Molecular Basis of Cancer

The development of cancer is a multi-step process that comprises gene mutations caused by environmental factors, biological agents or hereditary predispositions. These mutations might cause cells to bypass cell cycle checkpoints. Normally, two groups of genes are involved in regulating cell division: tumour suppressor genes and proto-oncogenes. Mutations in either or both of these groups of genes may lead to the development of cancer. Cancer has a much higher incidence in Singapore compared to other diseases and accounts for as much as 30 per cent of the deaths in this country. The recorded incidence of cancer is on the rise and this could be due to the accumulation of mutations from one generation to the next, although other reasons have also been proposed: increased exposure to carcinogens and increased detection rates as a result of effective cancer screening programmes. As such, an understanding of how cancer develops is important as this would set the platform for discussion of developing anti-cancer drugs.

Learning Outcomes

Candidates should be able to:

2(p) identify the causative factors, including genetic, chemical carcinogens, ionising radiation and loss of immunity, which may increase the chances of cancerous growth

2(q) explain how the loss of function mutation of tumour suppressor genes, including p53, and gain in function mutation of proto-oncogenes, including *ras*, results in uncontrolled cell division

2(r) describe the development of cancer as a multi-step process that includes accumulation of mutations, angiogenesis and metastasis

Use the knowledge gained in this section in new situations or to solve related problems

Learning Experiences

2.5.1 Uncontrolled Cell Division

This SLS lesson explains how dysregulation of the cell cycle can result in uncontrolled cell division. In addition, it explains how mutation of two groups of genes (tumour suppressor gene and proto-oncogene) can result in uncontrolled cell division.

2.5.2 Cancer

This SLS lesson describes the factors that increase the chance of cancer development, and explains that cancer development is a multi-step process.

2.5.3 Useful information about Cancer

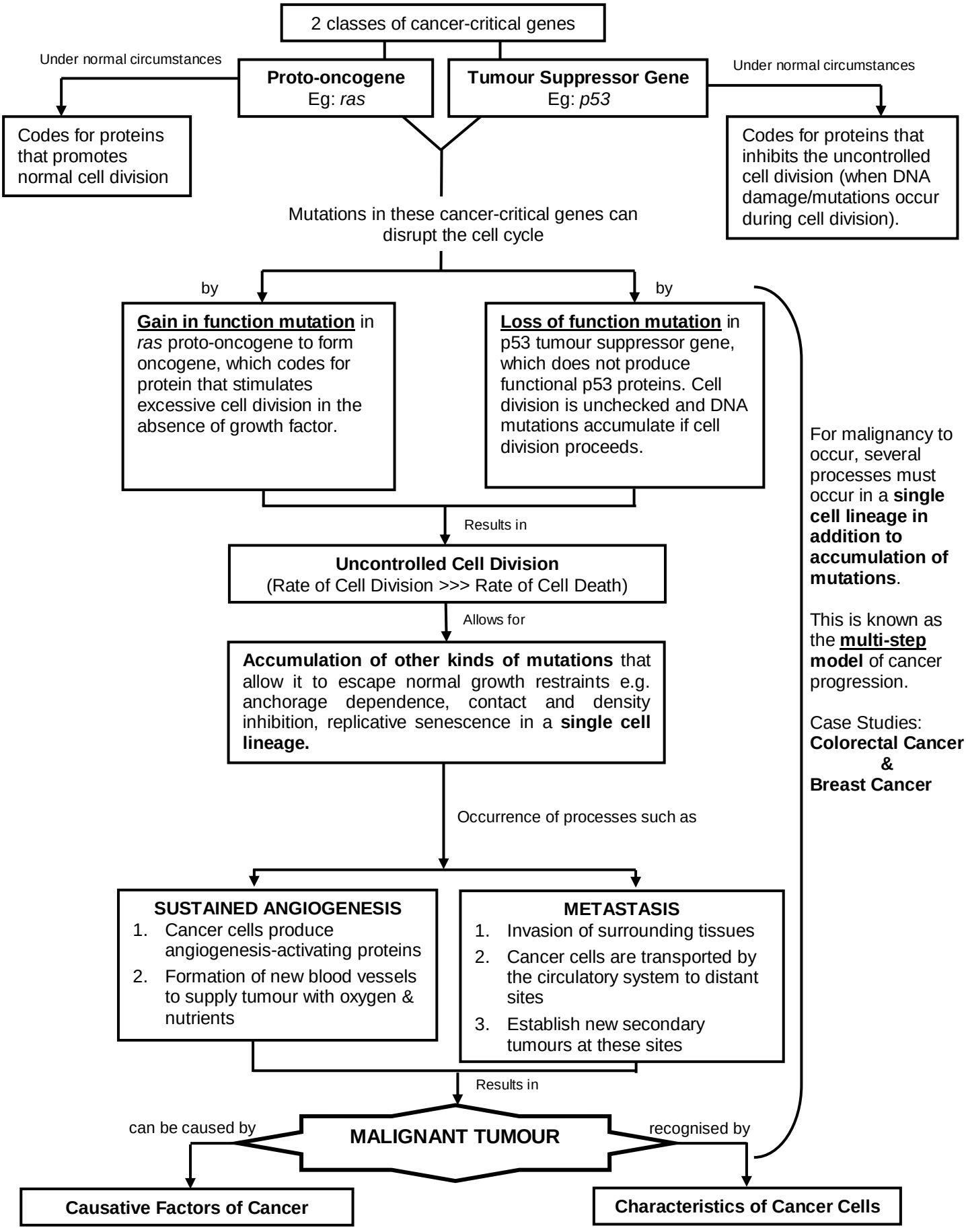
About 30 percent of deaths in Singapore are related to cancer (the principal cause of death in 2011). Through studies on families with high incidence of cancer, certain cancer genes such as BRCA have been elucidated. The following websites provide useful information about cancer:

The following websites provide useful information about cancer:

i. General cancer information – Singapore cancer statistics

ii. Principal causes of death

CONCEPT MAP


2 classes of cancer-critical genes
Under normal circumstances
Proto-oncogene
Eg: ras
Tumour Suppressor Gene
Eg: p53
Under normal circumstances
Codes for proteins that promotes normal cell division
Codes for proteins that inhibits the uncontrolled cell division (when DNA damage/mutations occur during cell division).
Mutations in these cancer-critical genes can disrupt the cell cycle
by
by
Gain in function mutation in ras proto-oncogene to form oncogene, which codes for protein that stimulates excessive cell division in the absence of growth factor.
Loss of function mutation in p53 tumour suppressor gene, which does not produce functional p53 proteins. Cell division is unchecked and DNA mutations accumulate if cell division proceeds.
Results in
Uncontrolled Cell Division
(Rate of Cell Division >>> Rate of Cell Death)
Allows for
Accumulation of other kinds of mutations that allow it to escape normal growth restraints e.g. anchorage dependence, contact and density inhibition, replicative senescence in a single cell lineage.
Occurrence of processes such as
SUSTAINED ANGIOGENESIS
1. Cancer cells produce angiogenesis-activating proteins
2. Formation of new blood vessels to supply tumour with oxygen & nutrients
METASTASIS
1. Invasion of surrounding tissues
2. Cancer cells are transported by the circulatory system to distant sites
3. Establish new secondary tumours at these sites
Results in
can be caused by
MALIGNANT TUMOUR
recognised by
Causative Factors of Cancer
Characteristics of Cancer Cells
For malignancy to occur, several processes must occur in a single cell lineage in addition to accumulation of mutations.
This is known as the multi-step model of cancer progression.
Case Studies:
Colorectal Cancer
&
Breast Cancer

CONTENT PAGE

1. INTRODUCTION TO CANCER

During normal development of healthy cells, there is intricate balance between the rate of cell division and rate of cell death in response to various growth signals, growth-inhibiting signals and death signals.

Cancer is a disease that results from **uncontrolled cell division**. Mutations in the genes that control cell division may result in a **dysregulation of cellular checkpoints**, such that a cell divides uncontrollably. Two important classes of genes, proto-oncogenes and tumour suppressor genes are involved in controlling cell division. In cancer cells, the **rate of cell division far exceeds the rate of cell death**, resulting in a net uncontrolled proliferation of new cells.

As this cell divides, it will pass on the damaged genes to the daughter cells, giving rise to a **clone of altered cells**. This uncontrolled proliferation of the cell allows the cell to possess an unstable genome and **accumulate more mutations** that allow it to escape normal growth restraints e.g. anchorage dependence, contact and density inhibition, replicative senescence.

These mutations occur at various stages and cancer is considered to develop via a **multi-step model** with accumulation of important mutations at each step. These mutations would eventually allow cancerous cells to invade tissues and **metastasise** (spread destructively) to **distant sites**, and causing death of the host organism. The death of the host organism occurs as normal healthy cells are replaced by cancerous cells at important organs.

1.1 CANCER TERMINOLOGY

Acute: Refers to symptoms that start and worsen quickly but do not last over a long time.

Biopsy: The removal of a small amount of tissue for examination under a microscope. Other tests can suggest that cancer is present, but only a biopsy can make a definite diagnosis. Learn more about biopsy.

Carcinoma: Cancer that starts in skin or tissues that line the inside or cover the outside of internal organs.

Chronic: Refers to a disease or condition that persists, often slowly, over a long time.

Leukemia: A cancer of the blood. Leukemia begins when normal white blood cells change and grow uncontrollably.

Lymphoma: A cancer of the lymphatic system. Lymphoma begins when cells in the lymph system change and grow uncontrollably. Sometimes a tumor is formed.

Polyp: A growth of normal tissue that usually sticks out from the lining of an organ, such as the colon.

Prognosis: Chance of recovery; a prediction of the outcome of a disease. Learn more about survival statistics used to estimate a patient's prognosis.

Sarcoma: A cancer that develops in the tissues that support and connect the body, such as fat and muscle.

Stage: A way of describing cancer, such as where it is located, whether or where it has spread, and whether it is affecting the functions of other organs in the body.

1.2 IMPORTANT CHARACTERISTICS OF CANCER CELLS

1. High rate of cell division

- Cell divides or proliferates in the absence of **growth factors**.

2. Genome Instability & Mutation

- Increased rate of **accumulation of mutations** at chromosomal and gene level.

3. Replicative Immortality

- In most human **somatic cells** that divide only a limited number of times, telomerase genes are switched off, i.e. not expressed.
- The **telomerase genes are reactivated** in most human **cancer / tumour cells**, where telomerase is produced and **maintains telomere lengths** so that the cancer cells divide indefinitely or are immortalised.
- Telomerase appears to allow cancer cells to **evade apoptosis**, and it is thought that the abnormal retention of telomeres is involved in the development of some types of cancer.
- That telomerase is likely to be an important factor in cancer implies its promising potential as a useful target for cancer diagnosis and treatment.
- This has stimulated the search for inhibitors of human telomerase as potential agents for treating cancer.

4. Loss of anchorage dependence

- Normal cells must make contact with a suitable substratum before they can attach, spread, and proliferate. This is known as **anchorage-dependent growth**.
- Cancer cells are able to proliferate in suspension cultures or in a semisolid medium without attachment to a surface. This is known as **anchorage-independent growth**.

5. Lack of contact inhibition and density-dependent inhibition (**Fig. 1**)

- Normal cells display **contact inhibition** while cancer cells do not.
- Normal cells proliferate in a culture dish until they make contact with neighboring cells.
- Cancerous cells, in contrast, continue dividing after contact with their neighbors, growing over adjacent cells in disordered, multilayered patterns.

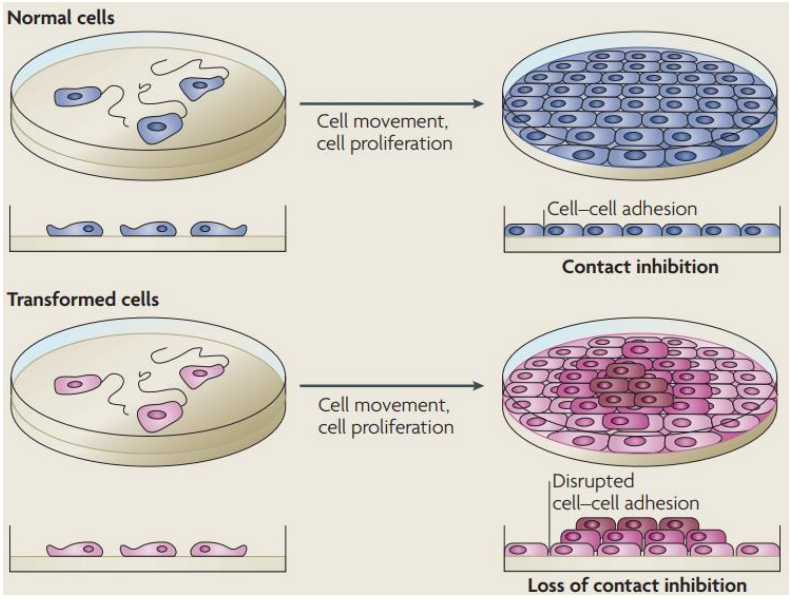


Fig.1: Density-dependent inhibition of cell division.

When grown in a cell culture, normal cells will divide only until they form a single layer. The availability of nutrients, growth factors and substratum for attachments limits the density of the cell population. If some cells are removed, those at the border will divide until the gap is filled with a single layer.

In contrast, cancer cells usually continue to grow after they are crowded, often piling up.

6. Inducing Angiogenesis

- **<u>Angiogenesis</u>** is the process by which **new blood vessels are formed.** It is a tightly regulated process which occurs only when necessary, such as during growth or repair. In the event of abnormal regulation, angiogenesis may result in diseases such as cancer.
- To develop into larger, potentially metastatic tumours, a growing tumour **stimulates the formation of new blood vessels**, a process termed **angiogenesis** or **vascularization** (**Fig. 2**).
- These blood vessels allow for an increased blood flow to the tumour, hence supplying **nutrients** and **oxygen** and removing **toxic waste products**. They also provide the pathways for cancer cells to spread to other sites in the body.
- In order to stimulate the formation of new blood vessels, a tumour cell expresses **angiogenesis-activating protein genes** to produce **angiogenesis activating proteins.**

7. Metastasis

- **<u>Metastasis</u>** is a process where the primary tumour cells invade local tissues and blood vessels, and **establish secondary tumours** called **metastases** at distant sites.

- The events are as follows (**Fig. 2**):
  1. Cancer cells **invade surrounding tissues** and penetrate through the walls of **lymphatic** and **blood** vessels, thereby gaining access to the bloodstream.
  2. The cancer cells are **transported by the circulatory system** throughout the body.
  3. Cancer cells leave the bloodstream and enter particular organs, where they **establish new secondary tumours at distant sites from the primary tumour**.

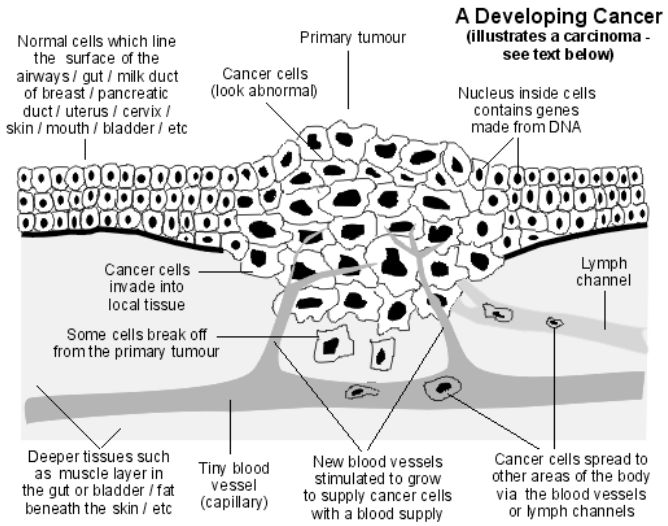


Fig. 2: This shows the spread of tumour cells to locations distant from the primary tumour site through the blood circulatory system.

8. Avoiding Immune Destruction

- The immune system has a surveillance role in monitoring for and eliminating the vast majority of incipient tumour cells.
- While majority of tumour cells are detected and eliminated, a small percentage however can **evade immune destruction** by disabling components of the immune system that have been dispatched to eliminate them.

1.3 TUMOURS CAN BE BENIGN OR MALIGNANT

When cells divide uncontrollably, it will give rise to a **tumour** or neoplasm. It may be benign or malignant.

- **Benign tumours** have few genetic mutations and do not cause serious health problems. They could be completely removed by surgery.

- **Malignant tumours** are invasive and could impair functions of organs and cancerous. Individuals with malignant tumours are considered to have cancer. Systemic treatment like radiation or chemotherapy is required in conjunction with surgery to ensure complete eradication of all malignant tumour cells.

Benign Tumour	Malignant Tumour

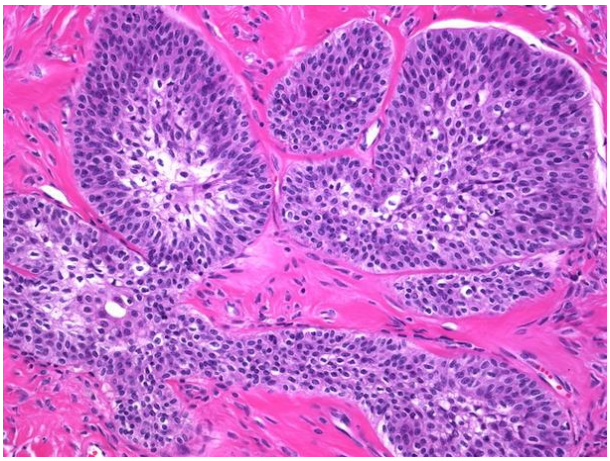
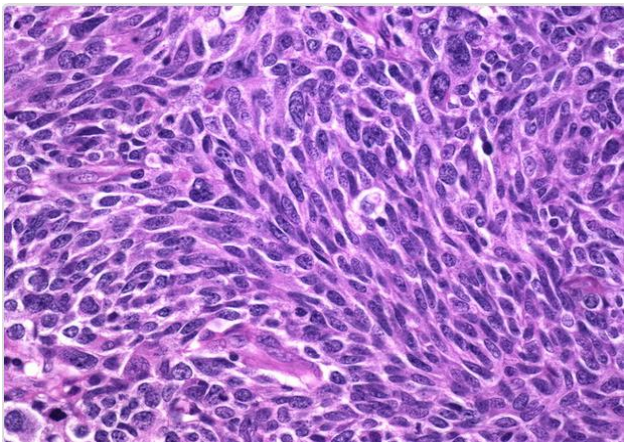

	

Fig. 3(a): Benign tumour with urothelial cells scattered in dense hyalinised stroma	**Fig. 3(b):** Malignant tumour with appearance of urothelial carcinomas of the urinary tract

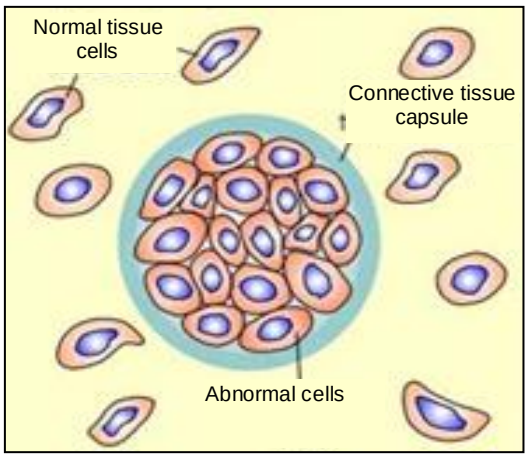
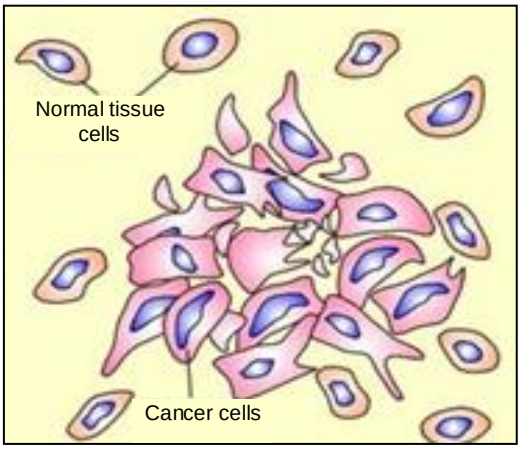

	

Fig. 4(a): Well-defined tumour boundary	**Fig. 4(b):** Poorly-defined tumour boundary

Benign Tumour	Malignant Tumour
Benign tumour cells have **small** and **regularly-shaped nucleus.** Cells are **well-differentiated**, resembles parental cells. They will still have most of the structural features of the cells from which they originated.	Malignant tumour cells have **large** and **irregularly-shaped nucleus.** Cells are **poorly differentiated** and does not resemble parental cells. Malignant cells may display varying stages of differentiation as de-differentiation may have occurred.
Cells have **low nuclear : cytoplasmic volume ratio.**	Cells have **high nuclear : cytoplasmic volume ratio.**
Low rate of mitosis as benign tumour cells divide less frequently than malignant tumour cells.	**High** rate of mitosis as malignant tumour cells rapidly divide.
Benign tumour have a **well-defined** tumour boundary. Tumour cells remain **clustered together** in a single mass in a localized region since they **do not have the ability to metastasise**. They grow slowly, with a **well-defined perimeter** and may be surrounded by a layer of connective tissue.	Malignant tumour have a **poorly-defined** tumour boundary. Tumour cells may or **may not cluster together**, as they have acquired the ability to **invade surrounding tissues, i.e. have an ability to metastasise**. They may form secondary tumours, called **metastases**, at distant sites in the body.

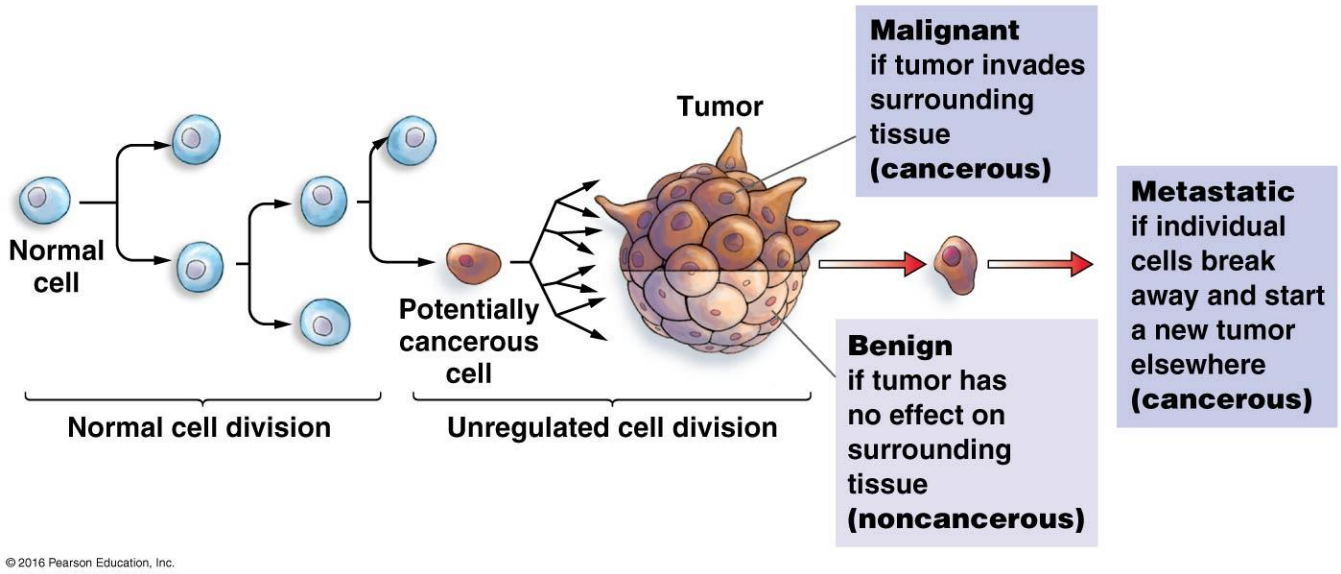


Fig. 5: Malignant tumour cells are invasive while benign tumours are not.

2. UNCONTROLLED CELL DIVISION COULD RESULT IN CANCER

2.1 CELL CYCLE

A cell may be stimulated to **divide**, **differentiate** or **die** by signals released from neighbouring cells. Clearly, in order for tissues and organs of a multi-cellular organism to grow to an appropriate size and develop in a coordinated manner in the body, **precise control of the cell cycle** of cells within different tissues and organs is required. This requires the **cell cycle checkpoints** to be in place**.**

2.2 CELL CYCLE CHECKPOINTS

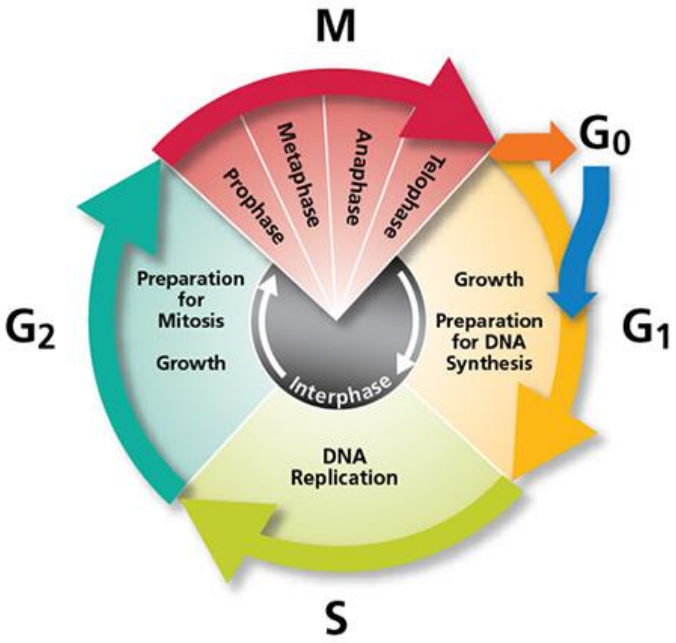
Cell cycle checkpoints are critical control points where stop and go-ahead signals can regulate the cycle. The checkpoints hence help ensure the orderly progression of the cell cycle. If some malfunction prevents the successful completion of the processes in each phase of the cell cycle, signals are sent to the control system to delay progression into the next phase.

Such delays provide time for the cell cycle machinery to be repaired and also prevent the disaster that might result if the cycle progressed prematurely to the next stage.

There are **three critical checkpoints** in the cell cycle that act as "stop/go ahead" switches to trigger subsequent phases in the cell cycle or to delay them **(Fig. 6)**.

M checkpoint – assessment of mitosis
- Occurs at metaphase.
- Checks if there is **successful formation of spindle fibres and attachment of spindle fibres to the kinetochores of chromosomes**.
- Ensure that there is successful separation of DNA to the two daughter cells.
- If spindle fibres are not formed or attachment to centromere is inadequate, mitosis is arrested.

G_2 checkpoint – assessment of DNA replication
- Occurs at G_2
- Checks if **DNA is successfully replicated without damage**.
- If there is irreparable damage, apoptosis occurs.
- Once checkpoint is passed, proteins will signal the cell to begin the molecular processes that will allow the cell to divide via mitosis.



G_1 checkpoint – assessment of cell growth
- Occurs at G_1
- Checks for the presence of proteins called **growth factors** that are required to stimulate cell division.
- Checks for **DNA damage** and **cell size**.
- Checks that **nutrients** are sufficient.
- Apoptosis will occur if DNA is damaged and cannot be repaired.
- Otherwise cell is committed to divide.
- Mature cells pass into the G_0 phase (resting state), these cells may never divide again.

Fig. 6: Checkpoints are dysregulated in tumours and mutations occur in the genes involved in cell cycle components, leading to genomic instability and cancer.

2.3 ROLE OF CYCLINS & CYCLIN-DEPENDENT PROTEIN KINASE IN CELL CYCLE

Cyclin-dependent kinases (**Cdks**) are a family of multifunctional enzymes that can modify various protein substrates involved in **cell cycle progression**. Specifically, Cdks **phosphorylate** their substrates by **transferring phosphate groups** from ATP to the substrates. As their name suggests, Cdks require the presence of **cyclins** to become active (**Fig. 7a**).

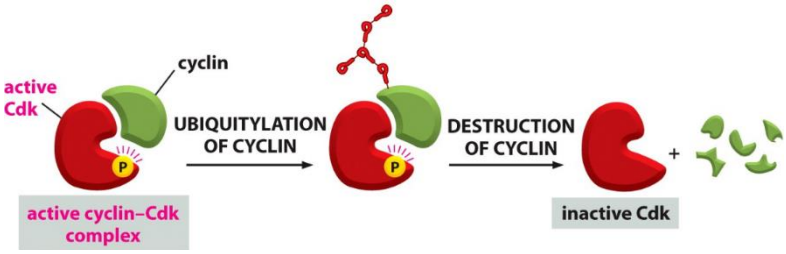


Fig. 7a: Inactivation of Cdk enzymes.

Cyclins are a family of proteins that have no enzymatic activity of their own but they **activate Cdks by binding** to them. Thus forming **cyclin-Cdk complexes** also called **maturation-promoting factor** (**MPF**).

Cyclins are named for their **cyclically fluctuating** cellular concentration according to the stages of the cell cycle (**Fig. 7b**). All eukaryotes have **multiple cyclins**, each of which acts during a specific stage of the cell cycle. In organisms with multiple Cdks, each Cdk is paired with a specific cyclin.

In general, **MPFs promote mitosis** by phosphorylating substrates that control the orderly progression of the cell cycle (**Fig. 8**).

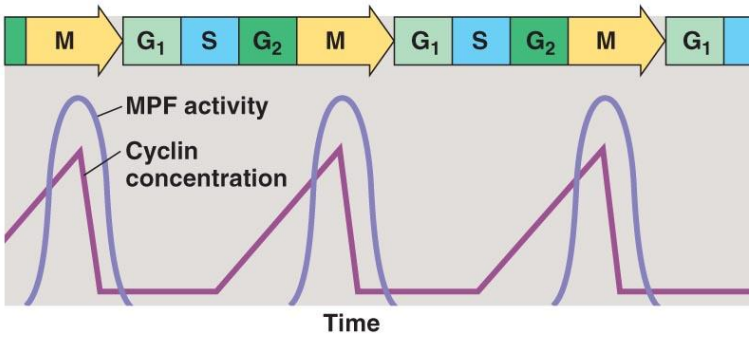
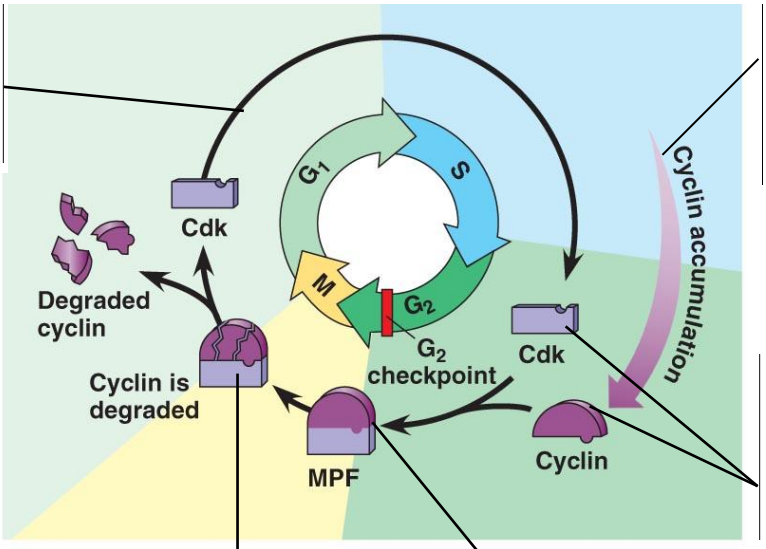


Fig. 7b: Fluctuations of cyclin concentration and MPF activity during the cell cycle.

(5) During G_1, conditions in the cell favour degradation of cyclic. Cdk component of MPF is recycled.



(1) Synthesis of cyclin begins in late S phase and continues through G_2. Because cyclin is protected from degradation during this stage, it accumulates.

(2) Accumulated cyclin combine with recycled Cdks, producing enough MPFs to pass the G_2 checkpoint and initiate the events of mitosis.

(4) During anaphase, the cyclin component of MPF is degraded, terminating the M phase. The cell enters the G_1 phase.

(3) MPFs promote mitosis by phosphorylating protein substrates. MPF's activity peaks during metaphase.

Fig. 8: Molecular control of the cell cycle at the G_2 checkpoint.

Although the exact signaling pathways that link Cdks to other molecules and event inside and outside of the cell remain unclear, it is clear that any disruption to the cell cycle operates directly or indirectly via the disruption to the function and levels of cyclins and/or cdks.

For example, in many cancers, when cell cycle control genes like **cyclin** and/or **Cdk genes are mutated**, this results in the **over-expression of cyclins and Cdks**.

Cancer cells have **escaped precise cell cycle control** such that they **proliferate indefinitely** and grow in inappropriate locations to hinder essential life processes in the organism.

IMPORTANT!

Cancer is based on the loss of cell cycle control. **Uncontrolled cell division** does not always mean that tumour cells divide more rapidly than normal cells. The crucial issue is the **relationship** between the rate of cell division and the rate of cell loss.

In **normal tissues**, **the rate of cell division and rate of cell loss are kept in balance so no net accumulation of new cells** occurs (Fig. 9).

In **cancer cells**, since they have escaped precise cell cycle control, the **rate of cell division <u>far exceeds</u> the rate of cell loss**. This results in net uncontrolled proliferation of new cells.

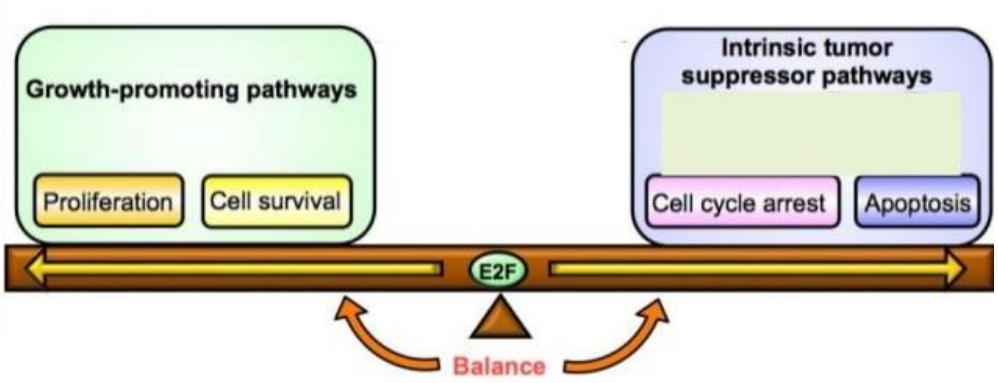


Fig. 9: A balance between rate of cell division and rate of cell loss.

2.4 MUTATIONS IN GENES CONTROLLING CELL CYCLE

Two classes of **cancer-critical genes**, **proto-oncogenes** and **tumour suppressor genes,** exert their effects by acting on the **cell cycle control machinery**. Mutations in these genes result in a net uncontrolled proliferation of cells where the rate of cell division far exceeds the rate of cell death.

1. **TUMOUR SUPPRESSOR GENE**

   Normal gene encodes a protein which **inhibits uncontrolled cell division**.

2. **PROTO-ONCOGENE**

   Normal gene encodes a protein which **stimulates normal cell division**.

2.4.1 TUMOUR SUPPRESSOR GENE

Tumour suppressor genes are a family of normal genes that code for proteins to **prevent uncontrolled cell division**. The loss of such proteins allows a cell to divide **through the absence of suppression in cell division**. Tumour suppressor genes are like the brake pedal of an automobile. The loss of a tumour suppressor gene is like having a defective brake pedal, thereby allowing the cell to divide continually.

The function of tumour-suppressor proteins can be categorised into the following:

1. **Take part in cell signaling pathways to inhibit the cell cycle**

2. **Halt cell division if DNA is damaged**

3. **Trigger DNA repair mechanisms, preventing cells from accumulating DNA damage**

4. **Initiate apoptosis**

   If the DNA damage **cannot** be repaired, the cell would initiate apoptosis (programmed cell death) to remove the threat it poses for the greater good of the organism.

5. **Maintain cell adhesion**

   Proteins known as metastasis suppressors help to maintain cell-to-cell adhesion or cell anchorage to the extracellular matrix, which is absent in most cancer cells.

2.4.1.1 LOSS OF FUNCTION MUTATION OF TUMOUR SUPPRESSOR GENE

A loss of function mutation is defined as one that results in **abolished protein function**. In a diploid organism, there are two copies of every gene. If the function of only one copy of a tumour suppressor gene is lost, cell cycle activity remains normal, as the **other copy of the gene** is still able to produce **sufficient quantity** of the **normal gene product** to regulate normal cell cycle.

Hence, in order to abolish the function of the tumour suppressor genes totally, **both copies** of the tumour suppressor gene must be **mutated so that no functional gene product** can be produced.

This is a **loss of function mutation** and thus described as mutated tumour suppressor genes **act in a recessive manner (Fig. 10a).**

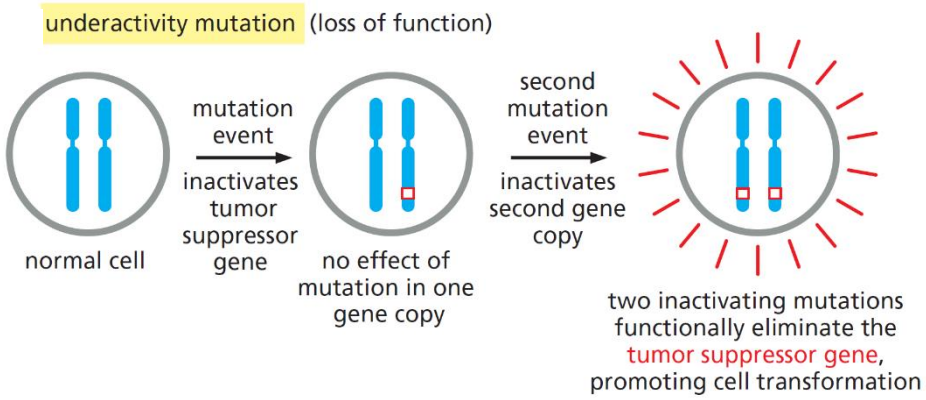


Fig. 10a: When a single copy of the tumour suppressor gene is mutated, the cell cycle is regulated normally since the second copy of the tumour suppressor gene is still functional and can code for the sufficient quantity of functional proteins to inhibit cell proliferation. However, when both copies of the tumour suppressor gene is mutated, no functional protein is produced and cell proliferation occurs.

2.4.1.2 *p53*, A TUMOUR SUPRESSOR GENE

The ***p53*** gene is a **tumour suppressor gene** that is found to be mutated in about half of all human cancers. It has been described to be one of the most important gene in human cancer.

It is so critical because of its triple involvement in cell cycle control, in apoptosis, and in maintenance of genetic stability – all aspects in protecting the organism against cellular damage and disorder (**Fig 10b**).

Thus, it is commonly known as the '**Guardian of the Genome**'.

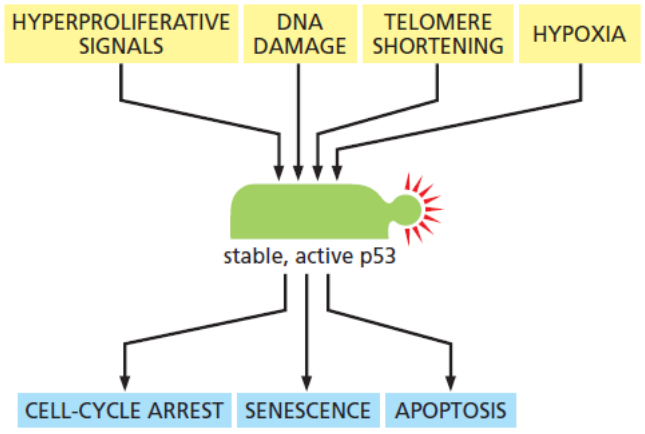


Fig. 10b: Modes of action of the p53 tumor suppressor. The p53 protein is a cellular stress sensor. In response to hyperproliferative signals, DNA damage, hypoxia, telomere shortening, and various other stresses, the p53 levels in the cell rise. As indicated, this may either arrest cell cycling in a way that allows the cell to adjust and survive, trigger cell suicide by apoptosis, or cause cell "senescence"—an irreversible cell-cycle arrest that stops damaged cells from dividing.

2.4.1.3 p53 PROTEIN IS INVOLVED IN THE CELL CYCLE – INHIBITING PATHWAY

p53 protein is a **transcription factor** that binds to DNA to trigger transcription of genes involved in **cell cycle inhibition** (**Fig. 10**). High levels of p53 proteins are only seen in cells when its **DNA is damaged**.

In its anti-cancer role, p53 protein works through several mechanisms:

- It can **activate DNA repair proteins** when DNA has sustained damage. Thus, it may be an important factor in aging.

- It can **arrest growth** by holding the cell cycle at the G1/S regulation point on DNA damage recognition. This ensures that the damaged DNA is not replicated and gives time for the cell to repair the DNA damage.
  - **Activated p53 protein** binds to specific DNA control elements and **promotes transcription** for the relevant genes e.g. ***p21*** gene.
  - **p21 protein stops the cell cycle** by binding to proteins that are involved in cell cycle progression such as Cdks.

- It can **initiate apoptosis** i.e. programmed cell death if DNA damage proves to be irreparable.
  - p53 protein activates "suicide" genes, to ensure that the cell with damaged DNA does not continue to proliferate.

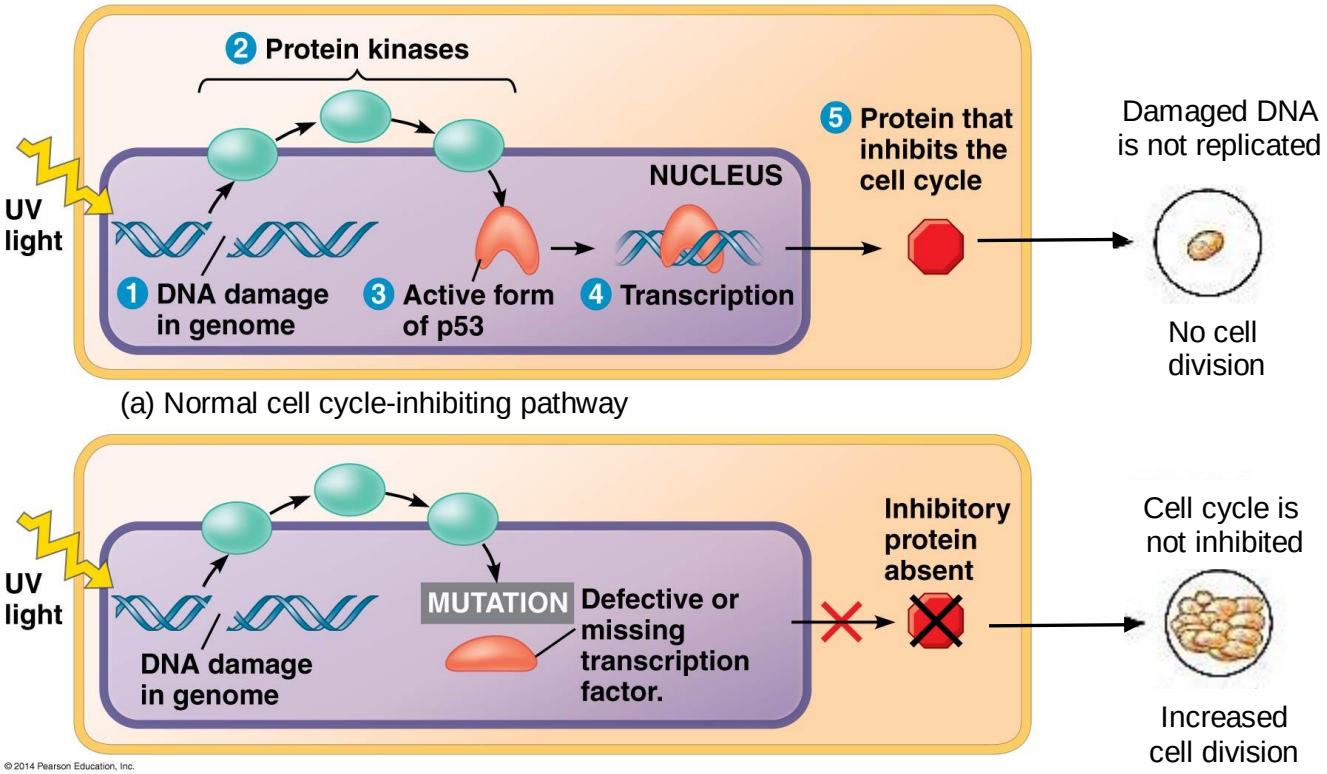


Fig. 11: Effect of normal p53 proteins and mutated form of p53 proteins on cell cycle.

2.4.2 PROTO-ONCOGENE AND ONCOGENE

Proto-oncogenes usually encode gene products that **promote normal cell division**. The function of proto-oncogene products can be categorised into the following:

1. **Growth factors** (GFs)
   External signals that stimulate cells to divide.

2. **Growth factor receptors**
   Membrane proteins that bind to growth factors.

3. **Protein kinases** *(KIV: Cell Signaling)*
   Enzymes that modify other proteins by chemically adding phosphate groups to them. The addition of a phosphate group usually results in the activation of a protein.

4. **Inhibitors of apoptosis**
   Proteins that inhibit the process of apoptosis and hence result in a reduced rate of cell death.

5. **Transcription factors**
   Proteins that bind to DNA to control the rate of transcription e.g. *myc* gene that regulates transcription of genes that induce cell proliferation.

Proto-oncogenes can **undergo gain of function mutations** and be converted to **oncogenes**.

Hence, an **oncogene** is a gene that **encodes a protein that promotes excessive cell division.** This will cause **cell division to be uncontrolled**.

2.4.2.1 GAIN IN FUNCTION MUTATION OF PROTO-ONCOGENE

In a diploid organism, there are two copies of every gene. A mutation of **one** of the two copies of a proto-oncogene into an oncogene is sufficient to cause abnormal cell proliferation.

In other words, the **oncogene acts** in a **dominant** manner. Such a mutation is a **gain in function mutation**. This type of mutation causes the genes to gain function, such as being **over-expressed**, or to encode for a **hyperactive protein**. (**Fig. 12**)

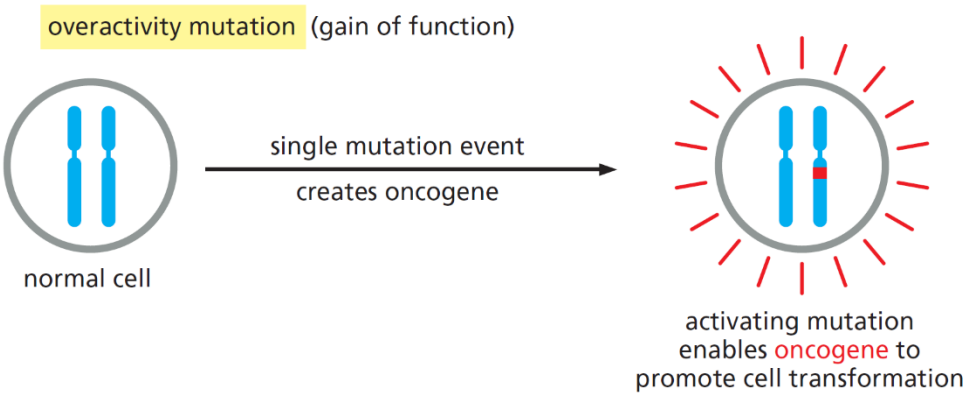


Fig. 12: Gain in function mutation. A mutation in a single copy of the proto-oncogene creates a oncogene which codes for a hyperactive protein or increased levels of a normal protein involved in cell cycle progression. Hence, allowing for cell proliferation.

2.4.2.2 *ras*, A PROTO-ONCOGENE

The *ras* gene encodes a small protein known as the **ras protein** which belongs to a super-family of proteins known as "**low-molecular weight G-proteins**".

G-proteins are a class of proteins that bind to guanine nucleotides (GTP and GDP). G-proteins are involved in **signal transduction**, which involves transmitting chemical signals from outside the cell to cause changes inside the cell. *(KIV: Cell Signalling)*

2.4.2.3 ras PROTEIN IS INVOLVED IN THE CELL CYCLE-STIMULATING PATHWAY

The ras protein, when activated, relays signals from a growth factor receptor to a series of protein kinases known as the phosphorylation cascade. The last protein kinase of the signal transduction pathway **activates transcription** of genes encoding proteins that **stimulate cell division**. The pathway is normally activated only when a growth factor binds to its receptor in the plasma membrane. Hence, activation of ras signalling causes **cell division** (**Fig. 13**).

Gain in function mutations in the ***ras* gene** lead to changes in the three-dimensional conformation of the ras protein. This causes GTP to remain bonded to the ras protein as a **ras-GTP complex** and thus it is in a constant "**active**" state, **even in the absence of growth factor.** This results in an **increase in cell signaling, transcription and consequently stimulates the cell cycle** (**Fig. 13**). Hence, a **hyperactive ras protein** leads to excessive cell division.

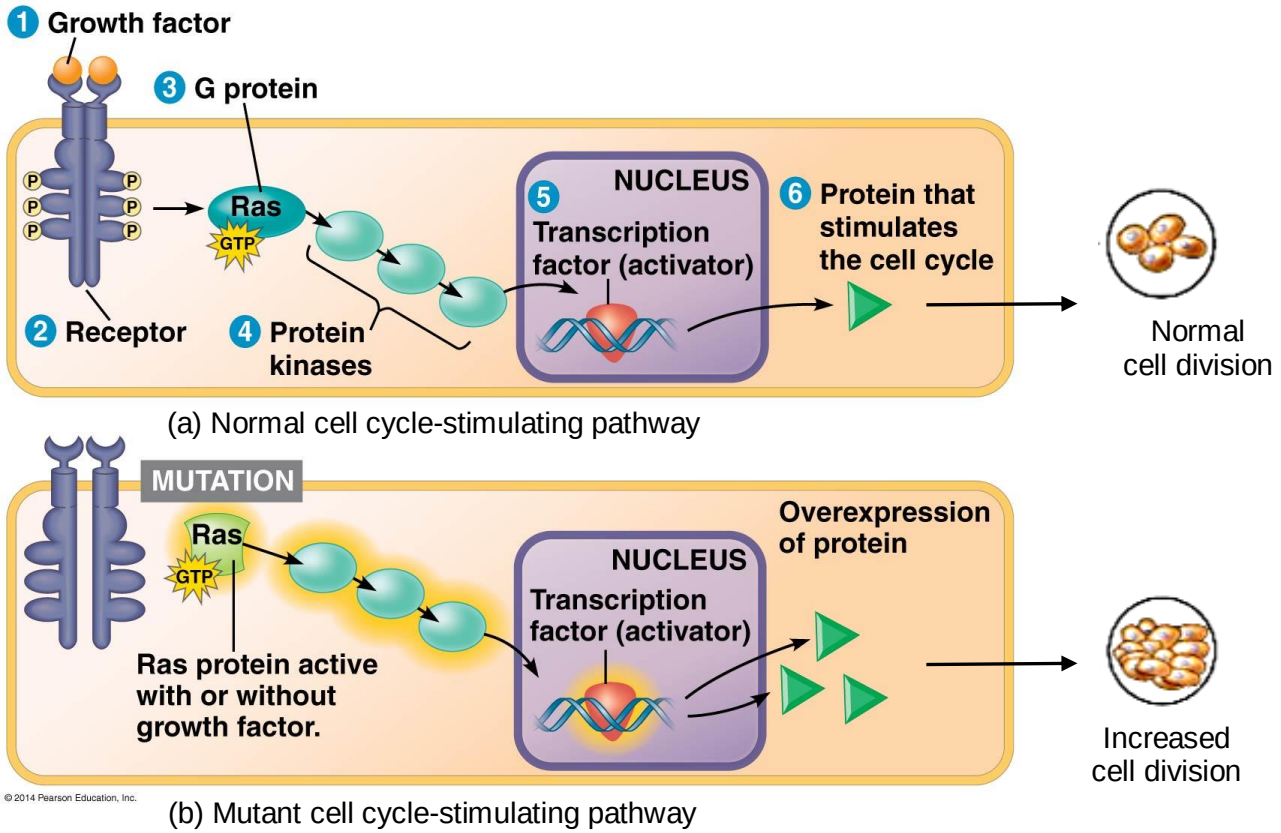


Fig. 13: Effect of normal ras proteins and mutated form of ras proteins on cell cycle.

2.4.2.4 CONSEQUENCES OF CONVERSION OF PROTO-ONCOGENES TO ONCOGENES

The conversion of a proto-oncogene to oncogene results in either of the following consequences (**Fig. 14**):

- **Quantitative change** [Points (b), (c) and (e) on page 19]
  Tumour formation is induced by an **increase in the absolute number (i.e. excessive amounts)** of oncogene product.

- **Qualitative change** [Points (a), (c), (d) and (e) on page 19]
  Tumour formation is induced by a **hyperactive** oncogene product.

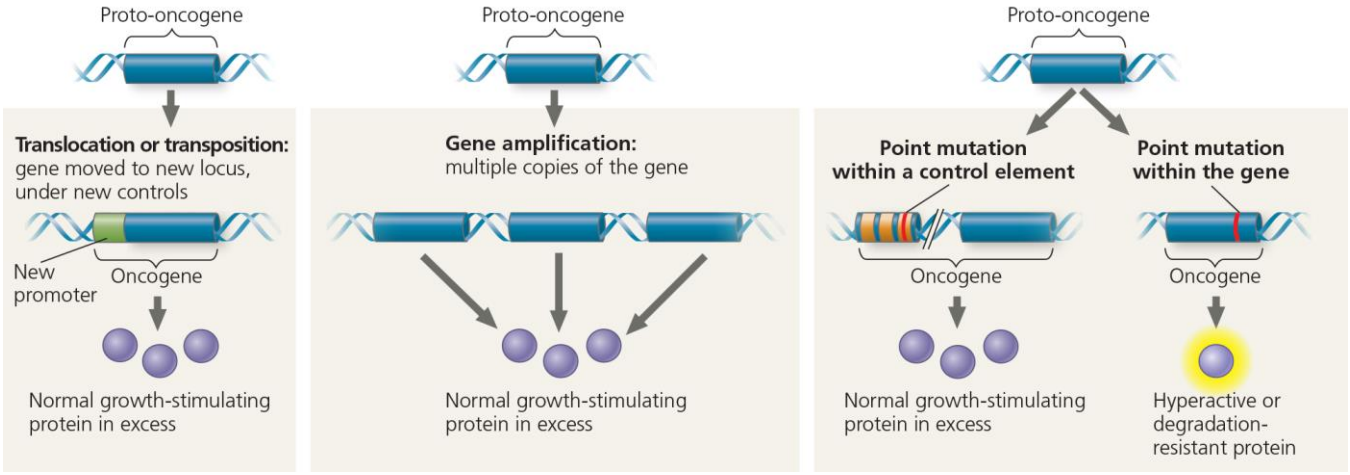


Fig. 14: The genetic changes that convert proto-oncogenes to oncogenes fall into three main categories: movement of DNA within the genome, amplification of a proto-oncogene, point mutations in a control element or in the proto-oncogene itself.

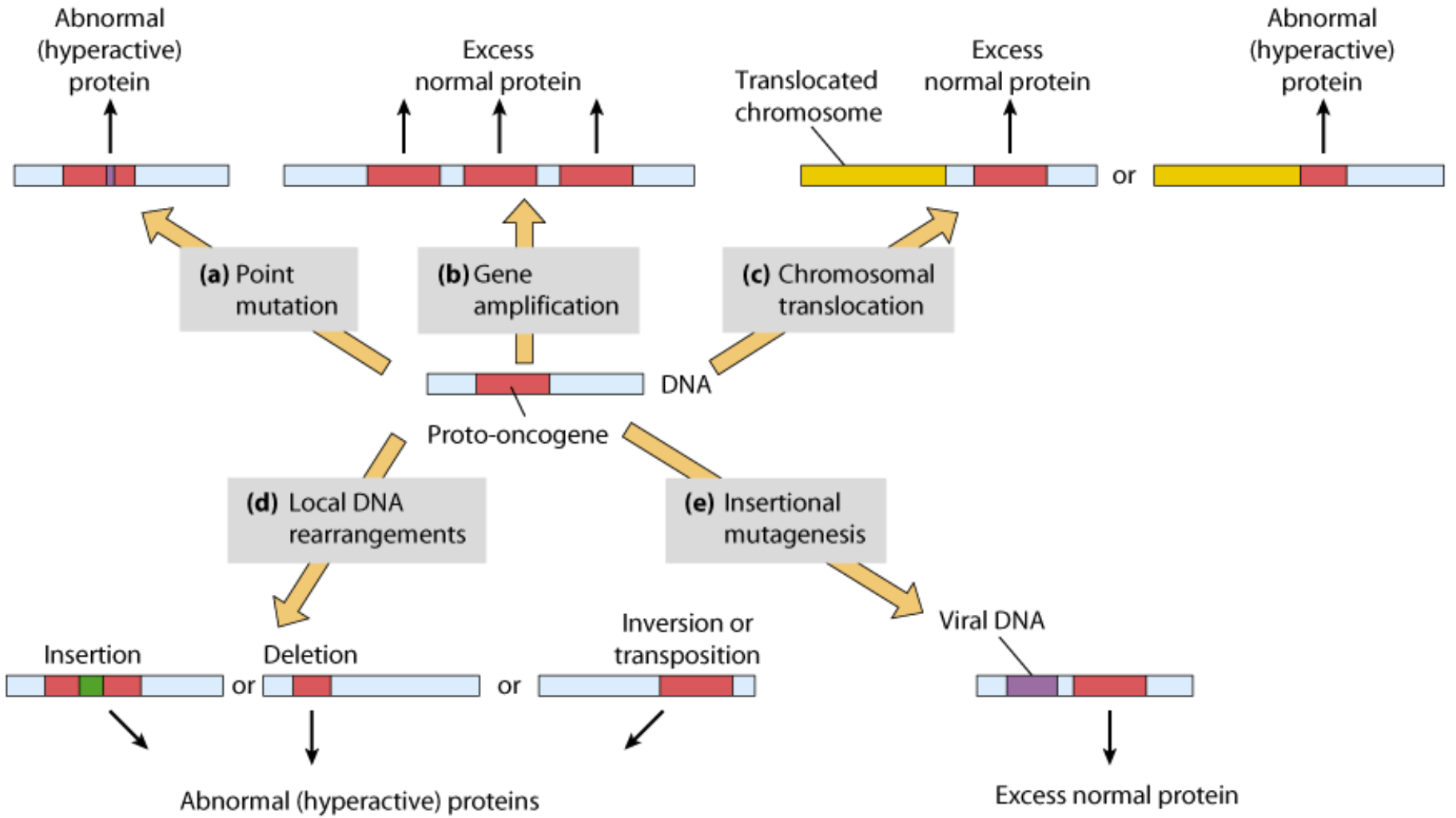


Figure 15: Mechanisms for converting proto-oncogenes to oncogenes. Some oncogenes produced by these mechanisms code for abnormal proteins, whereas others produce normal proteins in excessive amounts**. (a)** Point mutation involves single nucleotide substitution that creates an oncogene coding for an abnormal protein differing in a single amino acid from the normal protein produced by the proto-oncogene. **(b)** Gene amplification creates multiple gene copies, thereby leading to excessive production of a normal protein. **(c)** Chromosomal translocations move chromosome segments from one chromosome to another. This may fuse two genes together to form an oncogene encoding an abnormal protein, or it may a proto-oncogene next to a highly active gene, thereby causing the translocated proto-oncogene to become more active. **(d)** Local DNA rearrangements such as insertions, deletions, inversions and transposition can disrupt the structure of proto-oncogenes and cause them to produce abnormal proteins. **(e)** Insertional mutagenesis is triggered by the integration of viral DNA into a host chromosome near a proto-oncogene, thereby creating an oncogene coding for an abnormal protein, or it may enhance expression of the proto-oncogene and cause it to produce too much protein.

3. MULTI-STEP MODEL OF CANCER PROGRESSION

The development of a **malignant tumour** (**tumourigenesis**) is a **multi-step process** characterised by **accumulation of mutations** in a **single cell lineage**.

As this cell divides, it will pass on the damaged genes to the daughter cells, giving rise to a **clone of altered cells**. They originate from a common ancestral cell that has accumulated numerous mutations in cancer-critical genes, and then proliferate to form a tumour.

This uncontrolled proliferation of the cell allows the cell to possess an **unstable genome** and **accumulate more mutations** that allow it to escape normal growth restraints e.g. anchorage dependence, contact and density inhibition, replicative senescence.

These mutations would eventually allow cancerous cells to **invade tissues and metastasise to distant sites**, and causing death of the host organism. The death of the host organism occurs as normal healthy cells are replaced by cancerous cells at important organs.

Inherent in this **multi-step model of cancer progression** are the following steps:

1. **Accumulation of mutations**
2. **Activation of telomerase**
3. **Angiogenesis**
4. **Metastasis**

Evidences supporting the multi-step model of cancer progression include the following:

- Incidences of most cancers rise exponentially with age.
- Delay between exposure to carcinogens and appearance of the cancer.
  E.g.: Radiation from atomic bomb explosions at Nagasaki and Hiroshima only causes leukaemias after 5 – 8 years.
- Cancers develop progressively, from benign tumours, to increasingly malignant cells.

3.1 ACCUMULATION OF MUTATIONS

A **<u>single</u> mutation is not enough to convert a healthy cell into a cancer cell**. The genesis of a cancer typically requires **gradual accumulation** of **several independent mutations in cancer-critical genes in a <u>single</u> cell lineage** (**Fig. 16**).

The cancer-critical genes include:

- Proto-oncogenes
- Tumour suppressor genes
- Telomerase gene
- Angiogenesis-activating protein genes

Every step / stage of cancer progression corresponds to one or a few mutations.

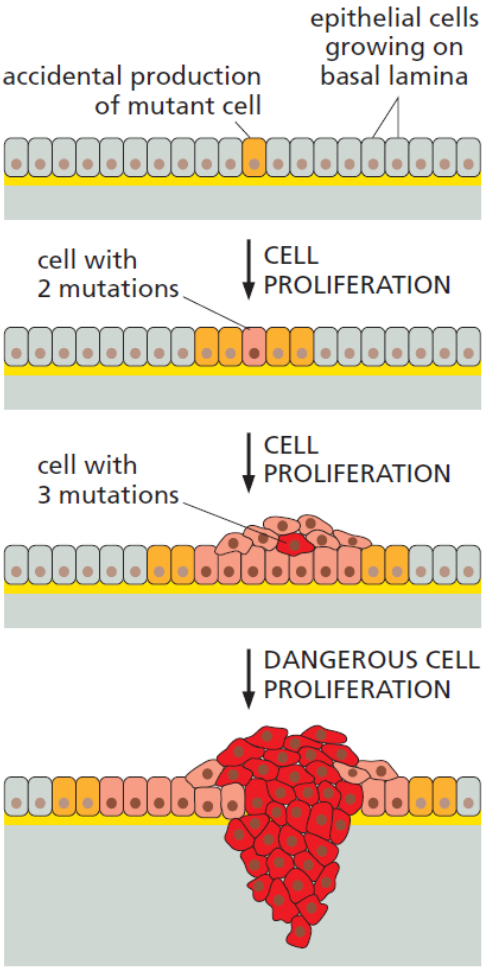


Fig. 16: Accumulation of mutation in a single cell lineage. A tumor develops through repeated rounds of mutation and proliferation, giving rise eventually to a clone of fully malignant cancer cells.

At each step, a single cell undergoes a mutation that either enhances cell proliferation or decreases cell death, so that its progeny become the dominant clone in the tumor. Proliferation of each clone hastens the occurrence of the next step of tumor progression by increasing the size of the cell population that is at risk of undergoing an additional mutation. The final step depicted here is invasion through the basement membrane, an initial step in metastasis. In reality, there are more than the three steps shown here, and a combination of genetic and epigenetic changes are involved.

3.2 ACTIVATION OF TELOMERASE

Please review Organisation of Eukaryotic Genome Lecture notes

Telomeres are regions of long stretches of **repetitive, non-coding DNA sequences** located at the **ends** of all eukaryotic chromosomes. During DNA replication, the ends of linear chromosomes shorten after each round of replication. Once telomeres are **shortened to a critical length** after a certain number of rounds of replication, the cell stops dividing and goes into **replicative cell senescence**. Hence telomere length appears to function as a natural check on the number of times a cell can divide i.e. **Hayflick limit**.

Telomerase is a **ribonucleoprotein complex** that adds telomere repeat sequences to the **3' ends** of DNA. The complex contains a reverse transcriptase enzyme and a single RNA molecule (AAUCCC in mammals), which acts as a template for synthesis of the telomere repeat (**Fig. 17**). Telomerases are usually not expressed in most human somatic cells. Telomerase genes are activated in most human cancer cells, where telomerase is produced and **maintains telomere lengths** so that the cancer cells **divide indefinitely**. Telomerase appears to allow cancer cells to **evade apoptosis** as well.

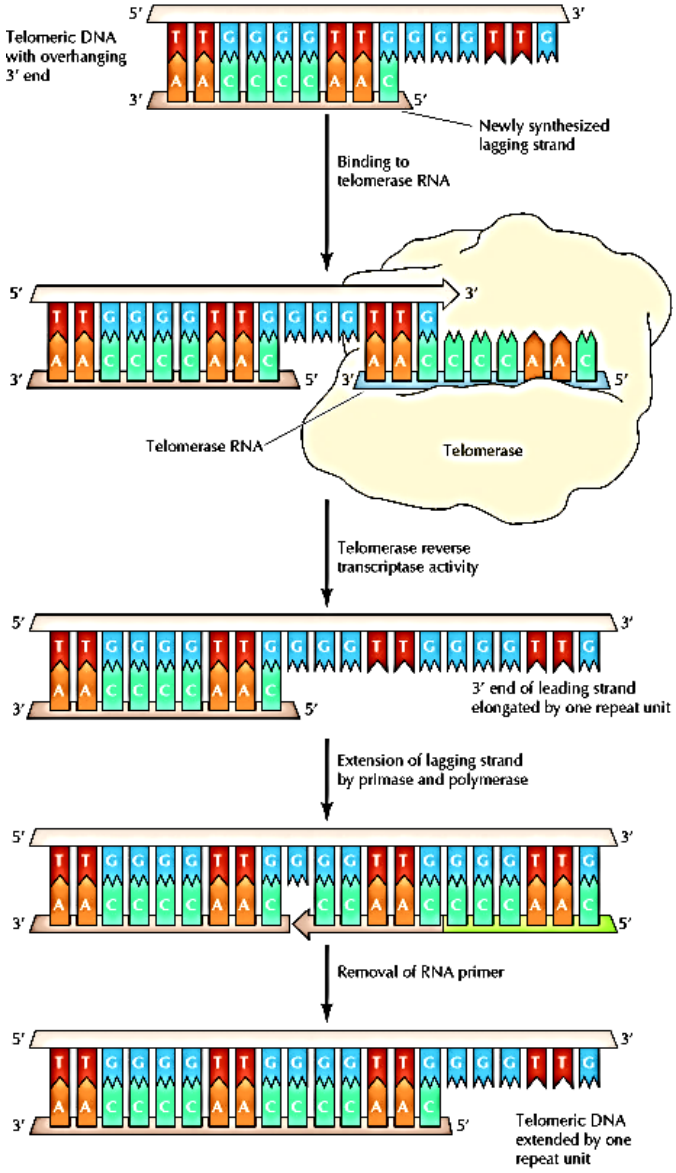


Fig. 17: Action of Telomerase. Telomeric DNA is a simple repeat sequence with an overhanging 3′ end on the newly synthesized leading strand. Telomerase carries its own RNA molecule, which is complementary to telomeric DNA, as part of the enzyme complex. The overhanging end of telomeric DNA binds to the telomerase RNA, which then serves as a template for extension of the leading strand by one repeat unit. The lagging strand of telomeric DNA can then be elongated by conventional RNA priming and DNA polymerase activity.

3.3 ANGIOGENESIS

Due to its more rapid proliferation, a tumour requires more nutrients and oxygen, and produces more toxic waste products that must be removed. Hence, to ensure its relentless growth, a growing tumour is able to **stimulate the formation of new blood vessels**, a process termed **angiogenesis** or **vascularisation**.

These blood vessels allow for an increased blood flow to the tumour, hence supplying nutrients and oxygen and removing toxic waste products. They also provide the pathways for cancer cells to spread to other sites in the body.

It is now clear that angiogenesis is critical for almost all cancers. In fact, many kinds of cancer cells produce angiogenesis-activating proteins such as:

- vascular endothelial growth factor (VEGF)
- fibroblast growth factor (FGF)

These angiogenesis-activating proteins must overcome the effects of **angiogenesis inhibitors** that normally restrain the growth of blood vessels. A finely tuned **balance** between the concentration of these **angiogenesis-activating proteins** (VEGF and FGF) and **angiogenesis inhibitors** determines whether a tumour will induce the growth of new blood vessels.

The angiogenic process, as currently understood, can be summarized as follows (**Fig. 18**):

1. A tumour cell releases **angiogenesis-activating proteins** that **attract endothelial cells** and promote their proliferation.
2. Endothelial cells secrete protein-degrading enzymes called **matrix metalloproteinases (MMPs)**.
3. These proteases break down the blood vessel wall and the components of the **extracellular matrix (ECM)**, allowing the endothelial cells to become **organised into new networks of blood vessels**.

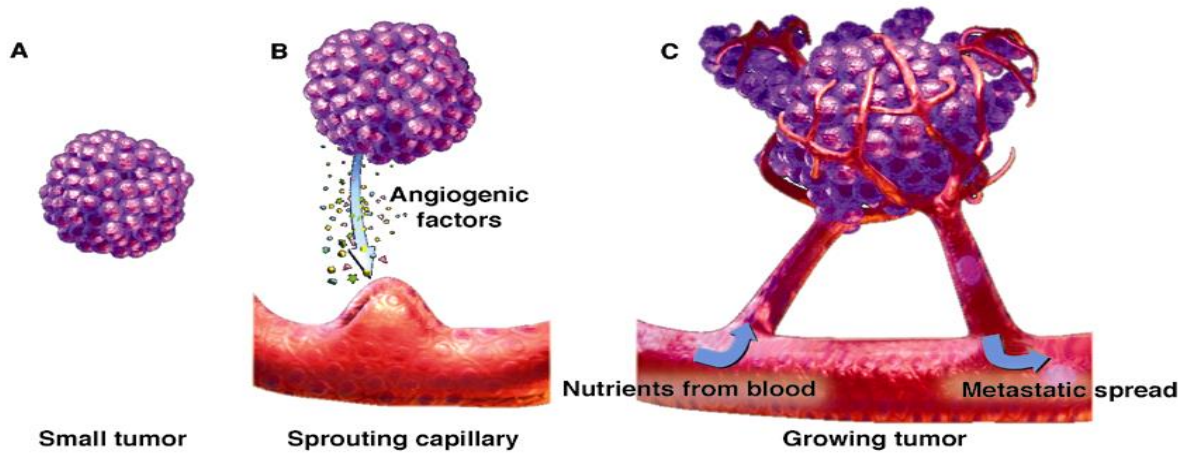


Fig. 18: Angiogenesis is associated with degradation and reformation of the blood vessel basement membrane (BM). In response to growth factors and matrix metalloproteinases (MMPs), the BM undergoes degradative and structural changes. Growth factors, such as VEGF, are produced by tumour cells. This induces the formation of an intermediate, and then a new (mature) BM. Together with the vascular endothelial, the BM mediates formation of a new blood vessel.

3.4 METASTASIS

Once angiogenesis has been triggered at an initial tumour site, the stage is set for the cancer cells to spread throughout the body. This ability to spread is based in two distinct mechanisms: **invasion** and **metastasis**.

Invasion refers to the acquired ability for cancer cells to perform **direct migration and penetration** into **neighboring tissues**.

Metastasis involves the ability of cancer cells to **enter the circulatory system** and **travel to distant sites,** where they form **secondary tumours** called **metastases** that are not physically connected to the primary tumour.

The ability of a tumour to metastasise depends on a complex cascade of events, beginning with angiogenesis. The events are as follows (**Fig. 19**):

1. Cancer cells **invade surrounding tissues** and penetrate through the walls of **lymphatic** and **blood** vessels, thereby gaining access to the bloodstream.
2. The cancer cells are **transported by the circulatory system** throughout the body.
3. Cancer cells leave the bloodstream and enter particular organs, where they **establish new secondary tumours at distant sites from the primary tumour**.

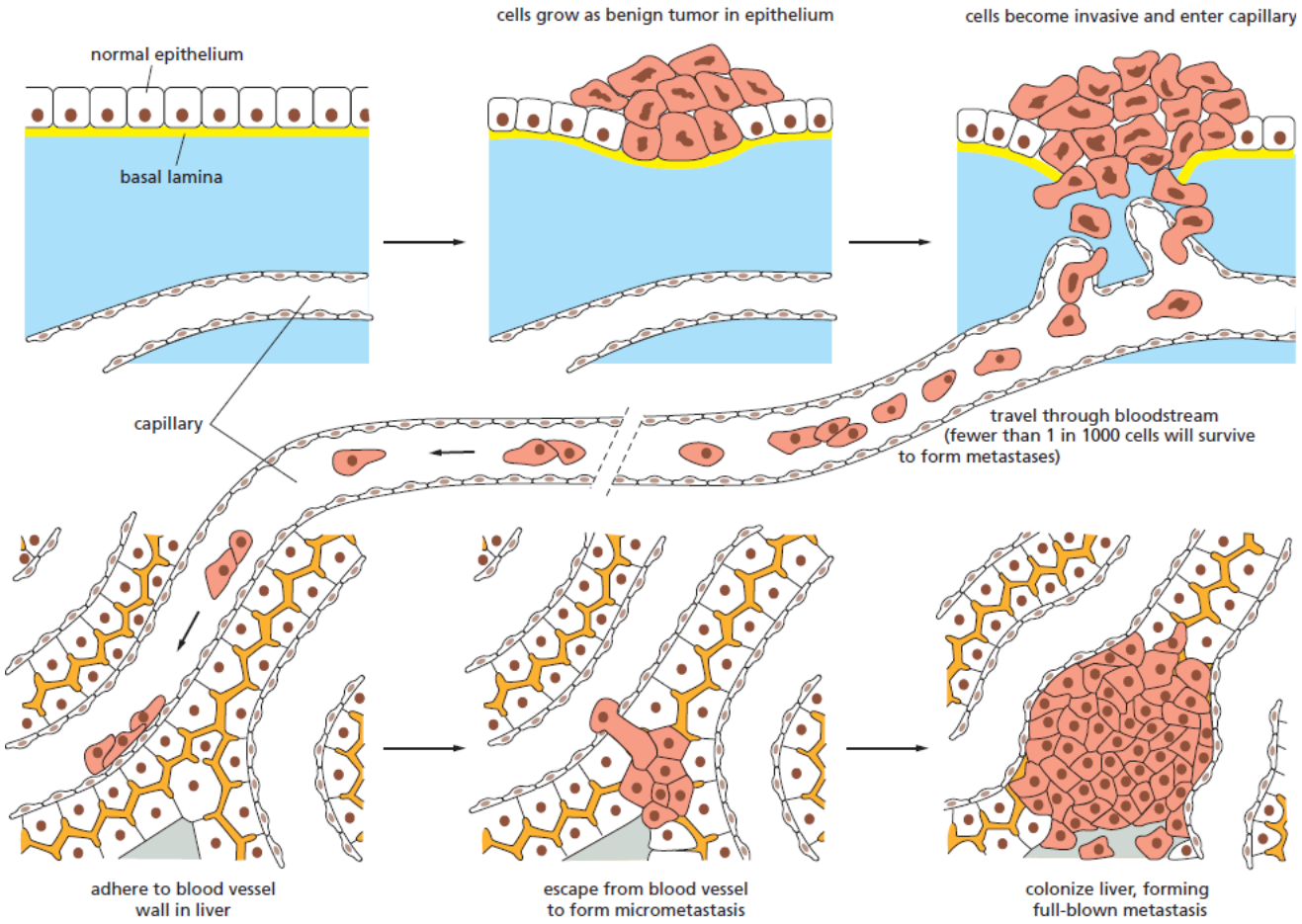


Fig 19: Steps in the process of metastasis. This example illustrates the spread of a tumor from an organ such as the bladder to the liver. Tumor cells may enter the bloodstream directly by crossing the wall of a blood vessel, as diagrammed here, or, more commonly perhaps, by crossing the wall of a lymphatic vessel that ultimately discharges its contents (lymph) into the bloodstream. Tumor cells that have entered a lymphatic vessel often become trapped in lymph nodes along the way, giving rise to lymph-node metastases.

3.5 CASE-STUDY – HEREDITARY COLORECTAL CANCER

In our case study to illustrate the multi-step model of cancer, we are interested in the hereditary familial colon cancer: **familial adenomatous polyposis (FAP)** which is one type of colorectal cancer. FAP is caused by ***Apc* gene** defects on chromosome 5.

It is important to note that **all the required mutations must occur in a single cell** lineage in order for the cancer to develop.

In FAP, a **small benign colonic polyp** forms first and will develop into a **larger benign growth (adenoma)** before finally becoming a **malignant tumour (carcinoma)**. Tumour cells are able to metastasise to other sites and invade other tissues.

As colon cancer progresses to full malignancy slowly, it is possible to describe the oncogene and tumour suppressor gene mutations at each stage in great molecular detail.

In such cancer, **at least three tumour suppressor gene mutations and one oncogene must be mutated in sequence** for an epithelial cell in the colon to become metastatic, thus, showing that different genes tend to be mutated at different stages of tumourigenesis (**Fig. 20**).

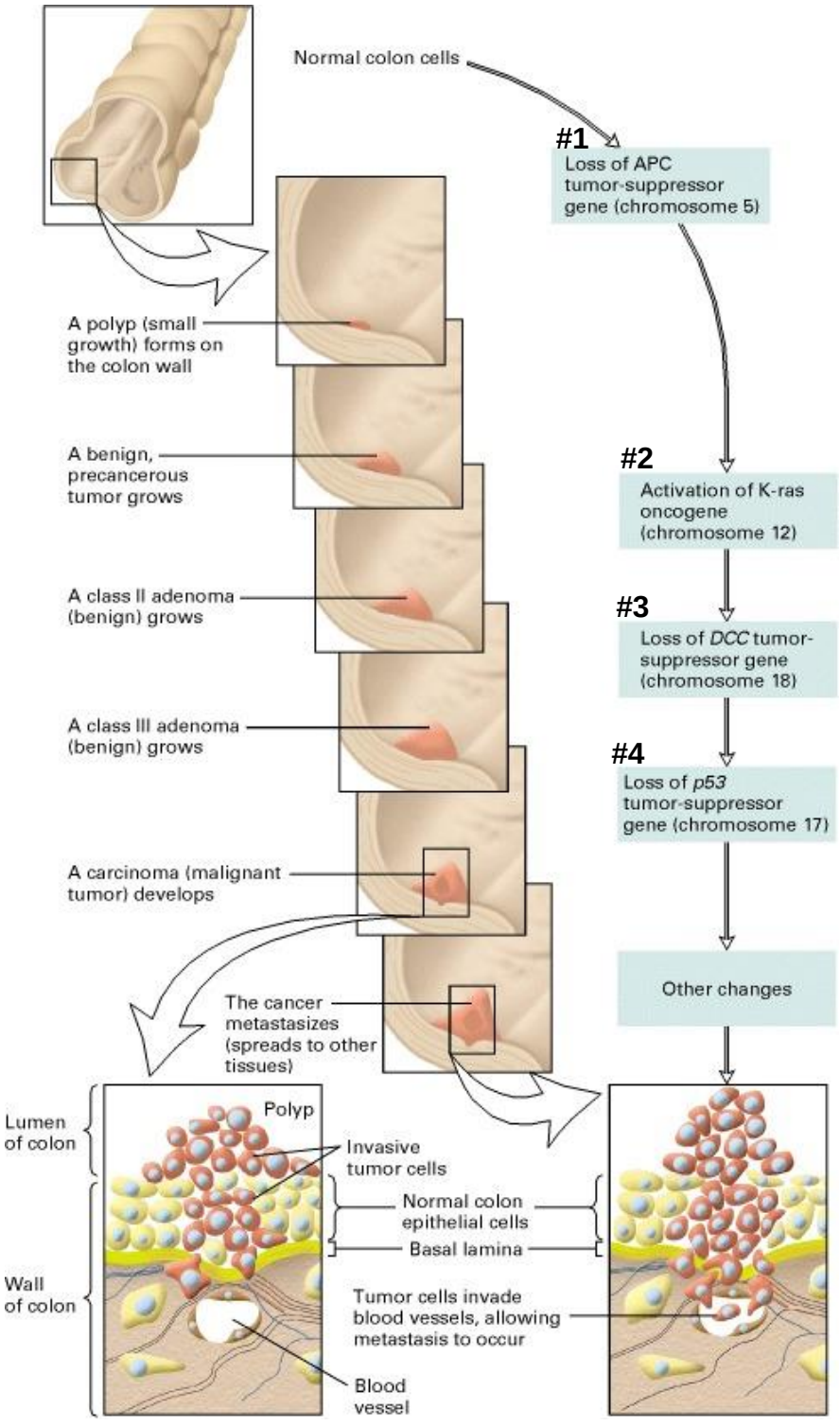


Fig. 20: Multiple mutations transform a normal colon epithelial cell into a cancer cell. In colon cancer, at least 5 genes are mutated in a single cell lineage.

3.5.1 SEQUENCE OF MUTATION ACCUMULATION IN COLORECTAL CANCER

MUTATION #1 - *APC* gene
(Tumour Suppressor Gene: controls proliferation, maturation, cell-to-cell contact and growth inhibition)

- Individuals inherit **one mutant copy** of *APC* (adenomatous polyposis coli) gene located on **chromosome 5**.
- The presence of a mutant *APC* allele causes the epithelial cells of the colon to partially escape cell cycle control, and the cell divides to form a small cluster of cells called a **polyp** or **adenoma**.
- People heterozygous for this condition develop hundreds to thousands of colon and rectal polyps early in life; however, not everyone develops cancer.
- Although it is not necessary for the second copy of the *APC* gene to be mutated in polyps at this stage, in the majority of cases, the second *APC* allele becomes mutant in a later stage of cancer development.

MUTATION #2 - *ras* gene
(Proto-oncogene: stimulates cell proliferation and division by transmitting growth signals from the cell surface to the nucleus)

- The second mutation in polyp cells that contain an *APC* gene mutation occurs in the *ras* proto-oncogene.
- The **gain in function mutation** of *ras* proto-oncogene results in it becoming *ras* oncogene, producing a **hyperactive Ras protein**.
- Results in the ras protein being permanently activated leading to continual signaling of the cell cycle to proceed into cell division.
- These allow an escape of the cell cycle control and the epithelial cells divide uncontrollably.
- The combined *APC* and *ras* gene mutations trigger the development of **intermediate adenomas**.
- These adenomas have defects in normal cell differentiation and will grow in culture, in the **absence of contact with other cells** and hence are described as **transformed**.

MUTATION #3 - *DCC* gene
(Tumour Suppressor Gene: involved in cell adhesion and differentiation)

- The third step toward malignancy requires **loss of function** of **both alleles** of the *DCC* gene (tumour suppressor gene).
- The *DCC* gene product is thought to be involved with cell adhesion and differentiation.
- Mutations in both copies of *DCC* genes result in the formation of **late stage adenomas** with a number of finger-like outgrowths called villi.

MUTATION #4 - *p53* gene
(Tumour Suppressor Gene - arrests cell cycle in response to DNA damage)

- In order for late adenomas to progress to **cancerous adenomas**, the cells need to lose both functional copies of the *p53* gene.
- This loss results in high mutation rates throughout the genome and loss of cell proliferation control.
- Subsequently, the final steps toward malignancy involve accumulation of mutations in an unknown number of genes associated with metastasis.

In summary, the development of a malignant tumour is paralleled by a gradual accumulation of mutations corresponding to an increasingly advanced stage of cancer (**Fig. 21**):

Event	Result
1. Loss of tumour suppressor gene (*APC*)	Small benign growth (polyp)
2. Activation of proto-oncogene (*ras*) 3. Loss of tumour suppressor gene (*DCC*)	Larger benign growth (adenoma)
4. Loss of tumour suppressor gene (*p53*)	Malignant tumour (carcinoma)

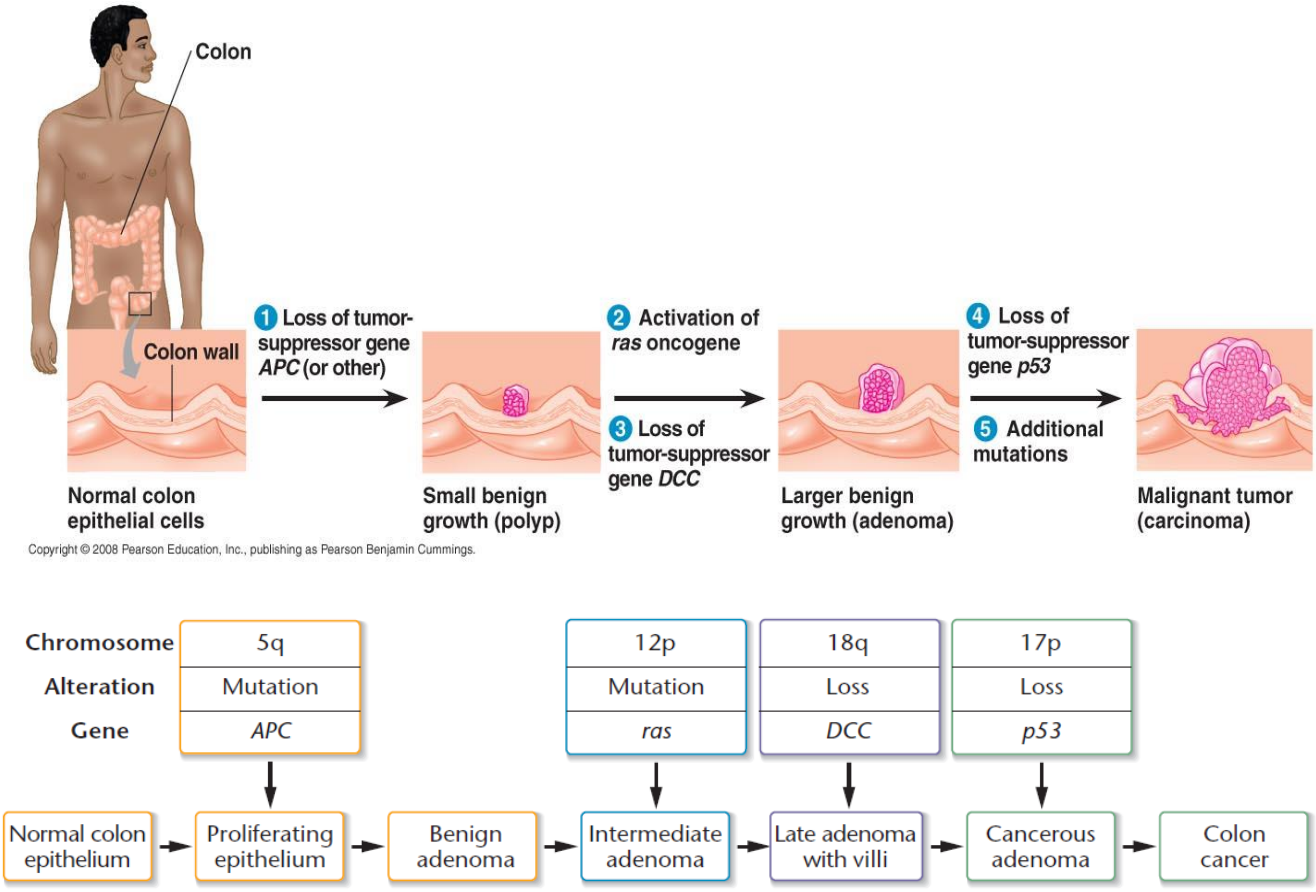


Fig. 21: A model for the multi-step development of colorectal cancer. The first step is the loss or inactivation of one allele of the APC gene on chromosome 5. In FAP cases, one mutant APC allele is inherited. Subsequent mutations involving genes on chromosomes 12, 17, and 18 in cells of benign adenomas can lead to a malignant transformation that results in colon cancer. Although the mutations on chromosomes 12, 17, and 18 usually occur at a later stage than those involving chromosome 5, the sum of changes is more important than the order in which they occur.

3.6 CASE-STUDY – BREAST CANCER

Breast cancer is not only the most common malignancy in women throughout the world and constitutes 22.9% of cancer in women, but it is also one of the major causes of death. The incidence rate varies according to geographic location.

Affected people have a 29% risk until age 50 and a 44% risk until age 70 to be affected by ovarian cancer. The major course of breast cancer incidence rate occurs in women after age 50; however, 5% to 12% of this cancer is seen in women under 45 years, is genetic, and proceeds from a mutation in genes predisposed to breast cancer.

What breasts are made of, and where and how cancer starts?

Breast tissue is found in the breast, upper chest and the armpit. Each breast contains 15-20 glands called **lobes**, where milk is produced in women who are breastfeeding. These lobes are connected to the nipple by tubes called **ducts**. Breast cancer usually begins within smaller structures of the lobes.

Much of the rest of the breast is **fatty tissue**. The breast and armpit also contain **lymph nodes** and **vessels** carrying lymph fluid, which are part of the immune system. Breast cancer can spread to other areas of the body through this **lymph system** or **blood vessels**.

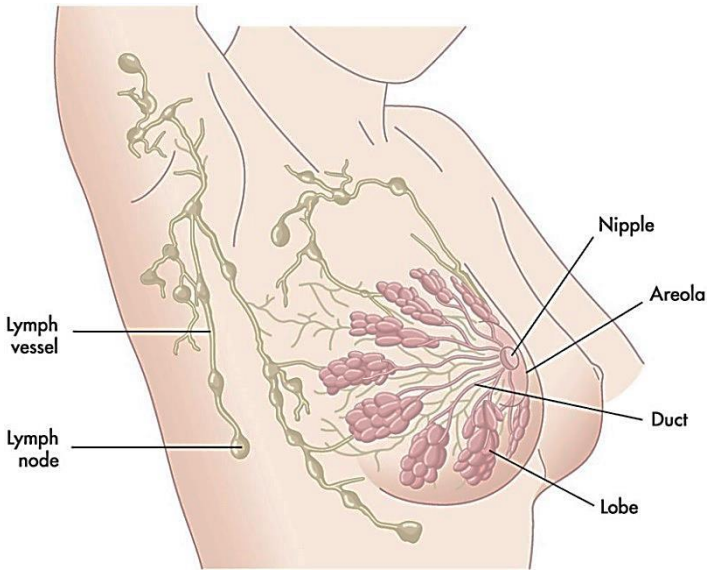


Fig. 22: Parts of the breast.

Being breast aware means getting to know how your breasts look and feel normally, so you can look out for any unusual changes and get them checked by your doctor.

Breast cancer is the **most frequent carcinoma** in females. Approximately 54 000 and 207 000 new cases of *in situ* and invasive breast carcinoma, respectively, are expected to be diagnosed in 2010 in the USA.

Early breast cancers that are only found only in the **milk lobes** (**lobular carcinoma**) or **ducts** (**ductal carcinoma**) of the breast and not other breast tissues are known as **non-invasive** breast cancers.

Invasive breast cancer is breast cancer that has **spread from where it began** in the breast ducts or lobules to surrounding normal tissue.
Breast cancer occurs in both men and women, although male breast cancer is rare.

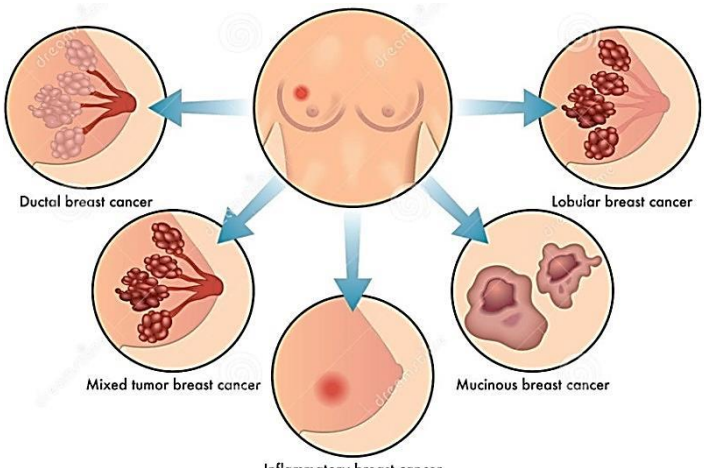


Fig. 23: Types of breast cancer.

BRCA Genes

BRCA1* and *BRCA2 (BReast CAncer genes 1 and 2) are the most well-known genes linked to breast cancer risk. *BRCA1/2* mutations can be passed to you from either parent and can affect the risk of cancers in both women and men. A person who has a ***BRCA1/2* mutation** is sometimes called a ***BRCA1/2* carrier**.

BRCA1 and *BRCA 2* are **tumor suppressor genes**. BRCA1 and BRCA2 proteins are normally expressed in the cells of breast and other tissues, where they help **repair damaged DNA**, or destroy cells if DNA cannot be repaired.

They are involved in the repair of **chromosomal damage** with an important role in the **error-free repair** of DNA double-strand breaks.

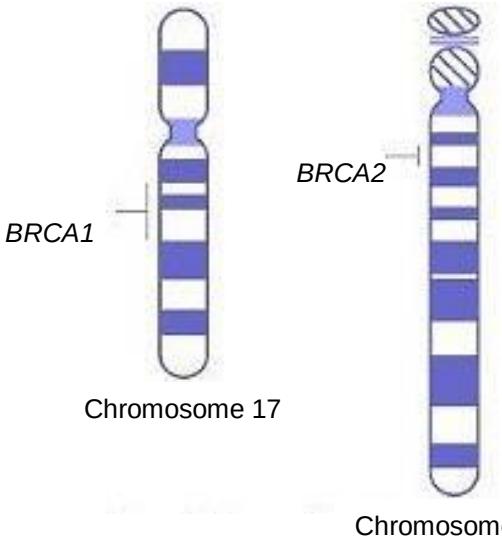


Fig. 24: The *BRCA* genes on their respective chromosomes. A damaged *BRCA* gene in either location can lead to increased risk of cancer, particularly breast or ovarian in women.
(National Library of Medicine, NCBI)

The cancer risk caused by *BRCA1* and *BRCA2* mutations are inherited in a dominant manner even though usually only one mutated allele is directly inherited. A mutated *BRCA* gene can be inherited from either parent, thus they are classified as **hereditary or germline mutations** rather than acquired or somatic mutations.

Cancer caused by a mutated gene inherited from an individual's parents is a **hereditary cancer** rather than a sporadic cancer.

Other genes

Changes in other genes are also associated with breast cancer. These abnormal genes are much less common and don't seem to increase risk as much as abnormal *BRCA1* and *BRCA2* genes, which are considered rare. Still, because these genetic mutations are rarer, they haven't been studied as much as the *BRCA* genes.

- ***ATM***: The *ATM* gene helps **repair damaged DNA**. DNA carries genetic information in cells. Inheriting two abnormal copies of this gene causes the disease ataxia-telangiectasia, a rare disease that affects brain development. Inheriting one abnormal *ATM* gene has been linked to an **increased rate of breast cancer** and pancreatic cancer in some families because the abnormal gene stops the cells from repairing damaged DNA.
- ***BRIP1***: The *BRIP1* gene also works to **repair DNA**. Inheriting one abnormal *BRIP1* gene is associated with **higher risk** of both breast and ovarian cancer.
- ***CDH1***: The *CDH1* gene makes a protein that **helps cells bind together** to form tissue. An abnormal *CDH1* gene increases the risk of a rare type of stomach cancer at an early age. The lifetime risk is up to 83%. Women with an abnormal *CDH1* gene also have a 39% to 52% lifetime risk of invasive lobular breast cancer.

3.6.1 DEVELOPMENT OF BREAST CANCER

Breast cancer development is the result of the **accumulation of genetic mutations** in the epithelial cells of the breast. The main genetic mutations occurring in breast cancer are amplification of a number of oncogenes (approximately 10) and the inactivation of tumor suppressor genes.

Since breast cancers undergo **clonal expansion,** a process where identical daughter cells arise from an original cell**,** and have almost certainly undergone **many more cell divisions** than normal cells, each tumour may have many millions of mutations, most of which are entirely innocent and some of which have accumulated in the cell of origin prior to tumourigenesis.

Despite some claims to the contrary, even at normal mutation rates, **clonal expansion** within a tumour is quite sufficient to account for the mutations of five or six genes that are generally supposed necessary for carcinogenesis to occur.

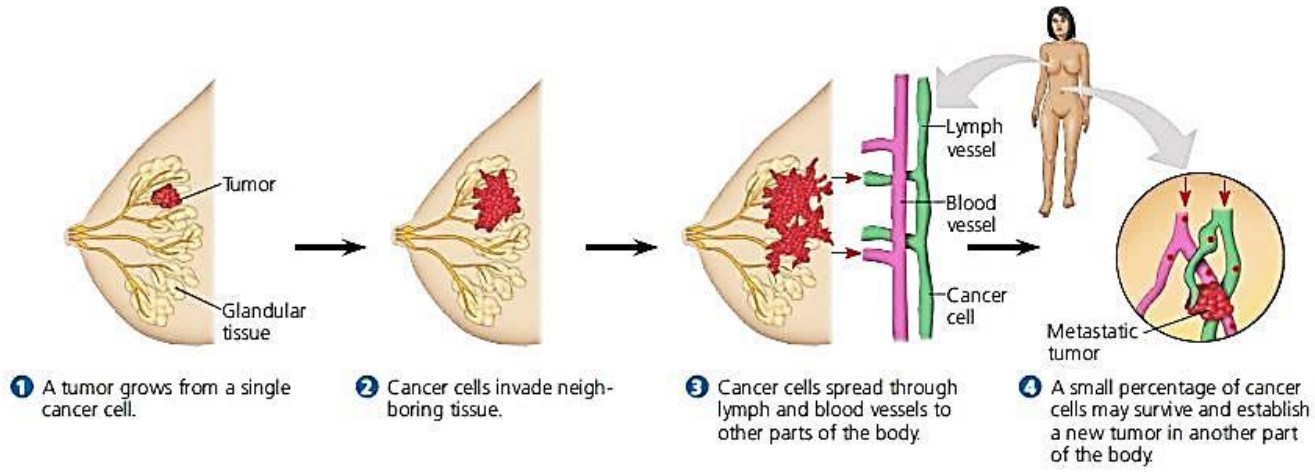


Fig. 25: The growth and metastasis of a malignant breast tumour (Reece *et al*., 2011).

There are approximately **five** known tumor suppressor genes in breast cancer, but in view of the high frequency of **loss of heterozygosity** found for various chromosomal locations, many more tumor suppressor genes must play a role in breast cancer development.

Recent findings…

More recently, **genetic abnormalities** have also been described in normal epithelial cells adjacent to invasive breast cancer, as well as in benign epithelial proliferations.

It is not known in what cell in the normal breast carcinomas develop. The candidates for these precursors include **ductal epithelial cells**, **myoepithelial cells** and **ductal 'stem cells'**; it has been hypothesised that the latter exist, but they have not been identified based on morphological or other characteristics. It is not known with certainty in what part of the ductal tree most carcinomas develop, but the most likely site is the terminal ductolobular unit.

It has been discovered that most genetic mutations found in invasive breast carcinoma are already present in carcinoma *in situ*. It has become clear that, unlike the situation for colorectal cancer, there is **not one linear route** from a normal epithelial cell to invasive carcinoma, but that there are several distinct pathways leading to distinct histological types of carcinoma.

For example in the development of **lobular carcinoma *in situ***:

- Normal epithelial cell → lobular carcinoma *in situ* (LCIS) → invasive lobular carcinoma (ILC).
- Loss-of-function mutations in the E-cadherin gene (CDH1). E-cadherin acts as a tumor suppressor protein, which prevents cells from growing and dividing too rapidly or in an uncontrolled way.

Inactivation of E-cadherin is already present in LCIS, indicating that this is an early step in the development of lobular cancer. Amplification and overexpression of cyclin D1 are also frequently found in ILC.

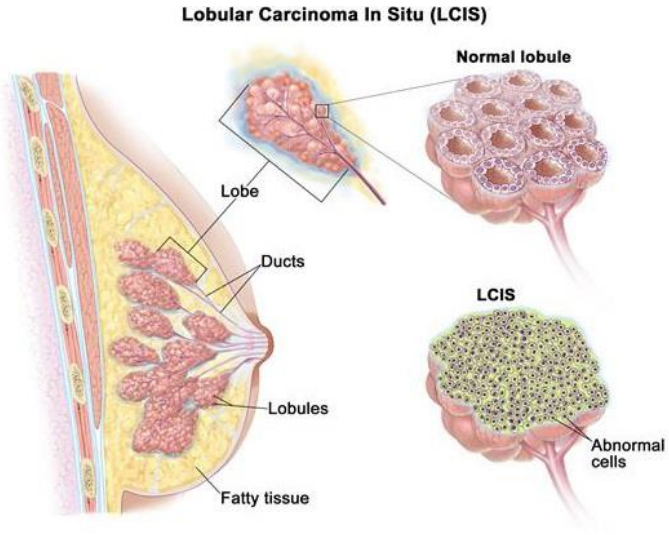


Fig. 26: Lobular Carcinoma *in situ*. A condition in which abnormal cells are found in the lobules of the breast. This condition seldom becomes invasive cancer. However, having LCIS in one breast increases the risk of developing breast cancer in either breast (adapted from National Cancer Institute).

Are genetic tests available to detect *BRCA1* and *BRCA2* mutations?

Yes. Several different tests are available, including tests that look for a known mutation in one of the genes i.e. a mutation that has already been identified in another family member and tests that check for all possible mutations in both genes.

DNA (from a blood or saliva sample) is needed for mutation testing. The sample is sent to a laboratory for analysis. It usually takes about a month to get the test results.

Who should consider genetic testing for BRCA1 and BRCA2 mutations?

Because harmful *BRCA1* and *BRCA2* gene mutations are **relatively rare** in the general population, most experts agree that mutation testing of individuals who do not have cancer should be performed only when the person's individual or family history suggests the possible presence of a harmful mutation in BRCA1 or BRCA2.

Several **screening tools** are now available to help health care providers with this evaluation. These tools assess family history factors that are associated with an increased likelihood of having a harmful mutation in *BRCA1* or *BRCA2*, including:

- Breast cancer diagnosed before age 50 years
- Cancer in both breasts in the same woman
- Both breast and ovarian cancers in either the same woman or the same family
- Multiple breast cancers
- Two or more primary types of *BRCA1*- or *BRCA2*-related cancers in a single family member

4. CAUSATIVE FACTORS OF CANCER

It has been estimated that two-thirds of all cancers can be prevented by lifestyle modification. Although the ultimate cause or causes of the many existing forms of cancer are still unknown, there are specific factors that are so often associated with the disease that they are considered either to increase a person's risk to cancer or to create a likely setting for cancer to develop. Most cancers are believed to develop only after **repeated contact with carcinogens**, substances that cause or promote the development of cancer.

Some examples of causative factors of cancer are described below:

1. Lifestyle & Diet

(a) Cigarette and tobacco smoking

Smoking remains the most common preventable cause of death in the developed world. Smoking causes cancer of the lung, upper respiratory tract, oesophagus, bladder and pancreas, all of which, can generally be considered "self-induced" if smoking related.

When you inhale smoke, chemicals such as **polycylic aromatic hydrocarbons**, or PAHs, an important class of carcinogens found in tobacco smoke enter your lungs and spread around the rest of your body via the bloodstream. These organic compounds can bind to DNA of cells to form a physical complex known as an **adduct**. Hence, causing damage to DNA. This damage can, under certain circumstances, be repaired by cellular DNA repair mechanisms.

However, if not repaired, these adduct cause mistakes in DNA synthesis during normal cell division, which introduce mutations into the DNA sequence, leading to gene mutations. PAHs tend to form adducts at several sites on the *p53* gene in the lung cells and other cells of smokers. The resulting mutations prevent the production of functional p53 protein in the affected cells. Therefore, eventually leading to a loss of normal growth control mechanisms and the cell divides uncontrollably leading to tumour formation.

(b) Exposure to chemical carcinogens

Salted, pickled and smoked foods such as smoked fish and meats treated with nitrites should be limited. Nitrites appear to contribute significantly to cancer. Meats that have been charred over grill should be eliminated from the diet because the charred part is carcinogenic.

When meats such as beef, pork, fish or poultry is cooked using high-temperature methods such as grilling, **heterocylic amines** (HCAs) and **polycyclic aromatic hydrocarbons** (PAHs) are formed. HCAs are formed when amino acids, sugars and creatine (a substance found in muscle) react at high temperatures. PAHs are formed when fat and juices from meat grilled directly over an open fire drip onto a fire, causing flames. These flames contain PAHS that then adhere to the surface of the meat. PAHs can also be formed during the smoking of meats. When the meat is consumed, HCAs and PAHs spread around the body system via the bloodstream.

These organic compounds can **bind to DNA** and **cause mistakes in DNA synthesis** during normal cell division, which introduce mutations into the DNA sequence, leading to gene mutations. PAHs tend to form adducts at several sites on the *p53* gene. The resulting mutations prevent the production of functional p53 protein in the affected cells (*p53* is a tumour suppressor gene). Therefore, eventually leading to a loss of normal grown control mechanisms and the cell divides uncontrollably leading to tumour formation.

Animal (saturated) fats, especially from red meat, are associated with several different types of cancer, including colon, rectum and prostate. A lack of dietary fiber is linked to increased risks of colorectal cancer. Obesity also increases one's risk of developing cancer.

Chemical carcinogens such as aromatic hydrocarbons and their derivatives, nitrosamines, asbestos, benzene, formaldehyde and diesel exhaust are dangerous in high concentrations. When they are

introduced into the body, these chemical carcinogens can act directly on cellular DNA, introducing mutations into the DNA sequence. Whilst diverse in structure, these chemicals share a common property of causing mutations, leading to gene mutations.

If these mutations occur in cancer critical genes e.g. tumour suppressor genes and proto-oncogenes, the cell can experience uncontrollable cell division which may eventually lead to tumour formation. Although a few of these chemical carcinogens can act directly on DNA but generally the more potent ones are relatively inert chemically, and become damaging only after they have been changed to a more reactive form by metabolic processes.

2. Radiation Exposure

(a) Ionising radiation

Potential sources of ionising radiation (X rays and γ rays) include nuclear explosion and medical diagnosis. Ionising radiation can result in the production of free radicals of water (the OH radical) which are chemically very reactive. These **free radical** can interact with cellular DNA to produce **double stranded breaks** leading to **chromosomal rearrangements** and **deletions**, hence affecting cancer critical genes e.g. tumour suppressor genes and proto-oncogenes. Consequently, the cell can experience uncontrollable cell division which may eventually lead to tumour formation.

(b) Ultraviolet (UV) radiation

A long history relates exposure to the sun to skin damage and skin cancer. Ultraviolet radiation is the radiation from the sun to the earth. The most harmful of this type of radiation are the high-frequency, **DNA-damaging** UV-B rays. These are the rays that cause 90% of all skin cancers as they lead to distinctive mutation patterns during DNA replication.

Excessive exposure to UV rays will allow for the production of a covalent attachment between adjacent pyrimidines in one strand (thymine dimers) or base pair substitutions, insertions and deletions in cellular DNA. These can cause mutations in cancer critical genes e.g. tumour suppressor genes and proto-oncogenes resulting uncontrollable cell division which may eventually lead to tumour formation.

3. Age

If cancer results from an **accumulation of mutations** and if mutations occur throughout life, then the longer we live, the more likely we are to develop cancer. Thus, the chances of developing cancer **increases with age** (refer to the multi-step model of cancer).

4. Genetic Predisposition

Certain types of cancer, such as **colon** and **breast cancer**, often run in families. An individual inheriting an oncognene or a mutant allele of a tumour-suppressor gene is one step closer to accumulating necessary mutations for cancer to develop. It is the predisposition to cancer (not the cancer itself) that is inherited.

For example, in familial colorectal cancer, individuals that inherit one mutant copy of the *APC* gene (tumour suppressor gene) have an increased disposition to develop colon cancer. In hereditary breast cancer, individuals that inherit one mutant copy of genes such as *BRCA1*, *BRCA2* and *p53* (tumour suppressor genes) have an increased disposition to develop breast cancer. Other additional mutations coupled with non-genetic (like environmental) factors must be present for cancer to develop. Having a family history of cancer means one is at higher risk, therefore it is more important for that person to take action to reduce risk.

5. Loss of Immunity

When the immune system is suppressed by drugs, viruses such as HIV, or even mental states of anxiety, stress, or depression, it is unable to detect and destroy cancerous cells due to the absence or inadequate numbers of immune cells. Hence, tumours may develop (**Fig. 26**).

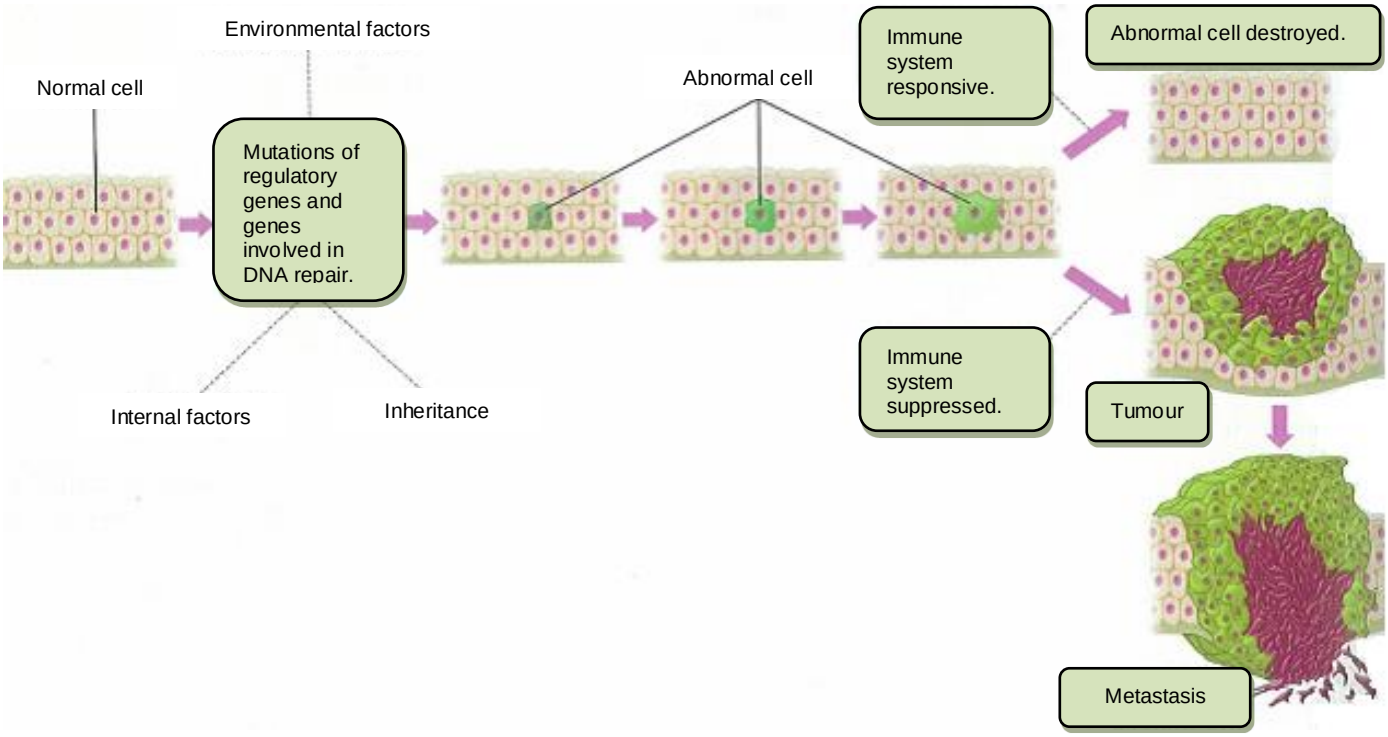


Fig. 27: Overview of the development of cancer. The progression to cancer requires multiple genetic changes in regulatory genes, some of which may be inherited and some of which may be caused by environmental or internal factors. A cancer cell reproduces rapidly and may invade and colonise distance tissues. Suppression of the immune system appears to be involved in many cancers.

6. Viral Infections

15% of human cancers involve certain viruses. Termed as **tumour viruses**, they can integrate their genetic material into the DNA of their host cells and transform a normal cell into a tumourigenic cell by:

- **inactivating** tumour suppressor genes or **converting** a proto-oncogene to an oncogene
- directing expression of viral proteins that can inactivate **p53** and other **tumour suppressor proteins** to render a host cell more susceptible to cancer
- introducing an **oncogene** into a normal cell e.g. retroviruses can carry a copy of a host-derived oncogene, which is reverse transcribed and inserted into the genome of the next infected host cell.

Note that on its own, a viral infection is insufficient to trigger cancer. For instance, only a small number of women infected with the human papillomavirus (HPV) develop cervical cancer, and that is only after a long incubation period. Viral infection requires other events like mutations in proto-oncogenes or tumour suppressor genes coupled with additional mutations in other genes and processes such as angiogenesis and metastasis to cause cancer.