth factor secreted by the tumour cells stimulates the endothelial cells present in the wall of capillaries. As a result, new outgrowth of the capillaries is formed into the tumour. These outgrowths of capillaries are also helpful

for metastasis of malignant cells. Therefore, angiogenic stimulation induces the growth of new blood capillaries which penetrate easily in the tumour tissue and provide the opportunity for the cancer cells to enter the circulatory system. As a result, metastasis process begins.

## (v) Failure to Differentiate:

Another general characteristic of most of the cancer cells is that they fail to differentiate. This property is closely related with the abnormal proliferation. Normal cells are fully differentiated. In most fully differentiated cells, cell division ceases. In case of cancer-cells, normal differentiation program is blocked at the early stages of differentiation. The relationship between defective differentiation and rapid proliferation is clearly noted in case of leukemia. All of the different types of blood cells develop from a common pluripotent stem cell in the bone marrow. Some of the descended cells develop erythrocytes but others differentiate to form lymphocytes, granulocytes and macrophages. Cells of each of these types become round as they differentiate but once they become fully differentiated cell division ceases But leukemia cells fail to undergo terminal differentiation. Instead, they become blocked at early stage of maturation at which they retain their capacity for proliferation and continue to divide.

## (vi) Auto stimulation of Cell Division:

Cancer cells produce growth factor that stimulates their own cell division. Such abnormal production of a growth factor by the cancer cell leads to continuous auto stimulation of cell division. This is known as autocrine growth stimulation. Hence the cancer cells are less dependent on general growth factor produced within the body physiologically from normal source for inducing growth of all normal cells. It is also noted that the reduced growth factor dependence of cancer cell results from abnormalities in intracellular signalling system.

## (vii) Apoptosis:

For every cell, there is a fixed span of life, i.e., time to live and time to die. This cell death is a very orderly process and so it is called Programmed Cell Death or PCD or Apoptosis. Apoptosis is a mechanism of programmed cell death or cell suicide which is essential for the survival of the organism, for the normal development of the organism as the programmed destruction of the organism as the programmed destruction of cells is found during embryo-genesis. It also protects the organism by removing damaged cells which may be due to viral infection or due to exposure to radiations. It also inhibits the tumour development and so any defect in the control of apoptosis may lead to cancer.

## There are two methods by which cells may die such as:

1. Death by injury that is through mechanical damage or due to toxic chemicals.

2. By Apoptosis, i.e., through programmed cell death.

## (viii) Density-Dependent Inhibition:

One of the primary distinguishing characteristic features between cancer cell and normal cell is that normal cells show density-dependent inhibition of cell division in culture but cancer cells continue to proliferate independent of cell density.

Proliferation of normal cell continues until they reach a finite cell density. Normal cells are very sensitive to cell density. So when they reach a finite density they enter the $G_0$ state of the cell cycle. But cancer cells continue to divide to high cell density.

## (ix) Cellular Characteristics:

Cancer cells can be distinguished from normal cells by microscopic examination. Cancer cells have a high nucleus to cytoplasm ratio, prominent nucleoli, many mitosis, and relatively little specialised structure. Normal cells have a cytoskeleton which consists of microtubules and microfilaments. But the cytoskeleton of cancer cells undergoes de-polymerisation and the microtubules disaggregate.

## (x) Chromosomal Change:

Normal cell contains normal chromosome number, e.g., normal cells of human beings contain 46 or 23 pairs chromosomes. But in cancer cell the chromosomes can undergo both structural and numerical changes. In human being the parent cell of any cancer has 46 chromosomes Later, after a series of abnormal divisions the cancer cells contain series of chromosome numbers and karyotype. The chromosomes swell up and the number of chromosome sets increase owing to the growth of cancer cells. This condition is known as aneuploidy. Earlier workers have suggested that in different cancer cell populations there are chromosomal stem lines involving a particular spectrum of chromosome structureand number.

An established cancer cell population will have a modal number in most of the cells over quite long periods and it is relatively stable. Generally speaking, no two karyotypes are identical in cancer cell and no typical chromosome group has been found to be involved. Therefore, the occurrence of any aneuploid cells in a particular tissue may have the possibility to become cancerous cell.

## (xi) Interaction with Immune System:

A few normal cells may be transformed in pre-cancer cells every day in each of us in

response to radiation, certain viruses or chemical carcinogens in the environment. Because they are abnormal cells, some of their surface proteins are different from those of normal body cells. Such proteins act as antigens and stimulate an immune response that generally destroys these abnormal pre-cancer cells.

If the pre-cancer cells are destroyed by the immune system, then how does cancer occur? Further investigation demonstrates that there are some transformed cancer cells whose surface proteins are not so changed. Hence such cancer cells may remain anti-genetically similar to normal cells. As a result, the immune system cells may fail to distinguish the cancer cell from normal cell. Some workers suggest that sometimes cells of the immune system do recognise cancer cells but are not able to destroy them. In such case, cancer cells can stimulate B cells to produce IgG antibodies that combine with antigens on the surface of the cancer cells. These blocking antibodies may block the T cells so that they are unable to adhere to the surface of the cancer cells and destroy them. For some unknown reason, the blocking antibodies are not able to activate the complement system that would destroy the cancer cells.

## Carcinogens:

Many agents including radiation, chemicals and viruses have been found to induce cancer in both experimental animals and humans. Agents which cause cancers are called carcinogens. Radiation (Solar ultraviolet ray, X-ray) and chemical carcinogens act by damaging DNA and inducing somatic mutations. These carcinogens are generally called initiating agent because the induction of mutations in key target genes is supposed to be the initial event leading to cancer development.

Some of the initiating agents that cause human cancers include solar ultraviolet radiation—the major causeof skin cancer. The exposure of the thyroid gland to X-rays greatly increases the incidence of thyroid cancers. Varieties of chemical carcinogen including tobacco smoke (containing benzo(a)pyrene, dimethyl nitrosamine and nickel compound) and aflatoxin produced by some molds are the major identified cause of human cancer. Other carcinogens induce the cancer development by stimulating cell proliferation rather than inducing mutations. Such compounds axe called tumour promoters. The first suggestion that chemicals can cause cancer dates back to 1761, when a doctor noted that people who use snuff suffer fromnasal cancer. A few years later a British physician observed a high incidence of cancer of the scrotum among the chimney-sweepers iii their youth. He explained the fact that the chimney soot became dissolved in the natural oil of the scrotum, irritating the skin and, consequently, initiates the development of cancer. On the basis of two separate observations it became evident that certain chemicals (Table 23.1) can cause cancer. Later, as the industrial revolution moved into twentieth century, more and more incidence of cancer were reported among the workers who were continuously

exposed to industrial chemicals.

**Table 23.1:** Gene/Factors

| (a) Initiating Stimuli | Function |
|---|---|
| Tumour Necrosis Factor $\alpha$ receptor family (TNF) | Death signal |
| Ceramide | gives signal for apoptosis induction. |
| FAS/Apo–1 | Death signal like TNF; For peripheral deletion of T lymphocytes. |
| Nur 77 (Zinc finger containing steroid receptor) | Death signal in thymocytes. |
| **(b) Inducing Cellular susceptibility** | |
| c-mye | produces myc protein which gives cell susceptibility for apoptosis |
| Rb–1 | Deficiency of Rb–1 gives susceptibility. |
| | Rb protein may inhibit P 53 mediated apoptosis |
| E2F1 | induces susceptibility |
| P 53 | apoptosis in response to cell injury is dependent on P 53. |
| **(c) Modulating factors** | |
| DAD 1 gene | gives signal for cell death |
| BCl–2 gene family | Some members inhibit cell death, such as bcl–2, BCl–X. Members which promote death like bax, bid and bad. |
| **(d) Effector mechanisms** | |
| Caspases, ICE, Ich–1 | Genes encoding cysteine proteases which are involved in the effector pathway of apoptosis. |

In the early 1940s Peyton Rous observed that repeated application of coal tar to rabbit skin causes tumour to develop, but the tumour disappears when application of the coal tar is stopped. It is also noted that when the skin is treated with turpentine, tumour again reappears.

Normally turpentine does not cause cancer itself. Therefore the coal tar and turpentine are playing two different roles. Some carcinogens induce some normal cells to become irreversibly altered to a pre- neoplastic state. This is known as initiation and the carcinogens are known as initiation agents. Here coal tar is an initiating agent. On the other hand, some carcinogens stimulate the pre-neoplastic cells to divide and form tumour. This is known as promotion and the carcinogens are termed promoting agents. Here turpentine behaves as promoting agents. Berenblum observed that painting the skin of a mouse a single time with methylcholanthrene rarely causes the development of tumours. But subsequently application of castor oil (an oil derived from seeds of Croton tiglium) triggers the formation of multiple tumours on the skin which has been exposed previously to methylcholanthren is acting as an initiator whereas castor oil acts as a promoter. Initiation is a quick, irreversible process that causes a permanent change in a cell's DNA. The carcinogenic chemicals that act as initiating agent are capable to bind with DNA. Hence they interfere with the normal function of DNA and induce somatic mutation and, consequently, bring about stable, inheritable changes in the cell's properties.

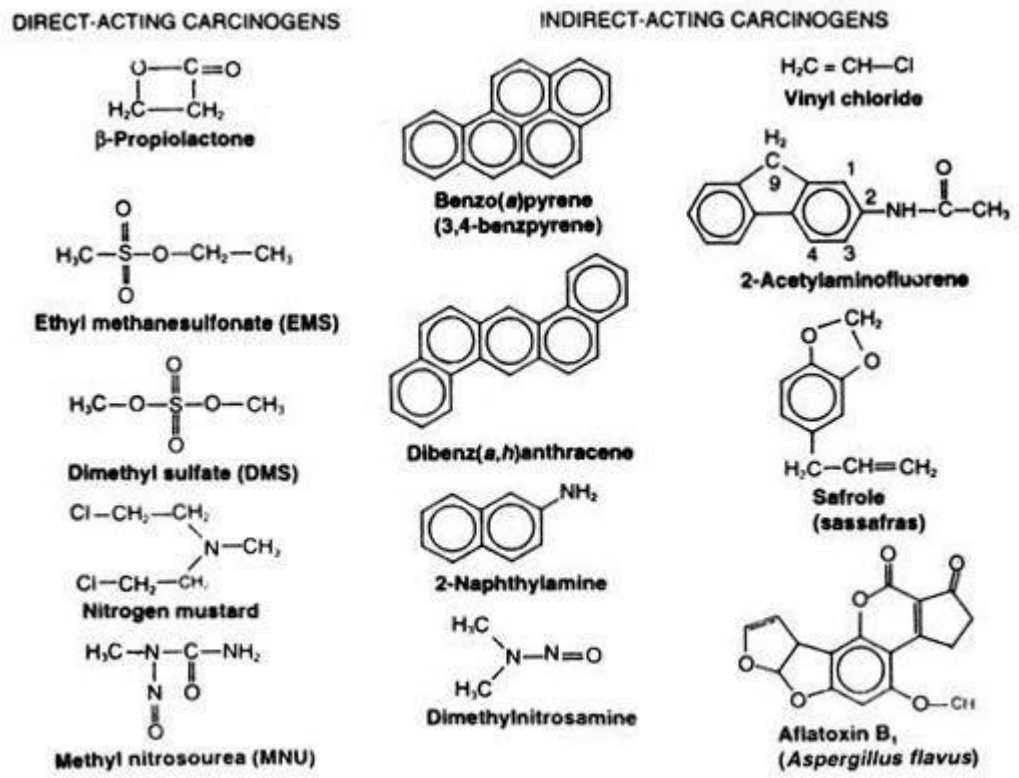


**Fig. 23.2:** Structure of some direct acting and indirect acting chemical carcinogens.

On the basis of action of chemical carcinogens on DNA, there are two broad categories of carcinogens— direct acting and indirect acting (Fig. 23.2). Direct acting carcinogens are highly electrophilic compounds that react with DNA. Indirect acting carcinogens are converted to ultimate carcinogens by introduction of electrophilic centres. In other words, indirect acting carcinogens must be metabolised before they can reactwith DNA.

**The steps of metabolic activation of benzo(a)pyrene—a polycyclic aromatic hydrocarbon—are shown in Fig. 23.3.:**

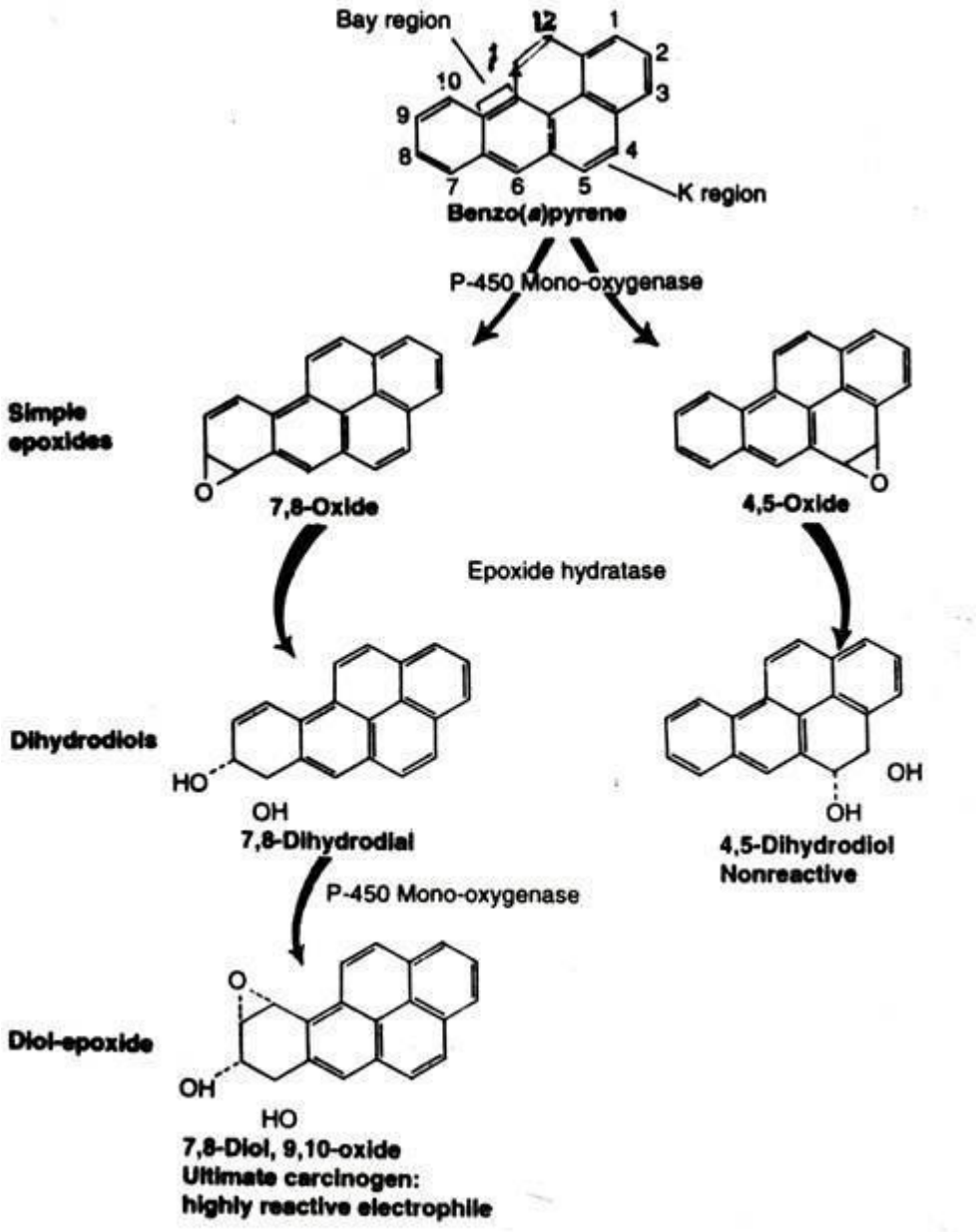


**Fig. 23.3:** Steps of metabolic activation of benzo(a)pyrene—a powerful carcinogen.

On the other hand, promotion is a gradual, partially reversible process that needs prolonged exposure to promoting agents. If a cell that has already undergone initiation is exposed to a promoting agent, the cell starts to divide and the number of genetically damaged cells goes up. As the damaged cells continue to divide, a gradual selection for cells showing higher growth rate and invasive properties occurs—leading to the formation of malignant tumour. The promotion phase continues for longer period. That is why cancer does not develop just after exposure to a carcinogenic agent. The mechanism of action of promoting agents has come from the studies of phorbol esters which are present in castor oil and act as tumour promoters. Phorbol esters bind to the plasma membrane and activate protein kinase C. Protein kinase C isa component of the phosphoinositide signalling pathway whose

activity is normally controlled by the second messenger, diacylglycerol. The activation of protein kinase C leads to phosphorylation of many target proteins and, consequently, activates the transcription factor API which switches on the transcription of genes involved in stimulating cell proliferation. Therefore, the mode of action of phorbol esters gives an insight into the possible mechanism of action of a promoting agent. Energy that travel through space is known as radiation. Natural source of radiation to which humans are generally exposed are ultraviolet rays, cosmic rays and emission from radioactive elements. We are also exposed to another high- energy radiation like X-ray. Medical, industrial and military activities generally create the high- energy radiation. Sunlight has the ability to cause skin cancer in people who spend long hours in the sunlight. Sunlight contains ultraviolet rays which are also absorbed by normal skin pigmentation. Hence, for this reason, dark-stained or black people usually have lower rates of skin cancer than fair- skinned individual.

Because ultraviolet radiation is very weak to pass through the skin, it does not induce any other type of cancer except skin cancer. It is more or less restricted superficially on skin because skin cancer rarely metastasizes. This type of cancer can be cured by easily removing the affected site surgically. Xeroderma pigmentosum is a type of inherited malignant disease. Individuals with this malignant disease develop extensive skin tumours after exposure to sunlight. Homozygotes for the autosomal recessive mutation responsible for xeroderma pigmentosum are less efficient in the repair of DNA damaged by exposure to ultraviolet light.

X-rays are high energy radiation. They are strong enough to penetrate the skin and reach internal organs. X-rays thus make a serious cancer hazard because they are able to induce gene mutation or DNA damage. Many radioactive elements emit radiation. It also acts as carcinogen and causes cancer. Marie Curie, the co-discoverer of the radioactive elements polonium and radium, died of a form of leukemia that appeared to be caused by her extensive exposure to radioactivity. Another example of radiation-induced cancer occurred in New Jersey in 1920. A group of women was employed by a factory that produced watch which glow in the dark. The luminescent point used to point the watch dial contained radium. The paint was applied with a fine-tipped brush that the employee frequently wetted with their tongue. During this process, minute quantities of radium were ingested through saliva in the digestive system from where they were readily abs, -bed and distributed in the different cells ana tissues thorough circulatory system. Several years later these women suffered from bone cancer caused by radioactive radium that had gradually become concentrated in their bone. The most well-known horrifying examples of radiation- induced cancer occurred in Japan and in Nevada of United States. In 1945 atomic bombs were exploded over Hiroshima and Nagasaki. The massive fallout of radioactive elements increased the incidence of leukemia, lymphomas and cancers of the thyroid, breast,' uterus and gastrointestinal tract.

Similarly, in Nevada, people suffered from cancer due to the radioactive fallout during nuclear bomb testing. It is suggested that radioactive carcinogen is thought to initiate malignant transformation by causing DNA damage. Alternatively, it is also explained that subsequent exposure of radiation damaged cells to promoting agents stimulates the cell to divide abnormally and form tumour. There are many viruses which are capable of causing tumour in animals, human as well as plants (Table 23.2). These viruses are called tumour viruses or oncovirus. Some tumour viruses have RNA genome and are known as DNA tumour viruses. Some tumour viruses have DNA genome and are known as retroviruses. Retrovirus replicates via synthesis of a DNA provirus in the infected cells. In addition, HIV is indirectly responsible for the cancer that develops in AIDS patient as a result of immunodeficiency.

**Table 23.2:** List of Chemical Carcinogens and Type of Cancer induced by such chemicals

| Carcinogen | Type of cancer induced |
|---|---|
| Acrylonitrile | Colon, lung |
| 4-Aminodiphenyl | Bladder |
| Aniline derivatives | Bladder |
| Arsenic compounds | Lung, skin |
| Asbestos | Lung, mesothelium |
| Benzene | Leukemia |
| Cadmium salts | Prostate, lung |
| Carbon tetrachloride | Liver |
| Chromium and chromates | Lung, nasal sinuses |
| Diethylstibestrol (DES) | Uterus, vagina |
| Lead | Kidney |
| Mustard gas | Lung, larynx |
| α-Naphthylamine | Bladder |
| Nickel | Lung, nose |
| Organochloride pesticides | Liver |
| Polychlorinated biphenyls | Liver |
| Radon | Lung |
| Soot and tars | Skin, lung, bladder |
| Vinyl chloride | Liver, lung, brain |
| Wood and leather dust | Nasal sinuses |
| Tobacco smoke, which contains the following: | Lung, oral cavity, larynx, esophagus, stomach, pancreas, others |
| Aminostilbene, arsenic, benz[*a*]anthracene, benz[*a*]pyrene, benzene, benzo[*b*]fluoranthene, benzo[*c*]phenanthrene, benzo[*j*]fluoranthene, cadmium, chrysene, dibenz[*a,c*]anthracene, dibenzo[*a,e*]fluoranthene, dibenz[*a,b*]acridine, dibenz[*a,j*]acridine,dibenzo[*c,g*]carbazone, N-dibutylnitrosamine, 2,3-dimethylchrysene, indeno[*1,2,3-c,d*]pyrene, S-methylchrysene, S-methylfluoranthene, α-naphthylamine, nickel compounds, N-nitrosodimethylamine, N-nitrosomethylethylamine, polonium-210, N-nitrosodiethylamine, N-nitrosonornicotine, N-nitrosoanabasine, N-nitrosopiperidine | |

The herpes viruses are the most complex animal viruses. The genome length of these viruses is 100-200 Kb. Many herpes viruses cause tumour in many animals such as

frogs, chickens, monkeys etc. Epstein- Barr virus, a member of herpes virus, can trigger the development of some human malignancies including Burkett's lymphoma in some region of Africa and nasopharyngeal carcinoma in China. It also causes B- cell lymphomas in AIDS patient and other immunosuppressed persons. Cell transformation by herpes viruses is not fully understood because of the complexity of their genome. But it is evident that some viral genes are required to induce transformation of lymphocytes.

Of the DNA tumour viruses, the papoviruses are the best studied DNA tumour viruses from the standpoint of molecular biology and have received particular attention because they have been critically important as models for understanding the molecular basis of cell transformation. The genome size of papoviruses is small (approximately 5 Kb). Simian virus 40 ($SV_{40}$) and polyomavirus are the important and commonly

known member of papoviruses. Both these viruses are similar in size and general structure. A virus usuallymultiplies in specific cells derived from animals in which the virus normally grows. Such cells are called permissive cells. Cells which do not allow the viruses to grow are called non-permissive cells. $SV_{40}$ and polyoma viruses, on entering their respective host cells, undergo one of the two types of behaviour—they enter the permissive cell of the host, undergo the lytic phase, and multiply within host cell, ultimately killing them. Since a permissive cell is killed as a consequence of virus replication, it cannot become transformed. Sometimes viruses enter non-permissive cells and are not able to multiply, i.e., virus replication is blocked. In this case, the viral genome sometimes integrates into cellular DNA and expression of specific viral genes results in transformation of the infected cells.

The $SV_{40}$ and polyoma virus genes that trigger cell transformation have been identified, isolated and sequenced by molecular analysis. The genome of $SV_{40}$ and polyomavirus are divided into early and late regions. The early region is expressed immediately after infection and is needed for synthesis of viral DNA. The late region is not expressed until after viral DNA replication has begun. The early region of $SV_{40}$ codes for two proteins which are known as small (17 Kd) and large (94 Kd) T-antigens. In additionto small and large regions, the genome of polyomavirus contains a third early region which is called as middle T region. It codes for a protein of about 55 Kd.

Experimentally, it has been shown that large T of $SV_{40}$ is sufficient to induce transformation and the middle T region of polyoma virus is primarily responsible for transformation. During lytic cycle, the early region proteins are needed to initiate viral DNA replication as well as to stimulate host cell gene expression and DNA synthesis. Since the replication viral DNA is dependent on host cell enzymes, therefore stimulation of gene expression of the host cell is a critical event in the viral

life cycle. Most of the cells of adult animal cells become non-dividing. So the enzymes required for cell division are not available within the cell. Therefore they must be stimulated to divide in order to induce the enzymes needed for viral DNA replication. This stimulation of cell division by the early gene products of virus can lead to transformation if the viral DNA becomes stably integrated and expressed in a non permissive cells. The early region proteins of $SV_{40}$ and polyoma virus induce transformation by interacting with host proteins that regulate cell division.

The papilloma viruses are small DNA viruses. The genome length of such viruses is approximately 8 Kd. Some of these viruses induce only benign tumours such as warts. But some others cause malignant carcinomas— particularly cervical and anogenital cancers. Cell transformation by papilloma viruses occurs from the expression of two early region genes $E_6$ and $E_7$. The hepatitis B viruses are another group of DNA virus. They have the smallest genomes which is approximately 3 Kb. These viruses mainly infect the liver cells and cause liver damage. But how they induce cell-transformation is not clearly known.

Possibly tumour results from expression of a viral gene. Alternatively, the chronic cell damage of liver simply induce the continuous cell division which, ultimately, causes the cell transformation. The retroviruses, one family of RNA viruses, also cause human cancer. For example, human T-cell lymphotropic virus type-I (HTLV- I), a RNA virus, is the causative agent of T-cell leukemia. A related virus (HTLV-II) causes a rare form of leukemia called hairy T- cell leukemia. HIV (Human immunodeficiency virus) is the causative agent of AIDS. These viruses, i.e., HTLV-I, HTLV-II, HIV, actually does not cause cancer by directly converting a normal cell into a tumour cell. The AIDS patients become susceptible to high incidence of some malignancies like lymphomas and Kaposi's sarcoma due to immunosuppression of the patient. RNA viruses have an RNA genome which is extended at either end bya long terminal repeat (LTR). The LTR contains many of the signals that allow retrovirus to function (Fig. 23.4). Retroviruses use their genomic RNA as a template to make DNA with the help of reverse transcriptase. This DNA is then integrated into host's DNA as DNA the provirus. The DNA provirus is transcribed to yield genome length RNA provirus directed transcription involves a promoter—a sequence that directs the RNA polymerase to a specific initiation site and an enhancer—a sequence that facilitates transcription.

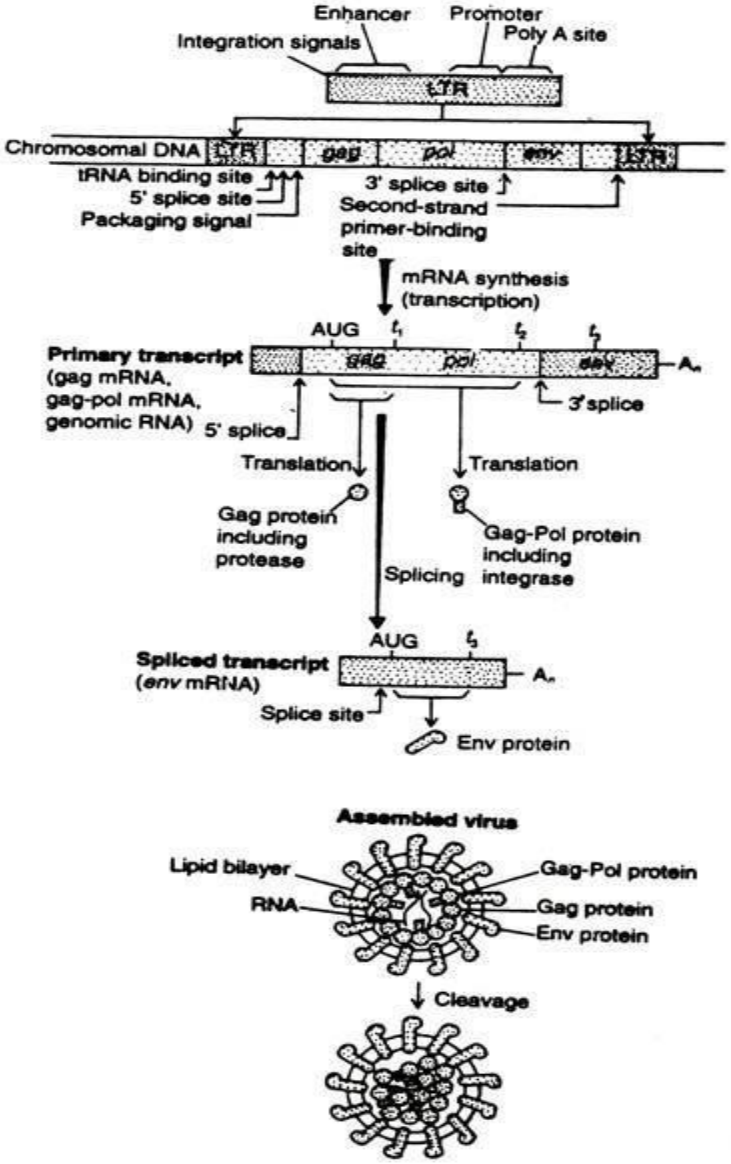


Fig. 23.4: Genetic elements of proviral DNA and the corresponding gene products.

The promoter and enhancer are located in the LTR. The primary transcript serves as the genomic RNA for progeny virus particles and as mRNA for the gag and pol genes. In addition the full length RNA is spliced to yield mRNA for env.

The gag gene encodes the viral protease and structural proteins of the virus particle, pol encodes reverse transcriptase and integrase and env encodes envelope glycoproteins. These three genes are only required for viral replication but play no role in cell transformation.
This type of retrovirus causes tumour only when any mutation results at the time of integration of pro-viral DNA within or adjacent to host's genome. But there are some other retroviruses which contain specific genes which are responsible for the induction of cell transformation and acts as potent carcinogens. The first cancer causing gene is f6und in the retrovirus called Rous Sarcoma virus (Fig. 23.5) that produces sarcomas in chicken. It was later named src gene. Genes like src which are capable of inducing malignant transformation, are referred to as oncogenes. The identification of the first viral oncogene has provided a model for understanding many aspects of cancer development at the molecular level.

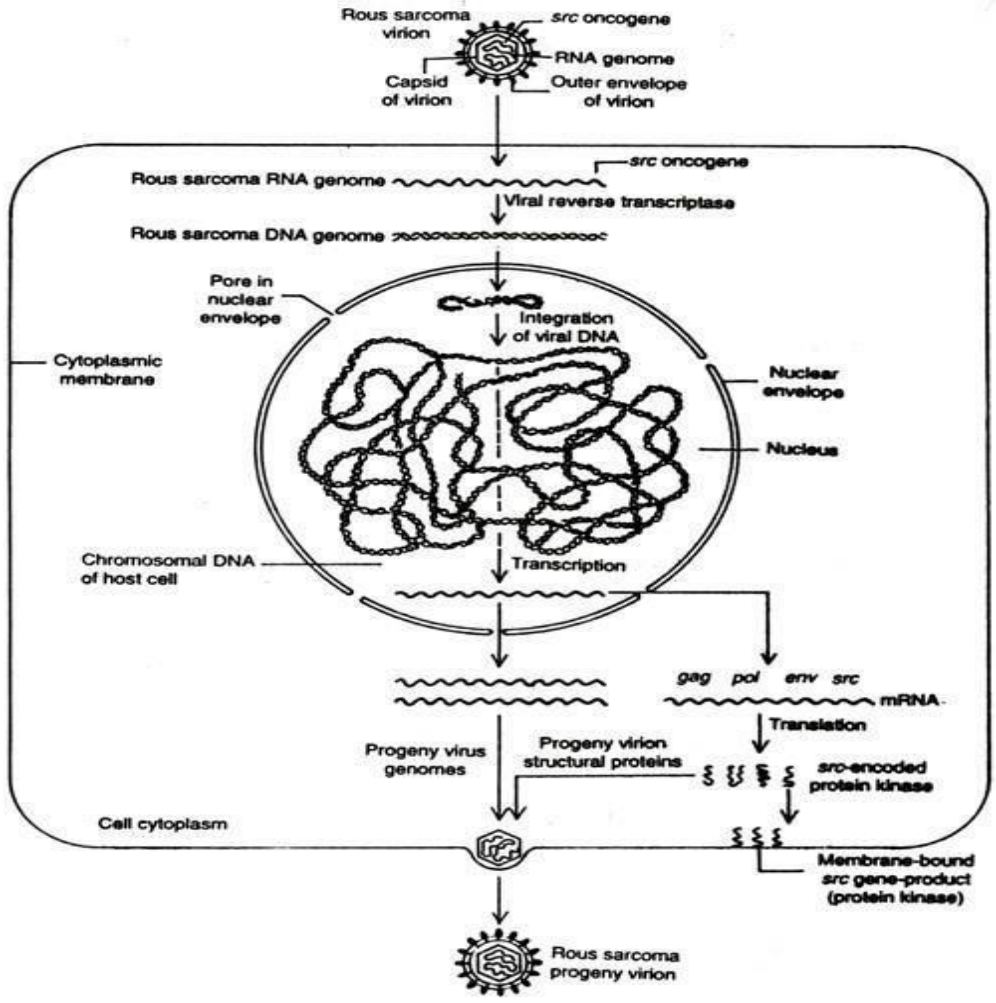


**Fig. 23.5:** Life cycle of Rous Sarcoma RNA tumour virus.

## Proto-oncogene:

All 20 viral oncogenes derive from cellular genes in normal cells. The normal cellular version of the gene is called a proto-oncogene. Retroviruses pick up into their genome sequences from mRNA population that will increase viral production (by increasing cell proliferation). These viruses can transmit genes from one species to another, thus breaking evolutionary barriers (Bishop, 1983).

Another agent that stimulates proliferation, epidermal growth factor (EGF), like Svc, increases phosphorylation of membrane proteins at tyrosines. It is thought that this tyrosine phosphorylation somehow controls cell proliferation. Cancer cells (also called transformed cells) contain ten times more phosphotyrosine than normal cells. About half of the oncogenes code for Tyr protein kinases and in all cases the proteins are integral components of the cell membrane. Most oncogenes seem to be related in one way or another with the same pathway of regulation of cell proliferation by protein growth factors.

EGF stimulates lung development and differentiation. In intact organisms, the production of growth factor induces multiplication at a short distance only, stimulating the cell that secretes it and the nearby cells. This is sometimes called autocrine secretion. When EGF reaches the membrane of a target cell it binds toa specific receptor protein of 170,000 Daltons, and the complex is subsequently internalized. The receptor protein becomes phosphorylated during this process and the phosphorus binds to tyrosine. Phosphorylation of tyrosine is rare in proteins except in cells that have been transformed by retroviruses and become cancerous. Both in EGF and in viral transformation phosphorylation of tyrosine results in increased cell proliferation. Cells start dividing only after many hours of adding the growth factors. Growth inhibitors, called chalones (Gr., to slow down) are numerous, which have been isolated from tissues. These are also numerous and important as growth factors.

## Oncogenes:

Oncogene is a type of specific viral gene that is capable of inducing cancer or cell transformation—either in the body of host or in the tissue in culture. After the discovery of src oncogene in RSV, more than 40 different highly oncogenic retroviruses have been isolated (Table 23.3) from a variety of animals like mice, rat, cat, chickens, turkeys, monkeys etc.

All these viruses contain at least one (in some cases two) oncogene like RSV. These oncogene are not needed for viral replication but is responsible for cell transformation. In some cases different viruses contain the same oncogenes. Many of these genes encode protein which, in turn, acts as the key components of signalling pathways that induces cell transformation.

Table 23.3: Examples of Tumour Viruses

| Class | Examples | Tumours induced | Organism |
|---|---|---|---|
| **DNA viruses:** | | | |
| Herpesviruses | Lucke virus | Kidney adenocarcinoma | Frogs |
| | Epstein-Barr virus (EBV) | Burkitt's lymphoma, nasopharyngeal carcinoma | Humans |
| | Marek's disease virus | Lymphoma | Chickens |
| Papovaviruses | Shope papilloma virus | Papillomas | Rabbits |
| | SV-40 | Subcutaneous, kidney and lung sarcomas | Hamsters |
| | Polyoma | Liver, kidney, lung, bone, blood vessels, nervous tissue, connective tissues | Mice |
| | Human papillomaviruses | Cervical cancer | Humans |
| Hepatitis B virus | | Liver cancer | Duck, Woodchucks, squirrels, human |
| Adenoviruses | Human adenoviruses | Subcutaneous, intraperitoneal, intracranial | Hamsters |
| **RNA viruses:** | | | |
| B-type viruses | Bittner mammary tumor virus | Mammary carcinoma | Mice |
| C-type viruses | Rous sarcoma virus | Sarcomas | Birds, mammals |
| | Murine leukemia viruses (Gross, Moloney, Friend, Rauscher, and others) | Leukemia | Mice |
| | Feline leukemia virus | Leukemia | Cats |
| | Murine sarcoma virus | Sarcoma | Mice |
| | Feline sarcoma virus | Sarcoma | Cats |
| | Avian leukemia viruses (avian myeloblastosis and others) | Leukemia | Chickens |
| | Human T-cell leukemia virus | Leukemias/Lymphomas | Humans |
| Plant viruses | Wound tumor virus | Roots and stems | Plants |

## Oncogene in Human Cancer:

Direct evidence for the involvement of cellular oncogenes (the term cellular oncogene is generally used to distinguish this group of cancer- causing genes from viral oncogenes) in human tumour was first derived from gene transfer experiment carried out in the laboratories of Robert Weinberg and Geoffrey Cooper in the early 1980s.

In this process, a DNA segment isolated from tumour cells is artificially introduced into normal cells to see its subsequent changes. DNA isolated from a human bladder carcinoma was found to efficiently induce malignant transformation of recipient mouse cells in culture. This experiment reveals that the human tumour contains a cellular oncogene. The first human oncogene identified in gene transfer experiment was the ras oncogene. The ras oncogenes are not present in normal cells, but they are generated in tumour cells as a consequence of point mutation of the ras proto-oncogene. This results in the change of a single amino acid at critical position of the ras protein molecule encoded by ras gene.

The first such mutation was the substitution of valine for glycine at position 12. A single nucleotide, change which alters codon 12 from GGC (Gly) to GTC(Val) is responsible for the transforming activity. This is detected in bladder carcinoma DNA.

The ras gene encodes membrane-bound guanine-nucleotide binding proteins (G- protein) that plays a central role in the transmission of singles from receptor-bound external growth factor to the cell interior. During this process, GTP is hydrolysed into GDP. Therefore, Ras protein alternates between active (GTP bound) and inactive (GDP bound) states. But oncogenic ras proteins remain in the active GTP bound state and drive unregulated cell proliferation leading to the development of malignancy. In human tumour, point mutation is an important mechanism by which proto-oncogenes are converted into oncogenes. Besides this, the gene rearrangement—resulting mainly from chromosome translocation—sometimes lead to the conversion of proto-oncogene to oncogene. The classical example regarding the conversion of proto- oncogene to oncogene due to translocation of chromosome is the Burkitt's lymphoma. It produces the malignancy of the antibody producing B-lymphocytes. In this case a piece of chromosome(s) 8 carrying c- myc proto-oncogene is trans-located to the immunoglobulin heavy chain locus on chromosome 14 (Fig. 23.6). Since the antibody genes are extremely active in lymphocytes, the transcriptional regulation of the adjacent myc proto-oncogene is disturbed, resulting in an abnormal pattern of synthesis of the myc protein product.

Such abnormal pattern of expression of the c-myc gene—which encodes transcription factor normally induced in response to growth factor stimulation—is sufficient to drive cell proliferation and contribute to tumour development.

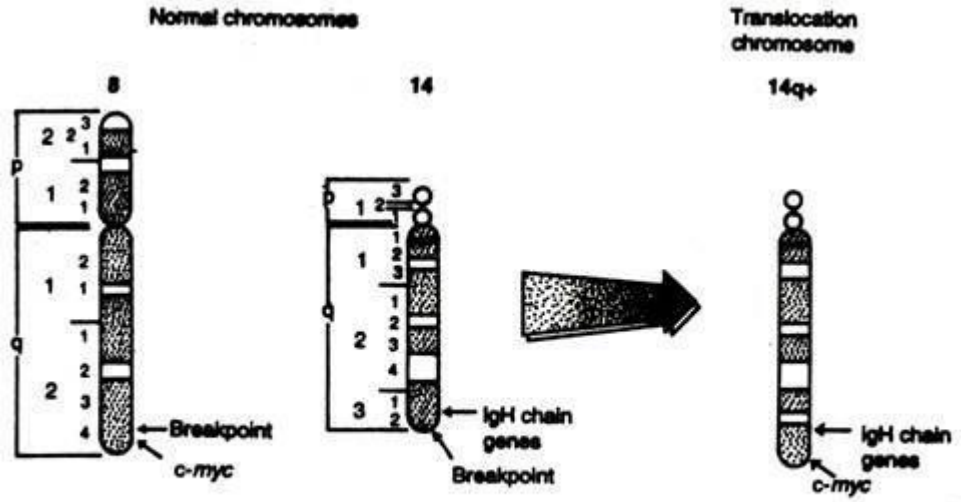


Fig. 23.6: Translocation of a c-myc protooncogene from chromosome 8 to 14.

Translocation of some proto-oncogene often causes the rearrangement of coding sequences which lead to the formation of abnormal gene products. In chronic myelegenous leukemia, the abl proto-oncogene is trans-located from chromosome 9 to chromosome 22 forming Philadelphia chromosome (Fig. 23.7).

The abl proto- oncogene which contains two alternative first exon (1A and IB) is joined to the middle to the bcr gene on chromosome 22. Exon IB is deleted as a result of the translocation. Transcription of the fused gene initiates at the bcr promotor and continues through abl. Splicing then generates a fused bcr/abl mRNA, in which abl exon 1A sequences are also deleted and bcr sequences are joined to abl Exon 2.

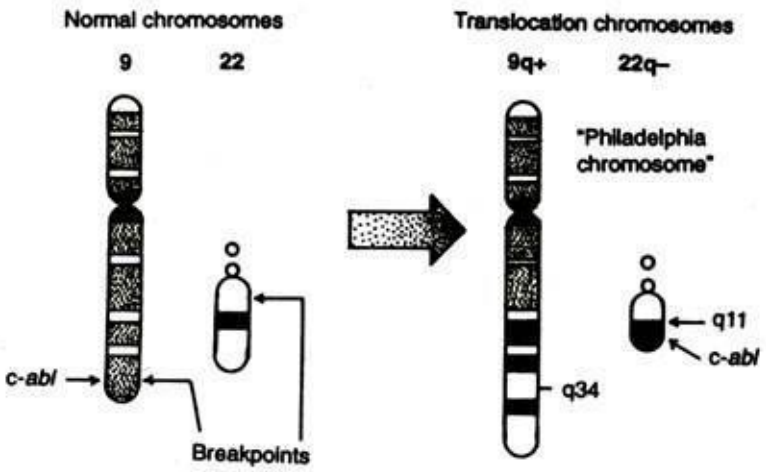


**Fig. 23.7:** Reciprocal translocation between chromosomes 9 and 22 that produce Philadelphia chromosome.

The bcr/abl mRNA is translated to yield a recombinant bcr/abl fusion protein in which the normal amino terminus of abl proto-oncogene has been replaced by bcr amino acid sequences. The fusion of bcr sequences results in aberrant activity and altered subcellular localisation of the abl protein tyrosine kinase, leading to cell transformation. Gene amplification occurring in the tumour cell is a common process by which proto- oncogenes are converted to oncogene. Gene amplification takes place due to an increase of the number of copies of a gene resulting from the repeated replication of a region of DNA. Therefore,gene amplification leads to the overproduction of a particular protein or enzyme from the amplified gene. A prominent example of oncogene amplification is the involvement of the N-myc gene in neuroblastoma,a tumour of embryonal neuronal cells.

Amplified copies of N-myc gene are frequently present in rapidly growing tumour. Hence it indicates that N-myc amplification is related with the development of neuroblastomas. Amplification of erb B-2 which encodes a receptor protein kinase is similarly associated to the development of breast and ovarian carcinomas.

Table 23.4: Retroviral Oncogenes

| Oncogene | Virus | Species |
|---|---|---|
| *abl* | Abelson leukemia | Mouse |
| *akt* | AKT8 virus | Mouse |
| *cbl* | Cas NS-1 | Mouse |
| *crk* | CT10 sarcoma | Chicken |
| *erbA* | Avian erythroblastosis-ES4 | Chicken |
| *erbB* | Avian erythroblastosis-ES4 | Chicken |
| *ets* | Avian erythroblastosis-E26 | Chicken |
| *fes* | Gardner-Arnstein feline sarcoma | Cat |
| *fgr* | Gardner-Rasheed feline sarcoma | Cat |
| *fms* | McDonough feline sarcome | Cat |
| *fos* | FBJ murine osteogenic sarcome | Mouse |
| *fps* | Fujinami sarcome | Chicken |
| *jun* | Avian sarcoma-17 | Chicken |
| *kit* | Hardy-Zuckerman feline sarcome | Cat |
| *maf* | Avian sarcoma-AS42 | Chicken |
| *mos* | Moloney sarcoma | Mouse |
| *mpl* | Myeloproliferative leukemia | Mouse |
| *myb* | Avian myeloblastosis | Chicken |
| *myc* | Avian myelocytomatosis | Chicken |
| *qin* | Avian sarcoma 31 | Chicken |
| *raf* | 3611 murine sarcoma | Mouse |
| *rasH* | Harvey sarcoma | Rat |
| *rasK* | Kirsten sarcoma | Rat |
| *rel* | Reticuloendotheliosis | Turkey |
| *ros* | UR2 sarcoma | Chicken |
| *sea* | Avian erythroblastosis-S13 | Chicken |
| *sis* | Simian sarcoma | Monkey |
| *ski* | Avian SK | Chicken |
| *src* | Rous sarcoma | Chicken |
| *yes* | Y73 sarcome | Chicken |

Subsequent studies have discovered a number of oncogenes (Table 23.4) which are associated with humantumour. Among them chromosomal location of some oncogenes are shown in Fig. 23.8.

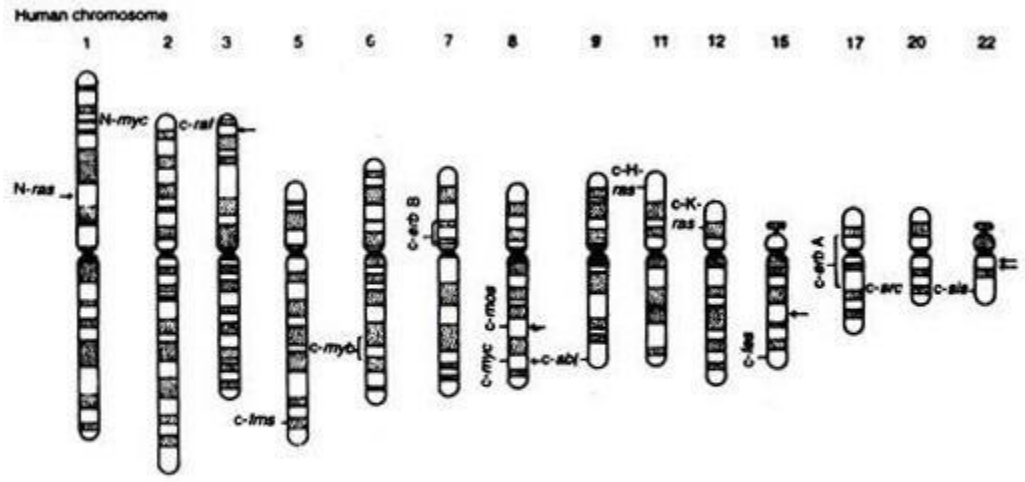


Fig. 23.8: Chromosomal location of some human protooncogenes.

## Functions of Oncogene Products:

We have understood that alternation in normal genes, proto-oncogenes, can convert them into oncogenes that code for proteins that are abnormal in structure or are produced in inappropriate amounts. The proteins encoded by the normal genes regulate normal cell proliferation. But the protein encoded by the corresponding oncogene proteins drives the uncontrolled proliferation of the cancer cells.

In addition, some oncogene products involved in other aspects of the behaviour of cancer cells such as defective differentiation and failure to undergo programmed cell death. Besides this, majority of oncogene proteins function as elements of the signalling pathways that regulate cell proliferation in response to growth factor stimulation. These oncogene proteins include polypeptide growth factors, growth factor receptors, elements of intracellular signalling pathway and transcriptional factors (Table 23.5).

Table 23.5: Representative Oncogenes of Human Tumours

| Oncogene | Type of cancer | Activation mechanism |
|---|---|---|
| *abl* | Chronic myelogenous leukemia, acute lymphocytic leukemia | Translocation |
| *bcl-2* | Follicular B-cell lymphoma | Translocation |
| *E2A/pbx1* | Acute lymphocytic leukemia | Translocation |
| *erb B-2* | Breast and overian carcinomas | Amplification |
| *gip* | Adrenal cortical and ovarian carcinomas | Point mutation |
| *gli* | Glioblastoma | Amplification |
| *gsp* | Pituitary and thyroid tumors | Point mutation |
| *hox-11* | Acute T-cell leukemia | Translocation |
| *lyl* | Acute T-cell leukemia | Translocation |
| *c-myc* | Burkitt's lymphoma | Translocation |
| *c-myc* | Breast and lung carcinomas | Amplification |
| *L-myc* | Lung carcinoma | Amplification |
| *N-myc* | Neuroblastoma, lung carcinoma | Amplification |
| *PML/RA/Rα* | Acute promyelocytic leukemia | Translocation |
| *PRAD1* | Parathyroid adenoma | Translocation |
| *PRAD1* | Breast carcinoma | Amplification |
| *rasH* | Thyroid carcinoma | Point mutation |
| *rasK* | Colon, lung, pancreatic, and thyroid carcinomas | Point mutation |
| *rasN* | Acute myelogenous and lymphocytic leukemias, thyroid carcinoma | Point mutation |
| *ret* | Thyroid carcinoma | DNA rearrangement |

If the oncogenes induce uncontrolled cell growth that leads to cancer then it is obvious that the products of these genes would act by stimulating all division in some manner. For example, the product of the v-sis oncogene (the v stands for virus) of simian sarcoma virus is closely related to a polypeptide growth hormone called platelet-derived growth factor (PDGF). This factor produced by platelets promotes wound healing by stimulating growth of cells at wound site.

Simian sarcoma virus with v-sis gene in their genome when injected into the body of woolly monkey, induce sarcoma. They are also able to transform fibroblasts growing in culture to a tumorous state. This type of cellular transformation occurs by a mechanism which is possibly related to the effect of normal PDGF on cells at the wound site. Other oncogenes encode products that are identical to growth hormone as well as hormone receptors. For example, oncogene erb B and fms encode proteins that are closely related to the receptors for epidermal growth factor (EGF) and colony stimulating factor-1 (CSF-1).

CSF-1 is a growth factor that stimulates growth and differentiation of macrophages. The receptor of this growth factor is a trans membrane-protein with growth factor domains on the outside of the cell andprotein kinase domains on the inside of the cell (Fig. 23.9).

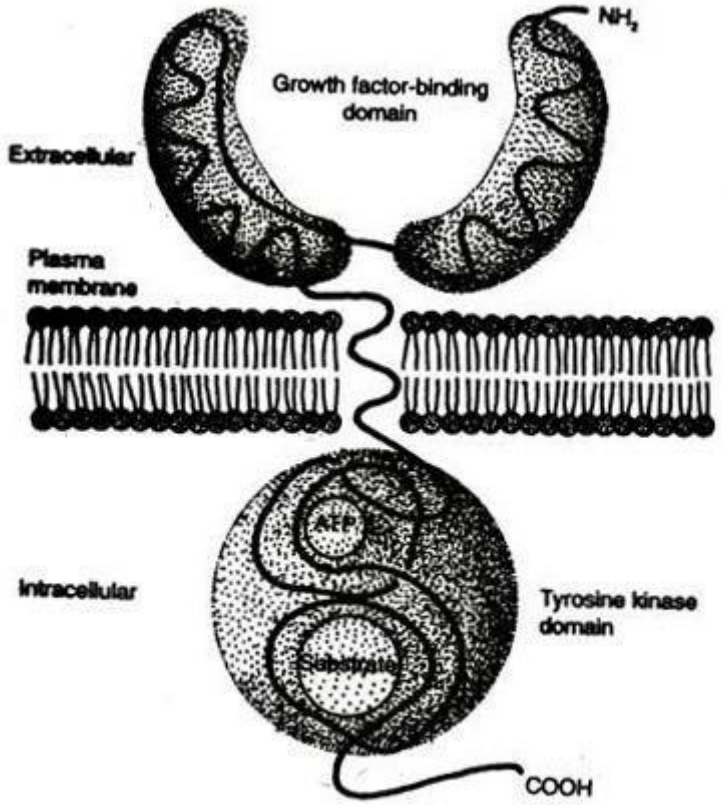


**Fig. 23.9:** Structure of transmembrane growth factor receptors.

These receptors are key components in trans-membrane signalling pathways. The erb A gene product is an analog of the nuclear receptor for the thyroid hormone $T_3$. Therefore, all of the gene products are undoubtedly involved in intercellular communication circuit which control cell division during the growth and development of highly differentiated tissue.

Protein tyrosine kinase is a trans-membrane receptor that is capable of transmitting a perfect signal instructing a cell to divide. Alternation in the structure and function of this enzyme will transmit a wrong signal instructing the cell to divide when it normally should not divide—the result will be tumour formation. Following the discovery that the src oncogene codes for a protein kinase, more than 20 other oncogenes have also been found to code for protein tyrosine kinases. These oncogene encoded tyrosine kinases can be subdivided into two main classes such as receptor protein tyrosine kinases and non-receptor protein tyrosine kinases. Receptor protein tyrosine kinases are trans-membrane proteins that contain a growth factor receptor domain which are exposed on the outer surface of the plasma membrane and a tyrosine kinase catalytic domain at the inner surface of the plasma membrane. In a normal receptor of this type, first appropriate growth such as PDGF, EGF, binds with receptors site and activates protein tyrosine kinase domain. Activation of protein tyrosine kinase stimulates cell proliferation through activation of the membrane associated G protein Ras (Fig. 23.10).

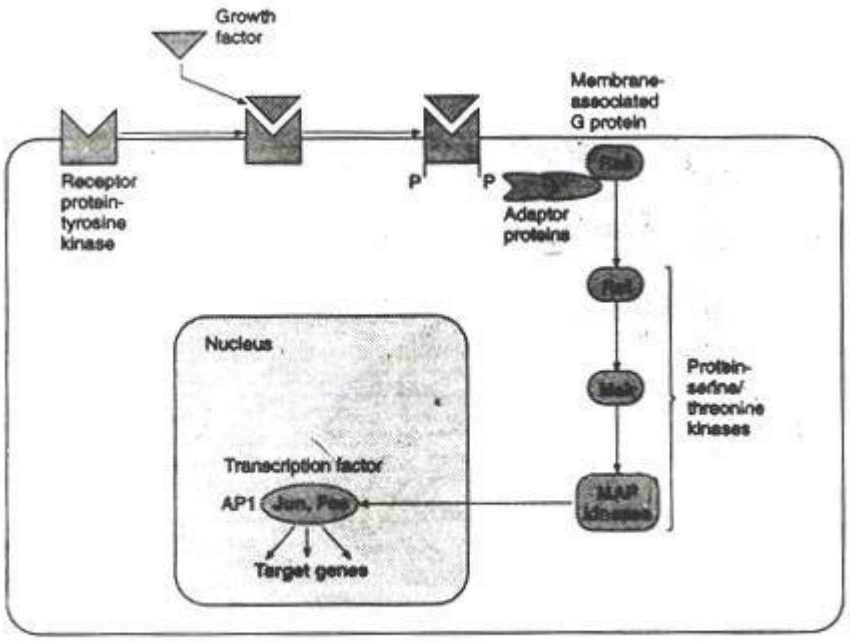


Fig. 23.10: Activation of protein tyrosine kinase that stimulates cell proliferation through activation of the membrane associated G protein Ras.

Activation of Ras triggers the phosphorylation of a series of cytoplasmic protein-serin/theronine kinase, thereby leading to phosphorylation of the nuclear API transcription factor which, in turn, activates genes involved in stimulating cell proliferation. Oncogenes can code for abnormal receptor protein- tyrosine kinases in which the growth factor binding site is disrupted leading to unregulated activity of the protein tyrosine kinase site.

Non-receptor protein tyrosine kinase are usually bound to the membrane's cytoplasm or free in the cytosol. The non-receptor protein tyrosine kinase is encoded by the src gene. ncogene-encoded non-receptor kinases often show excessive unregulated protein-tyrosine kinase activity. Another group of oncogenes code for plasma membrane associated G proteins. In human cancer, ras oncogene shows almostresemblance with cellular ras gene of the host except that ras oncogene is the mutant form in contrast to cellular ras gene. Hence mutant ras G proteins are produced. They retain bound GTP instead of hydrolyzing it to GDP. As a result mutant ras protein in its active form mislead the transmission of signal from external growth factors. Hence the host cells undergo abnormal cell division.

Most of the protein kinase activity showed by mammalian cells catalyses the phosphorylation of the amino acids serine and theonin, not tyrosine. These protein-serine/threonine kinase like protein-tyrosine kinase can be encoded by oncogene. The most important oncogene belonging to this group is the raf oncogene. It codes for a protein serine/threonine kinase that transmits signals from plasma membrane Ras protein to the cell interior. Some oncogenes code for proteins that function within the nucleus, particularly in the regulation of gene transcription. The examples of such oncogenes are the jun and fos oncogene which code for proteins that make up the AP! transcription factor. The $AP_1$ factor regulates the expression of a

group of genes that are involved in stimulating cell proliferation. The myc oncogene, associated with several kinds of human cancer, also appears to code for a transcription factor.

## Proto-Oncogene:

It is well-established that oncogenic virus contains a relatively small number of genes which has facilitated the identification of the viral genes that cause cell to become malignant. The first cancer- causing gene to be identified occurs in Rous sarcoma virus, a small retrovirus that produces sarcomas in chickens.

An unexpected feature of retroviral oncogene is their lack of involvement in virus replication while other viral gene involves efficiently in the same process. Again, the existence of viral oncogene is not an integral part of the virus life cycle. Therefore, the origin and existence of viral oncogene leads to a new line of investigation. Such investigations have led to the surprising discovery that the src gene is not present only in cancer cells. Using nucleic acid hybridisation techniques, it has been shown that DNA sequence that Eire homologous to— but not identical with—the Rous src gene can be detected in the genome of normal cells of a wide variety of organisms including salmon, mice, cows, birds and humans.

The unexpected discovery that cells contain DNA sequences that are closely related to viral oncogenes has been substantiated by studies on a variety of other tumour viruses and, in each case, they resemble genes present in the genome of normal cell. The term proto- oncogene has been introduced to refer to these normal cellular genes that closely resemble oncogenes. The resemblance of viral oncogenes to proto- oncogene suggests that viral oncogenes may have originally been derived from normal cellular genes. According to this concept, the first step in the creation of retro-viral oncogenes took place million years ago when the ancient virus infected cells and became integrated in the host chromosomal DNA adjacent tonormal cellular proto-oncogenes.

When the integrated pro-viral DNA was later transcribed to regenerate new viral RNA molecules, the adjacent proto-oncogene sequences might have been transcribed as well. In this way, a viral RNA molecule containing normal proto- oncogene sequences could have been created. Since a proto-oncogene would initially serve no useful purpose for a virus, it would be free to mutate during subsequent cycles of viral infection. Such mutation would eventually convert proto-oncogene into an oncogene. Therefore, the realisation that oncogenic viruses contain genes that cause cell to become malignant raise the question of whether genetic alteration are also involved in non-virus induced

cancers. The ability of many carcinogens to act as mutagens provides the reason to believe that genetic changes play a role in non-viral carcinogenesis. Besides this, recent research suggests that cellular oncogenes are derived from normal proto-oncogenes by at least five mechanisms:

## (i) Point Mutation:

The simplest mechanism for converting a proto- oncogene into an oncogene, it involves a single base pair substitution or point mutation.

### (ii) Local DNA Rearrangement:

The second mechanism for creating oncogenes is based on DNA rearrangements that cause either deletions or base sequence exchanges between proto-oncogene and surrounding genes.

### (iii) Insertional Mutagenesis:

The evidence of third mechanism comes from the findings that some cancer-causing retrovirus lack oncogenes and these particular viruses cause cancer by integrating a DNA copy of their genetic information into a host chromosome in a region where a proto-oncogene is located and thus disrupt the structure of the host proto-oncogene and thereby convert it into an oncogene.

### (iv) Gene Amplification:

The fourth mechanism for creating oncogenes uses gene amplification to increase the number of copies ofa particular protogene. This overproduction of copies of a particular proto- oncogene leads to malignant transformation.

### (v) Chromosomal Translocation:

The fifth mechanism for creating an oncogene involves chromosomal translocation. It is a process where aportion of one chromosome is physically broken and joined to another chromosome. As a result, the broken segment containing proto-oncogene is transferred from its normal location to a new location whereit is converted as oncogene.

## Tumour Suppressor Genes:

We have now seen how the presence of an oncogene can stimulate uncontrolled cell growth and division, thereby fostering the development of malignancy. Cancer can also be induced by the loss of tumour suppressor genes that normally inhibit cell

proliferation.

The term tumour suppressor gene implies that the normal function of gene of this type is to restrain cell growth and division. In other words, tumour suppressor genes act as brakes on the process of cell proliferation and inhibits tumour development. In many tumours these genes are lost or inactivated, thereby removing negative regulators of cell proliferation and contributing to the abnormal proliferation of tumour cells. Normally, the function of tumour suppressor gene is just opposite to oncogene. The first evidence of the activity of tumour suppressor gene came from somatic cell function experiment done by Henry Harris etal in 1969. The fusion of tumour cells with normal cell yields hybrids that contain chromosomes from both parents. Such hybrids are usually non-tumerogenic. Suppression of tumourigenicity by cell fusion indicates that genes derived from the normal cell definitely suppress the tumour development.

The first suppressor gene to be identified is involvement in hereditary retinoblastoma, a rare type of eye cancer that develops in children who have a family history of the disease. Such children inherit a chromosomal deletion in a specific region of one copy of chromosome 13. Although the deletion occurs in all cells, only a few in the retina actually become malignant because the initial deletion in chromosome 13 does not cause cancer by itself; for cancer to develop, a subsequent mutation must also occur in the same region of the homologous chromosome 13.

It has, therefore, been concluded that chromosome 13 contains a gene on homologous chromosome of a normal diploid cell where such gene normally functions to inhibit retinoblastomas. In inherited retinoblastoma one defected copy of gene is genetically transmitted. The loss of this single copy of gene is compensated by the identical second copy of the gene present on the same region of the second copy of chromosome 13. Therefore loss of a single copy of gene is not by itself sufl5cient to trigger tumour development, but retinoblastoma almost always develops in these individuals as a result of a second somatic mutation leading to further loss of the function of the remaining second copy of normal gene.

The gene lost in hereditary retinoblastoma is called RBI. It is a tumour suppressor gene that codes for the nuclear protein $p^{RB}$ that inhibits expression of a group of genes whose products are needed for uncontrolled cell proliferation. In hereditary retinoblastoma a defective or copy of the RBI gene is inherited from the affected person. Hence a lack of $p^{RB}$ resulting from loss of both copies of RBI (one due to deletion and other due to a second somatic mutation) can lead to uncontrolled proliferation which ultimately causes the development of retinoblastoma. In nonhereditary cases, two normal RBI genes are

inherited and retinoblastoma develops only if two somatic mutations in adult inactivate both copies of RBIin the same cell.

**Table 23.6:** Main classes of oncogenes categorised by nature of their Protein Products

| Nature of Protein Product | Examples of Oncogenes | Comments |
|---|---|---|
| Growth factors | it sis | Platelet-derived growth factor (PDGF) |
| Protein-tyrosine kinases | *erb B* | Membrane receptor of epidermal growth factor (EGF) |
| | *fms* | Membrane receptor for colony-stimulating factor-1 (CSF-1) |
| | *src, yes, fgr* | Membrane nonreceptor proteintyrosine kinases |
| Membrane-associated G proteins | *ras* | Membrane-associated GTP-binding protein |
| | *gsp* | $G_s$($\alpha$ sub-unit) |
| | *gip* | $G_1$($\alpha$ sub-unit) |
| Protein-serine/threonine kinases | *raf, mos* | Cytoplasmic protein-serine/threonine kinases |
| Transcription factors | *jun, fos* | Components of AP1 transcription factor |
| | *erb A* | Thyroid hormone receptor |

Following the discovery of the RBI gene several other tumour suppressor genes have been identified (Table 23.6). The second suppressor gene is $p^{53}$ which is frequently inactivated in a wide variety of human cancer including leukemia's, lymphomas, sarcomas, brain tumour and carcinomas of many tissues including breast, colon and lung. The $p^{53}$ protein is a nuclear transcriptional factor that switches on the activity of genes that arrest cells in the $G_1$ phase of the cell cycle. Normally, the production of the $p^{53}$ protein is stimulated when DNA is damaged due to exposure to ultraviolet ray or DNA damaging agents.

Hence $p^{53}$ appears to act like a molecular policeman that checks the cell for DNA damage and prevents the cell from proliferation if damage is detected. The loss of $p^{53}$ function allows the survival and reproduction of cells in which DNA damage has led to the production of oncogenes and/or the loss of other tumour suppressor genes. In addition to mediating cell cycle arrest $P^{53}$ is required to apoptosis induced by DJNA damage. Unrepaired DNA damage normally induces apoptosis that eliminates cells which might develop into cancer. Cells lacking p fail to undergo apoptosis.

This failure contributes to the resistance of many tumours to chemotherapy. The failure of function of p is thought to account for the high frequency of $p^{53}$ mutations that lead to inactivation of $p^{53}$. Like $p^{53}$, the INK4 is a tumour suppressor gene that prevents lung cancer. Similarly, two other tumour suppressor genes such as APC and DCC prevent

colon cancer. When these genes are deleted or mutated, such cancers develop. The product of RBI and INK4 tumour suppressor genes regulate cell cycle progression at the same point. These genes inhibit passage through the restriction point in $G_1$ by suppressing transcription of a number of genes involved in cell cycle progression and DNA synthesis.

A rare hereditary form of colon cancer, familial adenomatous polyposis, is produced due to inherited mutation of the APC gene. In this type of cancer hundreds of polyps or benign colon adenomas are produced within the colon of an individual. Some of these polyps are transformed into malignancy. Inactivated or mutated form of some additional tumour suppressor genes is also associated with the development of breast, ovarian and pancreatic carcinomas as well as in some rare inherited cancer syndromes such as Wilm's tumour (a childhood kidney tumour). The tumour suppressor gene of Wilm's tumour is WT1 which is frequently inactivated in Wilm's tumour. The product of WT1 gene appears to suppress transcription of a number of growth factor inducible genes.

## Prevention and Treatment of Cancer:

There is a general belief among the common people that cancer cannot be cured. Although this is partially true, it depends on several aspects of the patient and the time of detection. In many cases, when it is clinically detected then it is already late and it goes beyond the treatment.

Actually, cancer is a disease that ultimately has to be understood at the molecular and cellular level. In factmany cancers can be cured if they are detected at the early stage of its development. In case of hereditary cancer, regular testing may allow early detection. Therefore, whether cancer is curable or not is a debatable question. With the help of modern and sophisticated technology, cell biologists are alwaystrying to improve the methods for prevention and treatment of cancer. The first step in preventing cancer is to identify the agents that cause cancer. For example, it is already known that tobacco smoke causes cancer. So just to prevent the possibility of this type of lung cancer, it is advisable simply to avoid tobacco smoke. Similarly the discovery of carcinogenic properties of X-ray and sunlight suggests that individuals should avoid unnecessary medical X-ray and use protective lotions during long time exposure to sunlight. Epidemiological data also allow potential carcinogens to be identified in exposed human population. The epidemiological approach is based on comparison of cancer rates among various groups of people exposed to different environmental conditions. For example, when Japanese individuals move to the United States heir susceptibility to developing stomach and lung cancer changes to reflect the rates for such cancers in the United

States. Therefore, the comparison of the frequency of stomach and lung cancer in Japan, in the United States and in Japanese immigrants to the United States suggests that environmental factors play a prominent role in causing cancer. Epidemiological data have played an important role in identifying some of the environmental factors that may cause cancer.

The Ames test is a rapid screening method tor identifying potential carcinogens This method is based on the rationale that most carcinogens act as mutagens, it measures the ability of potential carcinogens to induce mutations in a strain of bacteria that lack the ability to synthesize the amino acids histidine. Each bacterial cell that has mutated to a form in which it no longer needs histidine will grow into a colony that can be counted. The number of colonies indicates the mutagenic potency of the substance to be tested. Chemicals to be tested m the Ames test are first incubated with a liver homogenate because many of the chemicals to which humans are exposed only become carcinogenic after they have undergone biochemicalmodification in the liver (Fig. 23.11) Cancer can be prevented in several other ways A person can modify his life style in order to reduce the risk of developing cancer. Change of life style sometimes requires minimizing the exposure to carcinogens. Tobacco smoking and extensive meat consumption are the probable causative factors of cancer. If any person method of treatment is most effective when the canceris detected at the early stage of development and when metastasis has not occurred. This method is not effective when the cancer has already been disseminated throughout the body by the process of metastasis.Therefore, early detection of cancer is very important for its treatment.

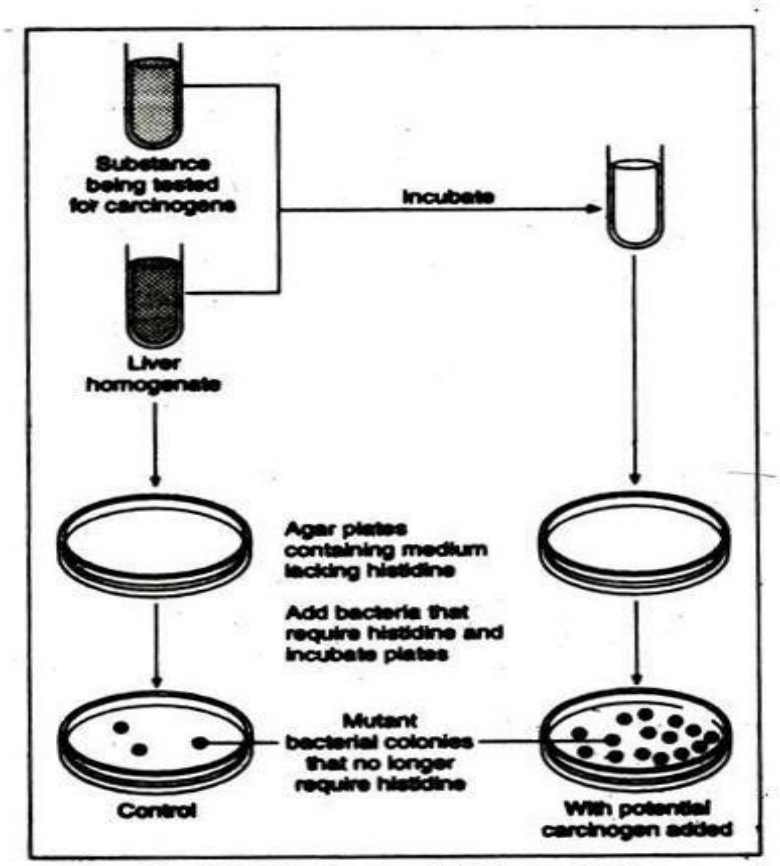


**Fig. 23.11:** Protocol of Ames Test.

Treatment of cancer-affected part of body with the help of X-radiation is another alternative method of curing cancer. X-ray is very effective for killing the cancer cells that are actively proliferating. The cells that are engaged in DNA synthesis prior to cell division, or are on the way of mitosis, are very sensitive to X-ray. But the main problem of using X- ray for the treatment of cancer is that normal and healthydividing cells of the body-such as blood-forming cells in the bone marrow are also destroyed along with the cancer cells. Moreover, X-radiation itself is carcinogenic. Hence there is always a chance of developing cancer after X-ray treatment. In spite of such risk posed by X-ray treatment, it is effective for the treatment of certain types of cancers like skin cancer, Hodgkin's disease and specific forms of testicular and bone cancer. Chemotherapy is another approach for treating cancer. This method is based onthe use of certain drugs that are designed to kill the proliferating cells as in radiation treatment. This method is also effective when the cancerous cells have already metastasized. The drugs are generally injected in the body and the circulatory system helps the drug to spread throughout the body. Some drugs used m cancer chemotherapy are given in the Table 23.7.

Table 23.7: Tumour Suppressor Genes

| Gene | Type of cancer |
|---|---|
| *APC* | Colon/rectum carcinoma |
| *BRCA1* | Breast and ovarian carcinomas |
| *BRCA2* | Breast carcinoma |
| *DCC* | Colon/rectum carcinoma |
| *DPC4* | Pancreatic carcinoma |
| *INK4* | Melanoma, lung carcinoma, brain tumours, leukemias, lymphomas |
| *NF1* | Neurofibrosarcoma |
| *NF2* | Meningioma |
| $P^{53}$ | Brain tumours, breast, colon/rectum, esophageal, liver, and lung carcinomas; leukemias and lymphomas |
| *Rb* | Retinoblastoma, sarcomas; bladder, breast, and lung carcinomas |
| *VHL* | Renal cell carcinoma |
| *WT1* | Wilm's tumour |

Like radiation, chemotherapeutic drugs also kill the normal and healthy cells along with cancer cells. This type of treatment has also some toxic side-effect-like loss of hair (caused by destruction of hair follicle cells), diarrhea (caused by destruction of cells of the intestinal lining) and susceptibility to infections (caused by destruction of blood cells). Sometimes two or more combination of drugs are also used for the treatment of cancer. Besides its side- effects and other disadvantages, it is true that, for certain types of cancer, chemotherapy is very successful for curing cancer like Burkitt s lymphoma, chorio carcinoma, acute lymphocytic leukemia, Hodgkin's disease, lymphomas, mycosis fungoides, Wilm tumour, Ewing s sarcoma, thabdomyosarcoma, retinoblastoma, and embryonal testicular tumours etc.

Table 23.8: Some drugs used in Cancer Chemotherapy

| Class | Examples | Mechanism of Action |
|---|---|---|
| 1. Antimetabolites | Methotrexate 5-Fluorouracil 6-Mercaptopurine | Inhibit enzymatic pathways for biosynthesis of nucleic acids by substituting for normal substrates |
| 2. Antibiotics (sybstances produced by microorganisms) | Actinomycin D Adriamycin Daunorubicin | Bind to DNA |
| 3. Alkylating agents | Nitrogen mustard Chlorambucil Cyclophosphamide Imidazole carboximides | Crosslink DNA |
| 4. Mitotic inhibitors | Vincristine Vinblastine Taxol | Interfere with mitotic spindle |
| 5. Hormones | Estrogen (for prostate cancer) Cortisone Progesterone Androgens | Inhibit growth of hormone-sensitive cells by interacting with hormone receptors |
| 6. Miscellaneous agents | L-Asparaginase | Hydrolyzes asparagine |

Although the use of surgery, radiation and chemotherapy has led to increased survival rates for certain kinds of cancer, many malignancies do not respond well to such treatment. Recent experimentation is attempting to exploit the ability of the immune system to recognize and kill tumour cells. This type of treatment is known as immunotherapy. The basic principle of immunotherapy is to exploit the ability of the immune system to recognise and kill tumour cells. Tumour cells tend to show cell surface antigens which make them recognisable by the immune system. Initially, some scientists attempted to utilise a person's own lymphocytes to kill cancer cells. For this experiment, lymphocytes were isolated from the blood of cancer patients and grown in culture in presence of Interlaken 2 to stimulate the cancersdestroying properties of the cell. The result was the isolation of a population of killer T-cells that were specifically targeted against the patient's tumour.

These cells, called tumour-infiltrating lymphocytes (TILs), were injected back into the patients from whom the blood was drawn. TILs axe more effective in inducing tumour regression. Recently TILs are made even more effective by using recombining DNA technique to insert some genes whose product enhances the additional potency of the TILs. A protein produced by march-panes called tumour necrosis factor (TNF) is effective in promoting the destruction of cancer cells if the TNF gene were inserted into the TILs. Obviously, the genetically engineered TILs would be more effective than normal TILs and would be more powerful to killing the tumour cells. Currently this technique is being tested in the hope of finding ways to promote immune destruction of cancer cells.

## Viral Cancers in Animals and Humans:

Viruses cause malignant tumors in animals, as such, tumors have been isolated from many animals such asfish, mice, rat, squirrels, dogs, deer and horses. The polyoma virus has been isolated from mice and the simian virus 40(SV 40) from monkeys.

Although we have no clear evidence that the viruses cause cancers in animals as no infective virus has been isolated from cell cultures, but some evidences are there to show the association of viruses with human cancers.

## At present, viruses have been implicated in the genesis of at least eight human cancers:

1. Electron microscopic and immunological studies show that there is association of Epstein-Barr virus (EB virus), a herpesvirus which is one of the best studied human cancer viruses, with Burkitt's lymphoma, a malignant tumour of Jaw and abdomen of children, occurring in certain regions of Africa.

2. EB virus has also been found associated with nasopharyngeal carcinoma found in certain Chinese populations.

3. Some strains of human papillomaviruses have been isolated from malignant tissues (not from normal tissues) from patients suffering from skin and cervical cancer.

4. Hepatitis B virus has been found associated with hepatocellular carcinoma (a type of liver cancer) and can be integrated into the human genome.
5. Hepatitis C virus results in cirrhosis of the liver that may lead to liver cancer.

6. Human herpesvirus-8 has been found associated with the development of Kaposi's sarcoma.

7. Human T-cell lymphotropic virus-1 (HTLV-1) seems able to cause T-cell leukemia.

8. Human T-cell lymphotropic virus-2 (HTLV-2) is found associated with hairy-cell leukemia.

## How Viruses Cause Human Cancer?

Although viruses are known to cause cancers in animals since many years, it is still uncertain that they cause cancers in humans. However, it is well established that some kinds of human tumors are strongly associated with infection by specific viruses.

The human T-cell lymphotropic viruses (HTLV-1 and HTLV-2) appear to transform T-cells into tumor cells by producing a regulatory protein that sometimes activates genes related to cell-division as well as virus reproduction. Some oncogenic viruses possess one or more very effective promoters or enhancers.

Whenever, these viruses integrate themselves next to an oncogene in cell-genome, the promoter orenhancer is thought to stimulate its transcription, resulting in a cancer. With the possible exception of HTLV-1, it is not yet known clearly how the viruses associated with human cancers actually aid in cancer development.

## DNA Oncogenic Viruses:

Oncogenic viruses are distributed in several families of DNA viruses. These include Herpesviridae, Poxviridae, Papovaviridae and Hepadnaviridae. The Herpesviridae include the Epstein-Barr Virus (EBV) which has been found as the cause of two forms of human cancers — Burkitt's lymphoma and nasopharyngeal carcinoma. EBV has also been implicated with Hodgkin's disease, a cancer of lymphatic system. Other herpes-viruses have been associated with human cancers of lip and cervix.

The Papilloma viruses belonging to the Papovaviridae cause benign tumours as well as cancer in several species including human. In humans, papilloma viruses cause uterine (cervical) cancer. Another member of Papovaviridae, the Simian Virus 40 (SV40) is among the best studied DNA tumour viruses. Natural host of SV 40 is cultured fibroblast cells of monkey. Such a cell culture is called permissive, because it allows viral multiplication and release of progeny viruses by cell lysis. On the other hand, when SV 40 is inoculated into non-permissive cell cultures e.g. the fibroblast cells of mice, the virus cannot multiply, but in a small number cells the viral DNA is, integrated with the host DNA causing their transformation into cancer cells. Due to integration into the host chromosome viral multiplication and cell lysis are absent. The phenomenon is comparable to lysogeny observed in temperate phage infection of bacteria. Integration of some DNA viruses is site-specific i.e. the viral DNA is inserted into a host chromosome at a specific site. But papova-viruses do not have such specificity and can be inserted at random. Hepatitis B virus (HBV) belonging to the Hepadnaviridae causes cancer of

liver. Many animal experiments have yielded results which clearly indicate a connection of HBV and liver cancer. Although direct proof is lacking in case of human beings, a survey revealed that all people with liver cancer had a previous infection of HBV.

## Oncogenic DNA Viruses:

### 1. Papova Viruses:

They induce benign warts and papilloma in natural hosts. Human papilloma virus (HPV) type 6 and 11 are incriminate in pre malignant lesions of the female and male genital tract SV 40 polyoma viruses produce tumours in mice when injected.

### 2. Herpes Virus:

Herpes simplex type 1 and 2 and Cytomegalovirus transform cultured cells at a very low frequency. They transformed hamster cells, when injected into another hamster, form tumours. In woman, Herpes simplex type 2 is responsible for cervical carcinoma.

No transforming gene is involved Epstein-Barr (EB) virus is associated with Burkitt's lymphoma in Africa and nasopharyngeal carcinoma in Chinese male population. It is believed that EB virus transforms normal lymphocytes into lymphoblast in immuno-compromised child.

### 3. Hepatitis B Virus:

It is implicated in hepato-cellular carcinoma. It does not carry any oncogene and is integrated in tumour cells as Hepatitis B virus DNA.

### 4. Pox Virus:

Yaba virus produces histiocytoma (benign tumour) in natural host (monkey) while shope fibroma virus induces fibroma in rabbit. Molluscum contagiosum virus produces benign growths in humans.

## RNA Oncogenic Viruses:

Among the RNA viruses only some members of the family Retro-viridae can cause cancer. Other RNA viruses which replicate by RNA replicase are non-oncogenic. Retroviruses which have a single- stranded RNA genome replicate via a double-stranded DNA produced by an RNA-dependent DNA polymerase (reverse transcriptase) and they

insert the DNA copy into the host chromosome as a provirus. Rous Sarcoma Virus (RSV) is of historical importance, because it was the first tumour-inducing virus to be studied. RSV is a retrovirus with a single-stranded RNA genome and its DNA copy is integrated into a specific site of the host chromosome as a provirus.

Research on RSV revealed identification of a cancer-inducing gene (an oncogene) in RSV genome. This gene, called src, is not essential for viral replication, as it does not code for any viral proteins. Later, it was discovered that a copy of the src gene is present in the host chromosome of normal cells and it was not oncogenic. Thus, the viral src gene which is oncogenic is derived from the host. How the non-oncogenic chromosomal src gene is converted to an oncogene in RSV is not clearly understood. It may occur through a mutation. The entry of a chromosomal gene into the viral genome possibly occurs through a process similar to that which operates in restricted transduction in bacteria.

It is thought that the RSV DNA produced through reverse transcription is inserted next to the chromosomal src gene and during transcription of the RSV RNA genome, src gene might be included. In this way, src gene might enter into the viral genome. RSV causes cancer in chicken. Similar retroviruses are known to cause cancer in other animals including monkey. But definite evidence of retroviruses causing cancer in humans was not available until 1980. In that year Gallo isolated a virus that could transform normal T-lymphocytes into cancerous T-lymphocytes causing a disease, called T-cell leukaemia.

The virus is known as Human T-cell Leukaemia Virus (HTLV). Later research during 1990s has confirmed the role of HTLV in causing human leukaemia. Another HTLV was later discovered causing hairy cell leukaemia in man. The malignant leucocytes develop hairy outgrowths on their surface. The second virus has been designated as HTLV-II. These retroviruses have been shown to transform normal T-cells by a regulatory protein which stimulates uncontrolled cell division. Besides leukaemia, HTLV is also known to cause neurological disorders, like spastic para-paresis. T-cell leukaemia is more or less restrictedin several countries, like Japan, West Indies and some parts of West Africa.

### i. Avian Sarcoma Leukosis Viral (ALV) Complex:

They are caused by antigenically related viruses which caused lymphoma, leukaemia and sarcoma.

### ii. Murine Leukosis Virus:

There are several strains of murine leukaemia and sarcoma viruses which are derived from Mo-Mu Lv during passages in mice and rats.

### iii. Mouse Mammary Tumour Viruses (MMTV):

They may be endogenous or exogenous viruses. The endogenous ones have no oncogenic role, whereas the exogenous ones are oncogenic. Mammary cancers develop only in susceptible females after a latent period of 6-12 months.

### iv. Sarcoma Leukaemia Viruses of Animals:

Leukaemia, lymphosarcoma in cat is induced by Feline leukaemia virus (Felv); Lymphosarcoma in cattleis due to bone leukaemia virus.

### v. Human T-cell Leukaemia Virus (HTLV):

Adult T-cell leukaemia is induced by HTLV- 1 but having cell leukaemia of T-cell type is due to HTLV- II.

## Probable Questions:

1. Classify Cancer on the basis of invasiveness and location.
2. Discuss Tumor suppressor gene's role in cancer control
3. Describe the role of oncogenes in cancer development?
4. What are the roles of cancer in cancer development?
5. Describe different DNA virus which causes cancer.
6. Describe different RNA virus which causes cancer.

## Suggested Readings:


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# UNIT-II

## Chromosomal abnormalities and molecular basis of cancer; Knudson's two-hit hypothesis in tumorigenesis; Genome instability in cancer; Epigenetics of Cancer; Cancer diagnosis, screening and treatment

**Objective:** In this unit we will discuss chromosomal basis of cancer. We will also discuss diagnosis, treatment approach of cancer and also role of epigenetics in cancer formation.

### What is Cancer?

Cancer is an abnormal and uncontrolled division of cells, known as cancer cells that invade and destroy the surrounding tissues. Generally cancer is defined as uncontrolled proliferation of cells without any differentiation. Cancer cells are different from normal cells in some aspects. They do not remain confined to one part of the body.

They penetrate and infiltrate into the adjoining tissues and dislocate their functions. Some of the cancer cells get detached from the main site of origin and travel by blood and lymph to sites distant from the original tumour and form fresh colonies, called metastasis or secondary growth.

### How Cancer Cells Differ from Normal Cells?

Normal cells have a limited life span. They are usually replaced by new cells through cell division and cell differentiation. Their production is regulated in such a manner that the number of a given cell type remains nearly constant. Normal cells show a property called contact inhibition.

Due to this property they contact with other cells, inhibit their uncontrolled growth. Cancer cells seem to have lost this property. But cancer cells do not respond to normal growth control mechanism. These cells proliferate in an unregulated manner and form clones of cells which can expand irregularly. This uncontrolled growth is called tumour or neoplasm.

**Types of Tumours:**
There are two types of tumours: benign and malignant.

**(i) Benign Tumour (= Non-malignant Tumour):**
It remains confined to the site of its origin and does not spread to other parts of the body. It causes limited damage to the body. It is non-cancerous.

**(ii) Malignant Tumour (= Cancerous Tumour):**
It first grows slowly. No symptoms are noticed. This stage is called the latent stage. The tumour later grows quickly. The cancer cells go beyond adjacent tissue and enter the blood and lymph. Once this happens, they migrate to many other sites in the body where the cancer cells continue to divide. A phenomenon in which cancer cells spread to distant sites through body fluids to develop secondary tumour is called metastasis. Only malignant tumours are properly designated as cancer.

Properties of Cancer Cells,

(i) Uncontrolled proliferative ability,

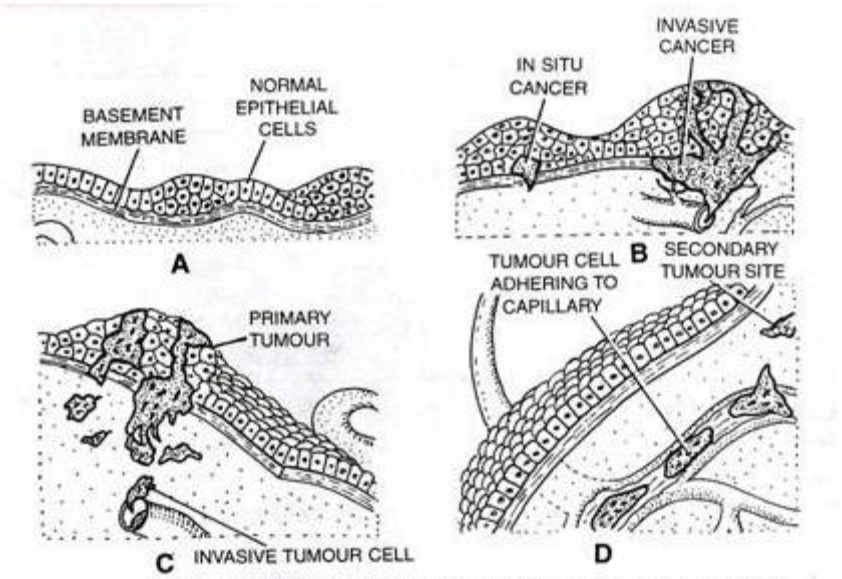
(ii) Extracellular growth factors are not required,

(iii) Overgrowth and ability to invade new sites (metastasis),

(iv) Nucleus becomes irregular with abundant granules,

(v) There is increase in number of lysosomes, reduction in mitochondrial cristae, more melanin and debris in cytoplasm,

(vi) Cancer cells resist induction of cell death which promotes development of tumours.



Stages in development of cancer. Primary tumour may become metastatic and get transformed into secondary tumour.

## Causes of Cancer:

Study of cancer cells is called oncology. Chemical and physical agents that can cause cancer are called carcinogens, which belong to three categories.

## (i) Oncogenic Transformations:

They are agents or factors which bring about changes in genetic material. They are of two types, radiations and chemicals.

## (ii) Tumour Promoters:

They promote proliferation of cells which have undergone oncogenic transformation, e.g., some growth factors, hormones.

## (iii) Tumour Viruses:

Some viruses are known to be connected with oncogenic transformations.

## Another classification of carcinogens is as follows:

## 1. Physical irritants:

(i) Use of Kangri (an earthen pot containing burning coal) by Kashmiris causes abdominal skin cancer as these people keep Kangri close to their abdomen during winter,

(ii) Betel and tobacco chewing causes oral cancer,

(iii) Heavy smoking causes lung cancer and may also cause cancer of oral cavity, pharynx (throat) and larynx,

(iv) Jagged teeth may cause tongue cancer,

(v) Excessive exposure to sun light can cause skin cancer.

## 2. Chemical Agents:

Several chemicals are known to cause cancer. These are caffeine, nicotine, products of combustion of coal and oil and pesticides; constant use of artificial sweetener can cause cancer. An animal protein-rich diet is known to cause cancer of large intestine.

Breast cancer has hormonal relationship. Thus, some sex hormones and steroids if secreted or given in large amounts may cause cancer. Chimney sweepers can develop cancer of scrotum. Dye workers have a high rate of bladder cancer.

## Carcinogens and Organs Affected:

## 3. Radiations:

The X-rays, cosmic rays, ultra-violet rays, etc. can cause cancer. Japanese people exposed to radiations from World War II nuclear bombing show five times the incidence of leukemia seen in the rest of the population.

## 4. Biological Agents:

Some viruses and other parasites, excessive secretion of certain hormones are believed to cause cancers.

## Cancer and Genes:

Cancer-associated genes are divided into the following three categories.

(i) Cancer causing viruses are called oncogenic viruses. The genes of oncogenic viruses are known as viral oncogenes. It is now held that all cells carry some cancer causing genes called oncogenes which when activated under certain conditions could change into oncogenic cells. Jumping genes are often involved in this conversion.

(ii) Tumour suppressor genes that inhibit cell proliferation.

(iii) Genes that regulate programmed cell growth.

## How Cancer Spreads?

Abnormal increase in number of cells in a tissue or organ forms a clone of proliferative cells. This excessive proliferation gives rise to a mass of cells which is initially known as benign tumour.

The benign tumour cells enter into the blood vessels and migrate to other sites in the body where these cells continue to divide, such tumour cells are known as malignant cells and tumours are called malignant tumours. The malignant tumours are designated as cancer.

## Detection and Diagnosis of Cancer:

It depends upon histological features of malignant structure,

(i) Bone marrow biopsy (a piece of the suspected tissue cut into thin sections is stained and examined under microscope by a pathologist) and abnormal count of WBCs in leukaemia,

(ii) Biopsy of tissue, direct or through endoscopy. Also endoscopic observation. Pap's test (cytological staining) is used for detecting cancer of cervix and other parts of genital tract,

(iii) Techniques such as radiography (use of X-rays), CT (computed tomography), MRI (magnetic resonance imaging) are very useful to detect cancers of the internal organs. In CT, X-rays are used to generate a three dimensional image of internal organs. In MRI strong magnetic fields and non-ionizing radiations are used to detect pathological and physiological changes in the living tissue. Antibodies against cancer specific antigens are also used for detection of certain cancers. Techniques of molecular biology can be applied to detect genes in individuals. Mammography is radiographic examination of breasts for possible cancer,

(iv) Monoclonal antibodies coupled to appropriate radioisotopes can detect cancer-specific antigens and hence cancer.

## Different Sites of Cancer:

Some of the important sites of cancer are skin, mouth, oesophagus, stomach, colon, rectum, liver, gall bladder, pancreas, blood, lymph, adipose tissue, lung, uterine cervix, breast, brain, penis, prostate, muscles, thyroid, kidney and bones.

## Possible Symptoms of Cancer:

(i) A persistent cough or hoarseness in a smoker,

(ii) A persistent change in digestive and bowel habits,

(iii) A change in a wart or mole,

(iv) A lump or hard area in the breast,

(v) Unexpected diminished or lost appetite,

(vi) Unexplained low- grade fever,

(vii) Unexplained loss of weight,

(viii) Any un-curable ulcer,

(ix) Bleeding in vagina at times other than the menstruation,

(x) Non-injury bleeding from the surface of the skin, mouth or any other opening of the body.

## Treatment of Cancer:

## Four general methods of treatment for cancer are currently available.

### 1. Surgery:

It involves the removal of the entire cancerous tissue.

### 2. Radiation therapy:

It involves the exposure of the cancerous parts of the body to X-rays which destroy rapidly growing cells without harming the surrounding tissue. Radon (Rn-220), Cobalt (Co-60) and Iodine (1-131) are radioisotopes which are generally used in radiotherapy.

### 3. Chemotherapy:

It involves the administration of certain anticancer drugs. These drugs check cell division by inhabiting DNA synthesis. These drugs may be more toxic to cancerous cells than to normal cells. Thus chemotherapeutic drugs kill cancerous cells. Majority of drugs have side effects like hair loss, anaemia etc.

Most cancers are treated by combination of surgery, radiotherapy and chemotherapy. Patients are given substances called biological response modifiers like a interferon which activates their immunity system and help in destroying the tumours. A common weed Catharanthus roseus is the source of two anticancer drugs, Vinblastine and Vincristine used in the treatment of leukaemia.

## 4. Immunotherapy:

It involves natural anti-cancer immunological defence mechanisms. Monoclonal antibodies are used in various ways, e.g., radio-immunotherapy for treatment of cancer. These therapies can be used either singly, or in a suitable combination. Efforts are being made to develop cancer vaccines.

## Two kinds of genes have been found to be associated with carcinogenesis:

These comprise tumor – suppressor genes and oncogenes.

## 1. Tumor – suppressor genes:

These are anti-oncogenes and encode proteins which restrain cell growth and prevent cells from becoming malignant. First tumor – suppressor gene resulting in eye cancer called retinoblastoma was discovered, designated as RB. It was caused due to deletion in one member of 13th pair of chromosome. Other cancers caused by tumor-suppressor genes include colon carcinoma, nephroblastoma, neurofibromas and thyroid carcinoma etc.

## 2. Oncogenes:

These genes encode proteins which promote the loss of growth control and conversion of a cell to a malignant state. The existence of oncogenes was discovered by investigations on RNA tumor viruses. An oncogene called Src which was carried by a RNA tumor virus – avian sarcoma virus (ASV) was actually present in the genome of uninfected cells.

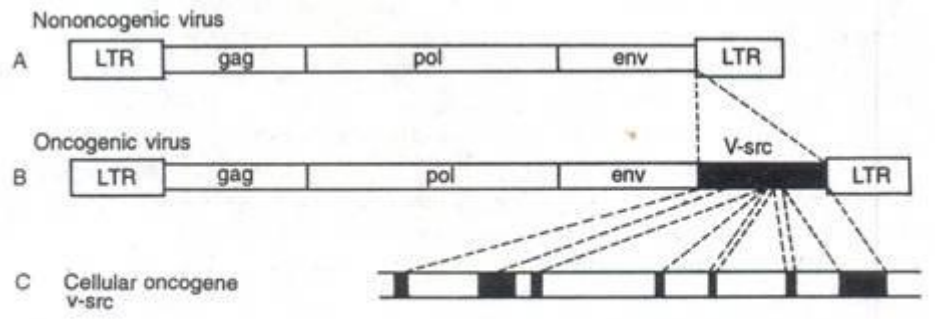



Fig. 1. Structure of (A) a retrovirus, (B) a retrovirus that has picked up oncogene *V-Svc*, and (C) cellular version of same oncogene *C-Svc*.

Therefore, cells possess a variety of genes — proto-oncogenes which have the potential to convert cell's activities toward malignant state.

A retrovirus has only three genes required for its life cycle and has terminal repeats at its ends. Some retroviruses, the oncogenic ones (i.e., producing cancer cells), carry an extra gene that induces cancer in animals. Many oncogenic viruses have been isolated, each one

having one oncogene, and these have been shown to be able to carry over 20 different oncogenes. Many independently isolated viruses have been found to carry the same gene.

The Src gene of Rous sarcoma virus codes for a plasma-membrane- bound protein kinase that phosphorylates the amino acid tyrosine on proteins on the cell membrane. In 1968 Huebner and Todaro predicted that normal cells contained enemies within in the form of oncogenes similar to the viral one, which could produce cancer when activated.

About 100 different oncogenes have been identified.

## These genes cause cancer in many ways as shown below:

## (a) Oncogenes encoding growth factor or their receptors:

Cancer-causing simian sarcoma virus (SIS) contained a gene SIS that codes for platelet-derived growth factor (PDGF) and secretes large amount of PGDF into blood and causing cells to proliferate in an uncontrolled fashion. Another oncogene erbB directs the formation of an altered EGF receptor and thus causing human skin cancer.

## (b) Oncogenes that encode cytoplasmic protein kniases:

There are large number of oncogenes which are responsible for the synthesis of several protein kinases resulting in cancer. These include Raf responsible for serine – threonine kniase. Another oncogene Src is also a protein kinase which phosphorylates tyrosine residues on protein substrates.

## (c) Oncogenes which encode nuclear transcription factors:

Among this category, there are oncogene myc responsible for carcinoma of lung, cervix and breast. Large amount of Myc protein is synthesized resulting in uncontrolled cell proliferation.

## (d) Oncogenes that encode products affecting apoptosis:

Apoptosis is one of the body's mechanisms to rid itself of tumor cells at an early stage of malignancy. Oncogenes — bcl-2 is closely related to apoptosis and encodes membrane-bound protein which inhibits apoptosis in certain tissues.

## Proto-oncogene:

All 20 viral oncogenes derive from cellular genes in normal cells. The normal cellular version of the gene is called a proto-oncogene. Retroviruses pick up into their genome sequences from mRNA population that will increase viral production (by increasing cell proliferation). These viruses can transmit genes from one species to another, thus breaking evolutionary barriers (Bishop, 1983).

Another agent that stimulates proliferation, epidermal growth factor (EGF), like Svc, increases phosphorylation of membrane proteins at tyrosines. It is thought that this tyrosine phosphorylation somehow controls cell proliferation. Cancer cells (also called transformed cells) contain ten times more phosphotyrosine than normal cells.

About half of the oncogenes code for Tyr protein kinases and in all cases the proteins are integral components of the cell membrane. Most oncogenes seem to be related in one way or another with the same pathway of regulation of cell proliferation by protein growth factors. EGF stimulates lung development and differentiation. In intact organisms, the production of growth factor induces multiplication at a short distance only, stimulating the cell that secretes it and the nearby cells. This is sometimes called autocrine secretion. When EGF reaches the membrane of a target cell it binds to a specific receptor protein of 170,000 Daltons, and the complex is subsequently internalized.

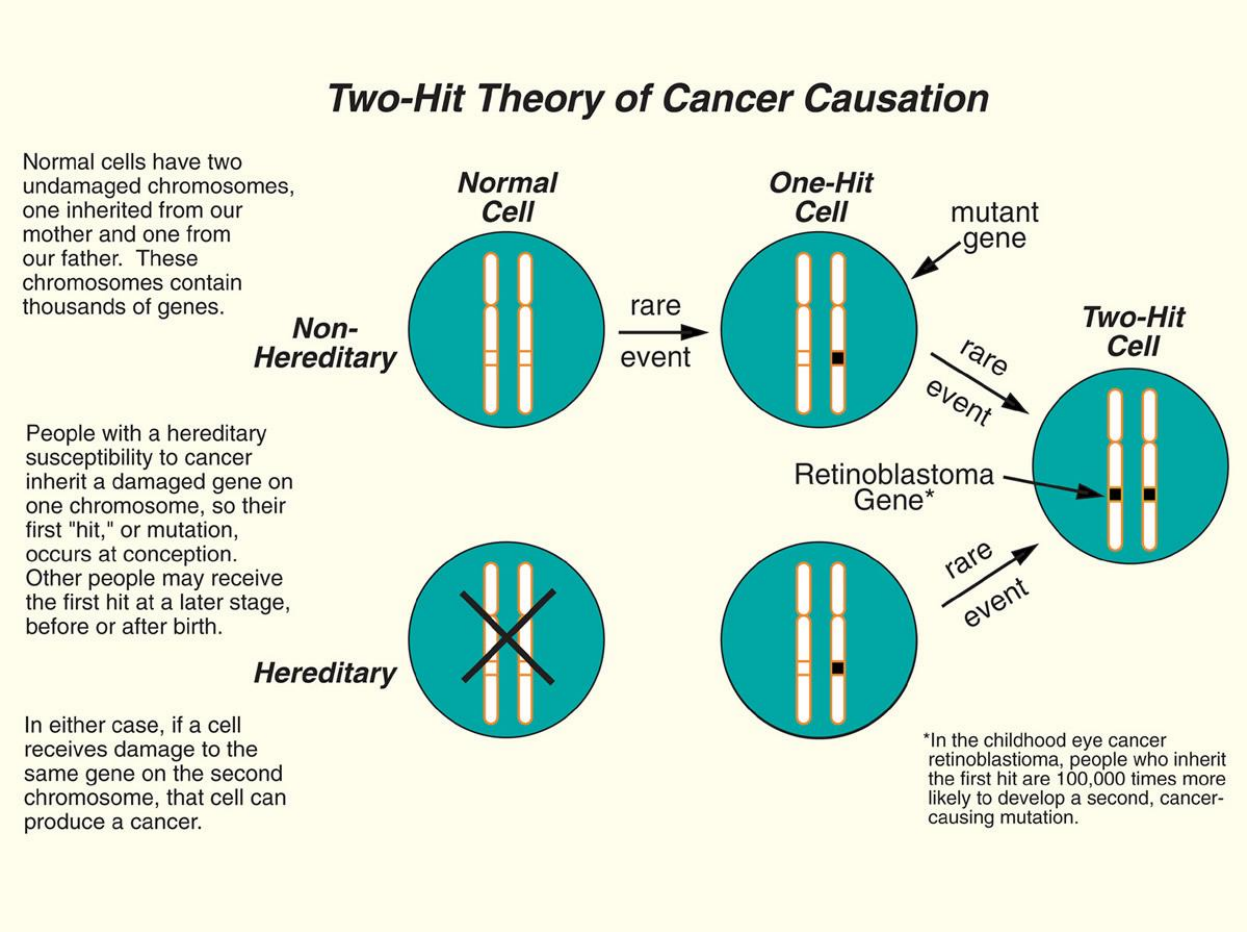
The receptor protein becomes phosphorylated during this process and the phosphorus binds to tyrosine. Phosphorylation of tyrosine is rare in proteins except in cells that have been transformed by retroviruses and become cancerous. Both in EGF and in viral transformation phosphorylation of tyrosine results in increased cell proliferation. Cells start dividing only after many hours of adding the growth factors. Growth inhibitors, called chalones (Gr., to slow down) are numerous, which have been isolated from tissues. These are also numerous and important as growth factors.

## Knudson's Two Hit Hypothesis:

The **Knudson hypothesis**, also known as the **two-hit hypothesis**, is the hypothesis that most tumor suppressor genes require both alleles to be inactivated, either through mutations or through epigenetic silencing, to cause a phenotypic change.[1] It was first formulated by Alfred G. Knudson in 1971 and led indirectly to the identification of tumor suppressor genes. Knudson won the 1998 Albert Lasker Clinical Medical Research Award for this work.

Knudson performed a statistical analysis on cases of retinoblastoma, a tumor of the retina that occurs both as an inherited disease and sporadically. He noted that inherited retinoblastoma occurs at a younger age than the sporadic disease. In addition, the children with inherited retinoblastoma often developed the tumor in both eyes, suggesting an underlying predisposition. Knudson suggested that two "hits" to DNA were necessary to cause the cancer. In the children with inherited retinoblastoma, the first mutation in what later came to be identified as the RB1 gene, was inherited, the second one acquired. In non-inherited retinoblastoma, instead two mutations, or "hits", had to take place before a tumor could develop, explaining the later onset.

It was later found that carcinogenesis (the development of cancer) depended both on the mutation of proto-oncogenes (genes that stimulate cell proliferation) and on the inactivation of tumor suppressor genes, which are genes that keep proliferation in check. Knudson's hypothesis refers specifically, however, to the heterozygosity of tumor suppressor genes. An inactivation of both alleles is required, as a single functional tumor suppressor gene is usually sufficient. Some tumor suppressor genes have been found to be "dose-dependent" so that inhibition of one copy of the gene (either via genetic or epigenetic modification) may encourage a malignant phenotype, which is termed haploinsufficiency.



## EPIGENETICS AND CANCER:

Chromatin structure defines the state in which genetic information in the form of DNA is organized within a cell. This organization of the genome into a precise compact structure greatly influences the abilities of genes to be activated or silenced. Epigenetics, originally defined by C.H.Waddington as 'the causal interactions between genes and their products, which bring the phenotype into being', involves understanding chromatin structure and its impact on gene function. Waddington's definition initially referred to the role of epigenetics in embryonic development; however, the definition of epigenetics has evolved over time as it is implicated in a wide variety of biological processes. The current definition of epigenetics is 'the study of heritable changes in gene expression that occur independent of changes in the primary DNA sequence'. Most of these heritable changes are established during differentiation and are stably maintained through multiple cycles of cell division, enabling cells to have distinct identities while containing the same genetic information. This heritability of gene expression patterns is mediated by epigenetic modifications, which include methylation of cytosine bases in DNA, posttranslational modifications of histone proteins as well as the positioning of nucleosomes along the DNA. The complement of these modifications, collectively referred to as the epigenome, provides a mechanism for cellular diversity by regulating what genetic information can be accessed by cellular machinery. Failure of the proper maintenance of heritable epigenetic marks can result in inappropriate activation or inhibition of various signaling pathways and lead to disease states such as cancer.

Recent advances in the field of epigenetics have shown that human cancer cells harbor global epigenetic abnormalities, in addition to numerous genetic alterations. These genetic and epigenetic alterations interact at all stages of cancer development, working together to promote cancer progression. The genetic origin of cancer is widely accepted; however, recent studies suggest that epigenetic alterations may be the key initiating events in some forms of cancer. These findings have led to a global initiative to understand the role of epigenetics in the initiation and propagation of cancer. The fact that epigenetic aberrations, unlike genetic mutations, are potentially reversible and can be restored to their normal state by epigenetic therapy makes such initiatives promising and therapeutically relevant.

In this review, we take a comprehensive look at the current understanding of the epigenetic mechanisms at work in normal mammalian cells and their comparative aberrations that occur during carcinogenesis. We also discuss the idea of cancer stem cells as the originators of cancer and the prospect of epigenetic therapy in designing efficient strategies for cancer treatment.

## Epigenetic mechanisms in normal cells

Chromatin is made of repeating units of nucleosomes, which consist of ~146 base pairs of DNA wrapped around an octamer of four core histone proteins (H3, H4, H2A and H2B). Epigenetic mechanisms that modify chromatin structure can be divided into four main categories: DNA methylation, covalent histone modifications, non-covalent mechanisms such as incorporation of histone variants and nucleosome remodeling and non-coding RNAs including microRNAs (miRNAs). These modifications work together to regulate the functioning of the genome by altering the local structural dynamics of chromatin, primarily regulating its accessibility and compactness. The interplay of these modifications creates an 'epigenetic landscape' that regulates the way the mammalian genome manifests itself in different cell types, developmental stages and disease states, including cancer. The distinct patterns of these modifications present in different cellular states serve as a guardian of cellular identity. Here, we will discuss the important aspects of the key epigenetic mechanisms present in normal cells.

## DNA methylation:

DNA methylation is perhaps the most extensively studied epigenetic modification in mammals. It provides a stable gene silencing mechanism that plays an important role in regulating gene expression and chromatin architecture, in association with histone modifications and other chromatin associated proteins. In mammals, DNA methylation primarily occurs by the covalent modification of cytosine residues in CpG dinucleotides. CpG dinucleotides are not evenly distributed across the human genome but are instead concentrated in short CpG-rich DNA stretches called 'CpG islands' and regions of large repetitive sequences (e.g. centromeric repeats, retrotransposon elements, rDNA etc.). CpG islands are preferentially located at the 5′ end of genes and occupy ~60% of human gene promoters. While most of the CpG sites in the genome are methylated, the majority of CpG islands usually remain unmethylated during development and in differentiated tissues. However, some CpG island promoters become methylated during development, which results in long-term transcriptional silencing. X-chromosome inactivation and imprinted genes are classic examples of such naturally occurring CpG island methylation during development. Some tissue-specific CpG island methylation has also been reported to occur in a variety of somatic tissues, primarily at developmentally important genes. In contrast, the repetitive genomic sequences that are scattered all over the human genome are heavily methylated, which prevents chromosomal instability by silencing non-coding DNA and transposable DNA elements. DNA methylation can lead to gene silencing by either preventing or promoting the recruitment of regulatory proteins to DNA. For example, it can inhibit transcriptional activation by blocking transcription factors from accessing target-

binding sites e.g. c-myc and MLTF. Alternatively, it can provide binding sites for methyl-binding domain proteins, which can mediate gene repression through interactions with histone deacetylases (HDACs). Thus, DNA methylation uses a variety of mechanisms to heritably silence genes and non-coding genomic regions.

The precise DNA methylation patterns found in the mammalian genome are generated and heritably maintained by the cooperative activity of the *de novo* methyltransferases—DNMT3A and DNMT3B, which act independent of replication and show equal preference for both unmethylated and hemimethylated DNA and the maintenance DNA methyltransferase—DNMT1, which acts during replication preferentially methylating hemimethylated DNA. While the role of CpG island promoter methylation in gene silencing is well established, much less is known about the role of methylation of non-CpG island promoters. Recent studies have shown that DNA methylation is also important for the regulation of non-CpG island promoters. For example, tissue-specific expression of MASPIN, which does not contain a CpG island within its promoter, is regulated by DNA methylation. Similarly, methylation of the non-CpG island Oct-4 promoter, strongly influences its expression level. Since CpG islands occupy only ~60% of human gene promoters, it is essential to elucidate the role of non-CpG island methylation in order to fully understand the global role of DNA methylation in normal tissue.

## Covalent histone modifications:

Histone proteins, which comprise the nucleosome core, contain a globular C-terminal domain and an unstructured N-terminal tail. The N-terminal tails of histones can undergo a variety of posttranslational covalent modifications including methylation, acetylation, ubiquitylation, sumoylation and phosphorylation on specific residues. These modifications regulate key cellular processes such as transcription, replication and repair. The complement of modifications is proposed to store the epigenetic memory inside a cell in the form of a 'histone code' that determines the structure and activity of different chromatin regions. Histone modifications work by either changing the accessibility of chromatin or by recruiting and/or occluding non-histone effector proteins, which decode the message encoded by the modification patterns. The mechanism of inheritance of this histone code, however, is still not fully understood.

Unlike DNA methylation, histone modifications can lead to either activation or repression depending upon which residues are modified and the type of modifications present. For example, lysine acetylation correlates with transcriptional activation, whereas lysine methylation leads to transcriptional activation or repression depending upon which residue is modified and the degree of methylation. For example, trimethylation of lysine 4

on histone H3 (H3K4me3) is enriched at transcriptionally active gene promoters, whereas trimethylation of H3K9 (H3K9me3) and H3K27 (H3K27me3) is present at gene promoters that are transcriptionally repressed. The latter two modifications together constitute the two main silencing mechanisms in mammalian cells, H3K9me3 working in concert with DNA methylation and H3K27me3 largely working exclusive of DNA methylation (Figure 1). A vast array of active and repressive histone modifications have been identified, which constitute a complex gene regulatory network essential for the physiological activities of cells. Genome-wide studies showing distinct localization and combinatorial patterns of these histone marks in the genome have significantly increased our understanding of how these diverse modifications act in a cooperative manner to regulate global gene expression patterns.

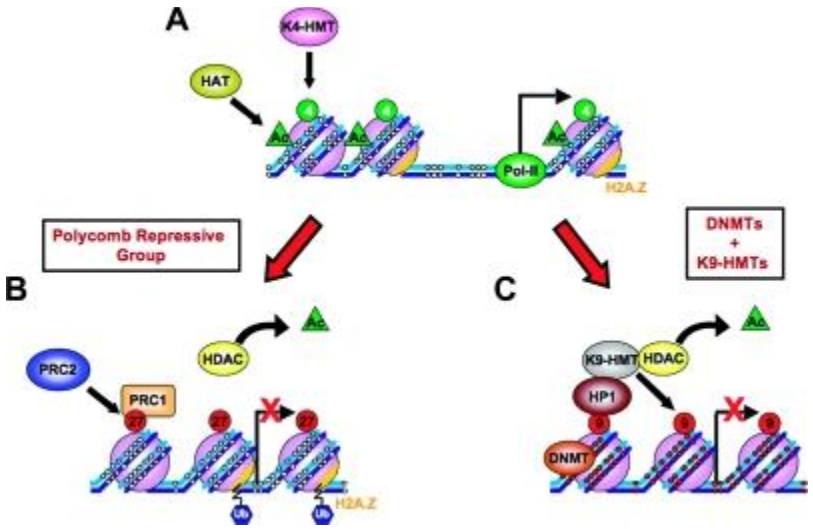



Fig. 1.

Epigenetic gene silencing mechanisms in mammals. (**A**) An active gene shows an open chromatin structure consisting of an unmethylated promoter region (small white circles on DNA strands), with no nucleosome upstream of the transcription start site (thick black arrow), an enrichment of active histone marks such as acetylation (green triangle, Ac) and H3K4 methylation (green circles, 4) and high levels of H2A.Z on nucleosomes (orange) surrounding the transcription start site. The open chromatin structure is permissible for binding of transcription factors and RNA Pol-II, which mediates active transcription on such promoters. Repression of such active genes (indicated by red arrows) can be achieved in normal cells by two main mechanisms: (**B**) Gene repression by the action of PRC1 and PRC2 that mediate the repressive H3K27 methylation (red circles, 27) is accompanied by the removal of acetylation by HDACs, loss of H3K4 methylation, chromatin compaction, nucleosome occupancy in the NFR and ubiquitylation of H2A.Z; (**C**) Long-term silencing through DNA methylation is performed by DNA methyltransferases. DNA methylation

(small red circles on DNA strands) is often accompanied by the repressive H3K9 methylation (red circles, 9), on promoters, which leads to chromatin compaction by recruitment of HP1. DNA Methylated-silenced promoters show a depletion of H2A.Z, loss of H3K4 methylation and histone de-acetylation. Ac, acetylation; EZH2, enhancer of zeste homolog 2; HP1, heterochromatin protein 1; K4-HMT, Histone H3 lysine 4 histone methyltransferase; K9-HMT, Histone H3 lysine 9 histone methyltransferase; Pol-II, RNA polymerase II; PRC1 and PRC2, polycomb repressive complex 1 and 2; Ub, ubiquitination.

Specific patterns of histone modifications are present within distinct cell types and are proposed to play a key role in determining cellular identity. For example, embryonic stem (ES) cells possess 'bivalent domains' that contain coexisting active (H3K4me3) and repressive (H3K27me3) marks at promoters of developmentally important genes. Such bivalent domains are established by the activity of two critical regulators of development in mammals: the polycomb group that catalyzes the repressive H3K27 trimethylation mark and is essential for maintaining ES cell pluripotency through silencing cell fate-specific genes and potentially the trithorax group that catalyzes the activating H3K4 trimethylation mark and is required for maintaining active chromatin states during development. This bivalency is hypothesized to add to phenotypic plasticity, enabling ES cells to tightly regulate gene expression during different developmental processes. Differentiated cells lose this bivalency and acquire a more rigid chromatin structure, which may be important for maintaining cell fate during cellular expansion. This hypothesis is supported by the recent discovery of large condensed chromatin regions containing the repressive H3K9me2 mark, termed 'LOCKs' (large organized chromatin K9 modifications), in differentiated ES cells that can maintain silencing of large genomic regions in differentiated tissues.

Histone modification patterns are dynamically regulated by enzymes that add and remove covalent modifications to histone proteins. Histone acetyltransferases (HATs) and histone methyltransferases (HMTs) add acetyl and methyl groups, respectively, whereas HDACs and histone demethylases (HDMs) remove acetyl and methyl groups, respectively. A number of histone-modifying enzymes including various HATs, HMTs, HDACs and HDMs have been identified in the past decade. These histone-modifying enzymes interact with each other as well as other DNA regulatory mechanisms to tightly link chromatin state and transcription.

## Interplay of DNA methylation and histone modifications:

In addition to performing their individual roles, histone modifications and DNA methylation interact with each other at multiple levels to determine gene expression status, chromatin organization and cellular identity. Several HMTs, including G9a, SUV39H1

and PRMT5, can direct DNA methylation to specific genomic targets by directly recruiting DNA methyltransferases (DNMTs) to stably silence genes. In addition to the direct recruitment of DNMTs, HMTs and demethylases also influence DNA methylation levels by regulating the stability of DNMT proteins. DNMTs can in turn recruit HDACs and methyl-binding proteins to achieve gene silencing and chromatin condensation. DNA methylation can also direct H3K9 methylation through effector proteins, such as MeCP2, thereby establishing a repressive chromatin state. The interactions between DNA methylation machinery and histone modifying enzymes further enhance the complexity of epigenetic regulation of gene expression, which determines and maintains cellular identity and function.

## Nucleosome positioning and histone variants:

Non-covalent mechanisms, such as nucleosome remodeling and replacement of canonical histone proteins with specialized histone variants, also play an important role in how chromatin structure regulates gene activity. In addition to serving as the basic modules for DNA packaging within a cell, nucleosomes regulate gene expression by altering the accessibility of regulatory DNA sequences to transcription factors. Genome-wide nucleosome mapping data for various eukaryotic organisms reveal a common organizational theme with precise positioning of nucleosomes around gene promoters, compared with the relatively random pattern found in gene bodies. Nucleosome-free regions (NFRs) present at the 5′ and 3′ ends of genes are thought to provide the sites for assembly and disassembly of the transcription machinery. The loss of a nucleosome directly upstream of the transcription start site is tightly correlated with gene activation. Furthermore, the presence of an NFR at gene promoters with basal level of transcription correlates with their ability for rapid activation upon stimulation. In contrast, occlusion of the transcription start site within the NFR by a nucleosome is associated with gene repression. Modulation of the NFRs is orchestrated by ATP-dependent chromatin-remodeling complexes, which modify the accessibility of DNA regulatory sites through both sliding and ejection of nucleosomes. The interaction of nucleosome remodeling machinery with DNA methylation and histone modifications plays a pivotal role in establishing global gene expression patterns and chromatin architecture (Figures 1 and and2).

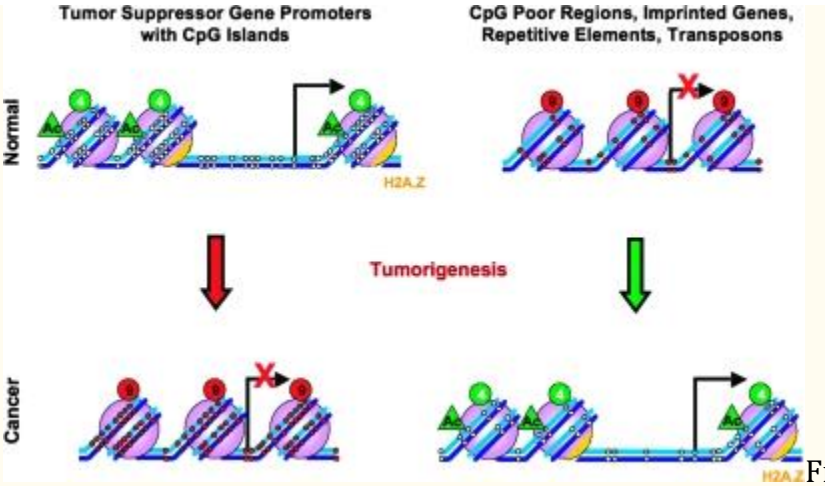


Fig. 2.

DNA methylation changes in cancer. In normal cells, CpG island promoters are generally unmethylated and when active, as in the case of tumor suppressor genes, are accompanied by active histone marks such as acetylation and H3K4 methylation (green circles, 4) allowing for a transcriptionally active open chromatin structure. However, repetitive regions, transposons, CpG poor intergenic regions and imprinted gene promoters are heavily methylated and accompanied by repressive histone marks such as H3K9 methylation (red circles, 9) that together form a silent chromatin state. During tumorigenesis, tumor suppressor gene promoters with CpG islands become methylated, resulting in the formation of silent chromatin structure and aberrant silencing (indicated by the red arrow). In contrast, the repetitive sequences, transposons and imprinted gene promoters become hypomethylated resulting in their aberrant activation (indicated by the green arrow).

In addition to physical alterations in nucleosomal positioning via nucleosome remodelers, the incorporation of histone variants, e.g. H3.3 and H2A.Z, into nucleosomes also influences nucleosome occupancy and thus gene activity. Unlike the major histone subtypes whose synthesis and incorporation is coupled to DNA replication in S phase, these variants are synthesized and incorporated into chromatin throughout the cell cycle. H3.3 and H2A.Z are preferentially enriched at promoters of active genes or genes poised for activation and can mediate gene activation by altering the stability of nucleosomes. H2A.Z incorporation may also contribute to gene activation by protecting genes against DNA methylation. In ES cells, H2A.Z colocalizes with bivalent domains where it may assist in maintaining key developmental genes in a poised state. Like canonical histones, histone variants undergo various posttranslational modifications, which determine their nuclear localization and function. For example, acetylated H2A.Z primarily associates with active genes in euchromatin, whereas ubiquitylated H2A.Z associates with facultative heterochromatin. Taken together, the inclusion of histone variants within nucleosomes provides an

additional epigenetic mechanism utilized by cells to modify chromatin structure according to the needs of diverse cellular processes.

## miRNAs:

miRNAs are small, ~22 nt, non-coding RNAs that regulate gene expression through posttranscriptional silencing of target genes. Sequence-specific base pairing of miRNAs with 3′ untranslated regions of target messenger RNA within the RNA-induced silencing complex results in target messenger RNA degradation or inhibition of translation. miRNAs are expressed in a tissue-specific manner and control a wide array of biological processes including cell proliferation, apoptosis and differentiation. The list of miRNAs identified in the human genome and their potential target genes is growing rapidly, demonstrating their extensive role in maintaining global gene expression patterns. Like normal genes, the expression of miRNAs can be regulated by epigenetic mechanisms. In addition, miRNAs can also modulate epigenetic regulatory mechanisms inside a cell by targeting enzymes responsible for DNA methylation (DNMT3A and DNMT3B) and histone modifications (EZH2). Such interaction among the various components of the epigenetic machinery re-emphasizes the integrated nature of epigenetic mechanisms involved in the maintenance of global gene expression patterns.

## Aberrant reprogramming of the epigenome in cancer:

The precise epigenomic landscape present in normal cells undergoes extensive distortion in cancer. These epimutations, along with widespread genetic alterations, play an important role in cancer initiation and progression. The cancer epigenome is characterized by global changes in DNA methylation and histone modification patterns as well as altered expression profiles of chromatin-modifying enzymes. These epigenetic changes result in global dysregulation of gene expression profiles leading to the development and progression of disease states. Epimutations can lead to silencing of tumor suppressor genes independently and also in conjunction with deleterious genetic mutations or deletions; thus, serving as the second hit required for cancer initiation according to the ‘two-hit’ model proposed by Alfred Knudson. In addition to inactivating tumor suppressors, epimutations can also promote tumorigenesis by activating oncogenes. The events that lead to initiation of these epigenetic abnormalities are still not fully understood. Nevertheless, since epigenetic alterations, like genetic mutations, are mitotically heritable, they are selected for in a rapidly growing cancer cell population and confer a growth advantage to tumor cells resulting in their uncontrolled growth.

## DNA methylation aberrations in cancer:

Cancer initiation and progression are accompanied by profound changes in DNA methylation that were the first epigenetic alterations identified in cancer. A cancer epigenome is marked by genome-wide hypomethylation and site-specific CpG island promoter hypermethylation (Figure 2). While the underlying mechanisms that initiate these global changes are still under investigation, recent studies indicate that some changes occur very early in cancer development and may contribute to cancer initiation.

Global DNA hypomethylation plays a significant role in tumorigenesis and occurs at various genomic sequences including repetitive elements, retrotransposons, CpG poor promoters, introns and gene deserts. DNA hypomethylation at repeat sequences leads to increased genomic instability by promoting chromosomal rearrangements. Hypomethylation of retrotransposons can result in their activation and translocation to other genomic regions, thus increasing genomic instability. Induction of genomic instability by hypomethylation is best exemplified in patients with the immunodeficiency, centromeric region instability and facial anomalies syndrome, which have a germ line mutation in the DNMT3B enzyme resulting in hypomethylation and subsequent chromosomal instability. Similar loss of DNA methylation and genomic instability is implicated in a variety of human cancers. In addition, DNA hypomethylation can lead to the activation of growth-promoting genes, such as *R-Ras* and *MAPSIN* in gastric cancer, *S-100* in colon cancer and *MAGE* (melanoma-associated antigen) in melanoma, and a loss of imprinting (LOI) in tumors. In Wilms' tumor, hypomethylation-induced LOI of *IGF2*, an important autocrine growth factor, results in its pathological biallelic expression. LOI of *IGF2* has also been linked with an increased risk of colorectal cancer. Thus, DNA hypomethylation leads to aberrant activation of genes and non-coding regions through a variety of mechanisms that contributes to cancer development and progression.

In contrast to hypomethylation, which increases genomic instability and activates proto-oncogenes, site-specific hypermethylation contributes to tumorigenesis by silencing tumor suppressor genes. Since the initial discovery of CpG island hypermethylation of the *Rb* promoter (a tumor suppressor gene associated with retinoblastoma), various other tumor suppressor genes, including *p16*, *MLH1* and *BRCA1*, have also been shown to undergo tumor-specific silencing by hypermethylation. These genes are involved in cellular processes, which are integral to cancer development and progression, including DNA repair, cell cycle, cell adhesion, apoptosis and angiogenesis. Epigenetic silencing of such tumor suppressor genes can also lead to tumor initiation by serving as the second hit in the Knudson's two-hit model. In addition to direct inactivation of tumor suppressor genes, DNA hypermethylation can also indirectly silence additional classes of genes by silencing

transcription factors and DNA repair genes. Promoter hypermethylation-induced silencing of transcription factors, such as *RUNX3* in esophageal cancer and *GATA-4* and *GATA-5* in colorectal and gastric cancers, leads to inactivation of their downstream targets. Silencing of DNA repair genes (e.g. *MLH1*, *BRCA1* etc.) enables cells to accumulate further genetic lesions leading to the rapid progression of cancer.

While the ability of DNA hypermethylation to silence tumor suppressor genes in cancer is well established, how genes are targeted for this aberrant DNA methylation is still unclear. One possibility is that silencing specific genes by hypermethylation provides a growth advantage to cells resulting in their clonal selection and proliferation. Tumor-specific CpG island methylation can occur through a sequence-specific instructive mechanism by which DNMTs are targeted to specific genes by their association with oncogenic transcription factors. Aberrant hypermethylation and silencing of specific target gene promoters by the PML–RAR fusion protein in acute promyelocytic leukemia is an example of such a mechanism. Large stretches of DNA can become abnormally methylated in cancer causing some CpG islands to be hypermethylated as a result of their location inside such genomic regions that have undergone large-scale epigenetic reprogramming. Another interesting mechanism proposes a role of histone marks in the tumor-specific targeting of *de novo* methylation and will be discussed in detail in the next section. Interestingly, regions that are hypermethylated in cancer are often pre-marked with H3K27me3 polycomb mark in ES cells (Figure 3) suggesting a link between the regulation of development and tumorigenesis. This observation also partially explains the theory of 'CpG island methylator phenotype' or CIMP that hypothesizes that there is coordinated methylation of a subset of CpG islands in tumors since many of these CIMP loci are known polycomb targets. Further understanding of how specific genomic regions are targeted for DNA hypermethylation in cancer will potentially lead to additional therapeutic targets.

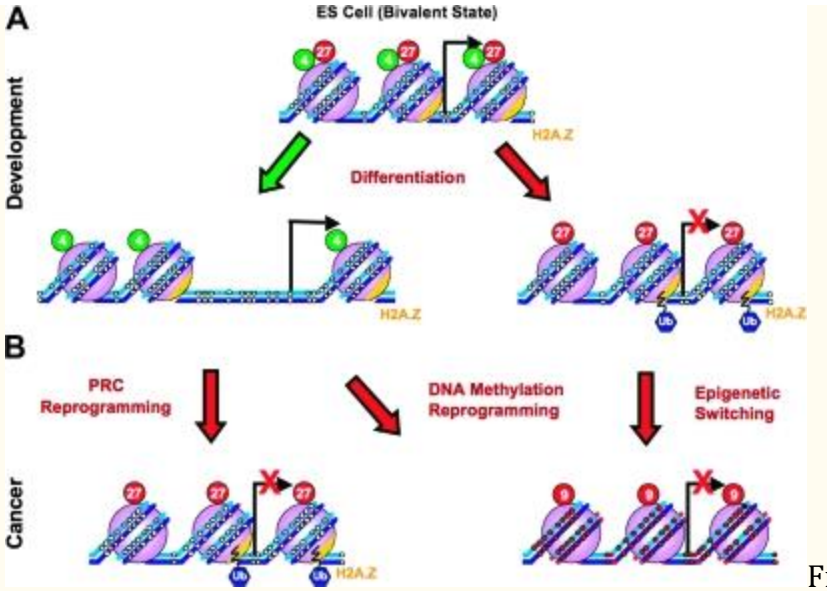


Fig. 3.

Reprogramming of the epigenome during development and tumorigenesis. (**A**) In ES cells, developmentally important genes are marked by a unique 'bivalent domain' structure, consisting of the active H3K4 methylation (green circles, 4) and repressive H3K27 methylation (red circles, 27) marks together with H2A.Z. Such bivalent domains are important for maintaining epigenomic plasticity that is required during development. During differentiation, the bivalent domains are lost, giving way to the establishment of a more rigid 'monovalent domain' structure that is either active (indicated by the green arrow) or repressive (indicated by the red arrow) depending upon which mark is maintained. (**B**) In cancer, cells undergo aberrant somatic reprogramming that results in gene silencing through formation of a compact chromatin structure. Silencing can occur through PRC (Polycomb Repressive Complex) reprogramming—silencing of active genes by the polycomb group; DNA methylation reprogramming—silencing through *de novo* hypermethylation (small red circles on DNA strands) accompanied by H3K9 methylation (red circles, 9) or epigenetic switching—replacement of gene repression by the polycomb mark with long-term silencing through DNA methylation; Ub, ubiquitylation.

## Changes in histone modifications in cancer:

Recent advances in high-throughput sequencing have enabled genome-wide mapping of chromatin changes occurring during tumorigenesis. These studies have revealed a global loss of acetylated H4-lysine 16 (H4K16ac) and H4-lysine 20 trimethylation (H4K20me3). Such loss of histone acetylation, which is mediated by HDACs, results in gene repression. HDACs are often found overexpressed in various types of cancer and thus, have become a major target for epigenetic therapy. HATs, which work in concert with HDACs to maintain histone acetylation levels, can also be altered in cancer. Aberrant formation of fusion

proteins through chromosomal translocations of HAT and HAT-related genes (e.g. MOZ, MORF, CBP and p300) occurs in leukemia. Mistargeting of such deleterious fusion proteins contributes to global alterations in histone acetylation patterns in cancer.

In addition to changes in histone acetylation, cancer cells also display widespread changes in histone methylation patterns. Alterations in H3K9 and H3K27 methylation patterns are associated with aberrant gene silencing in various forms of cancer. Dysregulation of HMTs responsible for repressive marks results in altered distribution of these marks in cancer and leads to aberrant silencing of tumor suppressor genes. For example, EZH2, which is the H3K27 HMT, is overexpressed in breast and prostate cancer. Increased levels of G9a, the H3K9 HMT, has been found in liver cancer and is implicated in perpetuating malignant phenotype possibly through modulation of chromatin structure. Chromosomal translocations of MLL, the H3K4 HMT, lead to ectopic expression of various homeotic (Hox) genes and play a key role in leukemic progression.

In addition to HMTs, lysine specific-demethylases that work in coordination with HMTs to maintain global histone methylation patterns are also implicated in cancer progression. LSD1, the first identified lysine demethylase, can effectively remove both activating and repressing marks (H3K4 and H3K9 methylation, respectively) depending on its specific binding partners, thus, acting as either a corepressor or a co-activator. After LSD1, several other histone lysine demethylases have been discovered including Jumonji C domain proteins. Several of these HDMs are upregulated in prostate cancer, thus, making them potential therapeutic targets. However, since HDMs, like LSD1, can perform both activating and repressive functions, it is essential to first understand their precise context-dependent roles before their therapeutic inhibition can be used as an effective cancer treatment strategy. Despite these challenges, targeting HDMs is a promising treatment option for the future as revealed by a recent study which showed that inhibition of LSD1 in neuroblastoma causes decreased proliferation *in vitro* and inhibition of xenograft growth.

## Epigenetic switching in cancer:

As mentioned previously, DNA methylation and histone modifications work independently and in concert to alter gene expression during tumorigenesis. A key facet of such silencing mechanisms is the formation of a rigid repressive chromatin state that results in reduced cellular plasticity. The recent discovery of tumor-specific *de novo* methylation of polycomb target genes, which are silenced by H3K27me3 in normal cells, is another example of this phenomenon. In ES cells, developmentally important genes are reversibly silenced by polycomb proteins through the establishment of the repressive H3K27me3 mark. After differentiation, these genes continue to be repressed through the maintenance of the

polycomb mark on their unmethylated promoters by EZH2. In cancer, the polycomb mark is replaced by *de novo* DNA methylation possibly through the recruitment of DNMTs via the polycomb complex. This tumor-specific 'epigenetic switching' of the plastic polycomb mark with more stable DNA methylation results in the permanent silencing of key regulatory genes that may contribute to cell proliferation and tumorigenesis (Figure 3). However, which transformation-associated factors trigger this switch is still unclear.

## Role of nucleosome positioning in cancer:

The roles of DNA methylation and histone modifications in cancer initiation and progression are well established; however, the changes in chromatin structure that accompany DNA methylation and histone modification changes are less well understood. Emerging data have revealed that nucleosome remodeling works in concert with DNA methylation and histone modifications and plays a central role in tumor-specific gene silencing. DNA methylation-induced silencing of tumor suppressor genes in cancer involves distinct changes in nucleosome positioning resulting in nucleosome occupancy at transcription start site (Figure 2). Reactivation of such silenced genes using DNMT inhibitors is accompanied by a loss of nucleosomes from the promoter region. In addition, nucleosome remodeling can lead to aberrant gene silencing via the transmission of repressive epigenetic marks to tumor suppressor gene promoters. Recent work by Morey *et al.* demonstrated that nucleosome remodeling and deacetylase (NuRD) corepressor complex plays a central role in aberrant gene silencing in leukemia via the oncogenic transcription factor, PML–RARa. The NuRD complex facilitates recruitment of the polycomb repressive complex 2 and DNMT3A to PML–RARa target gene promoters leading to their permanent silencing by establishing a repressive chromatin state. NuRD can also be recruited to methylated promoters through its interaction with the methyl-binding domain2 protein. Sustained binding of NuRD to such promoters may assist in preserving their repressive state through maintenance of DNA methylation.

Alterations in the SWI–SNF complex, an ATP-dependent chromatin-remodeling complex, are also associated with cancer development. Abrogation of SWI–SNF function through alterations in its various subunits can result in malignant transformation. The BAF47 (hSNF5) subunit of the SWI–SNF complex is a *bona fide* tumor suppressor and its inhibition in rhabdoid tumors causes inactivation of the p21 and p16 pathways leading to oncogenic transformation. Furthermore, BRG1 and BRM, the catalytic subunits of SWI–SNF, are silenced in ~15–20% of primary non-small-cell lung cancers. Treatment of BRM null cell lines with HDAC inhibitors has been shown to restore its expression, thus, making it a promising target for epigenetic therapy. However, such treatment also resulted in acetylation of BRM protein that abrogated its function. Development of specific HDAC

inhibitors, which can circumvent BRM acetylation, is essential for successful induction of functional BRM in tumors, which can be used as a prospective therapeutic target in the future.

Interestingly, a context-dependent oncogenic role of BRG1 has also been proposed. Work by Naidu *et al.* reveals that BRG1 contributes to cancer development by constraining p53 activity through the destabilization of the p53 protein. Opposing roles of SWI–SNF subunits highlight the requirement for a deeper understanding of the role of nucleosome remodeling in cancer development in order to develop effective tumor-specific therapies. In addition to remodeling complexes, the histone variant H2A.Z has also been implicated in tumorigenesis. H2A.Z is overexpressed in several types of cancer and has been associated with the promotion of cell cycle progression. Interestingly, loss of H2A.Z has also been implicated in tumor progression through possible destabilization of chromosomal boundaries resulting in spreading of repressive chromatin domains and *de novo* hypermethylation of tumor suppressor gene promoters.

## Deregulation of miRNAs in cancer:

Accumulating evidence from studies comparing miRNA expression profiles in tumors and corresponding normal tissues indicate widespread changes in miRNA expression during tumorigenesis. Since miRNAs regulate genes involved in transcriptional regulation, cell proliferation and apoptosis (the most common processes deregulated in cancer), alteration in their expression can promote tumorigenesis. miRNAs can function as either tumor suppressors or oncogenes depending upon their target genes. Many tumor suppressor miRNAs that target growth-promoting genes are repressed in cancer. For example, *miR*-15 and *16* that target *BCL2*, an antiapoptotic gene, are downregulated in chronic lymphocytic leukemia, whereas *let*-7 that targets the oncogene, *RAS*, is downregulated in lung cancer. Furthermore, *miR-127*, which targets BCL6, is significantly downregulated in prostate and bladder tumors and *mir-101*, which targets polycomb group protein EZH2, is downregulated in bladder transitional cell carcinoma. In contrast to tumor suppressor miRNAs, oncogenic miRNAs, which target growth inhibitory pathways, are often upregulated in cancer. For example, *miR-21*, which targets *PTEN*, is upregulated in human glioblastoma. *miRNA-155* is upregulated in breast, lung and several hematopoietic malignancies. While the exact mechanism of action of *miR-155* is still unclear, there are suggestions that it may play a role in the class switch recombination process by targeting activation-induced cytidine deaminase. The oncogenic *miR-17–miR-92* cluster, which targets pro-apoptotic gene *Bim*, is found overexpressed in several kinds of cancer.

Changes in miRNA expression can be achieved through various mechanisms including chromosomal abnormalities, transcription factor binding and epigenetic alterations. The initial report by Saito *et al.* demonstrated that *miR-127*, a tumor suppressor miRNA embedded in a CpG island, was silenced in cancer by DNA methylation and has led to subsequent discovery of several other miRNAs that are also silenced by epigenetic mechanisms in cancer (117,118). Since such epigenetic repression of tumor suppressor miRNAs can be potentially reversed by treatment with chromatin modifying drugs, they can serve as promising targets for epigenetic therapy. Saito *et al.* successfully demonstrated reactivation of *miR-127* in T24 bladder cancer cells following treatment with chromatin modifying drugs including DNA methylation and HDAC inhibitors. Such drug-induced activation of tumor suppressor miRNAs holds great promise for the future of cancer therapeutics.

## The cancer stem cell model:

Recent work suggests that the global epigenetic changes in cancer may involve the dysregulation of hundreds of genes during tumorigenesis. The mechanism by which a tumor cell accumulates such widespread epigenetic abnormalities during cancer development is still not fully understood. The selective advantage of these epimutations during tumor progression is possible, but it is unlikely that the multitude of epigenetic alterations that reside in a cancer epigenome occur in a random fashion and then accumulate inside the tumor due to clonal selection. A more plausible explanation would be that the accumulation of such global epigenomic abnormalities arises from initial alterations in the central epigenetic control machinery, which occur at a very early stage of neoplastic evolution. Such initiating events can predispose tumor cells to gain further epimutations during tumor progression in a fashion similar to accumulation of the genetic alterations that occurs following defects in DNA repair machinery in cancer. The 'cancer stem cell' model suggests that the epigenetic changes, which occur in normal stem or progenitor cells, are the earliest events in cancer initiation. The idea that these initial events occur in stem cell populations is supported by the common finding that epigenetic aberrations are some of the earliest events that occur in various types of cancer and also by the discovery that normal tissues have altered progenitor cells in cancer patients. This stem cell-based cancer initiation model is consistent with the observation that tumors contain a heterogenous population of cells with diverse tumorigenic properties. Since epigenetic mechanisms are central to maintenance of stem cell identity, it is reasonable to speculate that their disruption may give rise to a high-risk aberrant progenitor cell population that can undergo transformation upon gain of subsequent genetic gatekeeper mutations. Such epigenetic disruptions can lead to an overall increase in number of progenitor cells along with an increase in their ability to maintain their stem cell state,

forming a high-risk substrate population that can readily become neoplastic on gain of additional genetic mutations.

Several findings have recently emerged in support of the cancer stem cell model. Mice with a LOI at the *IGF2* locus and an *Apc* mutation show an expansion in the progenitor cell population of the intestinal epithelium, with the epithelial cells showing higher expression of progenitor cell markers and shifting toward a less-differentiated state. These mice were also at a higher risk for intestinal tumors relative to control mice. Interestingly, humans with LOI of *IGF2* also show a similar dedifferentiation of normal colonic mucosa cells along with a higher risk for colorectal cancer. Also, stem cell-like characteristics of tumor cells were displayed through successful cloning of mouse melanoma and medulloblastoma nuclei to form blastocysts and chimeric mice. DNA methylation-induced silencing of genes involved in the regulation of stem/precursor cells' self renewal capacity, such as *p16*, *APC*, *SFRPs* etc., is commonly observed in the early stages of colon and other cancers. Aberrant silencing of these so called 'epigenetic gatekeeper' genes in conditions of chronic stress, such as inflammation, enables stem/precursor cells to gain infinite renewal capacity thereby becoming immortal. These preinvasive immortal stem cells are selected for and then form a pool of abnormal precursor cells that can undergo further genetic mutations leading to tumorigenesis. Human ES cells with cancer cell characteristics including higher frequency of teratoma-initiating cells, growth factor and niche independence have also been found. These partially transformed stem cells display a higher expression of pluripotency markers suggesting their enhanced 'stemness' along with high proliferative capacity.

Polycomb proteins, which control the silencing of developmental regulators in ES cells, provide another link between stem cell biology and cancer initiation. Polycomb proteins are commonly upregulated in various forms of cancer. In addition, genes that are marked by polycomb repressive mark H3K27me3 in ES cells are often methylated in cancer suggesting the presence of a shared regulatory framework, which connects cancer cells with stem/progenitor cell populations. Such findings support the hypothesis of epigenetics playing a central role in early neoplasia and cancer stem cells being the key perpetuators of cancer.

## Epigenetic therapy of cancer:

The reversible nature of the profound epigenetic changes that occur in cancer has led to the possibility of 'epigenetic therapy' as a treatment option. The aim of epigenetic therapy is to reverse the causal epigenetic aberrations that occur in cancer, leading to the restoration of a 'normal epigenome'. Many epigenetic drugs have been discovered in the recent past that

can effectively reverse DNA methylation and histone modification aberrations that occur in cancer. DNA methylation inhibitors were among the first epigenetic drugs proposed for use as cancer therapeutics. The remarkable discovery that treatment with cytotoxic agents, 5-azacytidine (5-aza-CR) and 5-aza-2′-deoxycytidine (5-aza-CdR), lead to the inhibition of DNA methylation that induced gene expression and caused differentiation in cultured cells led to the realization of the potential use of these drugs in cancer therapy. These nucleoside analogs get incorporated into the DNA of rapidly growing tumor cells during replication and inhibit DNA methylation by trapping DNA methyltransferases onto the DNA, leading to their depletion inside the cell. This drug-induced reduction of DNA methylation causes growth inhibition in cancer cells by activating tumor suppressor genes aberrantly silenced in cancer. 5-Aza-CR (azacitidine) and 5-aza-CdR (decitabine) have now been FDA approved for use in the treatment of myelodysplastic syndromes and promising results have also emerged from the treatment of other hematological malignancies such as acute myeloid leukemia and chronic myeloid leukemia using these drugs. The possible clinical use of other improved DNA methylation inhibitors such as zebularine, which can be orally administered, is currently under investigation.

The ability of these drugs to be incorporated into DNA raises concerns regarding their potential toxic effect on normal cells. However, since these drugs only act on dividing cells, one can argue that treatment with these drugs should mainly target rapidly dividing tumor cells and should have minimal effects on slowly dividing normal cells. This argument has been supported by studies demonstrating minimal side effects of long-term treatment with DNA methylation inhibitors. Nevertheless, an alternative approach involving the development of non-nucleoside compounds, which can effectively inhibit DNA methylation without being incorporated into DNA, is also being actively pursued. Development of several small molecule inhibitors such as SGI-1027, RG108 and MG98 is a step in that direction. These molecules can achieve their inhibitory effects by either blocking catalytic/cofactor-binding sites of DNMTs or by targeting their regulatory messenger RNA sequences; however, the weak inhibitory potential of these drugs indicates a need for the development of more potent inhibitory compounds in future.

Aberrant gene silencing in cancer is also associated with a concomitant loss of histone acetylation. Re-establishing normal histone acetylation patterns through treatment with HDAC inhibitors have been shown to have antitumorigenic effects including growth arrest, apoptosis and the induction of differentiation. These antiproliferative effects of HDAC inhibitors are mediated by their ability to reactivate silenced tumor suppressor genes. Suberoylanilide hydroxamic acid (SAHA), which is an HDAC inhibitor, has now been approved for use in clinic for treatment of T cell cutaneous lymphoma. Several other HDAC inhibitors such as depsipeptide and phenylbutyrate are currently under clinical trials. The

interaction between different components of the epigenetic machinery has led to the exploration of effective combinatorial cancer treatment strategies, which involve use of both DNA methylation and HDAC inhibitors together. Such combination treatment strategies have been found to be more effective than individual treatment approaches. For example, the derepression of certain putative tumor suppressor genes was only seen when 5-Aza-CdR and trichostatin A were combined. Antitumorigenic effects of depsipeptide were enhanced when leukemic cells were simultaneously treated with 5-Aza-CdR. Synergistic activities of DNA methylation and HDAC inhibitors were also demonstrated in a study showing greater reduction of lung tumor formation in mice when treated with phenylbutyrate and 5-Aza-CdR together.

Apart from DNA methylation and HDAC inhibitors, HMT inhibitors have also been actively explored recently. One such inhibitor compound, DZNep, was shown to successfully induce apoptosis in cancer cells by selectively targeting polycomb repressive complex 2 proteins, which are generally overexpressed in cancer. While the specificity of DZNep was challenged in a subsequent study, these findings reinforce the potential of HMT inhibitors and the need for further development of specific histone methylation inhibitors.

miRNAs also represent promising targets for epigenetic therapy. The finding by Saito *et al.* demonstrated that downregulation of the oncogene BCL6 via reactivation of *miR-127* following treatment with 5-Aza-CdR and 4-phenylbutyric acid strongly advocates in favor of the potential of a miRNA-based treatment strategy. In addition, the introduction of synthetic miRNAs, which mimic tumor suppressor miRNAs, can be used to selectively repress oncogenes in tumors. miRNAs, such as *miR-101* that targets EZH2, can be used to regulate the aberrant epigenetic machinery in cancer that may assist in restoring of the normal epigenome. However, the lack of efficient delivery methods is a major hurdle in the effective use of this strategy. Development of efficient vehicle molecules for targeted delivery of synthetic miRNAs to tumor cells is of prime importance in future.

## Future prospects and challenges:

The epigenetic revolution that has come about in the field of biology during the last few decades has challenged the long-held traditional view of the genetic code being the key determinant of cellular gene function and its alteration being the major cause of human diseases. Advances made in the field of cancer epigenetics have led to the realization that the packaging of the genome is potentially as important as the genome itself in regulating the essential cellular processes required for preserving cellular identity and also in giving rise to disease states like cancer. Deeper understandings of the global patterns of these epigenetic modifications and their corresponding changes in cancer have enabled the

design of better treatment strategies. A combinatorial approach utilizing different epigenetic therapeutic approaches along with standard chemotherapy holds significant promise for successful treatment of cancer in future. Such approaches might also help in sensitizing cancer cells, especially cancer stem cells, which are refractory to standard chemotherapy. Further understanding of cancer stem cells along with development of more specific epigenetic drugs may hold the key to our ability to successfully reset the abnormal cancer epigenome.

## Probable Questions:

1. Classify cancer on the basis of tissue involved.
2. Discuss different types of carcinogens with suitable examples.
3. Describe Knudson's two hit hypothesis with suitable diagram.
4. How cancers are diagnosed?
5. What are the symptoms of cancer?
6. State the cancer strategies of cancer.
7. How proto oncogenes are converted into oncogenes?
8. How tumor suppressor genes are related to cancer?
9. How DNA methylation alteration causes cancer?
10. How histone modifications causes cancer?
11. Describe Role of nucleosome positioning in cancer.
12. How miRNA deregulation causes cancer?
13. How epigenetic therapy can be used in cancer treatment?

## Suggested Readings:

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# UNIT-III

# Genetic disease diagnosis and treatment: Application of medical genetics; Genetic Testing, Cytogenetic, Biochemical and Molecular; Gene therapy

**Objective:** In this unit we will discuss about genetic disease diagnosis and treatment procedure including gene therapy. We will also discuss various types of genetic testing such as cytogenetic, biochemical and molecular processes.

## Introduction:

Genetic testing is a type of medical test that identifies changes in genes, chromosomes, or proteins. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's chance of developing or passing on a genetic disorder. More than 77,000 genetic tests are currently in use, and others are being developed.

## Genetic testing involves looking for changes in:

Genes: Gene tests study DNA sequences to identify variations (mutations) in genes that can cause or increase the risk of a genetic disorder. Gene tests can be narrow or large in scope, analyzing an individual DNA building block (nucleotide), one or more genes, or all of a person's DNA (which is known as their genome).

Chromosomes: Chromosomal genetic tests analyze whole chromosomes or long lengths of DNA to see if there are large genetic changes, such as an extra copy of a chromosome, that cause a genetic condition.

Proteins: Biochemical genetic tests study the amount or activity level of proteins or enzymes; abnormalities in either can indicate changes to the DNA that result in a genetic disorder. Genetic testing is voluntary. Because testing has benefits as well as limitations and risks, the decision about whether to be tested is a personal and complex one. A geneticist or genetic counselor can help by providing information about the pros and cons of the test and discussing the social and emotional aspects of testing.

## Different types of genetic tests:

Many types of genetic tests are available to analyze changes in genes, chromosomes, or proteins. A health care provider will consider several factors when selecting the appropriate test, including what condition or conditions are suspected and the genetic variations typically associated with those conditions. If a diagnosis is unclear, a test that looks at many genes or chromosomes may be used. However, if a specific condition is suspected, a more focused test may be done.

## There are several types of genetic tests:

Molecular tests look for changes in one or more genes. These types of tests determine the order of DNA building blocks (nucleotides) in an individual's genetic code, a process called DNA sequencing. These tests can vary in scope:

Targeted single variant: Single variant tests look for a specific variant in one gene. The selected variant is known to cause a disorder (for example, the specific variant in the HBB gene that causes sickle cell disease). This type of test is often used to test family members of someone who is known to have a particular variant, to determine whether they have a familial condition. Also, direct-to-consumer genetic testing companies typically analyze a number of specific variants in particular genes (rather than finding all the variants in those genes) when providing health or disease risk information.

Single gene: Single gene tests look for any genetic changes in one gene. These tests are typically used to confirm (or rule out) a specific diagnosis, particularly when there are many variants in the gene that can cause the suspected condition. Gene panel: Panel tests look for variants in more than one gene. This type of test is often used to pinpoint a diagnosis when a person has symptoms that may fit a wide array of conditions, or when the suspected condition can be caused by variants in many genes. (For example, there are hundreds of genetic causes of epilepsy.)

Whole exome sequencing/whole genome sequencing: These tests analyze the bulk of an individual’s DNA to find genetic variations. Whole exome or whole genome sequencing is typically used when single gene or panel testing has not provided a diagnosis, or when the suspected condition or genetic cause is unclear. Whole exome or whole genome sequencing is often more cost- and time-effective than performing multiple single gene or panel tests.

Chromosomal tests analyze whole chromosomes or long lengths of DNA to identify large-scale changes. Changes that can be found include an extra or missing copy of a chromosome (trisomy or monosomy, respectively), a large piece of a chromosome that is added (duplicated) or missing (deleted), or rearrangements (translocations) of segments of

chromosomes. Certain genetic conditions are associated with specific chromosomal changes, and a chromosomal test can be used when one of these conditions is suspected. (For example, Williams syndrome is caused by a deletion of a section of chromosome 7.)

Gene expression tests look at which genes are turned on or off (expressed) in different types of cells. When a gene is turned on (active), the cell produces a molecule called mRNA from the instructions in the genes, and the mRNA molecule is used as a blueprint to make proteins. Gene expression tests study the mRNA in cells to determine which genes are active. Too much activity (overexpression) or too little activity (underexpression) of certain genes can be suggestive of particular genetic disorders, such as many types of cancer. Biochemical tests do not directly analyze DNA, but they study the amount or activity level of proteins or enzymes that are produced from genes. Abnormalities in these substances can indicate that there are changes in the DNA that underlie a genetic disorder. (For example, low levels of biotinidase enzyme activity are suggestive of biotinidase deficiency, which is caused by BTD gene variants.)

## How is genetic testing done?

Once a person decides to proceed with genetic testing, a health care provider can arrange testing. Genetic testing is often done as part of a genetic consultation.

Genetic tests are performed on a sample of blood, hair, skin, amniotic fluid (the fluid that surrounds a fetus during pregnancy), or other tissue. For example, a procedure called a buccal smear uses a small brush or cotton swab to collect a sample of cells from the inside surface of the cheek. The sample is sent to a laboratory where technicians look for specific changes in chromosomes, DNA, or proteins, depending on the suspected disorder. The laboratory reports the test results in writing to a person's doctor or genetic counselor, or directly to the patient if requested.

Newborn screening tests are done on a small blood sample, which is taken by pricking the baby's heel. Unlike other types of genetic testing, a parent will usually only receive the result if it is positive. If the test result is positive, additional testing is needed to determine whether the baby has a genetic disorder. Before a person has a genetic test, it is important to understand the testing procedure, the benefits and limitations of the test, and the possible consequences of the test results. The process of educating a person about the test and obtaining permission is called informed consent. Individuals interested in direct-to-consumer genetic testing do not need to go through a health care provider to obtain a test, but they can get it directly from the testing company. After undergoing direct-to-consumer genetic testing, people who test positive for a condition or are found to be at higher risk of developing a disorder are encouraged to follow-up with a genetic counselor or other health care provider.

## Uses of genetic testing:

Genetic testing can provide information about a person's genetic background. The uses of genetic testing include:

## Newborn screening:

Newborn screening is used just after birth to identify genetic disorders that can be treated early in life. Millions of babies are tested each year in the United States. The U.S. Health Services and Resource Administration recommends that states screen for a set of 35 conditions, which many states exceed.

## Diagnostic testing:

Diagnostic testing is used to identify or rule out a specific genetic or chromosomal condition. In many cases, genetic testing is used to confirm a diagnosis when a particular condition is suspected based on physical signs and symptoms. Diagnostic testing can be performed before birth or at any time during a person's life, but is not available for all genes or all genetic conditions. The results of a diagnostic test can influence a person's choices about health care and the management of the disorder.

## Carrier testing:

Carrier testing is used to identify people who carry one copy of a gene mutation that, when present in two copies, causes a genetic disorder. This type of testing is offered to individuals who have a family history of a genetic disorder and to people in certain ethnic groups with an increased risk of specific genetic conditions. If both parents are tested, the test can provide information about a couple's risk of having a child with a genetic condition.

## Prenatal testing:

Prenatal testing is used to detect changes in a fetus's genes or chromosomes before birth. This type of testing is offered during pregnancy if there is an increased risk that the baby will have a genetic or chromosomal disorder. In some cases, prenatal testing can lessen a couple's uncertainty or help them make decisions about a pregnancy. It cannot identify all possible inherited disorders and birth defects, however.

## Preimplantation testing :

Preimplantation testing, also called preimplantation genetic diagnosis (PGD), is a specialized technique that can reduce the risk of having a child with a particular genetic or

chromosomal disorder. It is used to detect genetic changes in embryos that were created using assisted reproductive techniques (ART) such as in-vitro fertilization (IVF). In-vitro fertilization involves removing egg cells from a woman's ovaries and fertilizing them with sperm cells outside the body. To perform preimplantation testing, a small number of cells are taken from these embryos and tested for certain genetic changes. Only embryos without these changes are implanted in the uterus to initiate a pregnancy.

## Predictive and presymptomatic testing:

Predictive and presymptomatic types of testing are used to detect gene mutations associated with disorders that appear after birth, often later in life. These tests can be helpful to people who have a family member with a genetic disorder, but who have no features of the disorder themselves at the time of testing. Predictive testing can identify mutations that increase a person's risk of developing disorders with a genetic basis, such as certain types of cancer. Presymptomatic testing can determine whether a person will develop a genetic disorder, such as hereditary hemochromatosis (an iron overload disorder), before any signs or symptoms appear. The results of predictive and presymptomatic testing can provide information about a person's risk of developing a specific disorder and help with making decisions about medical care.

## Forensic testing:

Forensic testing uses DNA sequences to identify an individual for legal purposes.Unlike the tests described above, forensic testing is not used to detect gene mutations associated with disease. This type of testing can identify crime or catastrophe victims, rule out or implicate a crime suspect, or establish biological relationships between people (for example, paternity).

## What do the results of genetic tests mean?

The results of genetic tests are not always straightforward, which often makes them challenging to interpret and explain. Therefore, it is important for patients and their families to ask questions about the potential meaning of genetic test results both before and after the test is performed. When interpreting test results, health care providers consider a person's medical history, family history, and the type of genetic test that was done.

A positive test result means that the laboratory found a change in a particular gene, chromosome, or protein of interest. Depending on the purpose of the test, this result may confirm a diagnosis, indicate that a person is a carrier of a particular genetic variant, identify an increased risk of developing a disease (such as cancer), or suggest a need for

further testing. Because family members have some genetic material in common, a positive test result may also have implications for certain blood relatives of the person undergoing testing. It is important to note that a positive result of a predictive or presymptomatic genetic test usually cannot establish the exact risk of developing a disorder. Also, health care providers typically cannot use a positive test result to predict the course or severity of a condition. Rarely, tests results can be false positive, which occur when results indicate an increased risk for a genetic condition when the person is unaffected.

A negative test result means that the laboratory did not find a change that is known to affect health or development in the gene, chromosome, or protein under consideration. This result can indicate that a person is not affected by a particular disorder, is not a carrier of a specific genetic variant, or does not have an increased risk of developing a certain disease. It is possible, however, that the test missed a disease-causing genetic alteration because many tests cannot detect all genetic changes that can cause a particular disorder. Further testing, or re-testing at a later date, may be required to confirm a negative result. Rarely, tests results can be false negative, which occur when the results indicate a decreased risk or a genetic condition when the person is actually affected.

In some cases, a test result might not give any useful information. This type of result is called uninformative, indeterminate, inconclusive, or ambiguous. Uninformative test results sometimes occur because everyone has common, natural variations in their DNA, called polymorphisms, that do not affect health. If a genetic test finds a change in DNA that has not been confirmed to play a role in the development of disease, known as a variant of uncertain significance (VUS or VOUS), it can be difficult to tell whether it is a natural polymorphism or a disease-causing variant. For these variants, there may not be enough scientific research to confirm or refute a disease association or the research may be conflicting. An uninformative result cannot confirm or rule out a specific diagnosis, and it cannot indicate whether a person has an increased risk of developing a disorder. In some cases, testing other affected and unaffected family members can help clarify this type of result.

Genetic testing has potential benefits whether the results are positive or negative for a gene mutation. Test results can provide a sense of relief from uncertainty and help people make informed decisions about managing their health care. For example, a negative result can eliminate the need for unnecessary checkups and screening tests in some cases. A positive result can direct a person toward available prevention, monitoring, and treatment options. Some test results can also help people make decisions about having children. Newborn screening can identify genetic disorders early in life so treatment can be started as early as possible.

## Risks and limitations of genetic testing:

The physical risks associated with most genetic tests are very small, particularly for those tests that require only a blood sample or buccal smear (a method that samples cells from the inside surface of the cheek). The procedures used for prenatal diagnostic testing (called amniocentesis and chorionic villus sampling) carry a small but real risk of losing the pregnancy (miscarriage) because they require a sample of amniotic fluid or tissue from around the fetus.

Many of the risks associated with genetic testing involve the emotional, social, or financial consequences of the test results. People may feel angry, depressed, anxious, or guilty about their results. In some cases, genetic testing creates tension within a family because the results can reveal information about other family members in addition to the person who is tested. The possibility of genetic discrimination in employment or insurance is also a concern. (Refer to What is genetic discrimination? for additional information.)

Genetic testing can provide only limited information about an inherited condition. The test often can't determine if a person will show symptoms of a disorder, how severe the symptoms will be, or whether the disorder will progress over time. Another major limitation is the lack of treatment strategies for many genetic disorders once they are diagnosed. A genetics professional can explain in detail the benefits, risks, and limitations of a particular test. It is important that any person who is considering genetic testing understand and weigh these factors before making a decision.

## Secondary findings from genetic testing:

Secondary findings are genetic test results that provide information about changes (variants) in a gene unrelated to the primary purpose for the testing.

When a clinician orders a genetic test to discover the genetic cause of a particular condition, the test will often sequence one or a few genes that seem most likely to be associated with that individual's set of signs and symptoms. However, if the individual's signs and symptoms do not have an obvious genetic cause, a clinician might order a test that sequences all of the pieces of an individual's DNA that provide instructions for making proteins (called an exome) or a test that sequences all of an individual's DNA building blocks (nucleotides), called a genome. These tests are called whole exome sequencing and whole genome sequencing, respectively.

Many more genetic changes can be identified with whole exome and whole genome sequencing than by sequencing just one or a few genes. Sometimes, testing finds a variant that is associated with a condition other than the one for which testing was originally

indicated. This is called a secondary finding. Some individuals with a secondary finding may not yet have any of the symptoms associated with the condition, but may be at risk of developing it later in life. For example, a person with a variant in the BRCA1 gene, which is associated with an increased risk of breast cancer and ovarian cancer, may not have developed cancer. Other individuals with secondary findings may have a known medical condition, such as extremely high cholesterol, but receive results that indicate a genetic cause for that condition, such as a variant in the LDLR gene.

In 2013, then again in 2017 and 2021, the American College of Medical Genetics and Genomics (ACMG) recommended that all labs performing whole exome and whole genome sequencing tests report particular secondary findings, in addition to any variants that are found related to the primary purpose of the testing. In the 2021 updated recommendations, ACMG proposed a list of 73 genes that are associated with a variety of conditions, from cancer to heart disease. The 73 genes for which secondary findings are reported were chosen because they are associated with conditions that have a definable set of clinical features, the possibility of early diagnosis, a reliable clinical genetic test, and effective intervention or treatment. The goal of reporting these secondary findings to an individual is to provide medical benefit by preventing or better managing health conditions. The variants that are reported are known to cause disease. Variants of unknown significance, whose involvement in disease at the current time is unclear, are not reported.

The information provided by secondary findings can be very important because it may help prevent a disease from occurring or guide the management of signs and symptoms if the disease develops or is already present. However, as with any type of medical diagnosis, the news of an unexpected potential health problem may lead to additional health costs and stress for individuals and their families. On the basis of secondary findings, additional testing to confirm results, ongoing screening tests, or preventive care may be advised. Individuals receiving whole exome or whole genome sequencing can choose to “opt out” of analysis of the 73 secondary finding genes and not receive variant results. As whole exome and whole genome sequencing become more common, it is important for individuals to understand what type of information they may learn and how it can impact their medical care.

## Can genes be patented?

A gene patent is the exclusive rights to a specific sequence of DNA (a gene) given by a government to the individual, organization, or corporation who claims to have first identified the gene. Once granted a gene patent, the holder of the patent dictates how the gene can be used, in both commercial settings, such as clinical genetic testing, and in noncommercial settings, including research, for 20 years from the date of the patent. Gene patents have often resulted in companies having sole ownership of genetic testing for patented genes.

On June 13, 2013, in the case of the Association for Molecular Pathology v. Myriad Genetics, Inc., the Supreme Court of the United States ruled that human genes cannot be patented in the U.S. because DNA is a "product of nature." The Court decided that because nothing new is created when discovering a gene, there is no intellectual property to protect, so patents cannot be granted. Prior to this ruling, more than 4,300 human genes were patented. The Supreme Court's decision invalidated those gene patents, making the genes accessible for research and for commercial genetic testing.

The Supreme Court's ruling did allow that DNA manipulated in a lab is eligible to be patented because DNA sequences altered by humans are not found in nature. The Court specifically mentioned the ability to patent a type of DNA known as complementary DNA (cDNA). This synthetic DNA is produced from the molecule that serves as the instructions for making proteins (called messenger RNA).

## Gene Therapy:

Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds. This technique is widely used to treat those defective genes which contribute to disease development. Gene therapy involves the introduction of one or more foreign genes into an organism to treat hereditary or acquired genetic defects. In gene therapy, DNA encoding a therapeutic protein is packaged within a "vector", which transports the DNA inside cells within the body. The disease is treated with minimal toxicity, by the expression of the inserted DNA by the cell machinery. In 1990 FDA for the first time approved a gene therapy experiment on ADA-SCID in the United States after the treatment of Ashanti DeSilva. After that, approximately 1700 clinical trials on patients have been performed with various techniques and genes for numerous diseases. Gene therapy is the process of inserting genes into cells to treat diseases. The newly introduced genes will encode proteins and correct the deficiencies that occur in genetic diseases. Thus, gene therapy primarily involves genetic manipulations in animals or humans to correct a disease, and keep the organism in good health. The initial experiments on gene therapy are carried out in animals, and then in humans. Obviously, the goal of the researchers is to benefit the mankind and improve their health.

An overview of gene therapy strategies is depicted in Fig. 13.1. In gene augmentation therapy, a DNA is inserted into the genome to replace the missing gene product. In case of gene inhibition therapy, the antisense gene inhibits the expression of the dominant gene.

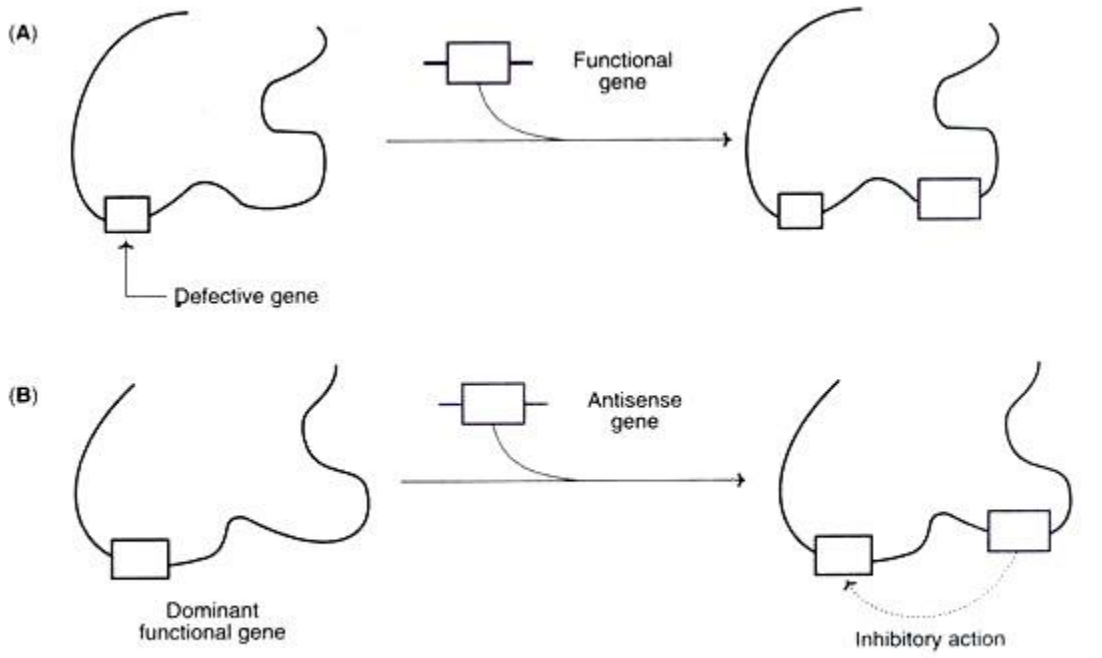


*Fig. 13.1 : Overview of two major gene therapy strategies*
*(A) Gene augmentation therapy (B) Gene inhibition therapy.*

## I. General gene therapy strategies

**a. Gene Augmentation Therapy (GAT):**

For diseases caused by loss of function of a gene, introducing extra copies of the normal gene may increase the amount of normal gene product to a level where the normal phenotype is restored (see Fig. 23.1). As a result GAT is targeted at clinical disorders where the pathogenesis is reversible. It also helps to have no precise requirement for expression levels of the introduced gene and a clinical response at low expression levels. GAT has been particularly applied to autosomal recessive disorders where even modest expression levels of an introduced gene may make a substantial difference.

Dominantly inherited disorders are much less amendable to treatment; gain-of-function mutations are not treatable by this approach and, even if there is a loss-of- function mutation, high expression efficiency of the introduced gene is required: individuals with 50% of normal gene product are normally affected, and so the challenge is to increase the amount of gene product towards normal levels.

**b. Targeted Killing of Specific Cells:**
This general approach is popular in cancer gene therapies. Genes are directed to the target cells and then expressed so as to cause cell killing. Direct cell killing is possible if the inserted genes are expressed to produce a lethal toxin (suicide genes), or a gene encoding a pro drug is inserted, conferring susceptibility to killing by a subsequently administered drug. Indirect cell killing uses immunostimulatory genes to provoke or enhance an immune response against the target cell.

**c. Targeted Mutation Correction:**
If an inherited mutation produces a dominant-negative effect, gene augmentation is unlikely to help. Instead, the resident mutation must be corrected. Because of practical difficulties, this approach has yet to be applied but, in principle, it can be done at different levels: at the gene level (e.g. by gene targeting methods based on homologous recombination); or at the RNA transcript level (e.g. by using particular types of therapeutic ribozymes — or therapeutic RNA editing).

**d. Targeted Inhibition of Gene Expression:**
If disease cells display a novel gene product or inappropriate expression of a gene (as in the case of many cancers, infectious diseases, etc.), a variety of different systems can be used specifically to block the expression of a single gene at the DNA, RNA or protein levels. Allele-specific inhibition of expression may be possible in some cases, permitting therapies for some disorders resulting from dominant negative effects.

## II. Approaches for Gene Therapy:

There are two approaches to achieve gene therapy.

**1. Somatic Cell Gene Therapy:**
The non- reproductive (non-sex) cells of an organism are referred to as somatic cells. These are the cells of an organism other than sperm or eggs cells, e.g., bone marrow cells, blood cells, skin cells, intestinal cells. At present, all the research on gene therapy is directed to correct the genetic defects in somatic cells. In essence, somatic cell gene therapy involves the insertion of a fully functional and expressible gene into a target somatic cell to correct a genetic disease permanently.

**2. Germ Cell Gene Therapy:**
The reproductive (sex) cells of an organism constitute germ cell line. Gene therapy involving the introduction of DNA into germ cells is passed on to the successive generations. For safety, ethical and technical reasons, germ cell gene therapy is not being attempted at present.

The genetic alterations in somatic cells are not carried to the next generations. Therefore, somatic cell gene therapy is preferred and extensively studied with an ultimate objective of correcting human diseases. Development of gene therapy in humans for any specific disease involves the following steps. In fact, this is a general format for introducing any therapeutic agent for human use.

a. **In vitro experiments and research on laboratory animals (pre-clinical trials).**

b. **Phase I trials with a small number (5-10) of human subjects to test safety of the product.**

c. **Phase II trials with more human subjects to assess whether the product is helpful.**

**d. Phase III trials in large human samples for a final and comprehensive analysis of the safety and efficacy of the product.**

As such, gene therapy involves a great risk. There are several regulatory agencies whose permission must be sought before undertaking any work related to gene therapy. Recombinant DNA Advisory Committee (RAC) is the supervisory body of the National Institute of Health, U.S.A., that clears proposals on experiments involving gene therapy. A large number of genetic disorders and other diseases are currently at various stages of gene therapy trials. A selected list of some important ones is given in Table 13.1.

**TABLE 13.1 Human gene therapy trials**

| *Disease* | *Gene therapy* |
|---|---|
| Severe combined immunodeficiency (SCID) | Adenosine deaminase (ADA). |
| Cystic fibrosis | Cystic fibrosis transmembrane regulator (CFTR). |
| Familial hypercholesterolemia | Low density lipoprotein (LDL) receptor. |
| Emphysema | $\alpha_1$-Antitrypsin |
| Hemophilia B | Factor IX |
| Thalassemia | $\alpha$- or $\beta$-Globin |
| Sickle-cell anemia | $\beta$-Globin |
| Lesch-Nyhan syndrome | Hypoxanthine-guanine phosphoribosyltransferase (HGPRT). |
| Gaucher's disease | Glucocerebrosidase |
| Peripheral artery disease | Vascular endothelial growth factor (VEGF) |
| Fanconi anemia | Fanconi anemia C |
| Melanoma | Tumor necrosis factor (TNF) |
| Melanoma, renal cancer | Interleukin-2 (IL-2) |
| Glioblastoma (brain tumor), AIDS, ovarian cancer | Thymidine kinase (herpes simplex virus) |
| Head and neck cancer | $p^{53}$ |
| Breast cancer | Multidrug resistance I |
| AIDS | *rev* and *env* |
| Colorectal cancer, melanoma, renal cancer | Histocompatability locus antigen-$B_7$ (HLA-$B_7$) |
| Duchenne muscular dystrophy | Dystrophin |
| Short stature* | Growth hormone |
| Diabetes* | Glucose transporter-2, (GLUT-2), glucokinase |
| Phenylketonuria* | Phenylalanine hydroxylase |
| Citrullinemia* | Arginosuccinate synthetase |

## III. Methods of gene therapy:

There are mainly two approaches for the transfer of genes in gene therapy:

1. Transfer of genes into patient cells outside the body (ex vivo gene therapy)

2. Transfer of genes directly to cells inside the body (in vivo).

### 1. Ex vivo gene therapy:

The ex vivo gene therapy can be applied to only selected tissues (e.g., bone marrow) whose cells can be cultured in the laboratory. The technique of ex vivo gene therapy involves the following steps (Fig. 13.2).

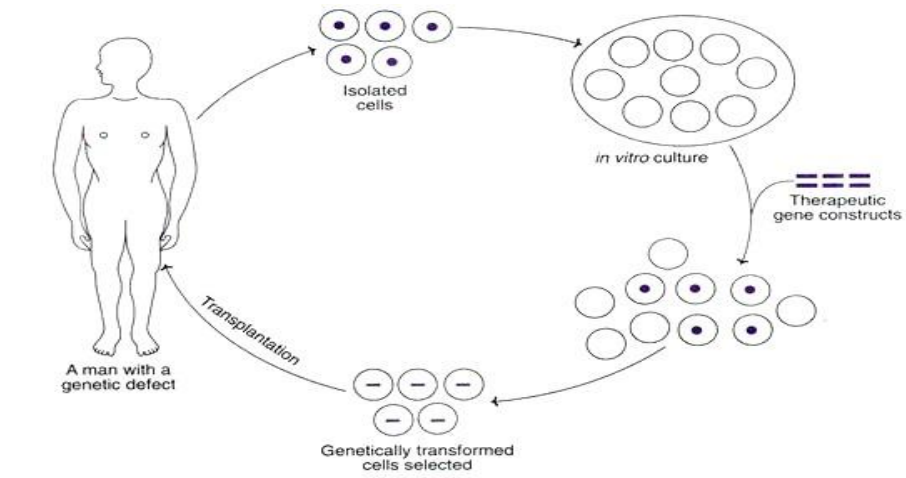


*Fig. 13.2 : The procedure for ex vivo gene therapy.*

1. Isolate cells with genetic defect from a patient.
2. Grow the cells in culture.
3. Introduce the therapeutic gene to correct gene defect.
4. Select the genetically corrected cells (stable trans-formants) and grow.
5. Transplant the modified cells to the patient.

The procedure basically involves the use of the patient's own cells for culture and genetic correction, and then their return back to the patient. This technique is therefore, not associated with adverse immunological responses after transplanting the cells. Ex vivo gene therapy is efficient only, if the therapeutic gene (remedial gene) is stably incorporated and continuously expressed. This can be achieved by use of vectors.

**Vectors in Gene Therapy:**

The carrier particles or molecules used to deliver genes to somatic cells are referred to as vectors. The important vectors employed in ex vivo gene therapy are listed below and briefly described next.

i. Viruses

ii. Human artificial chromosome

iii. Bone marrow cells.

**i. Viruses:**

The vectors frequently used in gene therapy are viruses, particularly retroviruses. RNA is the genetic material in retroviruses. As the retrovirus enters the host cell, it synthesizes

DNA from RNA (by reverse transcription). The so formed viral DNA (referred to as provirus) gets incorporated into the DNA of the host cell.
The proviruses are normally harmless. However, there is a tremendous risk, since some of the retroviruses can convert normal cells into cancerous ones. Therefore, it is absolutely essential to ensure that such a thing does not happen.

## Making retroviruses harmless:

Researchers employ certain biochemical methods to convert harmful retroviruses to harmless ones, before using them as vectors. For instance, by artificially removing a gene that encodes for the viral envelope, the retrovirus can be crippled and made harmless. This is because, without the envelope, retrovirus cannot enter the host cell. The production of a large number (billions) of viral particles can be achieved, starting from a single envelope defective retrovirus (Fig. 13.3).

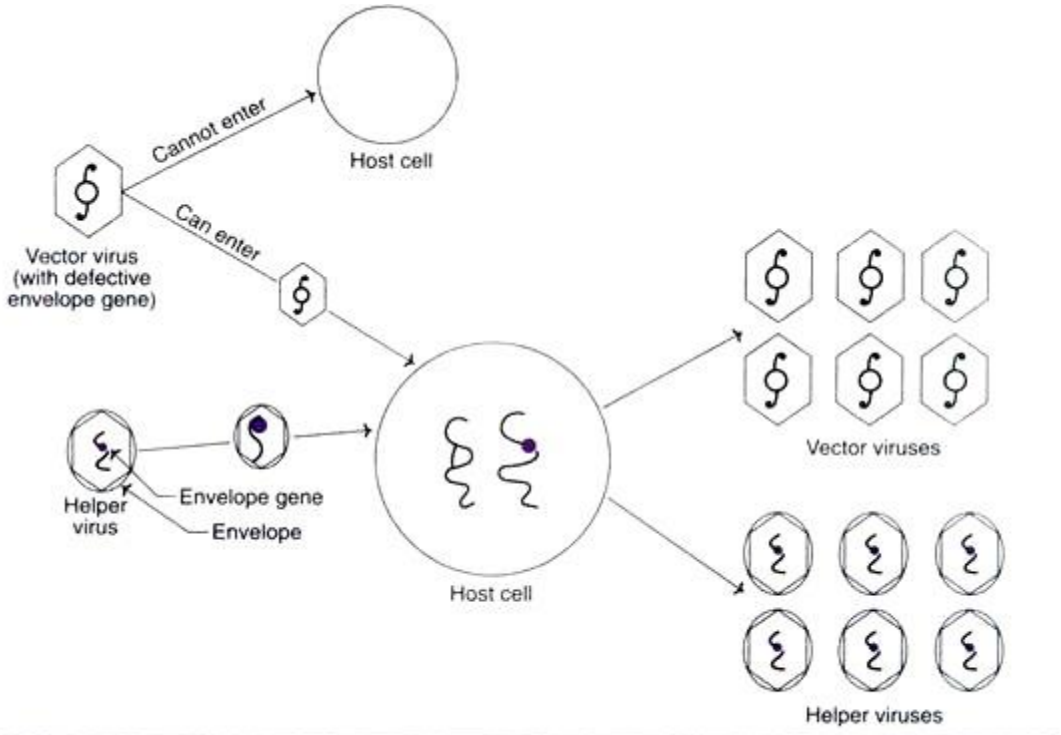


*Fig. 13.3 : Large scale production of vector viruses by using helper viruses.*

This is made possible by using helper viruses which contain normal gene for envelope formation. Along with the helper virus, the vector (with defective envelope gene) can enter the host cell and both of them multiply. By repeated multiplication in host cells, billions of vector and helper viruses are produced.
The vector viruses can be separated from the helper viruses and purified. Isolation of vector viruses, totally free from helper viruses, is absolutely essential. Contamination of helper viruses is a big threat to the health of the patients undergoing gene therapy.

## Retroviruses in gene therapy:

The genetic map of a typical retrovirus is depicted in Fig. 13.4A. In general, the retrovirus particle has RNA as a genome organized into six regions. It has a 5′-long terminal repeat (5′-LTR), a non-coding sequence required for packaging RNA designated as psi (Ψ), a gene gag coding for structural protein, a gene pol that codes for reverse transcriptase, a gene env coding for envelope protein and a 3-LTR sequence.

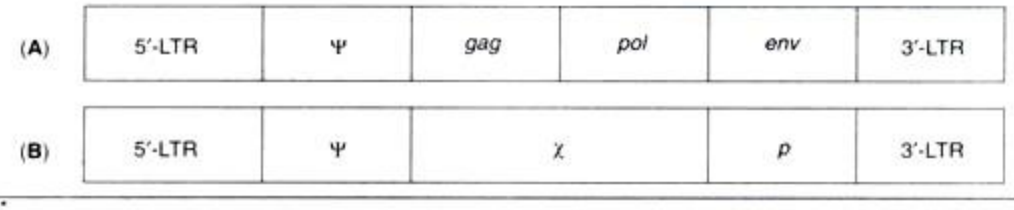


*Fig. 13.4 : A retrovirus used in gene therapy. (A) General map of a typical retrovirus (B) Gene map of a modified retrovirus for use in gene therapy (LTR-Long terminal repeat; Ψ-Packaging signal sequence;* gag-*Coding sequence for structural protein;* pol-*Coding sequence for reverse transcriptase;* env-*Envelope protein coding sequence; χ-Therapeutic gene; p–Promoter gene).*

For use of a retrovirus as a vector, the structural genes gag and pol are deleted. These genes are actually adjacent to Ψ region. In addition, a promoter gene is also included (Fig. 13.4B). This vector design allows the synthesis of cloned genes. A retroviral vector can carry a therapeutic DNA of maximum size of 8 kb.

A retroviral vector DNA can be used to transform the cells. However, the efficiency of delivery and integration of therapeutic DNA are very low. In recent years, techniques have been developed to deliver the vector RNA to host cells at a high frequency. For this purposes, packaged retroviral RNA particles are used. This technique allows a high efficiency of integration of pharmaceutical DNA into host genome.

Several modified viral vectors have been developed in recent years for gene therapy. These include onco-retrovirus, adenovirus, adeno-associated virus, herpes virus and a number of hybrid vectors combining the good characters of the parental vectors.

## Murine leukaemia viruses in gene therapy:

This is a retrovirus that causes a type of leukaemia in mice. It can react with human cells as well as the mouse cells, due to a similarity in the surface receptor protein. Murine leukaemia virus (MLV) is frequently used in gene transfer.

## AIDS virus in gene therapy?

It is suggested that the human immunodeficiency virus (HIV) can be used as a vector in gene transfer. But this is bound to create public uproar. Some workers have been successful in creating a harmless HIV (crippled HIV) by removing all the genes related to reproduction. At the same time, the essential genes required for gene transfer are retained. There is a distinct advantage with HIV when compared with MLV. MLV is capable of

bringing out gene transfer only in dividing cells. HIV can infect even non-dividing cells (e.g., brain cells) and do the job of gene transfer effectively. However, it is doubtful whether HIV can ever be used as a vector.

## ii. Human Artificial Chromosome:

The details of human artificial chromosome (HAC) are described elsewhere .HAC is a synthetic chromosome that can replicate with other chromosomes, besides encoding a human protein. As already discussed above, use of retroviruses as vectors in gene therapy is associated with a heavy risk. This problem can be overcome if HAC is used. Some success has been achieved in this direction.

## iii. Bone Marrow Cells:

Bone marrow contains totipotent embryonic stem (ES) cells. These cells are capable of dividing and differentiating into various cell types (e.g., red blood cells, platelets, macrophages, osteoclasts, B- and T-lymphocytes). For this reason, bone marrow transplantation is the most widely used technique for several genetic diseases.

And there is every reason to believe that the genetic disorders that respond to bone marrow transplantation are likely to respond to ex vivo gene therapy also (Table 13.2). For instance, if there is a gene mutation that interferes with the function of erythrocytes (e.g., sickle-cell anaemia), bone marrow transplantation is done. Bone marrow cells are the potential candidates for gene therapy of sickle-cell anaemia. However, this is not as simple as theoretically stated.

**TABLE 13.2 Selected list of genetic diseases that are likely to be cured by using bone marrow cells (potential candidates for gene therapy)**

| |
|---|
| Severe combined immunodeficiency (SCID) |
| Sickle-cell anemia |
| Fanconi anemia |
| Thalassemia |
| Gaucher's disease |
| Hunter disease |
| Hurler syndrome |
| Chronic granulomatous disease |
| Infantile agranulocytosis |
| Osteoporosis |
| X-linked agammaglobulinemia |

## Selected Examples of Ex Vivo Gene Therapy:

### a. Therapy for Adenosine Deaminase Deficiency:

The first and the most publicized human gene therapy was carried out to correct the deficiency of the enzyme adenosine deaminase (ADA). This was done on September 14, 1990 by a team of workers led by Blaese and Anderson at the National Institute of Health, USA (The girl's name is Ashanti, 4 years old then).

### b. Severe combined immunodeficiency (SCID):

This is rare inherited immune disorder associated with T-lymphocytes, and (to a lesser extent) B-lymphocytes dysfunction. About 50% of SCID patients have a defect in the gene (located on chromosome 20, and has 32,000 base pairs and 12 exons) that encodes for adenosine deaminase. In the deficiency of ADA, deoxyadenosine and its metabolites (primarily deoxyadenosine 5′-triphosphate) accumulate and destroy T-lymphocytes.

T-Lymphocytes are essential for body's immunity. Besides participating directly in body's defence, they promote the function of B-lymphocytes to produce antibodies. Thus, the patients of SCID (lacking ADA) suffer from infectious diseases and die at an young age. Previously, the children suffering from SCID were treated with conjugated bovine ADA, or by bone marrow transplantation.

### c. Technique of therapy for ADA deficiency:

The general scheme of gene therapy adopted for introducing a defective gene in the patient has been depicted in Fig 13.2. The same procedure with suitable modifications can also be applied for other gene therapies. A plasmid vector bearing a pro-viral DNA is selected. A part of the pro-viral DNA is replaced by the ADA gene and a gene (G 418) coding for antibiotic resistance, and then cloned. The antibiotic resistance gene will help to select the desired clones with ADA gene. A diagrammatic representation of the treatment of ADP deficient patient is depicted in Fig. 13.5.

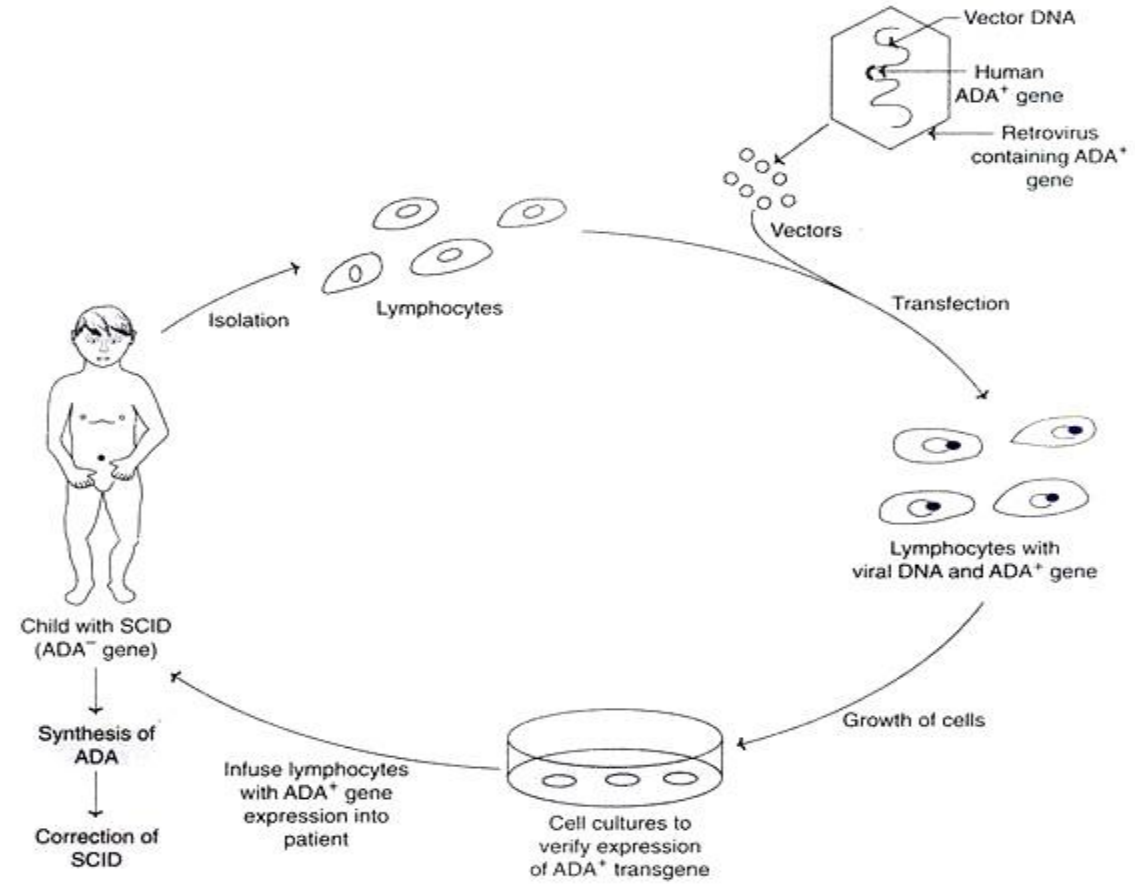


*Fig. 13.5 : Treatment of adenosine deaminase (ADA) deficient patient by somatic ex vivo gene therapy (SCID-Severe combined immunodeficiency disease).*

Circulating lymphocytes are removed from a patient suffering from ADA deficiency. These cells are transfected with ADA gene by exposing to billions of retroviruses carrying the said gene. The genetically-modified lymphocytes are grown in cultures to confirm the expression of ADA gene and returned to the patient. These lymphocytes persist in the circulation and synthesize ADA.

Consequently, the ability of the patient to produce antibodies is increased. However, there is a limitation. The lymphocytes have a short life span (just live for a few months), hence the transfusions have to be carried out frequently.

## Transfer of ADA gene into stem cells:

In 1995, ADA gene was transferred into the stem cells, obtained from the umbilical cord blood, at the time of baby's delivery. Four days after birth, the infant received the modified cells back. By this way, a permanent population of ADA gene producing cells was established.

## d. Therapy for Familial Hypercholesterolemia:

The patients of familial hypercholesterolemia lack the low density lipoprotein (LDL) receptors on their liver cells. As a result, LDL cholesterol is not metabolised in liver. The accumulated LDL- cholesterol builds up in the circulation, leading to arterial blockage and heart diseases.

Attempts are being made by gene therapists to help the victims of familial hypercholesterolemia. In fact, there is some success also. In a woman, 15% of the liver was removed. The hepatocytes were transduced with retroviruses carrying genes for LDL receptors. These genetically modified hepatocytes were infused into the patient's liver.

The hepatocytes established themselves in the liver and produced functional LDL-receptors. A significant improvement in the patient's condition, as assessed by estimating the lipid parameters in blood, was observed. Further, there were no antibodies produced against the LDL-receptor molecules, clearly showing that the genetically modified liver cells were accepted.

## e. Therapy for Lesch-Nyhan Syndrome:

Lesch-Nyhan syndrome is an inborn error in purine metabolism due to a defect in a gene that encodes for the enzyme hypoxanthine-guanine phosphoribosyl transferase (HCPRT). In the absence of HGPRT, purine metabolism is disturbed and uric acid level builds up, resulting in severe gout and kidney damage. The victims of Lesch- Nyhan syndrome exhibit symptoms of mental retardation, besides an urge to bite lips and fingers, causing self-mutilation.

By using retroviral vector system, HGPRT producing genes were successfully inserted into cultured human bone marrow cells. The major problem in humans is the involvement of brain. Experiments conducted in animals are encouraging. However, it is doubtful whether good success can be achieved by gene therapy for Lesch-Nyhan syndrome in humans, in the near future.

## f. Therapy for Haemophilia:

Haemophilia is a genetic disease due lack of a gene that encodes for clotting factor IX. It is characterized by excessive bleeding. By using a retroviral vector system, genes for the synthesis of factor IX were inserted into the liver cells of dogs. These dogs no longer displayed the symptoms of haemophilia.

## g. Ex Vivo Gene Therapy with Non-Autologous Cells:

The ex vivo gene therapies described above are based on the transplantation of genetically modified cells for the production of desired proteins. However, there are several limitations in using the patient's own cells (autologous cells) for gene therapy. These include lack of enough cells from target tissues, defective uptake of genes and their

inadequate expression. To overcome these problems, attempts are on to develop methods to use non-autologous cells (i.e., cells from other individuals or animals). The outline of the procedure is briefly described below.

Tissue-specific cells capable of growing in culture are selected. These include fibroblasts from skin, hepatocytes from liver, and myoblasts from muscle and astrocytes from brain. These cells are cultured and genetically modified with the therapeutic gene. They are then encapsulated in artificial membrane composed of a synthetic polymer (e.g., polyether sultone, alginase-poly L-lysine-alginate).The polymeric membranes are non-immunogenic, therefore the patient can accept non-autologous encapsulated cells. Further, being semipermeable in nature, these membranes allow the nutrients to enter in, and the encoded protein (by the therapeutic gene) to pass out.

Experiments conducted in animals have shown some encouraging results for using non-autologous cells in gene therapy. The encapsulated cells were found to proliferate and produce the required protein. However, the success has been very limited in human trials.

## 2. In Vivo Gene Therapy:

The direct delivery of the therapeutic gene (DNA) into the target cells of a particular tissue of a patient constitutes in vivo gene therapy (Fig. 13.6). Many tissues are the potential candidates for this approach. These include liver, muscle, skin, spleen, lung, brain and blood cells. Gene delivery can be carried out by viral or non- viral vector systems. The success of in vivo gene therapy mostly depends on the following parameters

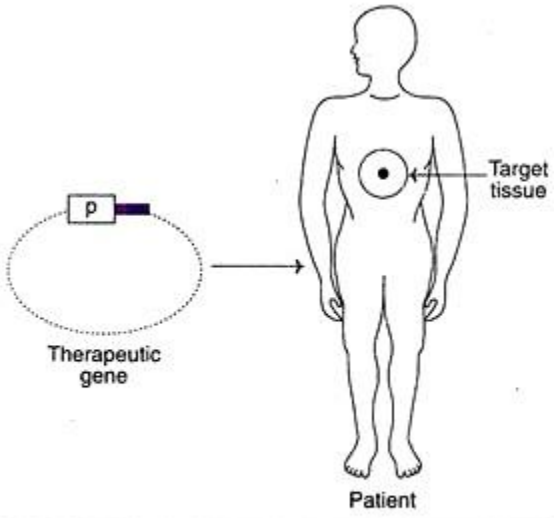


*Fig. 13.6 : Diagrammatic representation of in vivo gene therapy. (p-Promoter gene specific for therapeutic gene)*

i. The efficiency of the uptake of the remedial (therapeutic) gene by the target cells.
ii. Intracellular degradation of the gene and its uptake by nucleus.
iii. The expression capability of the gene.
In vivo gene therapy with special reference to gene delivery systems (viral, non-viral) with suitable examples is described.

## Gene Delivery by Viruses:

Many viral vector systems have been developed for gene delivery. These include retroviruses, adenoviruses, adeno-associated viruses and herpes simplex virus.

## Retrovirus vector system:

Replication defective retrovirus vectors that are harmless are being used. A plasmid in association with a retrovirus, a therapeutic gene and a promoter is referred to as plasmovirus. The plasmovirus is capable of carrying a DNA (therapeutic gene) of size less than 3.4 kb. Replication defective virus particles can be produced from the plasmovirus.
As such, for the delivery of genes by retroviral vectors, the target cells must be in a dividing stage. But majority of the body cells are quiescent. In recent years, viral vectors have been engineered to infect non-dividing cells. Further, attempts are on to include a DNA in the retroviral vectors (by engineering env gene) that encodes for cell receptor protein. If this is successfully achieved, the retroviral vector will specifically infect the target tissues.

## Adenoviral vector system:

Adenoviruses (with a DNA genome) are considered to be good vectors for gene delivery because they can infect most of the non-dividing human cells. A common cold adenovirus is a frequently used vector. As the target cells are infected with a recombinant adenovirus, the therapeutic gene (DNA) enters the nucleus and expresses itself.
However, this DNA does not integrate into the host genome. Consequently, adenoviral based gene therapy required periodic administration of recombinant viruses. The efficiency of gene delivery by adenoviruses can be enhanced by developing a virus that can specifically infect target cells. This is possible by incorporating a DNA encoding a cell receptor protein.

## Adeno-associated virus vector system:

Adeno-associated virus is a human virus that can integrate into chromosome 19. It is a single-stranded, non-pathogenic small DNA virus (4.7 kb). As the adeno-associated virus enters the host cell, the DNA becomes double- stranded, gets integrated into chromosome and expresses.

Adeno-associated viruses can serve as good vectors for the delivery of therapeutic genes. Recombinant viruses are created by using two plasmids and an adenovirus (i.e., helper virus) by a special technique. Some attempts were made to use therapeutic genes for the treatment of the human diseases-haemophilia (for production of blood clotting factor IX) and cystic fibrosis (for synthesis of cystic fibrosis trans membrane regulator protein) by employing adeno-associated viruses.

## Therapy for cystic fibrosis:

Cystic fibrosis (CF) is one of the most common (frequency 1: 2,500) and fatal genetic diseases. It is characterized by the accumulation of sticky, dehydrated mucus in the respiratory tract and lungs. Patients of CF are highly susceptible to bacterial infections in their lungs and most of them die before reaching the age of thirty.

## Biochemical basis:

In the normal persons the chloride ions of the cells are pushed out through the participation of a protein called cystic fibrosis trans membrane regulator (CFTR). In the patients of cystic fibrosis, the CFTR protein is not produced due to a gene defect. Consequently, the chloride ions concentrate within the cells which draw water from the surroundings. As a result, the respiratory tract and the lungs become dehydrated with sicky mucus, an ideal environment for bacterial infections.

## Gene therapy for Cystic Fibrosis:

As the defective gene for cystic fibrosis was identified in 1989, researchers immediately started working on gene therapy for this disease. Adenoviral vector systems have been used, although the success has been limited. The major drawback is that the benefits are short-lived, since the adenoviruses do not integrate themselves into host cells. Multiple administration of recombinant adenovirus caused immunological responses that destroyed the cells.

By using adeno-associated virus vector system, some encouraging results were reported in the gene therapy of CF. In the phase I clinical trials with CF patients, the vector persisted for about 70 days and some improvement was observed in the patients. Some researchers are trying to insert CF gene into the developing fetal cells (in experimental animals such as mice) to produce CFTR protein. But a major breakthrough is yet to come.

## Herpes simplex virus vector system:

The retroviruses and adenoviruses employed in in vivo gene therapy are engineered to infect specific target cells. There are some viruses which have a natural tendency to infect a particular type of cells. The best example is herpes simplex virus (HSV) type I, which infects

and persists in non-dividing nerve cells. HSV is a human pathogen that causes (though rarely) cold sores and encephalitis.

These are a large number of diseases (metabolic, neurodegenerative, immunological, tumours) associated with nervous system. HSV is considered as an ideal vector for in vivo gene therapy of many nervous disorders. The HSV has a double-stranded DNA of about 152 kb length as its genome. About 30 kb of HSV genome can be replaced by a cloned DNA without loss of its basic characteristics (replication, infection, packaging etc.). But there are some technical difficulties in dealing with large-sized DNAs in genetic engineering experiments. Some modified HSV vectors with reduced genomic sizes have been developed. Most of the work on the gene therapy, related to the use of HSV as a vector, is being conducted in experimental animals. And the results are quite encouraging. HSV vectors could deliver therapeutic genes to the brain and other parts of nervous system. These genes are well expressed and maintained for long periods. More research, however, is needed before going for human trials. If successful, HSV may help to treat many neurodegenerative syndromes such as Parkinson's disease and Alzheimer's disease by gene therapy.

## Gene Delivery by Non-Viral Systems:

There are certain limitations in using viral vectors in gene therapy. In addition to the prohibitive cost of maintaining the viruses, the viral proteins often induce inflammatory responses in the host. Therefore, there is a continuous search by researchers to find alternatives to viral vector systems.

## a. Pure DNA constructs:

The direct introduction of pure DNA constructs into the target tissue is quite simple. However, the efficiency of DNA uptake by the cells and its expression are rather low. Consequently, large quantities of DNA have to be injected periodically. The therapeutic genes produce the proteins in the target cells which enter the circulation and often get degraded.

## b. Lipoplexes:

The lipid-DNA complexes are referred to as lipoplexes or more commonly liposomes. They have a DNA construct surrounded by artificial lipid layers. A large number of lipoplexes have been prepared and used. They are non-toxic and non-immunogenic.

The major limitation with the use of lipoplexes is that as the DNA is taken up by the cells, most of it gets degraded by the lysosomes. Thus, the efficiency of gene delivery by lipoplex is very low. Some clinical trials using liposome-CFTR gene complex showed that the gene expression was very short-lived.

## c. DNA-molecular conjugates:

The use of DNA-molecular conjugates avoids the lysosomal breakdown of DNA. Another advantage of using conjugates is that large-sized therapeutic DNAs (> 10 kb) can be delivered to the target tissues. The most commonly used synthetic conjugate is poly-L-lysine, bound to a specific target cell receptor. The therapeutic DNA is then made to combine with the conjugate to form a complex (Fig. 13.7).

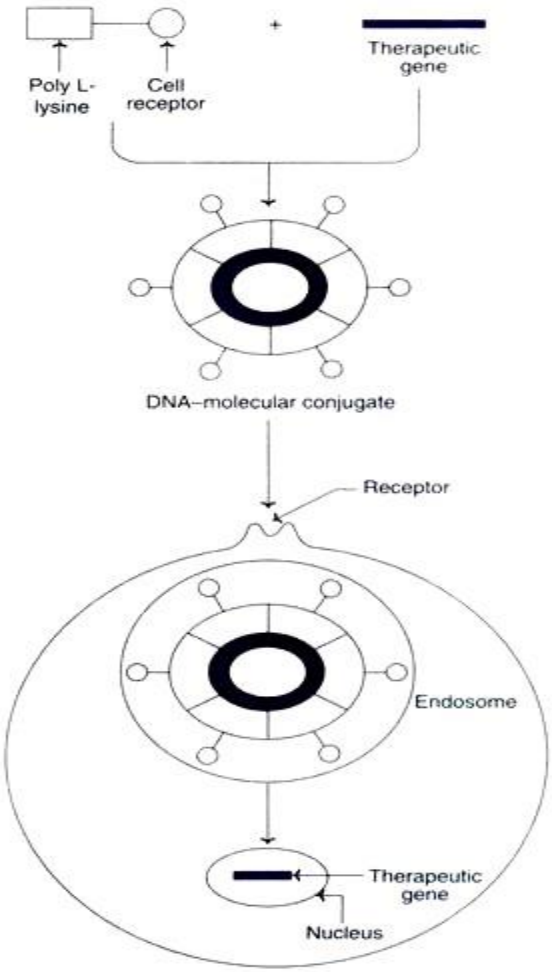


*Fig. 13.7 : DNA-molecular conjugate in the delivery of therapeutic gene.*

This DNA molecular conjugate binds to specific cell receptor on the target cells. It is engulfed by the cell membrane to form an endosome which protects the DNA from being degraded. The DNA released from the endosome enters the nucleus where the therapeutic gene is expressed.

## d. Human artificial chromosome:

Human artificial chromosome (HAC) which can carry a large DNA one or more therapeutic genes with regulatory elements is a good and ideal vector. Studies conducted in cell cultures using HAC are encouraging. But the major problem is the delivery of the large-sized chromosome into the target cells. Researchers are working to produce cells containing genetically engineered HAC. There exists a possibility of encapsulating and implanting these cells in the target tissue. But a long way to go!

## Efficiency of gene delivery by non-viral vectors:

Although the efforts are continuously on to find suitable non-viral vectors for gene delivery, the success has been very limited. This is mainly due to the following two reasons.

1. The efficiency of transfection is very low.

2. The expression of the therapeutic gene is for a very short period, consequently there is no effective treatment of the disease.

## Gene Therapy Strategies for Cancer:

Cancer is the leading cause of death throughout the world, despite the intensive treatment strategies (surgery, chemotherapy, radiation therapy). Gene therapy is the latest and a new approach for cancer treatment. Some of the developments are briefly described hereunder.

## Tumour necrosis factor gene therapy:

Tumour necrosis factor (TNF) is a protein produced by human macrophages. TNF provides defence against cancer cells. This is brought out by enhancing the cancer-fighting ability of tumour- infiltrating lymphocytes (TILs), a special type of immune cells.

The tumour-infiltrating lymphocytes were transformed with a TNF gene (along with a neomycin resistant gene) and used for the treatment of malignant melanoma (a cancer of melanin producing cells usually occurs in skin). TNF as such is highly toxic, and fortunately no toxic side effects were detected in the melanoma patients injected with genetically altered TILs with TNF gene. Some improvement in the cancer patients was observed.

## Suicide gene therapy:

The gene encoding the enzyme thymidine kinase is often referred to as suicide gene, and is used for the treatment of certain cancers. Thymidine kinase (TK) phosphorylates nucleosides to form nucleotides which are used for the synthesis of DNA during cell division. The drug ganciclovir (GCV) bears a close structural resemblance to certain nucleosides (thymidine). By mistake, TK phosphorylates ganciclovir to form triphosphate-

GCV, a false and unsuitable nucleotide for DNA synthesis. Triphosphate-GCV inhibits DMA polymerase (Fig. 13.8).

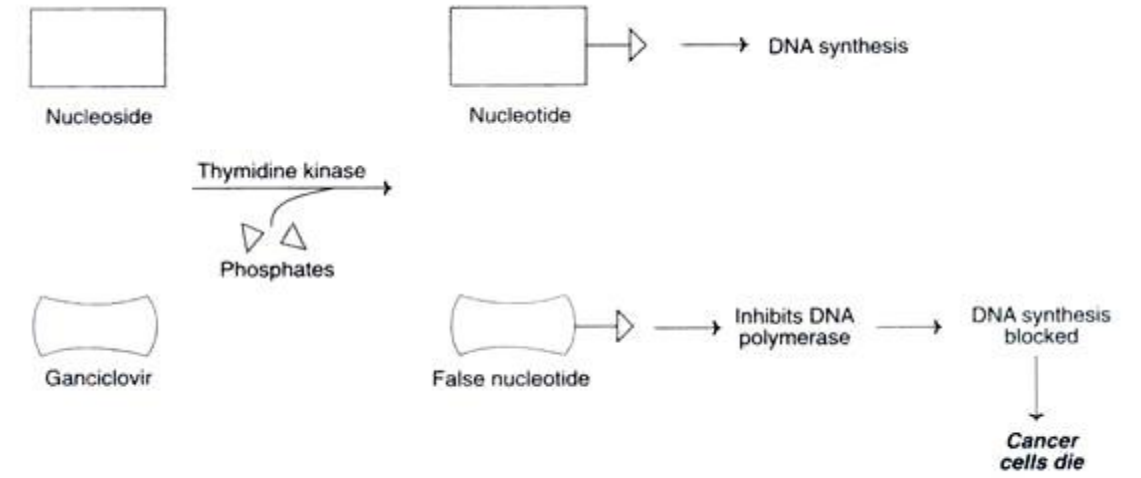


*Fig. 13.8 : The action of ganciclovir mediated by thymidine kinase to inhibit the growth of cancer cells.*

The result is that the elongation of the DNA molecule abruptly stops at a point containing the false nucleotide (of ganciclovir). Further, the triphosphate-GCV can enter and kill the neighbouring cancer cells, a phenomenon referred to as bystander effect. The ultimate result is that the cancer cells cannot multiply, and therefore die. Thus, the drug ganciclovir can be used to kill the cancer cells.

Ganciclovir is frequently referred to as a pro-drug and this type of approach is called pro-drug activation gene therapy. Ganciclovir has been used for treatment of brain tumours (e.g., glioblastoma, a cancer of glial cells in brain), although with a limited success.

In the suicide gene therapy, the vector used is herpes simplex virus (HSV) with a gene for thymidine kinase (TK) inserted in its genome. Normal brain cells do not divide while the brain tumour cells go on dividing unchecked. Thus, there is a continuous DNA replication in tumour cells. By using GCV-HSVTK suicide gene therapy, some reduction in proliferating tumour cells was reported. Several new strategies are being developed to increase the delivery of HSVTK gene to all the cells throughout a tumour.

## Two-gene cancer therapy:

For treatment of certain cancers, two gene systems are put together and used. For instance, TK suicide gene (i.e., GCV-HSVTK) is clubbed with interleukin-2 gene (i.e. a gene promoting immunotherapy). Interleukin-2 produced mobilizes immune response. It is believed that certain proteins are released from the tumour cells on their death.

These proteins, in association with immune cells, reach the tumour and initiate immunological reactions directed against the cancer cells. Two-gene therapies have been

carried out in experimental animals with colon cancer and liver cancer, and the results are encouraging.

## Gene replacement therapy:

A gene named $p^{53}$ codes for a protein with a molecular weight of 53 kilo Daltons (hence $p^{53}$). $p^{53}$ is considered to be a tumour-suppressor gene, since the protein it encodes binds with DNA and inhibits replication. The tumour cells of several tissues (breast, brain, lung, skin, bladder, colon, bone) were found to have altered genes of $p^{53}$(mutated $p^{53}$), synthesizing different proteins from the original.

These altered proteins cannot inhibit DNA replication. It is believed that the damaged $p^{53}$ gene may be a causative factor in tumour development. Some workers have tried to replace the damaged $p^{53}$gene by a normal gene by employing adenovirus vector systems .There are some encouraging results in the patients with liver cancer.

## Gene Therapy for Aids:

AIDS is a global disease with an alarming increase in the incidence every year. It is invariably fatal, since there is no cure. Attempts are being made to relieve the effects of AIDS by gene therapy. Some of the approaches are discussed hereunder.

## a. rev and env genes:

A mutant strain of human immunodeficiency virus (HIV), lacking rev and env genes has been developed. The regulatory and envelope proteins of HIV are respectively produced by rev and env genes. Due to lack of these genes, the virus cannot replicate.

Researchers have used HIV lacking rev and env genes for therapeutic purposes. T-Lymphocytes from HIV-infected patients are removed, and mutant viruses are inserted into them. The modified T-lymphocytes are cultivated and injected into the patients. Due to lack of essential genes, the viruses (HIV) cannot multiply, but they can stimulate the production of $CD_8$ (cluster determinant antigen 8) cells of T-lymphocytes. $CD_8$ cells are the killer lymphocytes. It is proved in the laboratory studies that these lymphocytes destroy the HIV-infected cells.

## b. Genes of HIV proteins:

Some genes synthesizing HIV proteins are attached to DNA of mouse viruses. These genetically-modified viruses are injected to AIDS patients with clinical manifestations of the disease. It is believed that the HIV genes stimulate normal body cells to produce HIV proteins. The latter in turn stimulate the production of anti-HIV antibodies which prevent the HIV replication in AIDS patients.

## c. Gene to inactivate gp120:

gp120 is a glycoprotein (molecular weight 120 kilo Daltons) present in the envelope of HIV. It is absolutely essential for binding of virus to the host cell and to bring replication. Researchers have synthesized a gene (called F105) to produce an antibody that can inactivate gp120.In the anti- AIDS therapy, HIV-infected cells are engineered to produce anti-HIV antibodies when injected into the organism. Studies conducted in experimental animals showed a drastic reduction in the synthesis of gp120 due to anti-AIDS therapy. The production of F1IV particles was also very reduced. There are some attempts to prevent AIDS by antisense therapy.

## Advantages of Gene Therapy

Gene therapy can cure genetic diseases by addition of gene or by removal of gene or by replacing a mutated gene with corrected gene.

Gene therapy can be used for cancer treatment to kill the cancerous cells.

Gene expression can be controlled.

Therapeutic protein is continuously produced inside the body which also reduces the cost of treatment in long ter

## The Future of Gene Therapy:

Theoretically, gene therapy is the permanent solution for genetic diseases. But it is not as simple as it appears since gene therapy has several inbuilt complexities. Gene therapy broadly involves isolation of a specific gene, making its copies, inserting them into target tissue cells to make the desired protein. The story does not end here.

It is absolutely essential to ensure that the gene is harmless to the patient and it is appropriately expressed (too much or too little will be no good). Another concern in gene therapy is the body's immune system which reacts to the foreign proteins produced by the new genes.The public, in general, have exaggerated expectations on gene therapy. The researchers, at least for the present, are unable to satisfy them. As per the records, by 1999 about 1000 Americans had undergone clinical trials involving various gene therapies.

Unfortunately, the gene therapists are unable to categorically claim that gene therapy has permanently cured any one of these patients. Some people in the media (leading newspapers and magazines) have openly questioned whether it is worth to continue research on gene therapy. It may be true that as of now, gene therapy due to several limitations, has not progressed the way it should, despite intensive research. But a break-through may come anytime, and of course, this is only possible with persistent research. And a day may come (it might take some years) when almost every disease will have a gene

therapy, as one of the treatment modalities. And gene therapy will revolutionize the practice of medicine.

## Probable questions:

1. Define Gene therapy.
2. Describe different strategies of gene therapy ?
3. Define somatic cell gene therapy and germ cell gene therapy?
4. What is ex vivo gene therapy and in vivo gene therapy?
5. How retroviruses are used in gene therapy?
6. How gene therapy is used in treatment of Cystic fibrosis ?
7. What is suicide gene therapy?
8. Describe gene therapy treatments for AIDS?
9. What is are the advantages of gene therapy?
10. Write about the future of gene therapy?
11. What is Differentiation therapy? Explain.
12. How differentiation therapy an be applied in cancer treatment ?

## Suggested Readings:


**1.** Robert C. Green MD, MPH, Jonathan S. Berg MD, PhD, Wayne W. Grody MD, PhD, Sarah S. Kalia ScM, CGC, Bruce R. Korf MD, PhD, Christa L. Martin PhD, FACMG, Amy L. McGuire JD, PhD, Robert L. Nussbaum MD, Julianne M. O'Daniel MS, CGC, Kelly E. Ormond MS, CGC, Heidi L. Rehm PhD, FACMG, Michael S. Watson PhD, FACMG, Marc S. Williams MD, FACMG & Leslie G. Biesecker MD. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 2013 Jul;15(7):565-74.

**2.** Sarah S. Kalia ScM, Kathy Adelman, Sherri J. Bale PhD, Wendy K. Chung MD, PhD, Christine Eng MD, James P. Evans MD, PhD, Gail E. Herman MD, PhD, Sophia B. Hufnagel MD, Teri E. Klein PhD, Bruce R. Korf MD, PhD, Kent D. McKelvey MD, Kelly E. Ormond MS, C. Sue Richards PhD, Christopher N. Vlangos PhD, Michael Watson PhD, Christa L. Martin PhD & David T. Miller MD, PhD ; on behalf of the ACMG Secondary Findings Maintenance Working Group. Recommendations for

reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017 Feb;19(2):249-255. doi: 10.1038/gim.2016.190.

3. Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, Stewart DR, Amendola LM, Adelman K, Bale SJ, Gollob MH, Harrison SM, Hershberger RE, McKelvey K, Richards CS, Vlangos CN, Watson MS, Martin CL; ACMG Secondary Findings Working Group. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021 May 20.

4. 1. Principles of Genetics. Snustad and Simmons.
5. 2. Genetics . Verma and Agarwal.
6. 3. Principles of Genetics by Tamarin.
7. 4. Biotechnology by V. Kumaresan

# UNIT-IV

# Concepts of Pharmacogenomics; Pharmacogenetics in cancer prognosis and treatment

**Objective:** In this unit we will discuss about pharmacogenomics. We will also discuss how pharmacogenomics can be used to treat cancer.

## Introduction:

Pharmacogenomics is the study of the impact of genetic variation on drug response. The terms 'pharmacogenetics' and 'pharmacogenomics' are often used interchangeably, but there are slight differences. As early as the 1930s, scientists recognized a connection between inborn variation in enzymes and drug response. In 1959, Friedrich Vogel of Germany coined the word 'pharmacogenetics' in reference to a single gene's effect on the actions of a drug. The field of pharmacogenetics continued to advance and by the time of the genomic era in the 1990s, scientists realized that many of the differences in drug response could be attributed to variation across multiple genes. The word 'pharmacogenomics' refers to the contribution of multiple variants and genes on drug response rather than on one or two genes in pharmacogenetics. Pharmacogenomics and pharmacogenetics are often used synonymously.

Drug response phenotypes examined by pharmacogenomics include efficacy (e.g., how well the medication works) and side effects (e.g., unintended responses to the medication). Toxicity and adverse reactions are the most commonly studied side effects that can be harmful to the patient. There are examples of non-harmful side effects and some can even be considered beneficial. For example, the glaucoma medication Lumigan was known to stimulate eyelash growth. This led to the development of Latisse, which the FDA approved for the purpose of promoting eyelash growth.

## Clinical Implementation of Pharmacogenomics:

Pharmacogenomics has the potential to influence clinically relevant outcomes in drug dosing, efficacy, and toxicity that can result in subsequent recommendations for testing. For many routinely used drugs, pharmacogenomics has provided inconclusive evidence for such testing. A probable reason could be the involvement of both genetic and nongenetic factors and their extent of contribution that determines the clinical relevance of some

drugs. Therefore, identification of genetic markers associated with drug responses does not always link to clinically useful predictors of adverse outcomes, and most of the time require independent replication of genotype–phenotype association before pursuing clinical implementation.

Lack of readily available resources, feasibility, utility, level of evidence, provider knowledge, cost effectiveness, and ethical, legal, and social issues further adds to the limitations and challenges to implementing pharmacogenomic testing in clinical practice. In order for a genetic marker to be implicated in clinical practice, an association of a genetic marker to a particular trait requires screening of tissues from several individuals, and corresponding functional studies are needed to establish probable association with the trait/phenotype. However, to overcome these challenges there are some pharmacogenomic tests for drugs currently used in clinical practice that have applied value in predicting ADRs and/or drug efficacy. These tests are based on distinct genetic variants that have well-validated reproducible and significant impact on the drug therapy. These tests have a strong causal association between genetic polymorphisms and drug responses: a strong indication for clinical utility and high prognostic value. The tests are available both commercially and in academic settings, with many of these tests having clinical guidelines for dose adjustment and alternative medications. In addition, various international pharmacogenomic consortia have been developed recently to supervise drug response studies.

The classic example of drug toxicity and pharmacogenetics involves the gene *TPMT* (thiopurine *S*-methyltransferase) and thiopurines, a class of drugs typically used to treat myeloid leukemias or as immunosuppressants. Depending on the genotype of the patient, thiopurines can be either an effective treatment or very harmful, even deadly, at regularly prescribed doses. While all patients on this medication require careful monitoring to avoid toxicity, patients who are heterozygous for variant *TPMT* alleles display intermediate enzyme activity levels and are more susceptible to toxicity, requiring extra monitoring and possibly reduced doses. Those homozygous for variant *TPMT* alleles can be enzyme deficient and require extremely reduced levels of the drug or an alternative treatment. Dosing guidelines for thiopurines based on *TPMT* pharmacogenetics were recently published by Mary Relling and colleagues as part of the Clinical Pharmacogenetics Implementation Consortium (CPIC).

CPIC is a collaborative effort formed in 2009 to help promote the use of pharmacogenomics in clinical practice. The key assumption underlying the CPIC guidelines is not whether tests should be ordered for genotyping, but rather, to help clinicians understand how available test results should be used to optimize drug therapy. The group uses well-defined criteria to evaluate the evidence for clinical applications of pharmacogenomics and publishes

corresponding drug dosing guidelines. By providing specific genotype-based dosing guidelines in addition to regularly used clinical parameters, CPIC aims to break down some of the barriers which impede routine use of pharmacogenomics in clinical settings. The initial set of pharmacogenomics guidelines that CPIC has published include well-known associations such as warfarin, CYP2C9, and VKORC1; clopidogrel and CYP2C19; codeine and CYP2D6; and HLA-B*5701 and abacavir.

The guidelines published by CPIC are freely available at the PharmGKB (the Pharmacogenomics Knowledge Base). The PharmGKB began in 2000 as a repository of pharmacogenetic and pharmacogenomic primary data and simple associations between drugs and genes found in the literature. PharmGKB was one of the first resources of its kind, and it remains a preeminent resource in the field. PharmGKB's mission has evolved over the past 10 years to reflect the changing field of pharmacogenomics. The PharmGKB now focuses on pharmacogenomics knowledge acquisition and manages consortia that examine important pharmacogenomic questions. Recently, PharmGKB has taken on a translational role and has become involved in the application of pharmacogenomics knowledge to the clinical setting.

Knowledge acquisition at PharmGKB entails aggregating and curating pharmacogenomics information, primarily in the form of drug-centered pathways (pharmacodynamic and pharmacokinetic), important pharmacogene summaries, and detailed annotations of the relationships among genetic variation, drugs, and diseases. All annotations, pathways, and summaries are manually curated by scientists with advanced degrees in various areas of biology. Pathways and gene summaries are peer-reviewed and published routinely. The PharmGKB staff have developed custom annotation tools that enable curators to capture complex pharmacogenomic relationships, including statistical measures of study significance (*p*-values, odds ratios, etc.) and study population parameters (study size, ethnicity/race, and other descriptive qualities such as disease status, gender-based studies, and pediatric studies) in a structured manner. Because of the size of the pharmacogenomic literature, manual annotation of every article is unrealistic. Therefore, automated and semiautomated methods for knowledge curation are in development.

A major contribution of the PharmGKB to the pharmacogenomics field is the standardization of pharmacogenomics knowledge. For example, PharmGKB maps all gene names and symbols to Hugo Gene Nomenclature Committee (HGNC) gene symbols, standardizing the genes in its database. It also standardizes the genetic variants across the genome. Although improvements have been made in published literature in the past several years, there remains a lack of standardization of gene names, haplotype terminology, and identification of genetic variation. Often, different papers refer to the same genetic variation differently. At times, amino acid changes are used to identify the

variation, while other times nucleotide base changes are used. Nucleotide changes are especially confusing because numbering depends entirely on the reference sequence being used. Cross-mapping variation between reference sequences with certainty can be quite challenging. Authors will use colloquial names for variants that may be well understood in that particular community but are not clear to scientists outside of that domain. In recent years, authors have begun to use Rapid Stain Identification (RSIDs), the identification used by the National Center for Biotechnology Information (NCBI)'s dbSNP website to refer to variants. The use of RSIDs is extremely helpful for identifying variants across publications. But many authors still do not utilize these identifiers and any literature before dbSNP will not have them. PharmGKB curators attempt to map all variants annotated from the literature to dbSNP RSIDs when possible, which serves to unify the body of knowledge about any particular variant. Connections can be made between papers that would not exist otherwise.

Another point of inconsistency in pharmacogenomics literature is the identification of the risk allele of the variant. Given an A/G variant that corresponds to a particular phenotype, knowing which base signifies risk of a particular toxicity is critical. In some papers, the risk allele is simply not identified. In other papers, the allele may be identified, but with A/T or C/G variants, the strand of the gene or chromosome makes a difference, and often this critical information is omitted. In these cases, PharmGKB curators investigate other literature and resources to identify the specifics of the risk allele.

The details of pharmacogenomic associations that PharmGKB maintains are extremely important for clinical applications of this knowledge. Study sizes, effect sizes, exact identification of the variant, and the risk allele are critical to employ pharmacogenomic knowledge in a patient setting. Clinically relevant associations are not derived from single studies, but rather are a result of an accumulation of research. A summary of the current research that gives an overall picture of the evidence for a particular association is useful. Therefore, PharmGKB creates 'clinical annotations' which provide summaries of one or more variant annotations in the database regarding a specific variant–drug association. Clinical annotations are written in the context of genotypes. For every variant–drug association, each genotype (e.g., AA, AG, GG) has a corresponding summary describing the effect of the genotype on drug response. The effect size for each genotype is written with respect to the other genotypes. That is, if the AA genotype corresponds with a lack of efficacy of a drug, then the AG and GG genotype descriptions will describe increased efficacy 'as compared to those with the AA genotype'. In this way, genotype summaries are comparable only within a given clinical annotation, or particular variant–drug pair. Each clinical annotation is assigned a 'strength-of-evidence' score. This score is primarily based on replication of the association in independent studies and/or conflicting studies, population size, effect size, and *p*-value of the association. The curation scientists

have the discretion to bump the score up or down based on other information, including but not limited to, effect size and clinical relevance.

In addition to clinical annotations, PharmGKB is involved in clinical applications through the consortia that they direct and manage. PharmGKB organizes research consortia and facilitates the sharing of pharmacogenomic data and knowledge across research groups. The International Warfarin Pharmacogenetics Consortium (IWPC), the International Warfarin Pharmacogenetics Consortium–Genome-Wide Association Studies (IWPC–GWA), and the Clinical Pharmacogenetics Implementation Consortium (CPIC), discussed above, are examples.

Knowledge in the field of pharmacogenomics is both broad and deep. Extensive knowledge of the genes, drugs, and phenotypes involved in many associations exists. Basic research in the field continues to increase the number of known associations and to replicate previous findings. Now, the most pressing challenge may be to translate pharmacogenomic knowledge from the bench to the bedside. For several drugs, the body of knowledge is large enough and deep enough to warrant incorporation of pharmacogenomics along with clinical factors when determining dosage on a routine basis. As genotyping and sequencing becomes cheaper, more patients will have genetic information available before beginning medications. In the age of direct-to-consumer genotyping, many individuals already have possession of their own genetic information. The challenge is for physicians to know how to use that information when prescribing medications. Dosing guidelines that incorporate genotypic knowledge are critical to bring pharmacogenomics into the clinic. The future of pharmacogenomics is the application of personalized medicine.

## Pharmacogenomics and Cancer:

Nowhere would pharmacogenomics appear more powerful than in the field of oncology. Here, a drug must interact with two genomes, and thus its effects are influenced not only by inherited or germline mutations but also by tumor-specific or somatic gene changes. With some exceptions, a common paradigm is to view germline mutations as heavily influencing drug metabolism and toxicity while somatic variants as strongly impacting clinical efficacy. An understanding of both genomes is required for optimizing tumor response, treatment regimens, and ultimately long-term survival.

One of the best known examples of tumor-specific therapeutics is the drug imatinib and its use in chronic myeloid leukemia (CML) . More than 95% of CML cases are associated with a reciprocal translocation and subsequent fusion between the *ABL* tyrosine kinase gene on chromosome 9 and the breakpoint cluster gene (*BCR*) on chromosome 22 [t(9:22)]. The resultant chimeric oncogene, *BCR-ABL*, encodes a constitutively active tyrosine kinase,

which further activates multiple downstream processes eventually leading to the malignant transformation of hematopoietic cells. Imatinib functions as a BCR-ABL specific tyrosine kinase inhibitor. The drug binds to the inactive conformation of the protein tyrosine kinase domain and blocks ATP binding, inhibiting conversion of the enzyme to its active form. By exploiting this particular molecular target, imatinib is able to induce a complete hematologic remission in greater than 95% of patients. Imatinib also inhibits tyrosine kinase for platelet-derived growth factor receptor A (PDGFRA) and stem cell growth factor receptor also known as proto-oncogene c-Kit. Thus, imatinib has also been used as an adjuvant therapy for gastrointestinal stromal tumors with *c-KIT* and *PDGFRA* mutations; in some studies, the response to imatinib has been shown to be dependent on the specific genotypes.

Therapeutic compounds have been developed targeting tyrosine kinase domains in several other oncogenes including somatic mutations in *EGFR*, the *EML4–ALK* fusion gene, and the V600E variant of *BRAF*. In addition to tumor-specific targets, some chemotherapeutics take advantage of tumor-specific defects by creating a "synthetic lethal" scenario. Hereditary breast and ovarian cancers, for example, are commonly associated with germline variants in *BRCA1* and *BRCA2*. Both gene products play an important role in the stable repair of double-strand breaks induced by DNA-damaging agents or breaks due to stalled replication forks. The proteins accomplish this task by facilitating repair via homologous recombination, a relatively error-free process. Loss-of-function variants in *BRCA1* or *BRCA2* thus force the cell to rely on other, often more error-prone repair mechanisms such as a single-strand break repair facilitated by poly (ADP-ribose) polymerases (PARPs). These enzymes are the target of a recently developed class of drugs known as PARP inhibitors (Olaparib, Niraparib, Rucaparib). In the presence of pathogenic variants in *BRCA1* or *BRCA2*, PARP inhibitors prevent a tumor cell from utilizing the backup single-stranded repair mechanisms leading to highly unstable DNA products and increased apoptosis. PARP inhibitors have been approved for the treatment of patients with confirmed germline or somatic mutations in *BRCA1* or *BRCA2*, and their improved efficacy over other treatment options has been a groundbreaking advancement.

While targeted therapeutics offer the opportunity for more efficient tumor treatment, an intimate knowledge of germline mutations in the metabolism of chemotherapeutics is equally as important. 6-Mercaptopurine (6-MP), for instance, is used in the treatment of leukemia for its ability to inhibit the formation of purine nucleotides during cellular replication and synthesis. The drug is inactivated by the enzyme thiopurine methyltransferase; however, nonsynonymous variants within the *TMPT* gene may lead to excessive level of 6-MP and life-threatening toxicity in both homozygous and heterozygous individuals.

## Pharmacogenetic testing:

Clinical pharmacogenetic testing is currently focused on detection of discrete genetic variants that are associated with well-defined aspects of pharmacokinetics and pharmacodynamics for a specific drug or class of drugs. As illustrated in Fig. 53.2, a laboratory test is designed to detect specific genetic variants (e.g., single nucleotide variants, insertions/deletions, and CNVs), referred to as genotypes. This information is used to assign alleles, often making assumptions about haplotypes/diplotypes (i.e., whether multiple variants occur on just one chromosome or whether such variants are divided between two chromosomes). The reason that haplotypes are often inferred is that most analytical methods can detect a gene variant but the method cannot determine the phase (chromosome position) of each variant. As such, specialized testing that can discriminate phase and frequency statistics is often used to predict the specific chromosome on which the pattern of genetic variants is likely to reside. The alleles are then assembled as a diplotype; this information is then used to predict the phenotype. Translation tables for both alleles and phenotypes are available through many resources, such as the Pharmacogenomics Knowledgebase (PharmGKB). Standardization of terms to define the predicted phenotype has also been proposed.

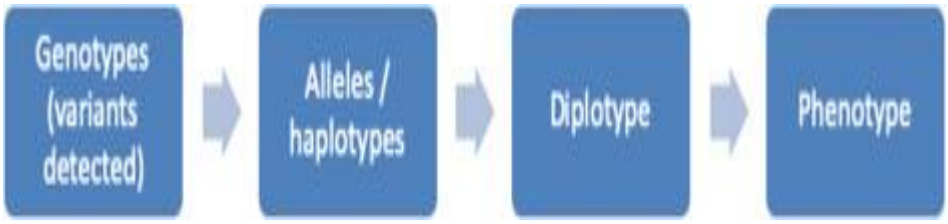


**Figure: Translation of pharmacogenetic results relies on comparison of results with consensus nomenclature to define alleles, haplotypes, diplotypes, and, ultimately, the predicted phenotype. The phenotype may also be influenced by nongenetic factors, such as drug–drug interactions.**

Some pharmacogenes are relatively simple to detect. For example, the association of many human leukocyte antigen (HLA) gene variants with potentially life-threatening drug hypersensitivity reactions requires a test that can identify the presence of at least one variant allele. The mode of inheritance is autosomal dominant for the well-known association between *HLA-B*57:01* and risk of hypersensitivity to abacavir; so, it does not matter whether one or two affected alleles are detected. The potentially life-threatening immune-mediated reaction that can precipitate when a carrier of the *HLA-B*57:01* allele is challenged with abacavir occurs regardless of dose or variation in pharmacokinetics,

making implementation a rather straightforward process. Thus a carrier that is naïve to abacavir is disqualified from initiating that therapy. Alternative drugs are offered instead.

The *SLCO1B1* gene is also relatively simple from an analytical perspective, in that a test designed to detect only one variant is sufficient to predict increased risk of myopathy from simvastatin therapy, a commonly prescribed drug used for cholesterol reduction. The organic anion transporter polypeptide 1B1, encoded by *SLCO1B1*, transports active simvastatin acid from the blood stream into the liver. Detection of the variant (rs4149056, c.521 T>C) is associated with reduced transporter function, resulting in an accumulation of the drug and subsequent muscle toxicity. One copy of the variant allele predicts decreased transporter function; two copies of the variant allele predict poor transporter function. This is in contrast to the *HLA-B*57:01* example above, because the mode of inheritance of *SLCO1B1* and most other pharmacogenes are autosomal codominant, leading to a range of phenotype predictions. A carrier of a variant *SLCO1B1* allele could minimize risk of the toxicity by using a lower dose or an alternate drug. Monitoring creatine kinase concentrations may also be useful for detecting and managing muscle toxicity associated with statin therapy.

Common nomenclature systems in pharmacogenetics classify combinations of sequence variants as star (*) alleles, where *1 usually reflects the reference allele (commonly referred to as "normal"), and numbered star alleles are assigned sequentially as new variants are identified. Variants are defined, and nomenclature is archived by repositories, such as the Pharmacogene Variation (PharmVar) Consortium. Few clinical laboratories offer complete gene sequencing, phase, and CNV determinations for pharmacogenes. Instead, most testing is targeted, designed to detect only select variants of known clinical significance. Pragmatically, the *1 allele is assigned by default when none of the targeted variants are detected. Therefore the true accuracy of a *1 allele designation is unlikely and depends on whether the assay detects all possible variants that an individual may carry. The star (*) approach to nomenclature has been adopted for many pharmacogenes.

Genes that code for drug metabolizing enzymes are among the best characterized and most commonly cited pharmacogenes. An example of nomenclature used to classify cytochrome P450 (CYP) drug metabolizing enzymes and the associated genes is shown in Fig. 53.3. *CYP2D6*, is one of the most clinically relevant pharmacogenes, but is also one of the most complicated. For example, the *CYP2D6*4* allele is defined primarily by the c.1847 G>A (rs3892097) variant, and subtypes are determined by combinations of additional variants. The *4A subtype (PharmVar *CYP2D6*4.001) is defined by a combination of seven variants. There are over 100 unique star (*) alleles and many more subtypes defined for *CYP2D6*, making this an extremely difficult gene to interrogate with accuracy and specificity. That

said, CYP subtypes do not usually affect the phenotype prediction and are most important for characterizing inheritance patterns in families, or for supporting research studies.

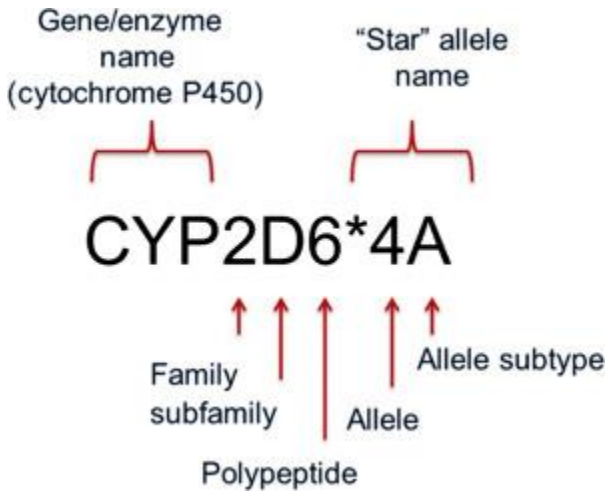


**Figure: Description of how the cytochrome P450 drug metabolizing enzymes and associated genes are named using the isozyme subtype 4A of CYP2D6 as an example.**

The *4 is the most common no-function *CYP2D6* allele. However, one of the variants used to define all, but one currently recognized *4 allele subtype (c.100 C>T, rs1065852), is also used to define dozens of other *CYP2D6* alleles, and some of which exhibit different phenotypic characteristics than the *4 allele (e.g., *CYP2D6*10*). This is an example wherein a genotype may be misclassified as the wrong allele and subsequent diplotype, such as assigning *4/*10 instead of *1/*4. Misclassification of alleles may occur with targeted testing and when consensus nomenclature is not consulted. Such misclassification has potential clinical significance if it affects the phenotype prediction. In this case, a *4/*10 would be classified as an intermediate metabolizer, whereas a *1/*4 would be classified as a normal metabolizer. Another analytical challenge is that many newly recognized *CYP2D6* alleles are gene hybrids that incorporate pieces of pseudogenes, such as *CYP2D7*, into a portion of the *CYP2D6* gene (e.g., *CYP2D6*36*). The *CYP2D6*36* is relatively common in Asian populations, but many gene hybrids are rare and are still being recognized, defined, and characterized.

Many important variants reside in noncoding regions or occur as a consequence of CNVs. Few traditional or massively parallel sequencing methods are optimized to detect all clinically relevant pharmacogenetic information. As a consequence of these analytical challenges, there is an overall lack of standardization in the specific alleles detected among laboratories for *CYP2D6*, and for some other complicated pharmacogenes. Complementary testing that provides more comprehensive detection of genetic variants (e.g., full gene

sequencing versus targeting only common variants) or testing that characterizes the phenotype directly (e.g., therapeutic drug monitoring, metabolic ratios, and enzyme activity testing) can refine the clinical value of pharmacogenetic testing, particularly for complicated pharmacogenes, such as *CYP2D6*.

Efforts to harmonize pharmacogenetic testing are underway, such as the Association for Molecular Pathology's recommendations for clinical *CYP2C19* testing. Well-characterized alleles were considered based on availability of accurate testing, reference materials, multiethnic allele frequencies, and clinical guidance documents for interpretation and implementation. As shown in Table 53.3, there are three alleles that were recommended for "Tier 1" testing. Tier 1 testing defined the minimum content that a clinical laboratory should offer, and could offer, based on meeting the criteria described above. "Tier 2" testing expands on that list to include variant alleles of clinical importance to a smaller group of individuals, or for which analytical characteristics are not yet mature. Consistent with the recommendations in Table 53.1, the variant alleles listed in Table 53.3 are defined based on primary causative variant [reference sequence (rs) number and common designation, with rf], along with the predicted function of each allele. Clinical laboratories that offer pharmacogenetic testing can be found through the voluntary National Institutes of Health Genetic Testing Registry.

In addition to concerns about accuracy of haplotype and diplotype assignments used to predict phenotype that were raised above, the actual drug response phenotype is affected by other genetic factors not detected by the test (e.g., other pharmacogenes involved in drug response), as well as demographic factors (e.g., age and sex), clinical factors (e.g., body size and kidney function), and nongenetic factors, such as drug–drug and food–drug interactions. For example, a person who inherits no *CYP2D6* variants would be anticipated to exhibit normal metabolism and achieve therapeutic concentrations of CYP2D6 drug substrates, but a drug may not be effective if the physiology to support the drug's mechanism of action is impaired or absent (e.g., opioid receptors that cannot respond to a opioid substrate that requires metabolic activation via CYP2D6). Also, a person who inherits no *CYP2D6* variants could exhibit a poor metabolizer phenotype if comedicated with an inhibitor of CYP2D6, such as fluoxetine. The US Food and Drug Administration (FDA) classifies drugs as strong, moderate, or weak inhibitors [10]. These coadministered drugs can elicit a poor metabolizer phenotype by inhibiting CYP2D6. A genetically predicted intermediate metabolizer is particularly vulnerable to drug–drug interactions. In addition, some supplements and food can inhibit CYP enzymes. For example, grapefruit juice is recognized to inhibit CYP3A4 activity, leading to reduced metabolism of associated drug substrates. As such, assignment of drug metabolizing enzyme pharmacogenetics to phenotype predictions and associated clinical implementation is multifactorial.

## Probable Questions:

1. How pharmacogenomics is introduced?
2. How pharmacogenetic testing is performed?
3. How pharmacogenetics is related to cancer?

## Suggested Readings:


**1.** Human molecular genetics. T Strachan and A P Read CRC press fifth edition

**2.** Molecular cell biology. H Lodish, CA Kaiser et al WH Freeman and Company

**3.** Nanotechnology: advances and real-life application. C Bhargava, A Sachdeva CRC press 2020 First edition

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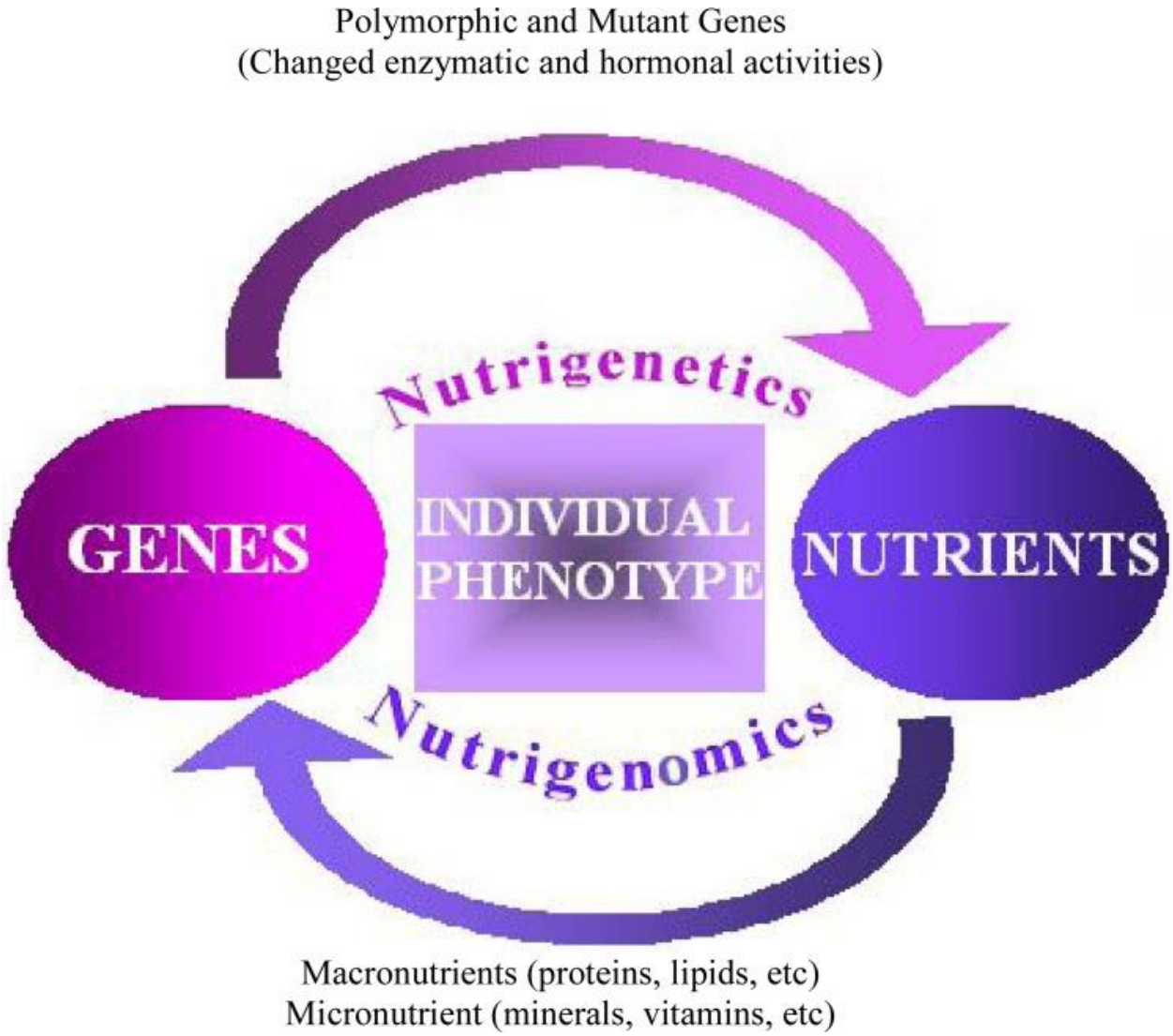
# UNIT-V

# Nutrigenomics and Personalized Medicine

**Objective:** In this unit we will discuss about Nutrigenomics and Personalized Medicine

**Introduction:** With the completion of human genome sequencing and entering the-Omics area, the new term "Nutritional Genomics" tends to replace the former "nutrient-gene interactions". It has been demonstrated that numerous genetic polymorphisms can influence protein structure function. The Nutritional genomic area includes two parts: first Nutrigenomics that is the study of interaction between dietary components and the genome, and the regulating changes in proteins and other metabolism; second Nutrigenetics that identify the response to dietary components with regard to genetic differences .

Nutrients are as environmental factors can interact with genetic material. It has been clearly demonstrated that DNA metabolism and repair depend on a wide range of dietary factors that act as cofactors or substrates in metabolic pathway, but much less is known about the impact of cofactors and/or micronutrients deficiency or excess on the fidelity of DNA replication and repair. Although the nutrients can influence the development of a particular phenotype, the response to a specific nutrient that determined by the individual genotype has also to be considered (Fig. 1).




The central role of genetic code in determining genome stability and related health outcomes such as developmental defects, degenerative diseases, and cancer is well-established. The etiology of complex chronic diseases obviously relates to both environmental and genetic factors. Specifically, the “fetal basis of adult disease” or “early origins hypothesis” postulates that nutrition and other environmental factors during prenatal and early postnatal development influence gene expression and cellular plasticity, which can alter susceptibility to adult diseases (cardiovascular diseases, diabetes, obesity. etc).

The concept of nutrients effects on DNA stability, repair and on the different gene expression processes, recently became more prominent in nutritional science. Numerous dietary components can alter genetic and epigenetic events and therefore influence health.

SNPs (single nucleotide polymorphisms) are the most common genetic variation, occur at about 500–2000 bp throughout the human genome, and normally found in at least 1% of the population. Many human studies have demonstrated the evidence for interaction between SNPs in various genes and the metabolic response to the diet. Moreover, SNPs analysis provides a potential molecular tool for investigating the role of nutrition in human health, diseases and identification of optimal diets.

Nutrients and genome interact at two levels: 1) Nutrients can induce or repress gene expression thereby altering individual phenotype. 2) Conversely, single nucleotide polymorphisms can alter the bioactivity of important metabolic pathways and mediators and influence the ability of nutrients to interact with them.

## Nutritional Genomics:

The interaction between the nutrients and cellular/genetic processes is being referred to as "nutritional Genomics". This term describes the interface of biochemistry genomics, human nutrition, understanding of reactions and interactions at the molecular genomic levels (11). The conceptual basis for this genomic research can be summarized with the following five principles: 1) Common dietary chemicals act on the human genome, either directly or indirectly, to alter gene expression and/or structure. 2) Under certain circumstances and in some individuals, diet can be a serious risk factor for a number of diseases. 3) Some diet-regulated genes (and their normal, common variants) are likely to play a role in the onset, incidence, progression, and/or severity of chronic diseases. 4) The degree to which diet influences the balance between healthy and disease states may depend on an individuals genetic background. 5) Dietary intervention based on knowledge of individual nutritional requirement, nutritional status, and genotype (i.e., "individualized nutrition") can be used to prevent, relieve, or cure chronic disease.

## Nutrigenetics:

Nutrigenetics term was used first time by Dr R.O Brennan in 1975 in his book Nutrigenetics. Nutrigenetics points to understanding how the genetic background of an individual impact to the diet.

The study of gene-nutrient interaction is a developing area of science. This idea that adverse diet/genome interaction can cause disease is not new and the unsuitable diet for any individual genotype could be a risk factor for monogenetic and polygenetic disease. Genetic polymorphisms can influence response to environmental elements, such as

enzymatic activities changes that affect circulating concentrations and ultimately the effectiveness of chemicals and their metabolites. Furthermore, metabolic disorders are other examples of influence of the genetic variations to diet such as PKU, defects associated with long chain fatty acid oxidation, iron absorption (haemochromatosis), which can be reasonably well managed with dietary restrictions.

As mentioned earlier SNPs study can be categorized in the field of Nutrigenetics. Some specific examples of the association between SNPs and specific food components such as enzymes deficiency are reviewed in this article. For example, different mutations in galactose-1-phosphate uridyltransferase (GALT) gene, phenylalanine hydroxylase gene, and Glucose-6-phosphate dehydrogenize (G6PD) gene resulted in Galactosemia, Phenylketonuria (PKU), and Favism diseases, respectively. Other examples of enzymes polymorphisms include Lactase-phlorizin hydrolase gene (LPH) polymorphisms that show how SNPs alter gene expression. This polymorphism is in the upstream of the lactase-phlorizin hydrolase gene (LPH) associated with hypolactasia and changes tolerance to dietary lactose (milk sugar, LPH hydrolyzes lactose into glucose and galactose) and allows different expression of the LPH.

Glutathione peroxide gene polymorphism is another example. The association between selenium supplementation and reduced incidence of liver, colon, prostate, and lung cancer in human has been shown. However, no individuals may respond equally. Glutathione peroxide is a selenium-dependent enzyme that acts as an antioxidant enzyme. Polymorphism at codon 198 of human glutathione peroxides results in a subsituation of proline to leucine amino acid, and has been associated with an increase risk of lung cancer. Investigators shown that persons with (Pro/Lue) genotype were at 80% greater risk for lung cancer and (Lue/Lue) genotypes were at 130% greater risk compared risk those with the (Pro/Pro) genotype. The leucine-coding allele was less responsive to increased activity because of selenium supplementation as compared with the prolin-containing allele.

Manganese super oxide dismutase (MnSOD) is a mitochondrial enzyme that plays a key role in detoxification of reactive oxygen species. A polymorphism valine to alanin subsituation in in this enzyme alters its transport into mitochondria, which has been associated with increased risk of breast cancer. Methylen tetrahydrofulate redoctase (MTHFR) enzyme catalyzes the reaction that produces 5-methyl tetrahydrofolate. The one-carbon units are carried on N-5 or N10 of tetrahydrofolate. One-carbone metabolism is needed for the denovo synthesis of purine nucleotides and thymidilate and for the remethylation of homocyisteine to methionine. With methionine adenylation S-adenosylmethionine (SAM) is formed, which is a cofactor for numerous methylation reactions such as DNA methylation that affect gene regulation. For the *MTHFR* gene tow

important SNPs has been well recognized: C677T (cytosine-to-thymidine subsituation resulting in the conversion of an alanine to valine) and A1298C (adenine-to-cytosine subsituation resulting in the conversion of an alanine to glotamic acid). The C677T polymorphism is the most common variant that occurs as homozygous T/T in 5–10% of the and as heterozygous C/T genotypes up to 40% general population (28). The presence of C677T or A1298C mutations is associated with reduction in MTHFR enzyme activity and impairs folate accumulation, which may cause increases homocysteine concentration in plasma, a risk factor for venous thromboembolic and ischemic arterial diseases.

Another polymorphism of *MTHFR* gene is Ala222Val that affects folate metabolism. It increases the conversion of dUMP to dTMP and leads to more folate-dependent thymidine biosynthesis and folate deficiency. This polymorphism is a risk factor for spontaneous abortions and decreased fetal viability, thus maternal folate supplementation can be useful for individuals with this polymorphism. MTHFR is also involved in maintenance genomic CpG methylation patterns and prevention of DNA strand breaks, these mutations are associated with increased risk of neural tube defects and some types of cancer.

Changes in the concentration of folate (the MTHFR substrate) and riboflavin (the MTHFR cofactor) can modulate the activity of *MTHFR* gene. Generally, folic acid supplementation can help the negative health effect of these SNPs with decrease in plasma homocysteine levels. Enzymes that utilize and metabolize vitamin B12 have been associated with NTDs, increased risk of Down syndrome and colon cancer. For example, a common polymorphism in the *HFE* gene (Cys282Tyr) is associated with iron storage disease hereditary haemochromatosis, leading to an iron accumulation in the liver, heart and endocrine glands. This protein is an important regulator of cellular iron homeostasis and has role in intestinal iron absorption by regulating the interaction of the transferrin receptor with transferrin.

Cytochrome P450s (CYPs) enzymes play a central role in the oxidative biotransformation of steroids, prostaglandins, nutrients, drugs, chemicals and carcinogens. Several dietary factors can alter the expression of CYP isoforms. CYP1A2 plays an essential role in the metabolism of wide range of drug and chemical substances. For example, CYP1A2 activates dietary carcinogens such as aromatic amines, but also detoxifies compounds such as caffeine. Low-activity CYP1A2 genotype with an increased risk of myocardial infarction suggests that this enzyme detoxify a substance, which may be an important risk factor in the population. Indeed, individuals with a low-activity CYP1A2 genotype are at a greater risk of coffee-associated heart disease. As caffeine is the main substance in coffee and is detoxified by CYP1A2, it may be an important risk factor for heart disease in certain population. Glutathione S transferase (GST) enzyme is a superfamily of enzymes that play

an important role in the detoxification of several dietary compounds. GSTM1, GSTT1 and GSTP1 are isoforms of this enzyme. The GSTM1 and GSTT1 null genotype have been associated with both an increased and a decreased risk of some types of cancers such as breast cancer. Some components such as dietary isothiocyanates that are found in cruciferous vegetables are eliminated with GSTs enzymes. Indeed, protective effect of the GSTM1 null genotype on colon and lung cancer has been related to lower urinary excretion of glutathione-conjugated phytochemicals indicating they are not rapidly excreted. GSTT1 plays a similar role to GSTM1 in eliminating beneficial phytochemicals found in cruciferous vegetables. Moreover, in vegetables rich in phytochemicals such as isothiocyanates the expression of GSTs is increased conjugating them to more water-soluble forms that are easily excreted.

Endothelial nitric oxide synthase (eNOS) is synthesized from the amino acid L-argenine by NO synthase (NOS). The eNOS is expressed in the endothelium and produces NO that diffuses to vascular smooth muscle cell, where it increases the concentration of cGMP, leading to vascular relaxation. NO has central role in the pathogenesis of coronary spasm and atherogenesis. Several polymorphisms of eNOS may be associated with specific phenotype. For example, a Glu298Asp polymorphism in the eNOS gene has been associated with ischemic heart disease, myocardial infarction, and coronary spasm. Genetic polymorphisms in catechol-O-methyltransferase, sulfotransferase, and UDP-glucuronosyltransferase result in differences in enzymatic activity. These enzymes metabolize some of dietary compounds. For example, green tea was associated with a lower risk of breast cancer only in women with the low-activity allele for catechol-O-methyltransferase. This enzyme catalyzes the methylation of catechins (a polyphenolic antioxidant plant secondary metabolite) in green tea making them more quickly eliminated.

Apolipoprotein E (ApoE) gene has three different alleles (ε2, ε3, ε4). Persons with ε4 variant respond to a high-fat diet negatively with an increased risk for coronary heart disease (CHD). In these individuals, low-fat diet should be useful (2). Moreover, there is an important relationship between allelic variants in the ApoA1/C3/ A4/A5 genes and the effect of dietary fats on lipoprotein metabolism and CVD (cardio vascular diseases) risk. Linkage disequilibrium within Apo A1/C3/A4/A5 cluster has been represented to affect plasma lipid concentration and CVD risk. Apolipoprrptein A-1 is and is a key component of high-density lipoprotein particles (HDL). The locus of gene encoding APOA-1 is on chromosome 11q and highly polymorph and has a specific SNP in its promoter region. An Adenin/Guanin subsituation in the promoter region (−75bp) of the ApoA1 gene is common in different populations. The presence of A allele (A/A and A/G) has been associated with incresed HDL-cholesterol. Moreover, mild increase in APOA-1 concentrations in subjects

with the G/G genotype was observed. APOA-5 gene is also an important regulator of triglyceride (TG)-rich lipoprotein (TRL) metabolism.

One of the Vitamin D receptor (VDR) polymorphism is Fok1. Individuals with F allele have three amino acids more than those without F allele in their VDR. The Ff or ff genotype is associated with 51% and 84% greater risk of colorectal cancer, respectively. Individuals that consumed low calcium and fat diet have more than double risk of colorectal cancer, specifically in persons with ff genotype rather than Ff genotype (8). VDR polymorphisms have been also associated with childhood and adult's asthma.

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptor supper family that plays an essential role in fatty acid oxidation, glucose, and extracellular lipid metabolism. PPARs are the best-known fatty-acid-regulated nuclear receptors. One of the three members of the PPARs family regulates many genes involved in fatty acid metabolism. PPR-α (PPARA) plays a central role in lipid oxidation and inflammation, whereas PPAR-γ is involved in adipocytes differentiation, glucose and lipid storage, and inflammation. PPAR-δ (also known as PPAR-β), may has a crucial role in development, lipid metabolism, and inflammation. These receptors bind to fatty acid and regulate the expression of genes involved in fatty acid transport and metabolism. PPARs family also involve in activation of about 300 genes.

The PPAR-α gene has a polymorphism at codon 162 (Lue162Val) that has been associated with changes in total cholesterol, LDL-associated cholesterol, and Apo B concentrations. The less common V162 allele is associated with significantly higher serum concentration of total cholesterol, LDL cholesterol, Apo B, and Apo C-III than in carriers of L162 allele, especially in men. For individuals with the common L162 allele, increased intake of polyunsaturated fatty acids (PUFAs) had little effect on fasting triacylglycerol concentrations. In those with the less common V162 allele, however, fasting triacylglycerol concentrations fell abundantly with increasing PUFA intake.

## Nutrigenomics:

Nutrigenomics aims to identify the effects of several nutrients, including macronutrients and micronutrients on the genome and explores the interaction between genes and nutrients or food bioactives and their effects on human health. The influence of nutrients on the transcription activity, gene expression, and heterogeneous response of gene variants is also referred to as "Nutrigenomics".

Nutrigenomics also describes the use of functional genomic tools to study a biological system to understanding of how nutritional molecules affect metabolic pathways and homeostatic control. This branch of science will reveal the optimal diet form within a series of nutritional changes, whereas Nutrigenetics will yield critically important information that assist clinicians in identifying the optimal diet for a given individual, i.e. personalized nutrition. Transcriptomics, proteomics, and metabolomics are also technologies that apply in Nutrigenomics research. According to numerous studies, nutrients can alter the expression of genes at the level of gene regulation, signal transduction, chromatin structure and protein function.

Epidemiological studies show association between food intake and the incidence and severity of chronic diseases. A large number of nutrition related pathologies (obesity, metabolic syndromes, type 2 diabetes, CVD, and some types of cancers) are polygenic and multifactorial and their onset and progression are related to multiple genes and their variants as well as several environmental factors, especially the diet. Dietary chemicals can affect gene expression directly or indirectly. At the cellular level nutrients may act as ligands for transcription factor receptors or be metabolized by primary or secondary metabolic pathways, thereby altering concentrations of substrates or intermediates, and finally positively or negatively affect signal pathways.

Transcription factors (TFs) are one of the key molecules through with nutrients can alter the gene expression. One of the most important groups of nutrient sensors is PPARs TFs with 48 members in the human genome. The majority of receptors in this superfamily bind nutrients, their metabolites, and influences expression of specific genes involved in numerous metabolic process in the liver, including fatty acid oxidation, ketogenesis, gluconeogenesis, amino acid metabolism, cellular proliferation, and acute-phase response. For example, the fatty acids palmitic (16:0), oleic (18:1n9), linoleic (18:2n6), and arachidonic acid (20:4n6), and the eicosanoids, 15deoxy-δ12, 14prostaglandinJ2 and 8-(S) hydroxyeicosatraenoic acid, are ligands for PPAR-δ. These nuclear receptors act as sensors for fatty acids. Lipid sensors usually heterodimerize with retinoid receptor, whose ligand is derived from another dietary chemical, vitamin A, and hyperforin, bind directly to nuclear receptors and influence gene expression. The liver X receptor-α (binding cholesterol metabolites), bind as a heteromers to specific nucleotide sequence (response elements) in the promoter regions of a large member of genes. During ligand binding, nuclear receptors undergo a conformational change that results in coordinated dissociation of corepressors and recruitment of coactivator proteins to prepare transcriptional activation. Thereby, a number of genes are induced such as those involved in fatty acid oxidation or fatty-acid storage, depending on the cellular metabolic state. In metabolically active organs, such as

the liver, intestine, and adipose tissue, these TFs act as nutrient sensors by changing the level of DNA transcription of specific genes in response to nutrients changes.

Dietary chemicals indirectly regulate some of TFs. The sterol regulatory element binding proteins (SREBPs), for example, are activated by protease cleavage, an event regulated by low levels of oxy sterols and changes in insulin/ glucose and PUFAS. The carbohydrate-responsive element-binding protein (chREBP) is a large TF, activated in response to high glucose levels, and is regulated by reversible phosphorylation events. This DNA binding protein serves as an effector of lipogenic gene expression. Moreover, dietary chemicals can directly affect signal transduction pathways. For example, green tea contains the polyphenol, 11-epigallocatechin-3-gallate (EGCG) that EGCG inhibits tyrosine phosphorylation of Her-2/neu receptor and epidermal growth factor receptor that reduces signaling via the phosphatidyl inositol 3-kinase (PI-3)-AKt kinase-NF-kB pathway. Activation of the NF-kB pathway is associated with some types of breast cancer.

PUFAs such as n-3 and n-6 are other micronutrients, which are also referred to as omega-3 and omega-6 fatty acids, may influence gene expression. Animal studies have demonstrated that PUFA intake can modulate the gene expression of several enzymes involved in lipid and carbohydrate metabolism. A significant interaction has also been shown for the PPARA Lue162Val polymorphism n-6 PUFA intake. Individuals with the less common V162 allele, increased n-6 PUFA intake is associated with a marked reduction in triacylglycerol concentration, whereas this association is not observed in L162 carriers. Conversely, in L162 and V162 carriers n-3 PUFA intakes results in triacylglycerol concentrations reduction.

Approximately 40 micronutrients are needed in the human diet. Suboptimal intakes of specific micronutrients have been associated with CVD (Vit B, E, and carotenoids), cancer (folate, carotenoids), neural tube defects (folate), and bone mass (Vit D). B6, B12 and folate deficiencies, for example, are associated with increased serum homocysteine levels. Hyperhomocysteinemia is a risk factor and marker for coronary artery disease. Deficiency of Vit B12, folic acid, B6, niacin, C or E, iron or zinc appears to imitate radiation in damaging DNA by causing single and double-strand breaks, oxidative lesions, or both Nutrient deficiencies are more important than radiation because of constancy of exposure to milieu promoting DNA damage. For example, folate deficiency breaks chromosomes due to substantial incorporation of uracil in human DNA (4 million uracil/cell).

Amino acids can play the role of nutritional signals in the modulation of expression of particular genes. Studies have shown that cells can detect variants in amino acid levels and respond by mechanism as control of transcription, mRNA stabilization ,as well as by up or

down regulation of translation initiation. For example, in human cells amino acid L-tryptophan in supraphysiologic concentrations is a powerful inducer of collagenase gene expression at a transcriptional level. The increase in collagenase mRNA levels was reversible, time and L-tryptophan dose-dependent. Simple and complex carbohydrates have differential effects on blood glucose concentrations. Foods with a high glycemic index (GI) would increase insulin production and, decrease synthesis of insulin receptors. High glucose concentration also induces the transcription of several genes of the glycolytic and lipogenic pathways. Therefore, dietary chemicals are regularly ingested and are involved indirectly and directly in regulation gene expression, it follows that a subset of genes regulated by diet must be involved in disease initiation, progression, and severity.

## Nutritional epigenetics:

The term “epigenetics” is used to gene expression that occurs without changes in the DNA sequence. Epigenetic regulation plays an important role in development and is needed to gain stable expression or repression of genes in specific cell types or at defined developmental stages. Epigenetic changes may influence cell cycle control, DNA damage, apoptosis, invasion, imprinting, and aging.

A majority of regulatory proteins including DNA methyltransferases, methyl-cytosine guanine dinucleotide binding proteins, histon-modifing enzymes, chromatin-remodeling factors, and their multimolecular complexes are involved in the overall epigenetic process. The best studied epigenetic modification is DNA methylation and in the mammals genome occurs at many of cytosine residues that are followed by guanine residue (CpG islands) and in most cases methylation in these regions induces gene repression. However, this phenomenon can lead to the expression of neighboring genes. Studies identify that DNA methylation is dependent on bioactive food components ranging from alcohol to zinc Several dietary factors may influence the provision of methyl groups available for the formation of S-adenosylmethinine. Moreover, dietary factors may modulate the use of methyl group by processes including change in DNA methyltransferase activity. The methyl groups’ status depends on B vitamins as cofactors including folate, Vit B12, and Vit B6. The folate-dependent biosynthesis of nucleotide precursors for DNA synthesis and of SAM for genome methylation is dependent on the availability of many vitamins including B12, B6, niacin, riboflavin and minerals (zinc, cobalt). Therefore, folate-mediated one-carbon metabolism mediates communication between the cellular nutrient environment and regulation of the genome. Impairments in one-carbon metabolism and the SAM cycle induced by nutritional deficiencies and/or SNPs in genes that encode folate-dependent enzymes, alter genome methylation patterns and gene expression levels. Disruptions in folate metabolism are common and increase risk cancers, cardiovascular disease,

neurological disorders and developmental anomalies such as spina bifida, cleft palate, and spontaneous abortion. Therefore, folate supplementation can reduce the risk of these disorders developing. DNA methylation patterns may effect on the response to bioactive food components and thereby account for differences in response in normal and neoplastic cells.

## Genome health and disease prevention:

It is clear that even the small damages in the genome can cause crucial effects in whole human life. DNA metabolism and repair is depending on a variety of dietary factors that act as cofactors or substrates. Nutritional requirements is important for the prevention of DNA oxidation (i.e. antioxidants such as carotenoids, Vit E and C), prevention of uracil incorporation into DNA (i.e. folate), maintenance methylation of CpG in DNA (methionine, cholin, folate and vitamin B12), as cofactors or as components of DNA repair enzymes (Zn, Mg), maintenance of telomere length (niacin, folate).

Many chronic diseases are polygenic and result from interaction between genes and environmental factor. Dietary intervention based on nutritional requirement, nutritional status, and genotype (i.e., "individualized nutrition"), can be used to prevent, control or treatment of chronic disease such as cardiovascular diseases (CVD), metabolic syndromes, and cancer. These disorders are partly mediated by chronic exposure to certain food components. Fore example, the association between amount of calories, the levels and types of vitamins, fat, and carbohydrates with atherosclerosis, diabetes, obesity, cancer, hypertension, and other chronic diseases is demonstrated.

## Genome damage and nutritional deficiency:

As mentioned earlier, nutritional status influences genome stability and deficiency of certain micronutrients can result in critical damages in the genome. Studies have shown that at least nine micronutrients (Vit E, Ca, folate, retinol, nicotinic acid, β-caroten, riboflavin, pantothenic acid, and biotin) affect genome stability in human *in vivo* . Folate and vitamin B12 are need for DNA replication, repair and maintenance of DNA methylation patterns. Both *in vivo* and *in vitro* studies with human cells clearly show that folate and vitamin B12 deficiencies and elevated plasma homocysteine are associated with the expression of chromosomal fragile sites, chromosomal breaks, excessive uracile in DNA and DNA hypomethylation. Nicotinic acid (niacin) also plays a fundamental role in chromosome integrity and reduction of cancer risk.

Reactive oxygen species (ROS) such as highly reactive hydroxyl radical and superoxide radical contributes to DNA damage. Antioxidants (Vit C and E) and enzymes such as superoxid dismutase, catalase and glutathionperoxidase may control lipid and protein oxidation induced by ROS. Since developmental, degenerative diseases and aging are partly caused by DNA damage, defining optimal requirements of key minerals and vitamins for preventing nuclear and mitochondrial DNA damage is important.

Infertility is another consequence of genome damage on human health. Genome damage results from specific micronutrient deficiencies may cause developmental defects in the fetus or increased risk of cancer in the child. For example, inadequate Vit C intake results in increased oxidation of sperm DNA; folate deficiency increases risk of NTDs and genome damage. Increased risk of childhood leukemia in children with mothers who did not intake enough folic acid supplementation during pregnancy has demonstrated. Additionally, zinc deficiency induces oxidative damage to DNA and impairs DNA repair, which has a teratogenic effects.

## Telomere and nutritional status:

Telomeres are nucleoprotein structures that cap the ends of chromosomes, and maintain chromosome stability. Degeneration of telomeres leads to whole chromosomal instability, and chromosomal fusion and therefore gene amplification, an important risk factor for cancer. Folate and nicotinic acid deficiency increased oxidative stress and telomere dysfunction. Under folate deficiency uracil is incorporated into DNA instead of thymidine, leading to chromosome breakage. Similarly, oxidative stress causes telomere shortening. Folate and other methyl donors such as Vit B12, cholin, and methionin have an important role in maintenance methylation of cytosine. Defects in the DNA methylation can cause excessive telomere elongation and homologous recombination between telomeres and telomeres fusion. Hypomethylation or hypermethylation of the CpG islands is in the promoter of telomerase may cause excessive expression of telomerase or silence the gene respectively. Telomere shortening has been observed in number of conditions including obesity, psychological stress, immune dysfunction, cancer, and CVD. In vitro studies antioxidant treatment has been found to prevent telomere damage.

## Conclusions

Nutritional genomics elucidate the interaction among nutrients, metabolic intermediates, and the mammalian genome. The response to bioactive food components is dependent on genetic background (Nutrigenetics effects) that can influence absorption and metabolism targets or sites of action. Likewise, the response to food components depends on DNA methylation and other epigenetic events. The ability of bioactive food components to influence gene expression patterns (nutrigenomics effects) is also a factor in determining the overall response. Finally, bioactive food components may influence protein synthesis, degradation, and posttranslational modification. Understanding the interrelationships among human genetics diversity, genome function, and dietary components will enable precise manipulation of genome function and stability throughout the life cycle for optimal human health and disease prevention. With greater insight in to the gene-nutrient interaction, alterations in diet and single nutrient interventions may help us to better protect against cancer, decrease the occurrence of cardiovascular and other chronic diseases, and perhaps increase human longevity.

# Personalized Medicine:

The application of emerging, high-throughput, data-intensive biomedical assays, such as DNA sequencing, proteomics, imaging protocols, and wireless monitoring devices, has revealed a great deal of inter-individual variation with respect to the effects of, and mechanisms and factors that contribute to, disease processes. This has raised questions about the degree to which this inter-individual variation should impact decisions about the optimal way to treat, monitor, or prevent a disease for an individual. In fact, it is now widely believed that the underlying heterogeneity of many disease processes suggests that strategies for treating an individual with a disease, and possibly monitoring or preventing that disease, must be tailored or ‘personalized’ to that individual's unique biochemical, physiological, environmental exposure, and behavioral profile. A number of excellent reviews on personalized medicine have been written, including a growing number of textbooks on the subject meant for medical students and clinicians. It should be noted that although many use the term ‘personalized’ medicine interchangeably with the terms ‘individualized’ and ‘precision’ medicine (as we do here), many have argued that there are some important, though often subtle, distinctions between them.

There are a number of challenges associated with personalized medicines, especially with respect to obtaining their approval for routine use from various regulatory agencies. In addition, there have many issues associated with the broad acceptance of personalized

medicines on the part of different health care stakeholders, such as physicians, health care executives, insurance companies, and, ultimately, patients. Almost all of these challenges revolve around a need to prove that personalized medicine strategies simply outperform traditional medicine strategies, especially since many tailored or personalized therapies, such as autologous CAR-T cell transplant therapies for certain types of cancer and mutation-specific medicines such as ivacaftor to treat cystic fibrosis, can be very expensive. In this review we consider the history and motivation of personalized medicine and provide some context on what personalized medicines strategies have emerged in the last few decades, what limitations are slowing their advance, and what is on the horizon. We also consider strategies for proving that personalized medicine protocols and strategies can outperform traditional medicine protocols and strategies. Importantly, we distinguish examples and challenges associated with personalized disease prevention, personalized health monitoring, and personalized treatment of overt disease.

## Archibald Garrod and The Precursors Of Personalized Medicine:

There is much in the history of western medicine that anticipates the emergence of personalized medicine. For reasons of brevity, we will not focus on all of these events, but rather only a few that we feel encompass the most basic themes behind personalized medicine. More than a century ago Archibald Garrod, an English physician, began studying in earnest diseases that would later become known as 'inborn errors of metabolism.' Garrod studied a number of rare diseases with overt, visible phenotypic manifestations including alkaptonuria, albinism, cystinuria and pentosuria. Of these, his focused work on alkaptonuria led to some notoriety when he observed that some members of families exhibiting alkaptonuria showed measurably outlying values for certain basic biochemical assays, e.g., from urine, relative to the values of family members who did not possess alkaptonuria. This led him to conclude that alkaptonuria was due to a specific 'altered course of metabolism' among affected individuals, which was subsequently proven correct. Further, in considering other rare diseases like alkaptonuria, Garrod argued that "…the thought naturally presents itself that these [conditions] are merely extreme examples of variation of chemical behavior which are probably everywhere present in minor degrees and that just as no two individuals of a species are absolutely identical in bodily structure neither are their chemical processes carried out on exactly the same lines". This more than hints at his belief that, at least with respect to metabolism, humans vary widely and that these differences in metabolism could help explain overt phenotypic differences between individuals, such as their varying susceptibilities to diseases and the ways in which they manifest diseases.

Garrod was working in the backdrop of a great deal of debate about the emerging field of genetics. Although the specific entities we now routinely refer to as genes (i.e., stretches of DNA sequence that code for a protein and related regulatory elements), were unknown to Garrod and his contemporaries, he and others often referred to 'factors' influencing disease possessed by certain individuals that were consistent with the modern notion of genes. Claims about the very presence of such factors were born out of discussions rooted in the findings of Mendel (later, it would be shown that many of the metabolic outliers Garrod observed in people with diseases like alkaptonuria were due to defects in genes possessed by people with those diseases). Mendel observed consistent connections between the emergence of very specific phenotypes only when certain breeding protocols were followed in peas that anticipated the modern field of genetics. Essentially, as discussed in an excellent book by William Provin. many in the research community at the time debated how genes or factors of the type Garrod and others were considering could explain the broad variation in phenotypic expression observed in nature. One group of academics and researchers, referred to as the 'Mendelians' in the historical literature, which included William Bateson and Hugo de Vries, focused on the discrete nature of the factors likely to be responsible for many observable inheritance patterns (such as those of focus in Mendel's studies and observations like Garrod's in the context of rare disease). In opposition to the 'Mendelians' were the 'Biometricians,' represented most notably by Karl Pearson, whose focus on continuous or graded phenotypes, like height, gave them concerns about how to reconcile such continuous variation with the overtly discrete ('either/or') factors and inheritance patterns considered by the Mendelians and researchers like Garrod.

The Mendelian vs. Biometrician debate was resolved to a great extent by the statistician Ronald Fisher in a series of seminal papers. Fisher argued that one could reconcile continuous phenotypic variation with discrete, heritable factors that contribute to this variation by suggesting that many factors (i.e., genes) might contribute in a small way to a particular phenotype. The collective effect, or sum total, of these factors could then create variation in phenotypes that give the appearance of continuity in the population at large (e.g., an individual who inherited only 1 of 25 genetic variants known to increase height would be shorter on average than someone who inherited 10 or 12, and much shorter, relatively speaking, than an individual who inherited 22 or 25). The belief that there might be many genes that contribute to phenotypic expression broadly, some with more pronounced effects and some with less pronounced effects, that interact and collectively contribute to a phenotype in a myriad of ways, has been validated through the application of modern high-throughput genetic technologies such as genotyping chips and DNA sequencing. As a result, much of the contemporary focus on personalized medicine is rooted in the findings of genetic studies, as it has been shown that individuals do in fact vary widely as each individual possesses subsets of literally many millions of genetic

variants that exist in the human population as a whole. In addition, subsets of these genetic variants may have arisen as *de novo* mutations and hence may be unique to an individual. These extreme genetic variations explains, in part, why individuals vary so much with respect to phenotypes, in particular their susceptibilities to disease and their responses to interventions. It should be emphasized that although personalized medicine has its roots in the results of genetic studies, it is widely accepted that other factors, e.g., environmental exposures, developmental phenomena and epigenetic changes, and behaviors, all need to be taken into account when determining the optimal way to treat an individual patient ( Figure 1).

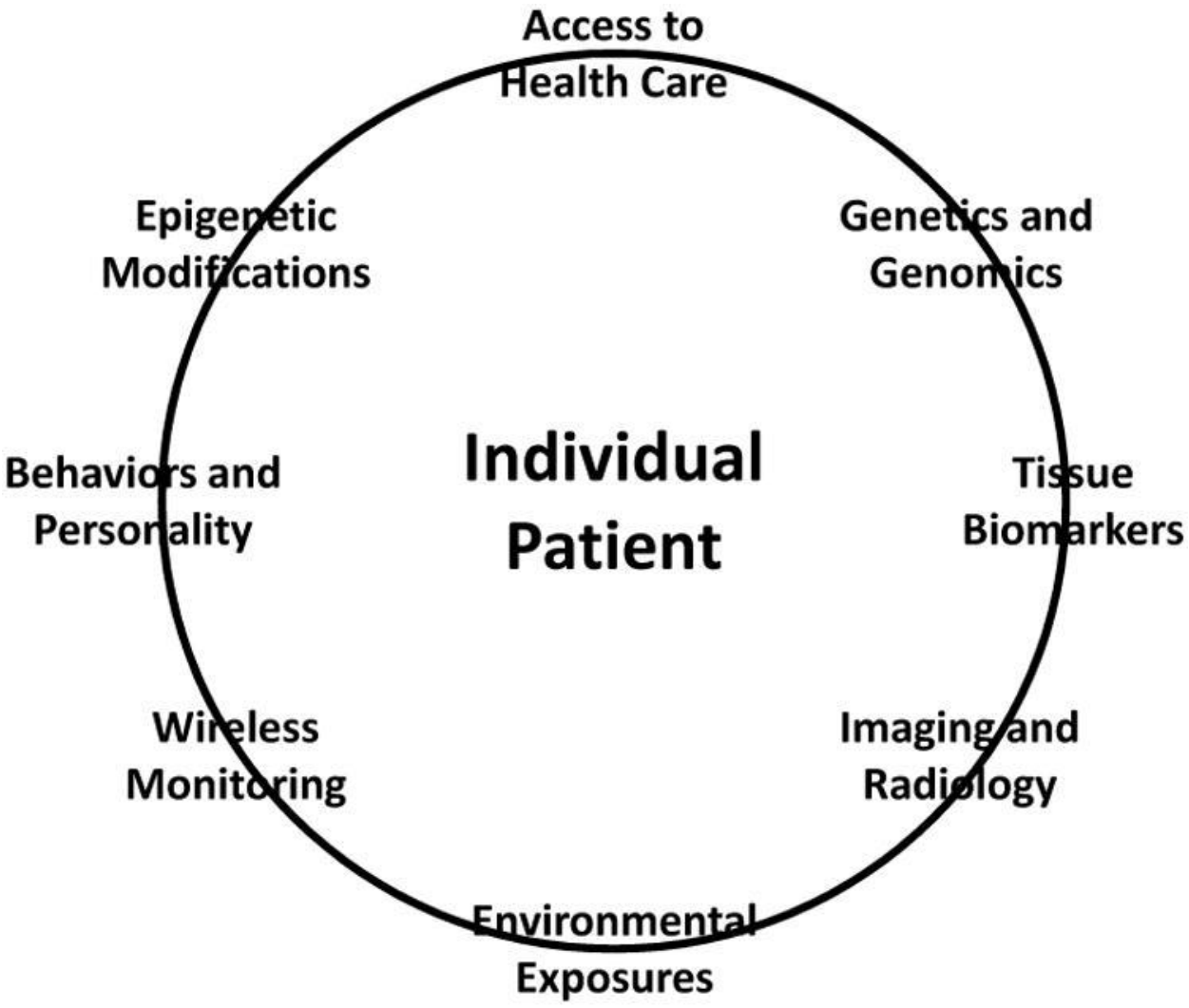



**Figure 1: Graphical depiction of elements in need of integration and assessment in pursuing truly personalized medicine. Access to health care is important since some individuals may not be able to access expertise and technologies due to geographic or economic barriers and therefore interventions might need to be crafted for those**

**individuals with this in mind. Inherited genetic information is really only predictive or diagnostic in nature however somatic changes to DNA can provide valuable insight into pathogenic processes. Tissue biomarkers (e.g., routine blood-based clinical chemistry panels) are useful for detecting changes in health status, as are imaging and radiology exams as well as data collected routinely via wireless monitors. Environmental exposures and behaviors can really impact the success of an intervention and exhibit great inter-individual variability. Epigenetic phenomena reshape gene function based on exposures and developmental or stochastic phenomena and should be monitored as well as indicators of a health status change.**

Another, sadly more obscure, publication was also prescient for personalized medicine, although this publication bears more on the need for clinical practices that are consistent with personalized medicine – rather than a scientific justification of personalized medicine. More than 60 years ago Hogben and Sim considered how clinical practice needs to pay attention to nuanced characteristics of patients in order to determine an appropriate intervention for them. Although more will be discussed about their paper in the section on 'Testing Personalized Medicines,' suffice it to say that the authors believed that in order to determine an optimal course of action for an individual patient in the absence of any *a priori* understanding of how best to treat that patient given his or her characteristics or profile, a number of items would need to be obtained. Thus, greater information about that patient would have to be gathered, a plan to vet the utility of an intervention chosen on the basis of that information would have to be pursued, and a strategy for incorporating the results of the patient-oriented study into future care would have to be crafted. Although simple in theory, the practical issues surrounding gathering more information about a patient and pursuing an the empirical assessment of a personalized intervention can be daunting. For example, questions surrounding how one can know that a chosen intervention works unless meticulous patient follow-up information is kept, how one would know if a patient satisfied with what they are experiencing with the intervention, and how one could assess the difference between other interventions that could have been chosen and the chosen personalized intervention, would all need to be addressed. In fact, practical issues surrounding the implementation of personalized medicine that Hogben and Sim considered are often overlooked in contemporary discussions about personalized medicine, especially since different technologies for profiling patients are constantly being developed and refined, and more and more evidence for inter-individual variation in factors associated with diseases (from technologies such as DNA sequencing, proteomics, sophisticated imaging protocols, etc.) is emerging.

## Early Examples of Personalized Medicine:

There have been a great many examples of interventions tailored to individual patient profiles, virtually all of them based on genetic profiles. Before providing a few classic examples, it should be emphasized that personalized medicine can be practiced not only for the treatment of disease, but also for the early detection and prevention of disease. We provide some historical examples of personalized disease treatments here and consider early detection and prevention in the next section, as developments in personalized disease detection and prevention are much more recent.

The human body deals with traditional pharmacotherapies (i.e., drugs) to treat disease in two general ways. Initially, the body must respond to a drug. This response occurs in steps, with the first step involving the absorption of the drug by the body. The drug must then be distributed throughout the body (during this process the drug might be 'biotransformed' or metabolized into useful components) and then begin to elicit effects. Finally, any remaining drug or drug components are excreted. These processes are often lumped under the heading of 'pharmacokinetics' and collectively referred to as the 'ADME' of a drug (Absorption, Distribution, Metabolism and Excretion). Pharmacokinetic activity is often under the control of a unique set of genes (e.g., drug metabolizing enzymes) that could harbor naturally-occurring genetic variants (or 'polymorphisms') that influence their function and hence how the body ultimately deals with a particular drug. Once a drug is within the body, how it interacts with its target (typically a gene or protein encoded by a gene) to elicit an effect is known as its 'pharmacodynamic' properties. These properties include the 'affinity' the drug has for its target(s), the drug's ability to modulate the target(s) (or its 'efficacy'), and the 'potency' of the drug, or how much of the drug is needed to induce a certain change in its target. Pharmacodynamic properties of a drug are also under genetic control.

Many early examples of personalized medicines were associated with genetically-mediated pharmacokinetic aspects of drugs. This was due in part to the biomedical science community's understanding of drug metabolizing enzymes and the role they play in the body's response to drugs. An excellent introduction to pharmacogenetic properties of drugs, as well genetic variants in genes that influence the efficacy and side effects of drugs (especially with respect to genetic variants in drug metabolizing enzymes) is the book by Weber. Warfarin is a widely used blood thinning medication that, if not dosed properly, could cause a potentially life-threatening adverse drug reaction. Warfarin targets a particular gene, VKORC1, and is metabolized in part by the gene CYP2C9. Naturally-occurring genetic variation in both the VKORC1 and CYP2C9 genes leads to variation in the pharmacodynamic and pharmacokinetic properties of Warfarin across individuals,

ultimately creating variation in individuals' responses to warfarin. The US Food and Drug Administration has therefore recommended that dosing for warfarin take into consideration an individual's genotype (i.e., the dose must be personalized to an individual based on the specific genetic variants they possess in the VKORC1 and CYP2C9 genes).

Another classic example of a drug that should only be provided to individuals with a certain genetic profile is primaquine (PQ). PQ has been used to manage malaria with some success in parts of the world where malaria is endemic. However, military doctors working in the past observed that some of the soldiers they treated for malaria that were provided the drug became jaundiced and anemic, and ultimately exhibited symptoms of what would later be termed 'acute haemolytic anaemia (AHA)'. It was later shown that the individuals exhibiting AHA after PQ administration carried variants in the G6PD gene. Current clinical practice with PQ therefore calls for the genotyping of individual patients to see if they carry relevant variants in the G6PD gene that might discourage PQ use for them.

A final, often-cited example of a personalized medicine is the drug imatinib. Imatinib is used to treat chronic myelogenous leukemia (CML). Imatinib inhibits an enzyme, tyrosine kinase,that is increased by the formation of a fusion of two genomic regions, one encompassing the Abelson proto-oncogene (abl) and the other the breakpoint cluster region (bcr). This fusion event arises in many tumors contributing to the development of CML and is referred to as the 'bcr-abl fusion' or 'Philadelphia chromosome.' However, not all individuals with CML have tumors harboring the bcr-abl fusion mutation. Therefore, imatinib is typically given only to individual CML patients with this fusion event.

## Contemporary Examples Of Personalized Medicine:

Drugs like warfarin, PQ and imatinib that appear to only work – or only work without side effects – when a patient possesses a certain genetic profile, have generated tremendous interest in identifying factors, such as genetic variants, that influence an individual patient's response to any number of drugs and interventions. This interest in crafting personalized medicines to treat diseases has expanded into personalized disease surveillance (i.e., early detection protocols) and personalized disease prevention strategies as well. We briefly describe a few very recent examples of this activity.

## Mutation-Specific Therapies:

Instead of developing a drug and then identifying factors that mitigate its efficacy or side effects through observational studies on individuals provided the drug, as with warfarin, PQ and imatinib, there are now attempts to identify, e.g., genetic profiles possessed by patients and then craft therapies that uniquely target those profiles. For example, the drug ivacaftor mentioned earlier was designed to treat individuals with cystic fibrosis (CF) that have very specific pathogenic mutations in the gene CFTR. The CFTR gene has many functions, but one set of functions is dictated by a 'gate-like' structure in the CFTR gene's encoded protein that can open and close to control the movement of salts in and out of cells. If the CFTR gene is dysfunctional, then the gate is closed, causing a build-up of mucus and other material in the lungs. Different mutations in the CFTR gene cause different types of dysfunction. For example, some mutations simply cause the CFTR gene to not produce anything, whether the gate is open or not. Other mutations cause the gate mechanism to dysfunction. Ivacaftor is designed to open the gate for longer periods of time in the presence of certain mutations that tend to cause the gate to be closed. Therefore, ivacaftor is only useful for the small subset of CF patients whose CFTR mutations lead to this specific gating problem. Connections between genetic variants and drug efficacy and side effects are growing in number, and in fact the US FDA provides a list of approved drug-based interventions that require a test to determine their appropriateness for an individual: Other publications consider the practical implications of approved personalized medicine interventions, such as the report produced by the Personalized Medicine Coalition (PMC).

A second example involves the emerging set of cancer treatments known as immunotherapies. Although there are many types of immunotherapies, all of them seek to prime or trigger an individual's own immune system to attack a cancer. One type of immunotherapy exploits potentially unique sets of genetic alterations that arise in a cancer patient's tumor cells, known as 'neo-antigens,' which are often capable of raising an immune response if recognized properly by the host's immune cells. Essentially, this type of immunotherapy works by harvesting cells from a patient that mediate that patient's immune reactions, such as T cells, then modifying those cells to specifically recognize and target the neo-antigens found to be present in the patient's tumor. These modified cells are then put back in the patient's body so these cells can attack the tumor cells giving off the neo-antigen signals. Cytotoxic T cell therapies like this, as well as immunotherapies in general, have had notable successes, but can be very patient-specific for two reasons. First, the neo-antigen profile of a patient might be very unique, such that cytotoxic T cells made to recognize and attack a specific set of neo-antigens will not work in someone whose tumor does not have those neo-antigens. Second, if 'autologous' constructs are used, then

the patient's own T cells are modified, and hence not likely to work as well in another patient, although attempts to make 'allogeneic' constructs in which one individual's T cells are modified and introduced into another patient's body are being pursued aggressively.

## Personalizing Early Detection Strategies:

If an individual is susceptible to a disease, or susceptible to recurrence of a disease, then that individual should be monitored. It is now believed that such monitoring should be pursued with use of 'personal thresholds,' as opposed to 'population thresholds,' to make claims about evidence or signs of disease or a pathogenic process. Population thresholds are derived from epidemiologic data and population surveys and include, for example, cholesterol levels > 200 being an indicator for risk of heart disease, or systolic blood pressure > 140 being an indicator of hypertension, risk of stroke or heart disease. Personal thresholds are established from legacy values of a measure collected on an individual over time that used to gauge how deviant future values of that measure might be for that individual. Significant deviations from historical or average legacy values are taken as a sign of a health status change, irrespective of whether or not those values are beyond a population threshold. As an example, Drescher et al. explored the utility of personal thresholds applied to longitudinal CA125 levels collected on a number of women, a subset of whom developed ovarian cancer. The authors found that in all but one instance, the application of personal thresholds would have captured the presence of ovarian cancer at the same time as, or importantly earlier than, the application of population thresholds. Further, the authors showed that the use of personal thresholds could have captured the ovarian cancer almost a year earlier, on average, then the use of population thresholds. As the costs and convenience associated with monitoring assays and technologies improves (i.e., they become cheap and non-intrusive, if not transparent, to an individual user, say through an easily implantable wireless device), then the use of personal thresholds will likely become the rule rather than the exception in health monitoring protocols.

## Personalizing Disease Prevention:

The use of genetic information to develop personalized disease prevention strategies is now well established in the scientific community, but not yet widely adopted in clinical practice. There are many excellent examples of how the use of genetic information can lead to both a decreased risk of disease development as well as decreased complications from standard treatment and screening strategies. A prime example relates to colorectal cancer, which remains the third leading cause of cancer deaths despite being a highly preventable illness. In 2012 Liao et al. reported an improvement in overall survival and a decreased risk

for cancer-specific deaths in patients taking postoperative aspirin if they exhibited a somatic mutation in the PIK3CA gene in their colorectal cancers compared with patients whose cancers had the wild-type PIK3CA gene. In 2015, Nan et al. reported varying effects of aspirin use on risk for development of colorectal cancer depending on an individual's genotype, with individuals possessing different genotypes having either lower, higher or no change in their risk of colorectal cancer development with aspirin use. Given that aspirin use can have serious side effects associated with intestinal and intracranial bleeding, it would be ideal to limit the use of this medication for those individuals predicted to have a side effect, based on genotype.

As another example, in 2018, Jeon et al. reported the use of expanded risk prediction models for determining when to begin colorectal cancer screening. Currently the guidelines use only age and family history as variables. Jeon et al. showed that by using information about an individual's environmental exposure and genetic profile, specifically the presence of colorectal cancer associated genetic variants, recommendations for when to start screening could change by 12 years for men and 14 years for women. The accuracy of relevant predictions about an individual's risk for colorectal cancer has been studied and suggests that the area under the curve (AUC) value for a model including environmental and genetic factors, where an AUC of 1.0 would suggest a model with perfect predictive accuracy, was 0.63 for men and 0.62 for women. This is compared to an AUC value of 0.53 (men) 0.54 (women) when only family history information was considered. Although there is still room for improvement given the AUCs were only ~0.62 for the model with patient environmental exposure and genetic information, the considerable improvement over models that did not include genetic or environmental information justifies their use.

## Emerging and Next-Generation Personalized Medicine Strategies:

There are a number of very recent research and clinical activities that are charting new territory for personalized medicine. We focus on four of these activities in the following, providing a brief overview of each. These activities include the use of patient-derived cell and organoid ‘avatars’ for determining the best therapies for that patient, the use of intense individualized diagnostic and monitoring protocols to detect signs of disease, the development of personalized digital therapeutics, and the use of personalized medicine approaches in treating patients with fertility issues.

## Patient-Derived Cellular Avatars:

It is now possible to harvest cells from individuals and use pluripotency induction (i.e., induced pluripotent stem cell or 'iPSC') methods on those cells to generate additional cell types of relevance to a patient's condition without having to directly biopsy the affected tissue. This allows researchers to essentially develop a 'disease in a dish' cellular model of a patient's condition. These *in vitro* cellular 'avatars' can be studied to identify key molecular pathologies that might give an indication as to how best to treat an individual patient of interest. The use of iPSC technologies in this manner can be extended with a few additional, very recently developed, technologies to create even better models of an individual's condition. For example, if the patient has a known mutation causing his or her condition, it is possible to use assays based on, e.g., Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and related constructs to create isogenic cells in which some cells have the mutation in question and some do not. Comparison of these cells allows direct insight into the effects of the mutation while controlling for all relevant genetic background effects associated with the patient's genome. In addition, it is possible to create partial organs or 'organoids' from cells obtained from an individual. Organoids can provide greater insight into molecular pathologies associated with an individual patient's condition since they can model cell:cell interactions and more global tissue function.

To achieve truly personalized medical care, the use of patient avatars derived from their own cells could be integrated with other pieces of information about a patient, as well as protocols for acting on that information. Schork and Nazor describe the motivation and integration of different aspects of patient diagnosis, intervention choice, and monitoring, using, among other things, patient avatars. One important aspect of the use of cell-based patient avatars in personalized medicine is that they can accommodate personalized drug screening: literally testing thousands of drugs and compounds against a patient's cells (or organoids, possibly modified with CRISPR technologies) to identify drugs or compounds that uniquely correct the patient's molecular defects. If the drug or compound has actually been approved for use, possibly for another condition, then it could be tested for efficacy with the patient in question under an approved drug 'repurposing' protocol. The use of patient-derived cells in personalized drug screening initiatives has shown some success in cancer settings, as tumor biopsies can yield appropriate material for drug screening. The biggest concern with this approach revolves around the question of whether or not the *in vitro* models capture relevant *in vivo* pathobiology and drug response information that may impact a patient's response to a chosen drug. A more direct strategy for *in vivo* experimental cancer intervention choice could involve implanting a device into a patient's tumor *in vivo* and then delivering different drugs through that device to see which ones have an effect.

## Intensive Personalized Health Monitoring:

The availability of inexpensive genotyping and sequencing technologies is allowing individuals and their health care providers to assess their genetically-mediated risk for disease and/or make a genetic diagnosis if they are already diseased. In addition, given the availability of health monitoring devices, online-ordered blood-based clinical assays, inexpensive imaging devices, etc. it is possible to continuously, or near continuously, monitor aspects of an individual's health. With this in mind, combining genetic risk or diagnostic assessment with intense health monitoring makes sense. A number of individuals with unique diseases and conditions have benefitted from a genetic diagnosis, as it uncovered potential genetically-mediated pathogenic mechanisms or revealed potential targets for pharmacotherapies for them. In addition, a number of individuals have monitored their health intensely for the express purpose of identifying signs of a health status changes, some of which might be attributable to genetic susceptibilities.

Monitoring individuals for health status changes are not trivial, however, if the measures being collected have not been evaluated in a population. This is because there will be no established norms that can be contrasted to the measures collected on an individual to know if those measures are abnormal. However, the community is quickly recognizing the utility of establishing 'personal thresholds' for measures as opposed to 'population thresholds,' as discussed in the 'Personalizing Early Detection Strategies' section above As noted, population thresholds are established from epidemiologic and population survey data and include often-used thresholds for determining disease status such as a cholesterol level greater than 200 for heart disease or a systolic blood pressure greater than 140 mmHg for hypertension. Personal thresholds are established from longitudinal or legacy values of a measure collected on an individual and may be unique to the individual in question and their use in some settings suggests that they work better than population thresholds.

## Digital Therapeutics and Personalized App Content:

The ubiquity of smart phones has attracted the interest of many researchers in the health professions as a vehicle for not only collecting health data through various 'apps' but also to provide advice, feedback, coaching, imagery, music, text-messages, or connections with other resources, that could benefit an individual with a particular condition or disease. This has led to the emergence of the concept of a 'digital therapeutic:' a smart phone app designed to treat and bring relief to an individual affected by a medical or psychological condition. The content provided by a digital therapeutic app to an individual could vary

depending on what is learned about that individual and his or her response to content provided in the app. In this way, the app can be personalized. Many digital therapeutics have undergone evaluation for their ability to engage users and benefit them. The US Food and Drug Administration (FDA) has created guidelines for registering digital therapeutics as bona-fide, insurance-reimbursable, approved health technologies, and has begun evaluating and approving many of them. The first approved digital therapeutic – an app for substance abuse – was approved by the FDA in 2017. How easily digital therapeutics will be assimilated into the care stream is an open question.

## Personalized Interventions Involving Fertility and Sterility:

Personalized medicine strategies and approaches can be applied to treatments for fertility, as many researchers have proposed. For example, it has been suggested that one could leverage 'real world' data of the type collected routinely on patients visiting reproductive medicine and fertility clinics (from, e.g., Electronic Medical Record (EMR) systems established at many hospitals and clinics), and use these data to in analyses exploring patterns, patient subgroups and individual patient profiles that could shed light on variation in fertility rates, responses to interventions to enhance fertility, etc. The results of these analyses could then guide future care for patients with fertility issues.(62) In the context of the use of digital medicine, proposals to develop smart phone apps that could provide personalized coaching content to enhance pregnancy have been put forth. Genetic variants known to influence fertility have also been identified and could be used to support diagnoses or personalized intervention plans. Finally, adaptive trial designs have been proposed that could be used to assess the utility of personalized approaches to raising awareness about time to conception and fertility.

In addition to these more traditional approaches to personalizing fertility interventions, there are a number of emerging strategies to enhance fertility in women that go beyond traditional ways of stimulating ovaries. For example, it is now possible to cryopreserve a set oocytes and ovarian tissue samples from a woman and then implant them in her at a later time that may suit her desire to become pregnant. Such a procedure would be highly personalized, since it would work with an individual's own cells and accommodate her preferences for becoming pregnant. However, this procedure would only work if the preserved tissues were viable and not damaged, although relevant cells in those tissues could, in theory, be corrected for genetic defects using gene editing techniques. A more futuristic and controversial personalized fertility intervention, involves the concept that one could use cell reprogramming technologies to generate sperm and egg cells from other cells obtained from an individual (e.g., skin cells) that could be edited to generate *de novo* gametes for fertilization – a concept known as '*in vitro* gametogenesis.'

## Conclusions:

Personalized Medicine, or the practice of characterizing an individual patient on a number of levels (e.g., genomic, biochemical, behavioral, etc.) that might shed light on their response to an intervention, and then treating them accordingly, is a necessity given the fact that clinically meaningful inter-individual variation has, and will continue to be, identified. The availability of modern biomedical technologies such as DNA sequencing, proteomics, and wireless monitoring devices, has enabled the identification of this variation, essentially exposing the need for the personalization of medicine at some level. The future challenges associated with this reality will be to not only improve the efficiency in the way in which individuals are characterized, but also in the way in personalized medicines are crafted and vetted to show their utility. This is not to say that interventions that work ubiquitously (i.e., the traditional single agent 'block buster' drugs) should be ignored if identified, but rather that they might be very hard to identify going forward.

There are a few other issues associated with personalized medicine that may hard to overcome in the near term. For example, the need for large data collections in order to identify factors that discriminate groups of individuals that might benefit more from one or another intervention, could create concerns about privacy and the data about those individuals possibly being used for nefarious purposes. Fortunately, this issue is not necessarily unique to health care settings, whether current or future, as it has plagued many other industries including the banking, marketing, and social media industries. Strategies exploited in these other industries could be used in health care settings as well. In addition, developing more efficient ways of developing personalized medicines (for example, with respect to cell replacement therapies or mutation-specific drugs that work for a small fraction of patients) is crucial to meet the demands of all patients. Also, paying for personalized medicine practices in the future may be complicated given that they might be initially more expensive. Finally, in order for various stakeholders to embrace personalized medicine, better strategies to educate and train health care professionals about personalized medicine must be developed and implemented.

## Probable Questions:

1. What is nutrigenetics?
2. Discuss nutritional genomics.
3. How nutrigenetics is related to epigenetics?
4. How nutritional deficiency and genome damage is interrelated?
5. How telomere shortening is related to nutrigenomics?
6. What is personalized medicine?
7. How personalized medicine concept was developed?
8. Give early and contemporary examples of personalized medicine.
9. Discuss mutation specific therapies.

## Suggested Readings:


**4.** Human molecular genetics. T Strachan and A P Read CRC press fifth edition

**5.** Molecular cell biology. H Lodish, CA Kaiser et al WH Freeman and Company

**6.** Nanotechnology: advances and real-life application. C Bhargava, A Sachdeva CRC press 2020 First edition

**4.** Handbook on nanotechnology application. K Faungnawakij et al Elsevier publication 2020 first edition

# UNIT-VI

## Concept of nanotechnology and nanomedicine in cancer treatment

**Objective**: In this unit we will discuss about role of nanotechnology in cancer treatment.

**Introduction:** Nano-biotechnology is a rapidly advancing area of scientific and technological opportunity that applies the tools and processes of Nano or micro fabrication to build devices for studying bio-systems. This particular discipline refers to the blending or intersection of nanotechnology with biology. Nano-biotechnology is often used to describe the overlapping multidisciplinary activities associated with bio-sensors, particularly photonics, chemistry, biology, biophysics, Nano-medicine, and engineering converge. Most of the scientific concepts in bio-nanotechnology are derived from other fields.

Nano-biotechnology takes most of its fundamentals from nanotechnology. Most of the devices designed for Nano-biotechnological use are directly based on other existing nanotechnologies. The most important objectives that are frequently found in Nano-biology involve applying Nano tools to relevant medical/biological problems and refining these applications. The difference between "Nano-biology" to **"Nano-biotechnology"** resides in the technology part of the term.

Anything that is "man-made" falls into the technology section of Nano-biotechnology. Nearly any molecular machinery that we can think of has its analog in biological systems and as for now, it appears that the first revolutionary application of Nano- biotechnology will probably be in computer science and medicine. As with nanotechnology and biotechnology, bio-nanotechnology has many potential ethical issues associated with it. Yet this innovative approach to biology allows scientists to imagine and create systems that can be used for extensive biological researches. Hence, biologically inspired nanotechnology uses biological systems as the inspirations for technologies not yet created. Nano-biotechnology is still in the early stages of development; however, its development is multidirectional and fast-paced. Nano-biotechnology research centres are being founded and funded at a high frequency, and the numbers of papers and patent applications is also rising rapidly. In addition, the Nano-biotechnology **"tool box"** is being rapidly filled with

new and viable tools for bio-Nano manipulations that will speed up new applications. Finally, an analysis of the total investment in Nano-biotechnology start-ups reveals that nearly 50% of the venture capital investments in nanotechnology is addressed to Nano-biotechnology.

One of the strongest driving forces in this research area is the semiconductor industry. Computer chips are rapidly shrinking according to Moore's law, i.e., by a factor of four every 3 yr. However, this simple shrinking law cannot continue for much longer, and computer scientists are, therefore, looking for the new ones. One approach is moving to single-molecule transistors. This shift is critically dependent on molecular Nano manipulations to form molecular computation that will write, process, store, and read information within the single molecule where proteins and DNA are some of the alternatives.

As medical research and diagnostics steadily progresses based on the use of molecular biomarkers and specific therapies aimed at molecular markers and multiplexed analysis, the necessity for molecular- level devices increases. Technology platforms that are reliable, rapid, low-cost, portable, and that can handle large quantities are evolving and will provide the future foundation for personalized medicine. These new technologies are especially important in cases of early detection, such as in cancer. Future applications of Nano-biotechnology will probably include Nano-sized devices and sensors that will be injected into, or ingested by, our bodies.

These instruments could be used as indicators for the transmission of information outside our bodies or they could actively perform repairs or maintenance. Nanotechnology-based platforms will secure the future realization of multiple goals in biomarker analysis. Examples for such platforms are the use of cantilevers, Nano mechanical systems (NEMS), Nano electronics (biologically gated nanowire), and nanoparticle in diagnostics imaging and therapy. The art of Nano manipulating materials and bio systems is converging with information technology, medicine, and computer sciences to create entirely new science and technology platforms. These technologies will include imaging diagnostics, genome pharmaceutics, bio systems on a chip, regenerative medicine, on-line multiplexed diagnostics, and food systems.

It is clear that biology has much to offer the physical world in demonstrating how to recognize, organize, functionalize, and assemble new materials and devices. In fact, almost any device, tool, or active system known today can be either mimicked by biological systems or constructed using techniques originating in the bio-world. Therefore, it is plausible that in the future, biological systems will be used as building blocks for the construction of the material and mechanical fabric of our daily lives.

Potential benefits of using Nano-objects (nanotubes, quantum dots, Nano rods and Nano prisms) and Nano devices (Nano capacitors, Nano pores and Nano cantilevers) leading to an expanded range of label multiplexing are described along with potential applications in future diagnostics and management of various diseases. It also speculates on further pathways in nanotechnology development and the emergence of order in this somewhat chaotic, yet promising, new field. For integration and establishment of a healthy society there are a lot of expectations from Nano-biotechnology to create tremendous expansion in the available technologies.

Cancer is a leading cause of death and a global health burden. It was estimated that there would be 18.1 million new cancer cases and 9.6 million cancer-related deaths by 2018. Cancer is a disease characterized by uncontrolled cell proliferation that spreads from an initial focal point to other parts of the body to cause death. For these reasons, it is key to ensure earlier detection and treatment of cancers to reduce disease spread and mortalities. Amongst the widely used strategies, today in cancer research is nanotechnology. Nanotechnology has led to several promising results with its applications in the diagnosis and treatment of cancer, including drug delivery, gene therapy, detection and diagnosis, drug carriage, biomarker mapping, targeted therapy, and molecular imaging. Nanotechnology has been applied in the development of nanomaterials, such as gold nanoparticles and quantum dots, which are used for cancer diagnosis at the molecular level. Molecular diagnostics based on nanotechnology, such as the development of biomarkers, can accurately and quickly detect the cancers[4]. Nanotechnology treatments, such as the development of nanoscale drug delivery, can ensure precise cancerous tissue targeting with minimal side effects. Due to its biological nature, nanomaterials can easily cross cell barriers. Over the years, nanomaterials have been used in the treatment of tumors, due to their active and passive targeting. Although many drugs can be used to treat cancers, the sensitivity of the drugs generally leads to inadequate results and can have various side effects, as well as damage to the healthy cells. In view of that, several studies have examined different forms of nanomaterials, such as liposomes, polymers, molecules, and antibodies, with the conclusion that a combination of these nanomaterials in cancer drug design can achieve a balance between increasing efficacy and reducing the toxicity of drugs[8]. However, due to the potential toxicity of nanomaterials, there is still a lot of advancement to be done on them before their readily acceptance in the clinic for cancer management. With the rapid development of nanotechnology, this paper will review its application in cancer diagnosis and treatment with focus on their benefits and limitations during use (Figure 1).

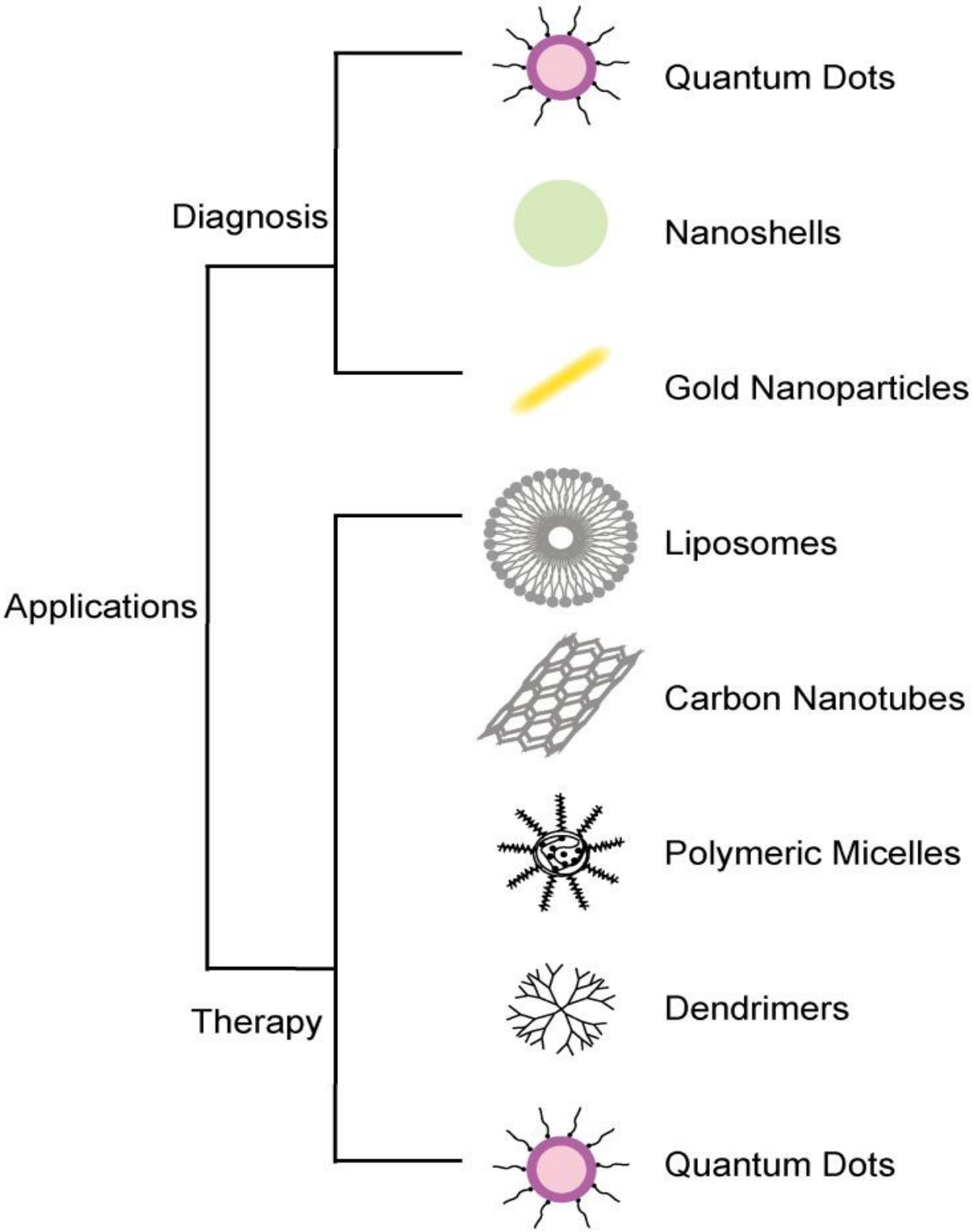



**Figure 1: Application of nanomaterials in cancer diagnosis and therapy**

## NANOTECHNOLOGY IN CANCER DIAGNOSIS:

Genetic mutations can cause changes in the synthesis of certain biomolecules leading to uncontrolled cell proliferation and ultimately cancerous tissues. Cancers can be classified as either benign or malignant. Benign tumors are confined to the origin of cancer while malignant tumors actively shed cells that invade surrounding tissues as well as distant organs. Cancer diagnostic and therapeutic strategies are targeted at early detection and inhibition of cancerous cell growth and their spread. Notable among the early diagnostic tools for cancers is the use of positron emission tomography (PET), magnetic resonance imaging (MRI), computed tomography (CT) and ultrasound. These imaging systems, however, are limited by their inadequate provision of relevant clinical information about different cancer types and the stage. Hence it makes it difficult to obtain a full evaluation of the disease state based on which an optimum therapy can be provided.

### Nanotechnology aids in tumor imaging:

In the past few decades, the application of nanoparticles in cancer diagnosis and monitoring has attracted a lot of attention with several nanoparticle types being used today for molecular imaging. Due to their advantages including small size, good biocompatibility, and high atomic number, they have gained prominence in recent cancer research and diagnosis. Nanoparticles used in cancer such as semiconductors, quantum dots and iron oxide nanocrystals possess optical, magnetic or structural properties that are less common in other molecules. Different anti-tumor drugs and biomolecules including peptides, antibodies or other chemicals, can be used with nanoparticles to label highly specific tumors, which are useful for early detection and screening of cancer cells.

For cancer diagnostics, imaging of tumor tissue with nanoparticles has made it possible to detect cancer in its early stages. In lung cancer, the detection of metastases can be determined by developing immune superparamagnetic iron oxide nanoparticles (SPIONs) that can be used in MRI imaging with the cancer cell lines as the target for the SPIONs[1]. Recent studies have shown a high specificity of SPIONs with no known side effects, making them suitable building blocks for aerosols in lung cancer MRI imaging.

Magnetic powder imaging has also been used in tomographic imaging technology where it has shown a high resolution and sensitivity to cancer tissues. In animal experiments, nebulization of the lungs has been achieved using magnetic nanoparticles (MNPs) with Epidermal growth factor receptor (EGFR), a commonly expressed protein in non-small cell lung cancer (NSCLC) cases as a target. Further, *in vitro* studies using nanosystem for

positron emission tomography (PET) have also been developed based on self-assembled amphiphilic dendritic molecules. These dendritic molecules spontaneously assemble into uniform supramolecular nanoparticles with abundant PET reporting units on the surface. By taking advantage of dendritic multivalence and the enhanced penetration and retention (EPR) effect, the dendritic nanometer system effectively accumulates in tumors, resulting in extremely sensitive and specific imaging of various tumors while reducing treatment toxicities.

## Nanotechnology Tools Used in Cancer Diagnosis:

In current research, nanotechnology can validate cancer imaging at the tissue, cell, and molecular levels. This is achieved through the capacity of nanotechnology applications to explore the tumor's environment, For instance, pH- response to fluorescent nanoprobes can help detect fibroblast activated protein-a on the cell membrane of tumor-associated fibroblasts. Hereon, we will discuss some nanotechnology-based spatial and temporal techniques that can help accurately track living cells and monitor dynamic cellular events in tumors.

## Near Infrared (NIR) Quantum Dots :

The lack of ability to penetrate objects limits the use of visible spectral imaging. Quantum dots that emit fluorescence in the near-infrared spectrum (i.e., 700-1000 nanometers) have been designed to overcome this problem, making them more suitable for imaging colorectal cancer, liver cancer, pancreatic cancer, and lymphoma. A second near-infrared (NIR) window (NIR-ii, 900-1700 nm) with higher tissue penetration depth, higher spatial and temporal resolution has also been developed to aid cancer imaging. Also, the development of a silver-rich Ag2Te quantum dots (QDs) containing a sulfur source has been reported to allow visualization of better spatial resolution images over a wide infrared range.

## Nanoshells :

Another commonly used nanotechnology application is the use of nanoshells. Nanoshells are dielectric cores between 10 and 300 nanometers in size, usually made of silicon and coated with a thin metal shell (usually gold). These nanoshells work by converting plasma-mediated electrical energy into light energy and can be flexibly tuned optically through UV-infrared emission/absorption arrays. Nanoshells are desirable because their imaging is devoid of the heavy metal toxicity even though their uses are limited by their large sizes.

## Colloidal Gold Nanoparticles :

Gold nanoparticle (AuNPs) is a good contrast agent because of its small size, good biocompatibility, and high atomic number. Research shows that AuNPs work by both active and passive ways to target cells. The principle of passive targeting is governed by a gathering of the gold nanoparticles to enhance imaging because of the permeability tension effect (EPR) in tumor tissues. Active targeting, on the other hand, is mediated by the coupling of AuNPs with tumor-specific targeted drugs, such as EGFR monoclonal antibodies, to achieve AuNP active targeting of tumor cells (Figure-2). When the energy exceeds 80kev, the mass attenuation rate of gold becomes higher than alternative elements like iodine, indicating a greater prospect gold nanoparticles. Rand et al. mixed AuNPs with liver cancer cells and found that using X-ray imaging, the clusters of liver cancer cells in the gold nanocomposite group were significantly stronger than those in the liver cancer cells alone. These findings have important implications for early diagnosis, with the technique allowing tumors as small as a few millimeters in diameter to be detected in the body.

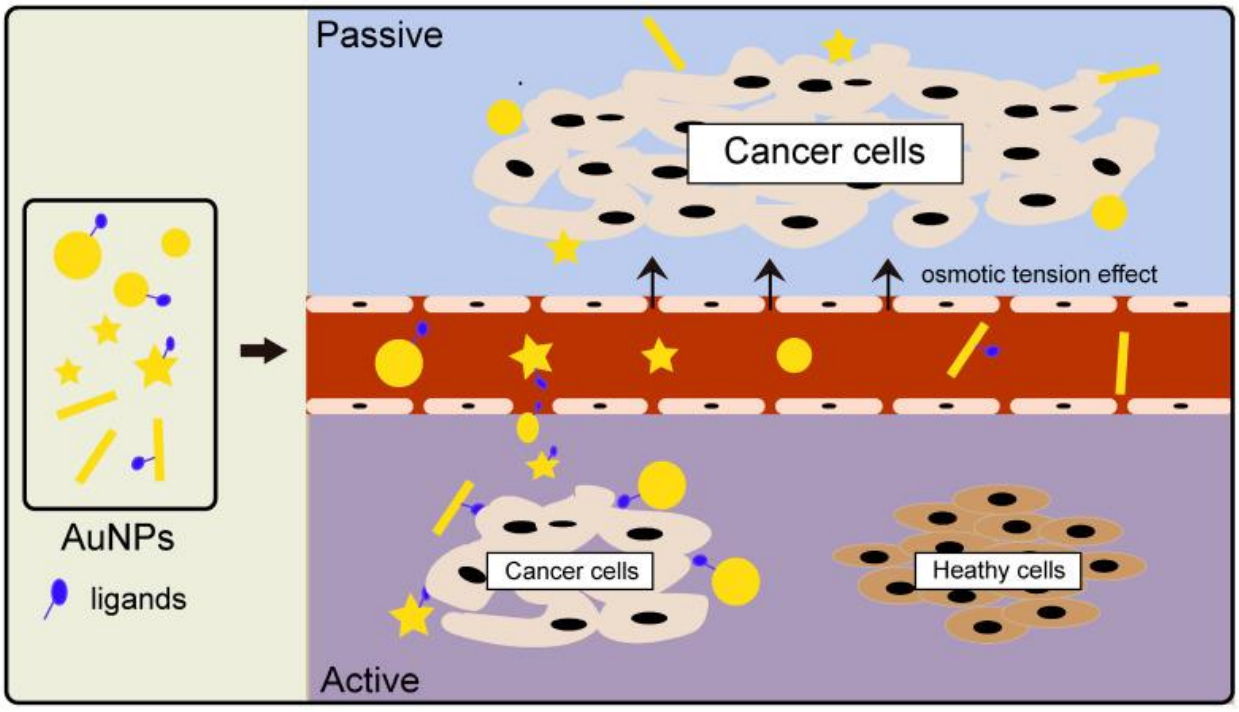



**Figure 2: Various types of gold nanoparticles (different sizes, morphologies, and ligands) accumulate in tumor tissues by the action of osmotic tension effect (termed Passive targeting) or localize to specific cancer cells in a ligand-receptor binding way (termed Active targeting).**

## Nanotechnology used in cancer biomarker screening:

Cancer biomarkers are biological features whose expression indicates the presence or state of a tumor. Such markers are used to study cellular processes, to monitor or identify changes in cancer cells, and these results could ultimately lead to a better understanding of tumors. Biomarkers can be proteins, protein fragments or DNA. Among them, tumor biomarkers, which are indicators of a tumor, can be tested to verify the presence of specific tumors. Tumor biomarkers ideally should possess a high sensitivity (>75%) and specificity (99.6%). Under current medical conditions, biomarkers from blood, urine, or saliva samples are used to screen individuals for cancer risk. But these biomarkers have not proven adequate for cancer screening. Therefore, several researchers have resorted to the study of extract patterns of abnormally expressed proteins, peptide fragments, glycans and autoantibodies from serum, urine, ascites or tissue samples from cancer patients. With the development of proteomics technology, protein biomarkers for many cancers have been discovered.

In general, protein profiling tests would remove the high molecular weight proteins such as albumin and immunoglobulins. However, the removal of these proteins also removes the low molecular weight protein biomarkers conjugated to them, resulting in the loss of the biomarkers of interest. These low molecular weight proteins represent a potential biomarker-rich population. Two studies led by Geho and Luchini came up with the method of capturing and enriching low molecular weight proteins by nanoparticles to obtain biomarkers from biological liquids, thus improving the screening of biomarkers. Nanoparticles compete with the carrier proteins by their surface characteristics, such as electric charge, or functional biomolecules, which are currently possessed by mesoporous silica particles, hydrogel nanoparticles, and carbon nanotubes.

Another method to improve screening with nanocarrier is to improve the sensitivity of mass spectrometry. The unique optical and thermal properties of carbon nanotubes enhance the energy-transfer efficiency of the analyte, contributing to the absorption and ionization of the analyte, and eliminate the interference of inherent matrix ions. A third approach is to use nanotechnology to make lab-on-chip microfluidics devices that can be used for immuno-screening or to study the properties of tumor cells. For example, a system showing great promise is lab-on-a-chip for high performance multiplexed protein detection using quantum dots made of cadmium selenide (CdSe) core with a zinc sulfide (ZnS) shell linked to antibodies to carcinoembryonic antigen, cancer antigen 125 and Her-2/Neu. Another example is that cells growing on the surface of different sized nanometres, which were discovered by these nanometres across can differentiate between tumor cells. Suffice it to say that there are still false-positive and false-negative results from screening of

biomarkers by nanotechnology, and we need to improve sensitivity without compromising specificity.

## NANOTECHNOLOGY IN CANCER THERAPY:

### Tools of Nanotechnology for Cancer Therapy:

The development of nanotechnology is based on the usage of small molecular structures and particles as tools for delivering drugs. Nano-carriers such as liposomes, micelles, dendritic macromolecules, quantum dots, and carbon nanotubes have been widely used in cancer treatment.

### Liposomes :

Liposomes are one of the most studied nanomaterials, which are nanoscale spheres composed of natural or synthesized phospholipid bilayer membrane and water phase nuclei. Because of the amphiphilicity of phospholipids, liposomes form spontaneously, allowing hydrophilic drugs to preferentially stay in the monolayer liposome while hydrophobic ones form before the multilayer liposome. Some drugs could be incorporated into liposomes by exchanging them from acidic buffer to the neutral buffer. Neutral drugs can be transported in liposomes also, but due to a poor avidity for acidic environments, they are not readily released from the inside of the liposomes. Other mechanisms of drug delivery are the combination of saturated drugs with organic solvents to form liposomes. Under the influence of the EPR effect, the vesicle of size around 4000 kDa or 500 nm can be allowed into the tumor by the gaps in vessels. In tumors they can fuse with cells, are internalized by endocytosis, and release drugs in the intracellular space. In the case of the appropriate pH, redox potential, ultrasonic and under the electromagnetic field, the liposome can also release the drug through passive or active ligand-mediated activity. The targeted therapy has an advantage in the vascular system, micrometastases, and blood cancers. It has been shown that the half-life of liposome is affected by size. The liposome up to 100 nanometers easily penetrate the tumor and stay longer, while the half-life of the bigger liposome is shorter because they are easily recognized and cleared by the mononuclear phagocyte system. Liposome-bound antibodies target tumor-specific antigens to ensure active targeting and then transport drugs to the tumor. With a lot of pharmacokinetic benefits, some liposomal drugs are approved for clinical therapy. For instance, liposomal forms of adriamycin have been used for the management of metastatic ovarian cancer where they have shown appreciable clinical benefit

## Carbon Nanotubes :

Based on the structure and the diameter, Carbon nanotubes (CNTs) can be categorized into two kinds, the single-walled CNTs (SWNTs) and the multiwalled CNTs (MWNTs). The SWNTs are composed of monolithic cylindrical graphene, and the MWNTs are composed of concentric graphene. Because of the physical and chemical properties of carbon nanotubes, that include surface area, mechanical strength, metal properties, electrical and thermal conductivity, it is a candidate well suited for large-scale biomedical applications. Carbon nanotubes also possess a property that allows them to absorb light from the near-infrared (NIR) region, causing the nanotubes to heat up by the thermal effect, hence can target tumor cells. The natural forms of carbon nanotubes promote noninvasive penetration of biofilms and are regarded as highly competent carriers for the transport of various drug molecules into living cells. Due to the suitability of carbon nanotubes, drugs such as paclitaxel are assembled with them and administered both *in vitro* and *in vivo* for cancer treatment.

## Polymeric Micelles :

Polymeric nanoparticles (PNPs) are the inventions that relate to a solid micelle with a particle size range of 10-1000 nm. PNPs are collectively known as polymer nanoparticle, nanospheres, nanocapsules or polymer micelles and they were the first polymers reported for drug delivery systems. PNPs serve as drug carriers for hydrophobic drugs and are widely used for drug discovery. The PNPs constructed from amphiphilic polymers with a hydrophilic and hydrophobic block can perform rapid self-assembly because of the hydrophobic interactions in an aqueous solution[69]. The PNPs can capture the hydrophobic drugs because of a covalent bond or the interaction via a hydrophobic core. Thus, to carry the hydrophilic charged molecules, such as proteins, peptides, and nucleic acids, these blocks are switched to allow interactions in the core and neutralize the charge.

The advantages of the higher thermodynamic stability and the smaller volume make the PNPs a suitable drug carrier with good endothelial cell permeability while avoiding kidney rejection. The hydrophobic macromolecules and drugs can be transferred to the center of the PNPs, hence, the injection of PNPs suspension after being separated in an aqueous solution could achieve therapeutic effect. Importantly, by oral or parenteral administration, drugs can reach the target cells in different ways, potentially provide alternative ways to lower cytotoxicity in healthy tissues compared to the cancer cells. However, the major challenges in the use of PNPs for cancer nanomedicine still exist in how to effectively deliver the drugs to the target site with limited side effects or drug resistance. Recently, the

PNPs have been used widely in the nanotechnology-based cancer drug design due to their excellent potential benefits for patient care. For example, adriamycin conjugated nanomaterial was used to treat several types of cancers where it achieved therapeutic effects to a decent degree. However, it also presented with many side-effects, such as toxicity and heart problems, thereby limiting its use. Such problems are overcome by Doxil (a liposomal form of doxorubicin), which is less associated with cardiotoxicity in patients, and hence may provide a safer nanomaterial synthetic approach for researchers in the future.

## Dendrimers :

The dendrimers are nanocarriers that have a spherical polymer core with regularly spaced branches. As the dendritic macromolecule diameter increases, the tendency to tilt towards a spherical structure increases. There are usually two ways to synthesize dendrimers, a divergent method in which the dendrimers can grow outward from the central nucleus, and a convergence method, where the dendrimers grow inward from the edges and end up in the central nucleus. Various molecules including polyacrylamide, polyglycerol-succinic acid, polylysine, polyglycerin, poly2, 2bis (hydroxymethyl) propionic acid, and melamine are commonly used to form dendrimers. These dendritic macromolecules exhibit different chemical structures and properties, such as alkalinity, hydrogen bond capacity and charge, which can be regulated by growing dendritic macromolecules or changing the groups on the surface of dendritic macromolecules. In general, the dendritic drug conjugates are formed by the covalent binding of antitumor drugs to dendritic peripheral groups. Thus, several drug molecules can attach to each dendritic molecule and the release of these therapeutic molecules is controlled in part by the nature of the attachment. The physicochemical and biological properties of the polymer including the size, charge, multi-ligand groups, lipid bilayer interactions, cytotoxicity, internalization, plasma retention time, biological distribution, and filtration of dendritic macromolecules, have made dendrimers potential nanoscale carriers. Several studies have further shown that cancer cells with a high expression of folate receptors could form foils from dendritic molecules bound to folate. An added advantage of dendrimers is their ability to bind to DNA as seen with the DNA-polyamides clustering DNA-poly(amidoamine) (DNAPAMAM), making them highly effective at killing cancer cells that express the folate receptor.

## Quantum Dots :

Quantum dots (QDs) are small particles or nanocrystals of semiconductor materials between 2 and 10 nanometers in size. The ratio of the height of the surface to the volume of these particles gives the QDs the intermediate electron property which is between a mass semiconductor and a discrete atom. Over the years, various QDs based techniques such as modification of QD conjugates and QD immunostaining have been developed. With the improvement of multiplexing capability, QDs conjugation greatly exceeds the monochromatic experiment in both time and cost-effectiveness. Moreover, at low protein expression levels and in a low context, QD immunostaining is more accurate than traditional immunochemical methods. In cancer diagnosis, QD immunostaining is a potential tool for the detection of various tumor biomarkers, such as a cell protein or other components of a heterogeneous tumor sample. Quantum dots can gather in specific parts of the body and transfer the drugs to those parts. The ability of the QDs to concentrate in a single internal organ makes them a potential solution against untargeted drug delivery, and possibly avoid the side effects of chemotherapy. The latest advancement in surface modification of QDs, which combine with biomolecules, including peptides and antibodies, *in vivo*, can be used to target tumors and make possible their potential applications in cancer imaging and treatment. Some studies combine QDs with prostate-specific antigen to label cancer, while others use QDs to make biomarkers that speed up the process with such immune markers having a more stable light intensity than traditional fluorescent immunomarkers. High sensitivity probes based on quantum dots have been reported for multicolor fluorescence imaging of cancer cells *in vivo* and can also be used to detect ovarian cancer marker cancer antigen 125 (CA125) in different types of specimens (such as fixed cells, tissue sections, and xenograft). Besides, the light stability of quantum dot signals is more concrete and brighter than that of traditional organic dyes. Chen et al. successfully detected BC using quantum-dot-based probes, confirming that unlike traditional immunohistochemistry, quantum dot immunohistochemistry (IHC) can detect the very low expressions of Human Epidermal Growth Factor Receptor 2 (HER2) as well as multichannel detection.

## CONCLUSION AND FUTURE DIRECTIONS :

Nanotechnology has shown a lot of promise in cancer therapy over the years. By their improved pharmacokinetic and pharmacodynamic properties, nanomaterials have contributed to improved cancer diagnosis and treatment. Nanotechnology allows targeted drug delivery in affected organs with minimal systemic toxicities due to their specificities. However, as with other therapeutic options, nanotechnology is not completely devoid of toxicities and comes with few challenges with its use including systemic and certain organ

toxicities, hence, causing setbacks with their clinical applications. Given the limitations with nanotechnology, more advancements must be done to improve drug delivery, maximize their efficacy while keeping the disadvantages to the minimum. By improving the interactions between the physicochemical properties of the nanomaterials employed, safer and more efficacious derivatives for diagnosis and treatment can be made available for cancer management. In sum, we sought to highlight the key advantages of nanotechnology and the shortfalls in their use to meet clinical needs for cancer. Adding to that, the therapeutic benefits of nanotechnology and future advancements could make them a therapeutic potential to be applied in other disease conditions. These may include ischemic stroke and rheumatoid arthritis which would require targeted delivery of a suitable pharmacologic agent at the affected site.

## Probable Questions:

1. How nanotechnology subject evolve?
2. How nanotechnology can be used in cancer diagnosis?
3. How nanotechnology can be used in cancer treatment?
4. What are the uses of carbon nanotubes?
5. What are the uses of quantum dots?
6. What are the uses of dendrimers?
7. What are the uses of polymeric micelles?
8. How Nanotechnology is used in cancer biomarker screening?
9. Discuss role of nanoshells in cancer diagnosis?
10. How nanotechnology is used in cancer imaging?

## Suggested Readings:


1. Human molecular genetics. T Strachan and A P Read CRC press fifth edition
2. Nanotechnology: advances and real-life application. C Bhargava, A Sachdeva CRC press 2020 First edition
3. Handbook on nanotechnology application. K Faungnawakij et al Elsevier publication 2020 first edition

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