ARULMIGU PALANIANDAVAR ARTS COLLEGE FOR WOMEN, PALANI.

P G DEPARTMENT OF ZOOLOGY

Learning Resources

CELL AND MOLECULAR BIOLOGY

Transmission Electron Microscope (TEM)

Electron beam is passed through the specimen to produce its image is called transmission electron microscope (TEM).The first TEM was designed by Max Knoll and Ernst Ruska in 1931. The TEM was first made available in the market in 1939. TEM has wide applications in the research on virology, oncologv,pollution studies, material science and semi-conductor research.

PRINCIPLE

The basic principle of electron microscopes is similar to the optical principle of ordinary compound microscope. Here, electron beam is substituted for light beam and electromagnetic coils are substituted for optical lenses.When high voltage current is passed through a filament of cathode ray tube, electron beams are produced from the filament.

If some voltage of electric current is applied to electromagnetic coils kept around the path of electron beam, the direction of electron beam can be changed suitably to focus on the specimen and objective.When an electron beam is passed through a specimen stained with metallic gold or osmium, the specimen absorbs some rays, reflects some other rays and leaves some rays to pass through it. This interaction between the specimen and electrons in the beam produces the image of the specimen.

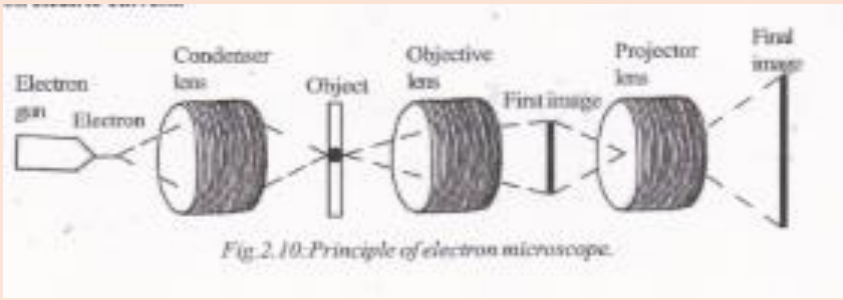


Fig.2.10:Principle of electron microscope.

The image of the specimen can be collected by an objective lens and magnified by an amplifier

The image due to electron distribution cannot be seen with naked eyes. So the image is recorded on a screen or photographic plate or camera. The wavelength of electron beam is very short (0.05A or 0.005 nm).The magnifying strength of microscopes is inversely proportional to the wavelength of the rays. TEM has higher resolving power than the compound microscope.

INSTRUMENTATION

The TEM consists of an electron gun, condenser lenses, objective lens, amplifier lens

fluorescent screen or photographic plate.Electron gun is the source of electron beam used in this microscope. It consists of a V- shaped filament, Whenelt cylinder and an anode plate with a hole at the centre.The Whenelt cylinder is a cup-like structure with a hole at the bottom.

The V-shaped filament is kept in the cup.The Whenelt cylinder acts as a cathode.

Electron gun :

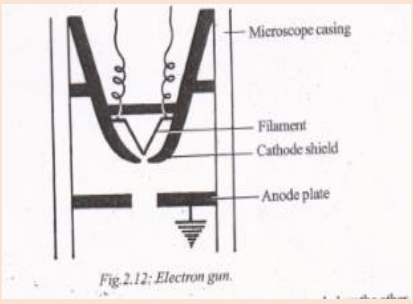


Fig.2.12: Electron gun.

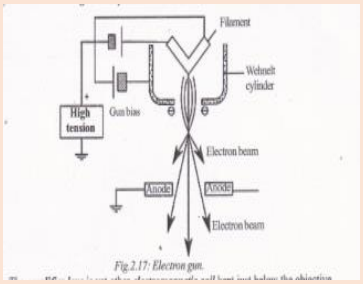
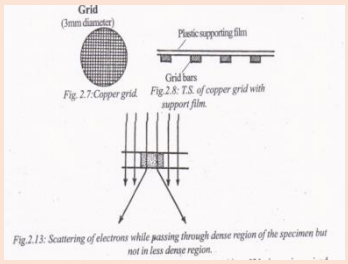


Fig.2.17: Electron gun.



When high voltage current is applied between the filament and anode plate, the filament is heated up to incandescence for emitting electrons.Since the electrons are attracted towards the anode, they are forced out through the hole in the anode plate by the cathode shield.Theelectron gun is placed at the top of the TEM.

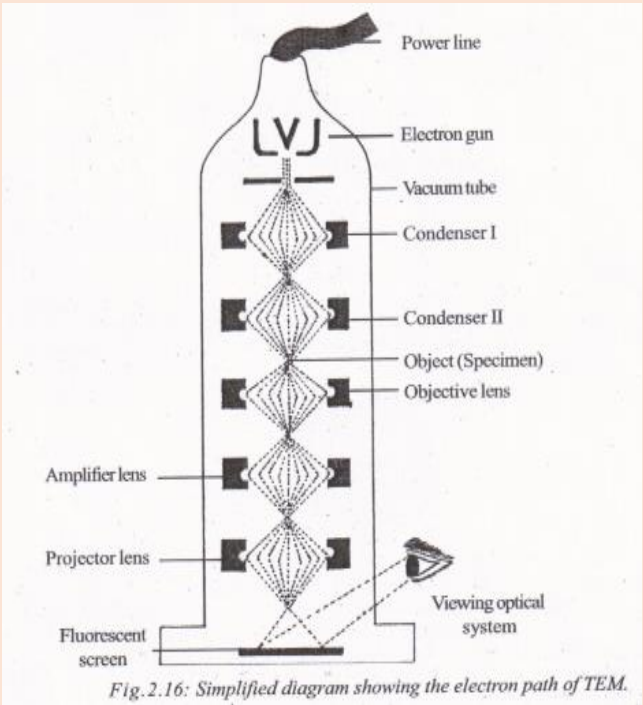


Fig.2.16: Simplified diagram showing the electron path of TEM.

There are two condenser lenses just below the electron gun.They are electromagnetic coils (coiI of wires). They collect and concentrate the electrons into a strong electron beam before focusing it onto the specimen. Just below the second condenser lens, there is a specimen stage.

A thin section of specimen is placed on a thin plastic film mounted on a copper grid. The grid size of 2.2nm or 3.2nm is used for this purpose.The specimen mounted grid is placed in the path of the electron beam.

The objective lens is another electromagnetic coil which is placed below the specimen stage. It collects the image of the specimen and focuses it towards the amplifier lens. The amplifier lens is other electromagnetic coil kept just below the objective lens.It magnifies the image produced by the objective lens to several 1000 times. A projector lens collects the magnified image and focuses it onto a fluorescent screen or photographic plate. The entire setup is placed in a vacuum tube, because electrons can move in a straight line only in a vacuum.While TEM is working, a large amount of heat is flowing through the electromagnetic coils. In order to keep the apparatus at a low temperature, cooling water is circulated through a cooling system around the TEM.

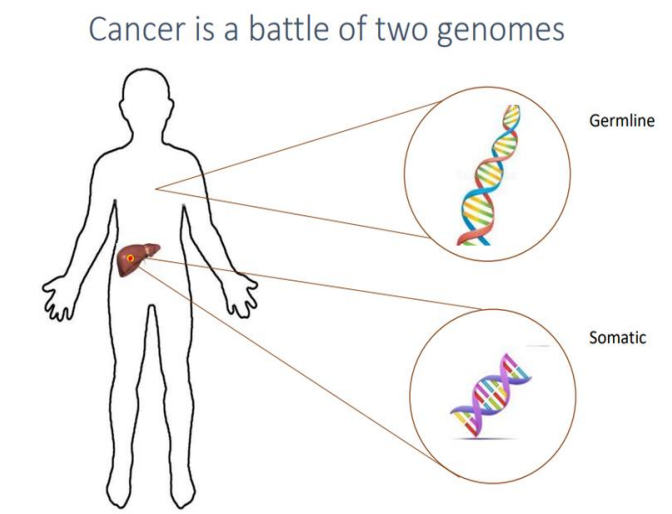
GENETIC BASIS OF CANCER

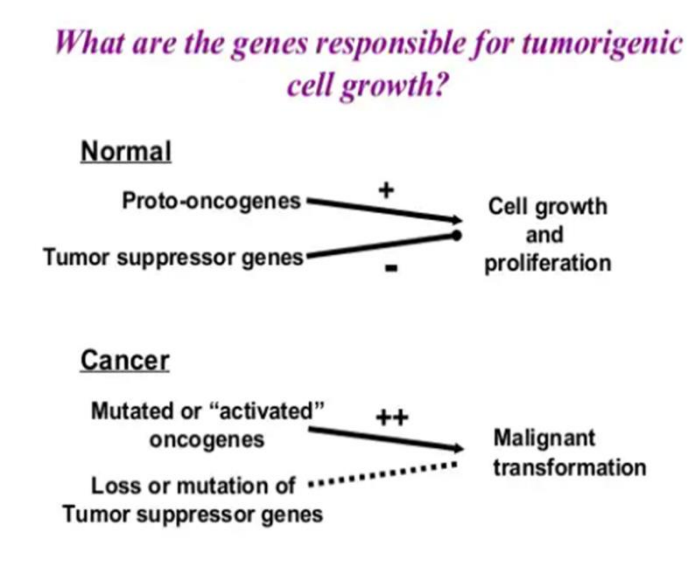
Cancer results from uncontrolled proliferation of single cell.It is monoclonal.

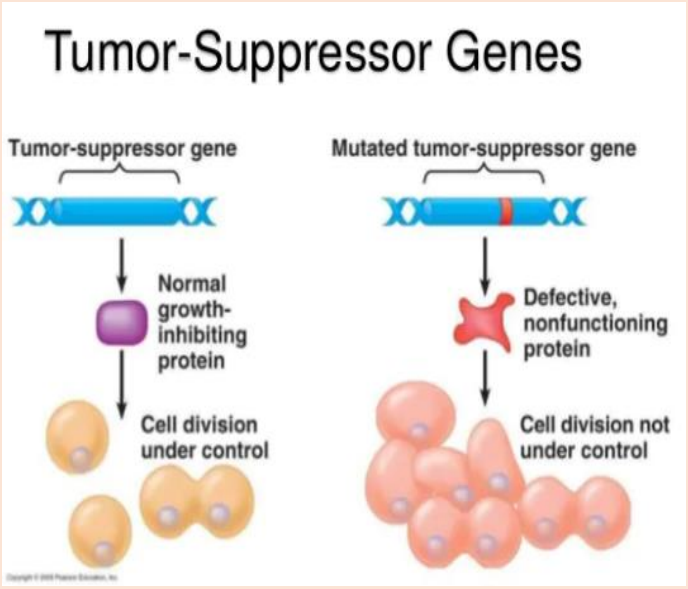
Malignant transformation requires more than a single genetic alteration.

a) Inherit from our parents (germ-line mutation)

b) Occur during our own lifetime (Somatic mutation)

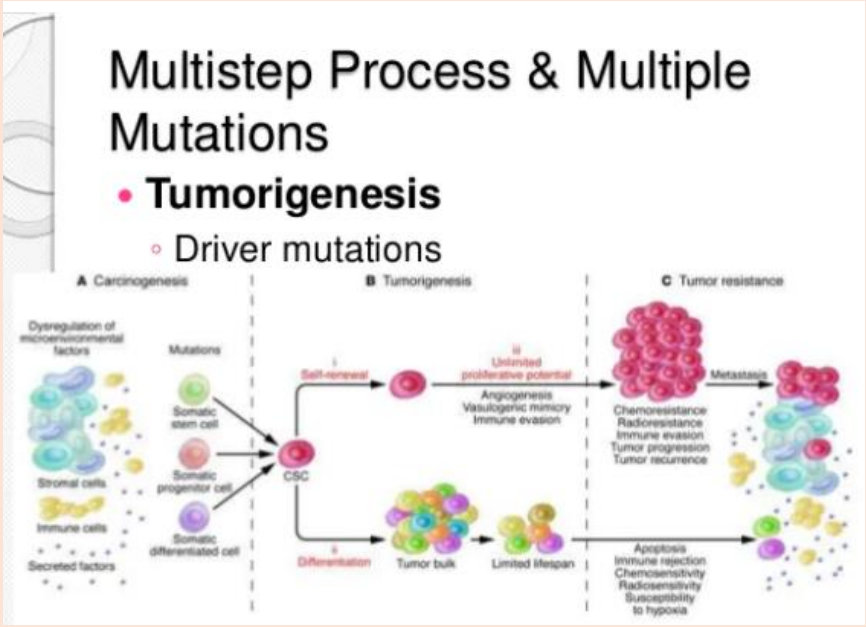






Tumorigenesis is a multistep process characterized by a

- Progression of **permanent genetic alterations** which may occur
- Many **successive cell divisions**
- Protection from **apoptosis**
- Expression of **telomerase**
- Change in the structure of chromatin in and around the gene
- Change in state of **methylation**
- **Histological changes**
- **Less responsive** to the body normal regulatory machinery



The cells of these tissues divided into **3 groups**

Stem cells-posses unlimited proliferation potential

Progenitor cells –from stem cells and posses limited proliferation potential

The differentiated end products of the tissue

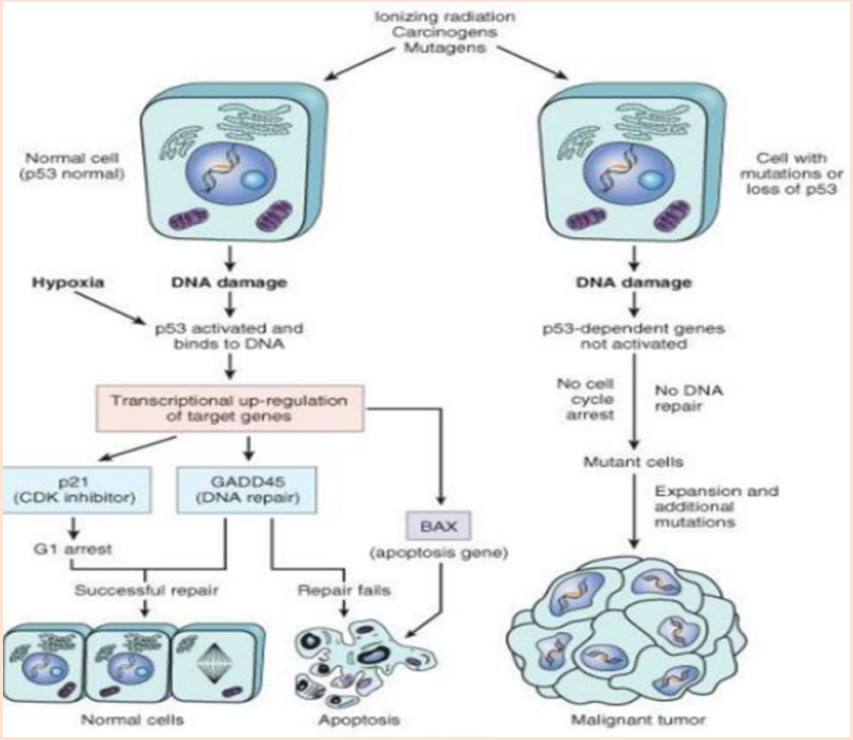
TUMOR –SUPPRESSOR GENES AND ONCOGENES:

Tumor –Suppressor genes act as a **cell brakes**;they encode proteins that restrain cell growth.

Oncogenes act as a cell **accelerators;**that promote loss of growth control & conversion of a cell to a malignant state.

Molecular Basis of Cancer

- Targeted genes:
  - 1. Proto-oncogenes (oncogenes)
  - 2. Tumor suppressor genes
  - 3. Genes controlling apoptosis
  - 4. Genes regulating DNA repair
- Other genes involved:
  - Genes regulating angiogenesis
  - Genes enhancing invasion and metastasis
- Carcinogenesis is a multistep process
  - At both genetic and phenotypic levels
  - Progression results from accumulation of genetic defects



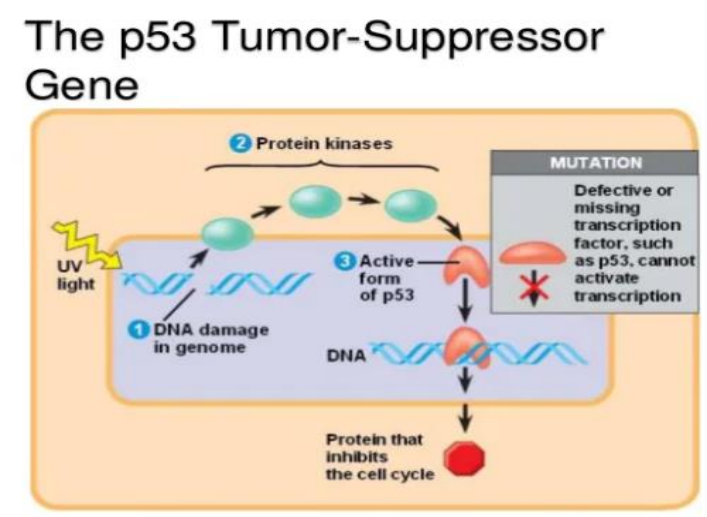
Tumor –Suppressor genes in humans are protective genes that help to maintain genetic stability and encode transcription factors (e.g.,TP53 and WT1),Cell cycle regulators (e.g.,RB and P16), components that regulate G proteins (NF1), BRCA1, BRCA2 and so on.

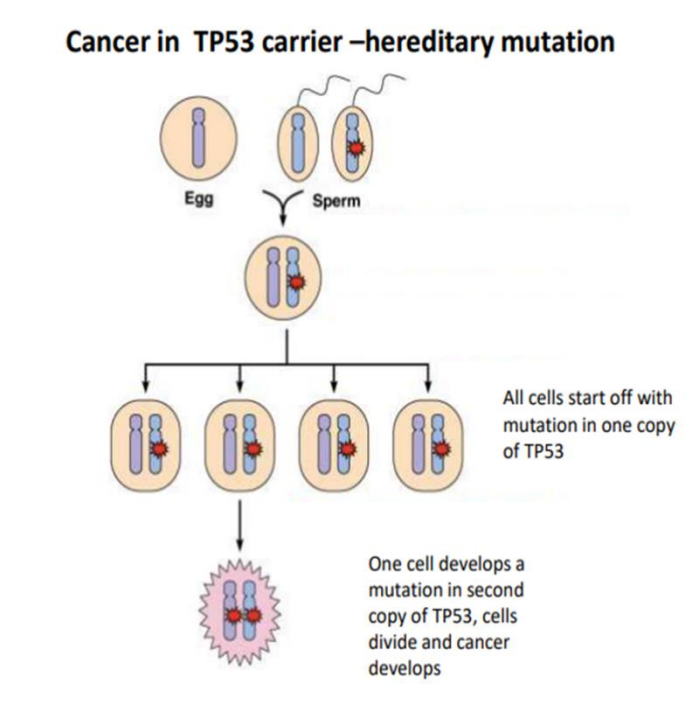
Germline mutations in BRCA1 or BRCA2 genes woman risk of hereditary breast or ovarian cancers man risk of hereditary prostate or breast cancers.

Women and men risk of pancreatic cancer and melanoma.

The concepts of TSGs were demonstrated first with the **Retinoblastoma gene (RB).**

Commonly affected TSGs include the **p53 gene**





The genetic basis of **Retinoblastoma** was **1st explained i**n 1971 by **Alfred Knudson** of the University of Texas.

Children suffering from Retinoblastoma revealed that a small piece from the interior portion of the thirteenth pair of homologous chromosome is missing.

The protein encoded by the RB gene ,pRB,helps regulate the passage of cells from G1 stage of the cell cycle into S phase.

Mutation of RB gene loses pRB activity.

1969 **Li and Fraumeni** describe four families from their studies of **childhood rhabdomyosarcoma.**

They observe that these families have multiple early onset cancers.

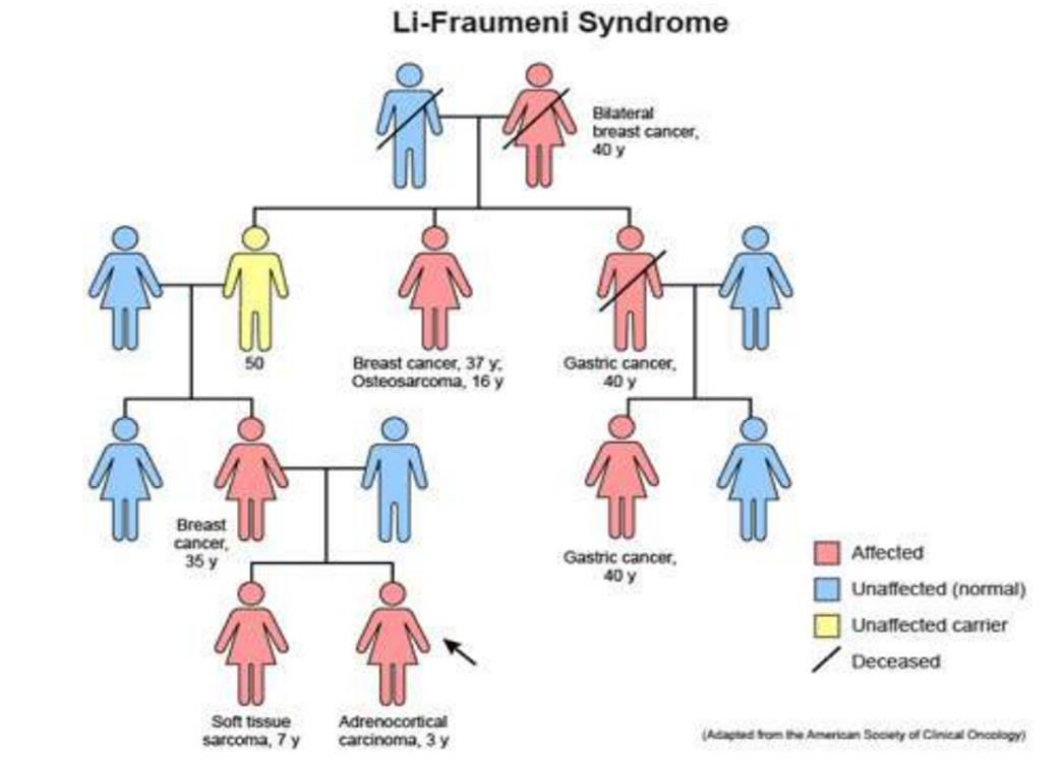
1982 the pattern of cancers is called **Li-Fraumeni syndrome**

In 1990,**TP53** was recognized as the TSG when absent,is responsible for rare inherited disorder called –**Li-Fraumeni Syndrome.**

Victims of this disease are affected with a very high incidence of various cancers including breast and brain and leukemia .

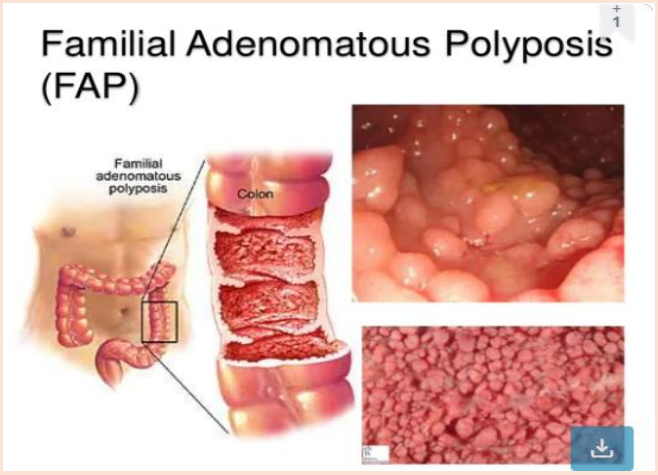
Persons of this Syndrome inherit one normal and one **abnormal allele of TP53 TSGene.**

Li and Fraumeni



Familial Adenomatous Polyposis Coli (FAP) is an inherited disorder, individuals develop hundreds or thousands of premalignant polyps (adenomas) from epithelial cells that line the colon wall.

The cells of **patients contain a deletion of a small portion of chromosome 5.**

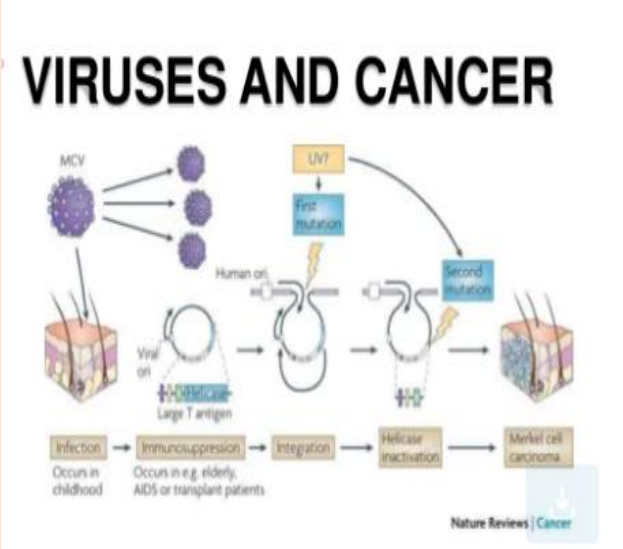


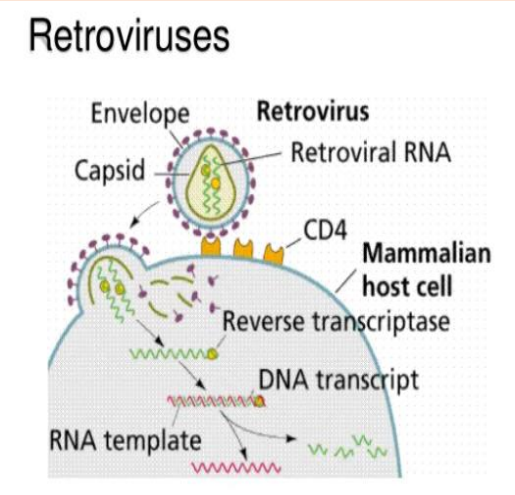
Numerous oncogenes were initially identified as part of the genomes of **RNA tumor viruses.**

The oncogene mutated most frequently in human tumors is **RAS ,encodes a GTP-biding protein (Ras).**

The first connection between oncogenes and growth factors was made in1983.

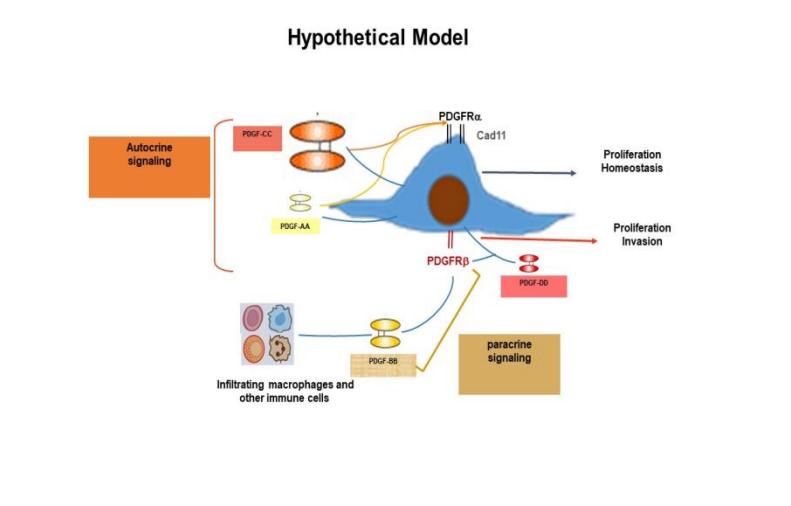
It was discovered in **cancer causing Simian sarcoma virus.**





It contains an **oncogene (sis)** derived from **the cellular gene for platelet-derived growth factor (PDGF),a protein present in human blood.**

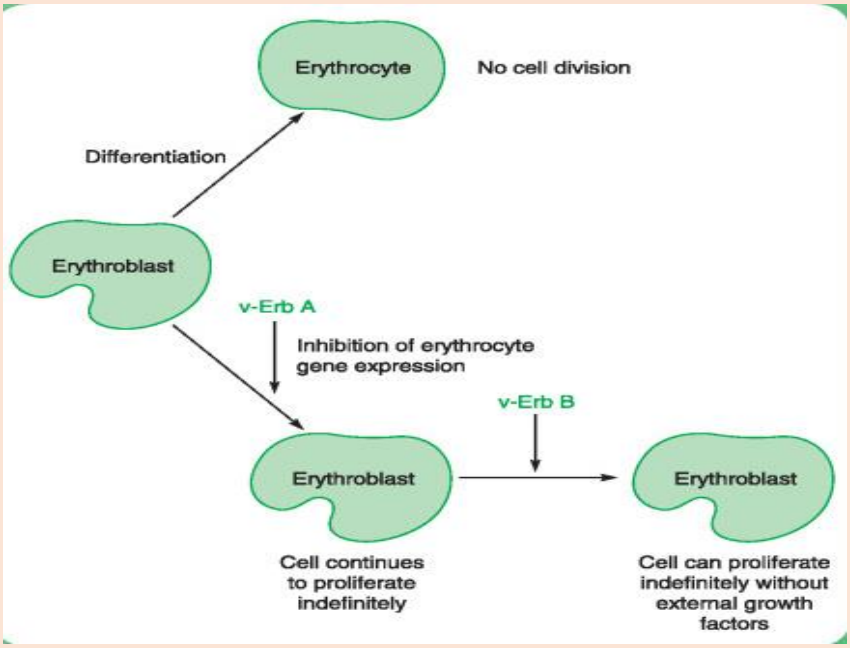
Overexpression PDGF has been implicated in the development of **brain tumors (Gliomas).**



Another **oncogenic virus ,avian erythroblastosis virus**,was found to carry an **oncogene (erb B) that encodes an EGF receptor.**

The malignant cells contain larger number of **receptors in their plasma membrane** than normal cells.

Mutations in EGFR occur commonly in lung cancers from patients who never smoked,but are not found in lung cancers from smokers.



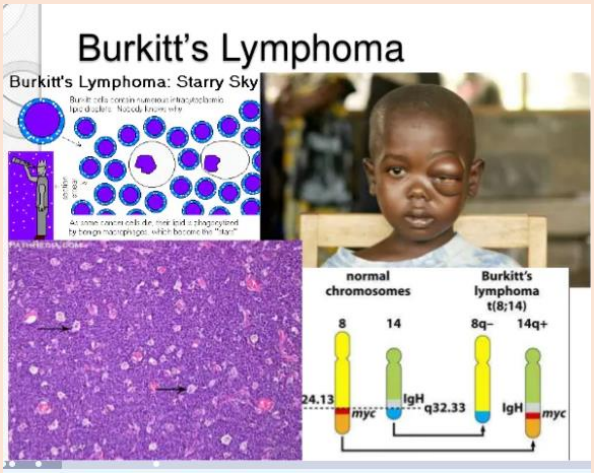
Mutations in **Raf (serine-threonine protein kinase) closely linked to melanoma.**

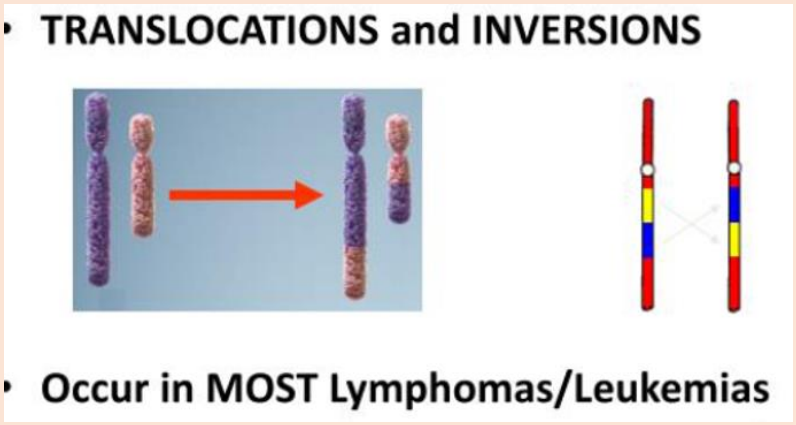
MYC gene is one of the proto-oncogenes commonly altered in human cancers.

Cancer among populations in **Africa, called Burkitt's lymphoma** results from the translocation of a MYC gene.

The oncogene closely **linked to apoptosis is BCL2 gene** when it is translocated causes lymphoid cancers.

In 2002 it was reported that the **locus that encodes two micro RNAs,miR-15a and miR-16,was either deleted** or underexpressed in most cases of chronic lymphocytic leukemia.





SPECIALIZATION OF PLASMA MEMBRANE

The plasma membrane shows here and there some specialized structures. These may be due to outgrowths or ingrowths or contact with adjacent membrane. Such structures include :

l. Microvilli

2.Desmosomes

3. Gap junction (Nexus)

4.Tightjunction(Zonaoccludens)

5.Interdigitations
6. Basal igfoldings

7. Plasmodesmata

MICROVILLI

Microvilli are minute finger-like projections arising from the surface of certain cells. They are found on the epithelial cells of intestine, kidney tubules, gall bladder, uterus, hepatic cells and yolk cells.

Each microvillus is cydindrical in shape. The microvillus is 0.6 to 0.8 micrometer long and has a diameter of 0. I micrometer.

It has a core of cytoplasm enclosed by the plasma membrane. The cytoplasmic core is traversed by fine micro filaments made up of actin.

The micro-filaments are attached to the tip of the microvillus by α actin.

The micro-filaments give rigidity to the microvilli.

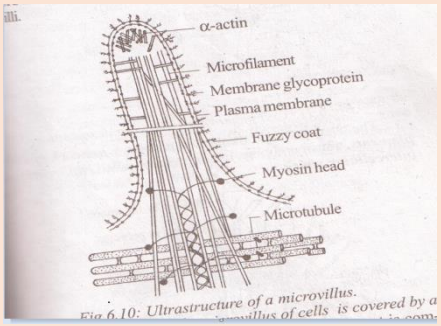


Fig 6.10: Ultrastructure of a microvillus.

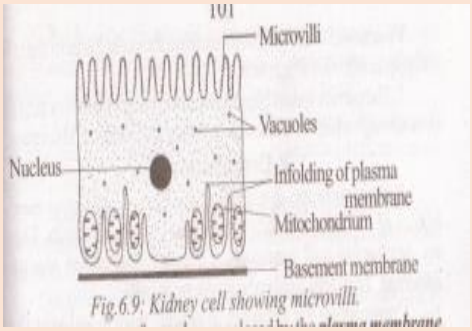


Fig.6.9: Kidney cell showing microvilli.

The outer surface of the microvillus of cells is covered by a coat of fine filaments called **fuzzy coat.**

The fuzzy coat is composed of **glycoproteins.At the base of microvillus,the microfilaments are joined to a transverse network of actin-myosin microtubules, that form the terminal web.**

Functions:

I. The microvilli **increase the surface area** of the cell and help in **effective absorption**.

2. The narrow spaces lying between the microvilli form a kind of sieve through which substances pass during the process of absorption.

2. Desmosomes

Desmosomes are disc-shaped thick areas plasma membranes of adjacent cells, which glue the adjacent cells.

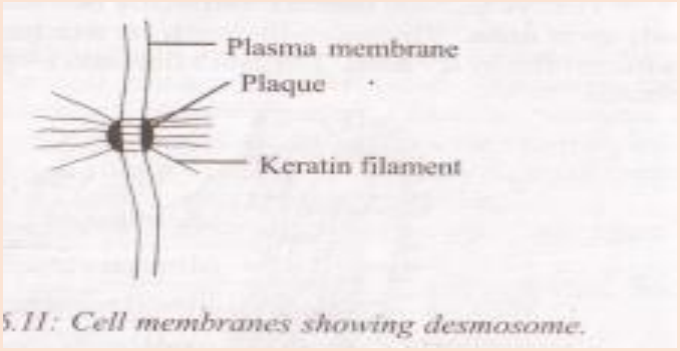
They are anchoring cell junctions. They are also called **macula adherens.**

They are found only in vertebrate cells. Desmosomes are abundant in cardiac muscle and skin that are subjected to severe mechanical stresses.

The desmosome consists of a plaque, a network of keratin filaments, transmembrane, linker proteins called cadherins and an intercellular space.

The plaque is a button-like structure attached the cytoplasmic side of each cell membrane.

It is made up of intracellular attachment proteins called placoglobins.



5.11: Cell membranes showing desmosome.

A network of keratin filaments, a type of intermediate filaments are attached to the plaque.

The free ends of keratin filaments are attached to the cytoskeleton. The **cadherins are transmembrane proteins present in the intercellular space.**

They are Ca++ dependent cell wall adhesion proteins. They link the plaques of adjacent cells. The intercellular space in the desmosome remains normal as in other places.

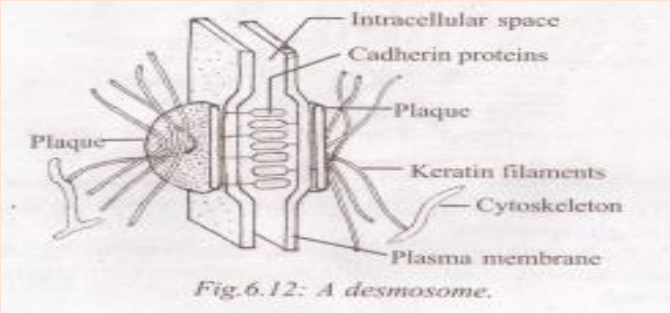


Fig.6.12: A desmosome.

There are four types of desmosomes. They are :

l. Belt desmosomes **2. Spot desmosomes**

3. Hemi-desmosomes **4. Septate desmosomes**

1.BeltDesmosomes

Belt desmosome is found just below the tight junction. It is in the form of a band encircling the borders on the inner side of the cell membrane of epithelial cells.

The Intercellular space is filled with fine filaments. The belt desmosomes help to close gaps and also help in the movement and change in shape of embryonic epithelial cells

2. SpotDesmosomes

Spot desmosomes are like rivets or spot welds between the plasma membrane of adjacent cells. The spot desmosome consists of an intercellular space, a disc-shaped intracellular plaque lying on the cytoplasmic surface of each cell membrane, keratin filaments and transmembrane linkers.

The transmembrane linkers arise from the plaques and traverse the intercellular space joining the plasma membranes. The spot desmosome is meant for mechanical attachment.

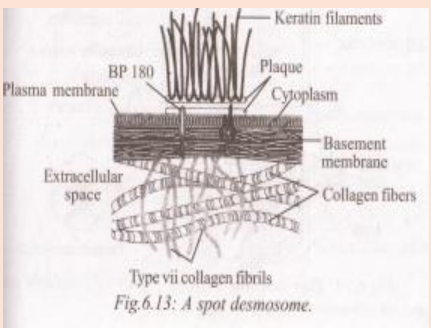


Fig.6.13: A spot desmosome.

3.HemiDesmosomes

Hemi desmosomes are half desmosomes. They have only one plague. They are present at the basal surface of certain epithelial cells. They resemble a typical desmosome, but their outer side remains coated with collagen fibrils.

They help to join the cell membrane of epithelial cells to the underlying basement membrane.

4. Septate Desmosomes

Septate desmosome is a desmosome containing many transverse septa between the plasma membranes in the intercellular space.

It is found in the epithelial cells of invertebrates. In these desmosomes, intercellular cementing substance and keratin filaments are absent. They help in attachment of cells for intercellular communication.

3.Gapjunction(Nexus)

Gap junction is a junctional complex between two cells at the point of contact.

It is a channel or pore through two cell membranes across the intercellular space between two adjacent cells. In the region of the gap junction, the intercellular space is narrow and has a width of only 20Å. The gap junction consists of a hollow containing a pair of hexagonal cylinders called connexons.

Each plasma membrane contains one connexon. The two connexons are arranged end to end to form a channel or pipe between the two cells.Each connexon is made up of six protein sub-units called connéxins. Calcium ions help to regulate the opening and closure of the

gap junctions.When the intracellular calcium ion concentration increases,the channels are closed.

Functions: I. In cardiac muscles and synapses, the gap junctions conduct electrical signals (ions).

2. Gap junctions allow passage of ions, sugars, vitamins, nucleotides and metabolites between cells.

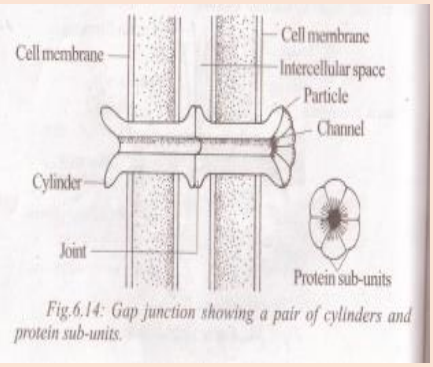


Fig.6.14: Gap junction showing a pair of cylinders and protein sub-units.

4.TightJunction

Tight junction is an occluding junctional complex where the plasma membranes of adjacent cells fuse together so Intimately, that the intercellular space disappears. The tight junctions occur in the epithelial cells of intestine, glands gall bladder and brain. Tight junctions are found in vertebrates. Tight junctions serve to seal the space between the cells and act as barriers for the diffusion of substances through these regions. The tight junction consists of an interlocking network of ridges on the inner surface of plasma membrane.

These ridges are called sealing strands. Each sealing strand is formed of a double row of proteinscalledoccludinsandclaudins.

Function:

1.Tight junctions prevent the passage of materials to and from the cells.

2. They prevent the leakage of pancreatic secretory products into the blood.

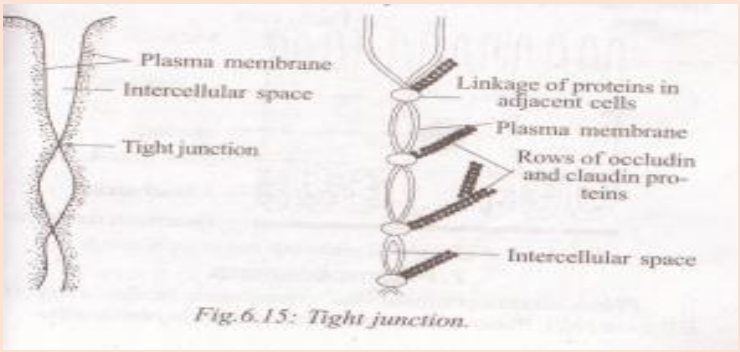


Fig.6.15: Tight junction.

5.Interdigitations

The plasma membranes of adjacent cells project into the cytoplasm as finger-like projections called interdigitations.The inter-digitations help to compartmentalise the cytoplasm.

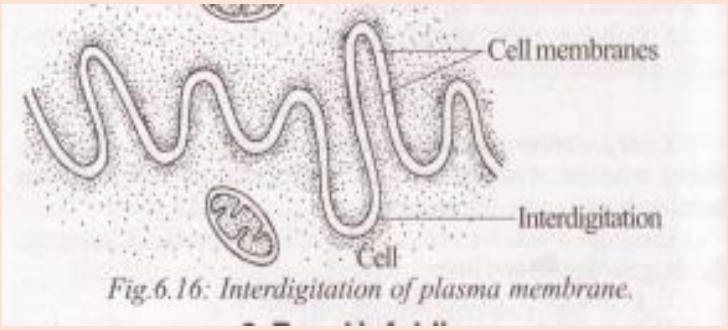


Fig.6.16: Interdigitation of plasma membrane.

6.Basalinfoldings

Basal infoldings are finger-like invaginations of the plasma membrane into the cytoplasm from the base of the cell. They arise from plasma membrane that faces the basement membrane.They are found in kidney cells. The basal infoldings function as septa and they split the cytoplasm into compartments. The basal infoldings enclose many mitochondria. They are concerned with the active transport of materials.

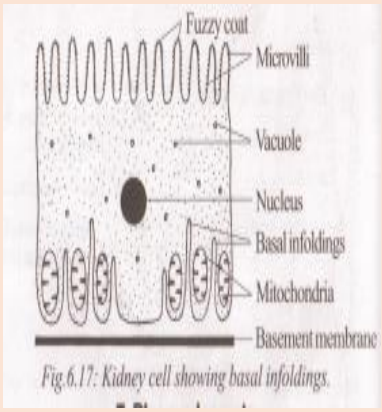


Fig.6.17: Kidney cell showing basal infoldings.

7.Plasmodesmata

Plasmodesma (singular) is a cytoplasmic bridge connecting adjacent cells. Plasmodesmata are found only in plant cells. The plasmodesmata form fine channels of 20 to 40 μm diameter between adjacent cells.

The centre of the plasmodesma has a narrow cylindrical structure called desmotubule.

The desmotubule is continuous with the endoplasmic reticulumof the adjoining cells. The cell wall contains many small openings called pits.

The adjacent cells are connected by cytoplasmic bridges through these pits. The cytoplasm and the endoplasmic reticulum of the adjacent cells make contact through the plasmodesmata. Plasmadesmata do the following functions:
Free movement of small metabolites and growth hormones between cells.

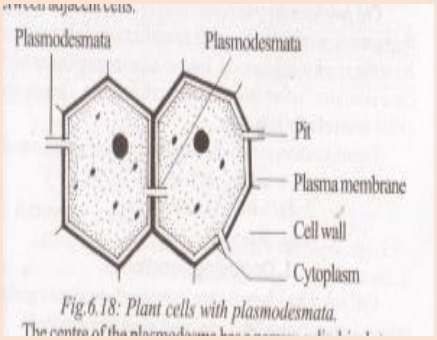


Fig.6.18: Plant cells with plasmodesmata.
