CORE IDEA 2: GENETICS AND INHERITANCE
TOPIC 2.8: Molecular Biology of Cancer

H2

Learning Outcomes:

You should be able to:

(o) (modified) explain the need to regulate the mitotic cell cycle tightly (knowledge that dysregulation of checkpoints of cell division can result in uncontrolled cell division and cancer is required, but details of the mechanism are not required)

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

(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

(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.

References:

Campbell, N. A., Reece, J. B., et al. (2018). Biology A Global Approach (Eleventh Edition)

- Chapter 12 Mitosis, p284 – 299
- Chapter 13 Sexual Life Cycles and Meiosis, p304 - 317

Note: Some of these references (including previous editions) are available in our library. You may wish to borrow them or visit the links to supplement your reading when necessary.

Lecture Outline

1 Introduction

The cell cycle is the sequence of events that occurs throughout the life of a cell, from its formation as a new cell by division, to its own division into two daughter cells.

- A cell cycle that involves mitosis will give rise to genetically identical cells and this is important for growth, repair and the asexual reproduction of organisms.
- This cycle is coupled intricately with DNA replication, which occurs during the synthesis phase of interphase.

The cell cycle compromises of two main stages

1. **Interphase:** Comprises of G_1 phase, S phase and G_2 phase
2. **Mitotic phase** (M phase): comprises mitosis (nuclear division) and cytokinesis (cytoplasmic division) [Covered in Topic 2.3: Cell Division]

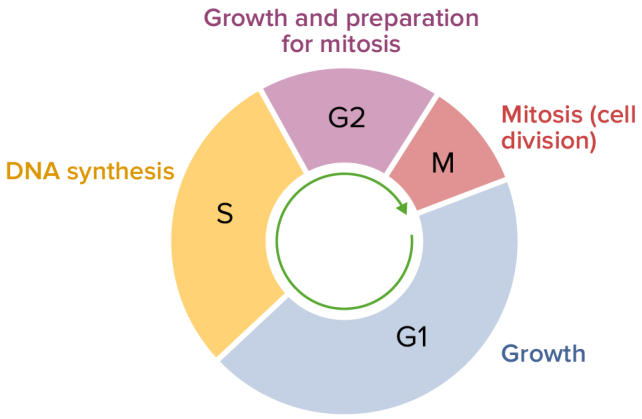


Fig. 1: The Mitotic Cell Cycle

Interphase

- The longest phase of the cell cycle and a period of intense metabolic activity, where synthesis and growth occurs in preparation for cell division
- Interphase can be divided into three subphases:
    - G_1 phase ("first gap")
    - S phase ("synthesis")
    - G2 phase ("second gap")

Checkpoint 1: RECAP

What occurs during the different stages of interphase?

G_1 phase	
S phase	
G_2 phase	

Asides from allowing the cell to grow, what do you think is the significance of **G_1 and G_2 phases?**

Learning Outcome 2(o) modified:

Explain the need to regulate the mitotic cell cycle tightly *(knowledge that dysregulation of checkpoints of cell division can result in uncontrolled cell division and cancer is required, but details of the mechanism are not required)*

2 Checkpoints In The Cell Cycle

The mitotic cell cycle is tightly regulated at various checkpoints that control the rate of cell division; uncontrolled cell division can result in cancer.

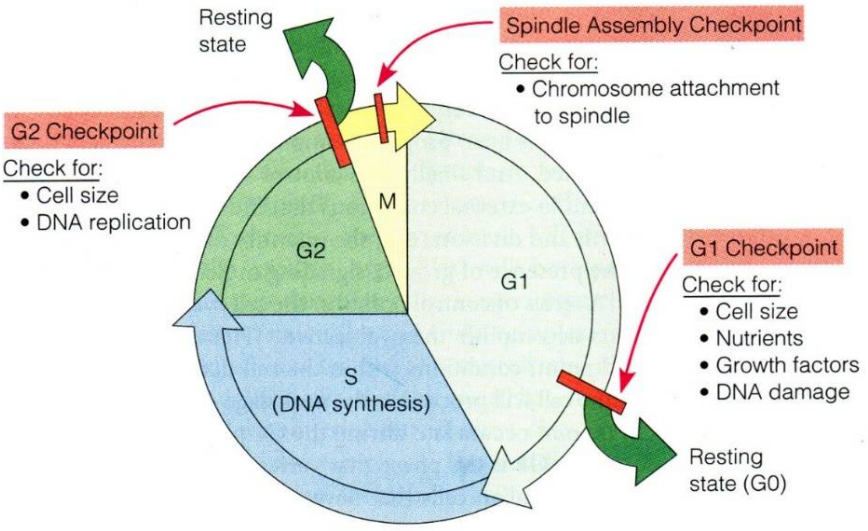


Fig. 2.1: Checkpoints in the Cell Cycle

The cell cycle is controlled at various **checkpoints**. These checkpoints are regulated by proteins such as **cyclin-dependent kinases** and **cyclins**. Such regulation which will lead to the activation and inhibition of other proteins to regulate the progression of cell division.

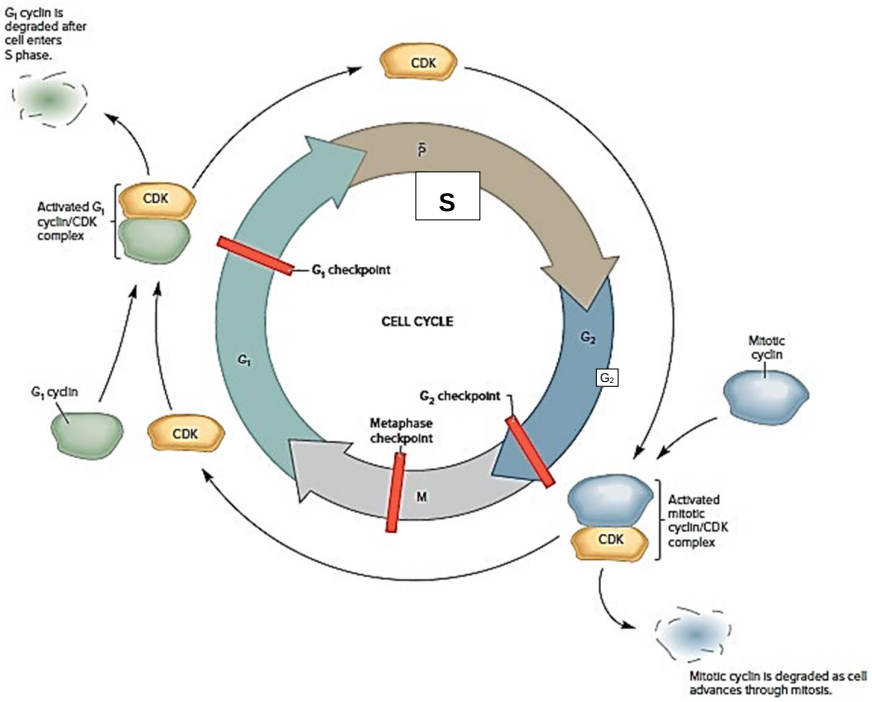


Fig. 2.2: Overview of cell cycle control by cyclin-CDK complexes

(you do not need to know the exact mechanism of how these proteins work)

2.1. G_1 checkpoint, also known as Restriction point

- Checks for presence of growth factors, nutrients, cell size and DNA damage before allowing the cells to proceed to the next phase.

2.2. G_2 checkpoint also known as DNA replication checkpoint

- Checks for DNA replication and DNA damage

  The G_2 checkpoint prevents cells from entering mitosis when DNA is not completely replicated or if DNA is damaged, providing an opportunity for repair and stopping the proliferation of damaged cells, hence the G_2 checkpoint helps to maintain genomic stability.

2.3. Metaphase (M) checkpoint, also known as the spindle assembly checkpoint

- Checks that chromosomes are all properly attached to the spindle fibres, to ensure that each daughter cell receives an equal set of chromosomes.

Dysregulation of these cell cycle checkpoints can lead to uncontrolled cell division and proliferation (increase on cell numbers due to cell division), leading to cancer.

Checkpoint 2: Significance of controlling the cell cycle via checkpoints

Uncontrolled cell division and proliferation of cells often lead to the formation of tumours, hence leading to cancer.

Using information from page 5, **how does the dysregulation of each checkpoint possibly lead to cancer?** [Hint, what is the function of each checkpoint?]

Checkpoint	Regulated check-point	Unregulated checkpoint
G_1 checkpoint: Restriction point		
G_2 checkpoint: DNA replication checkpoint		
M (metaphase) checkpoint: Spindle assembly checkpoint		

If the control system detects unfavourable internal or external conditions, it blocks progression through each of these checkpoints. Such delays are crucial because

- Provide time for errors or malfunctioning cellular machinery to be repaired
- Prevent errors in cell division that may result if cell cycle progressed prematurely to the next stage when conditions are still unfavourable

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

3 Causative Factors Leading To Cancerous Growth

Cancer is caused by changes in a cell's DNA, i.e. **mutations**. Factors that result in such mutations are called **causative factors of cancer**. Factors may be genetic (hereditary), inherited from our parents, while others may be due to environmental factors. Environmental factors include chemical carcinogens *(substances that can lead to cancer)*, ionising radiation, infectious agents leading to loss of immunity etc.

3.1. Genetics (Hereditary)

Genetics is a causative factor of cancer when defective alleles of genes are inherited from parents, resulting in increased predispositions to cancer in the offspring. As the cancer is due to an inherited gene mutation, it is referred to as hereditary cancer

- *E.g. children who inherited mutated BRCA1/BRCA2 genes has a higher risk of breast and ovarian cancer.*

3.2. Chemical Carcinogens

Chemical carcinogen causes mutations in DNA by forming covalent bonds with DNA to form DNA-carcinogen complex. This distorts the DNA double helix hence causes replication errors. It may also result in the removal of DNA nucleotides or cause DNA strands to break.

- *E.g.: tar in cigarettes*

3.3. Ionising Radiation

Ionising radiation causes mutations in DNA via strand breakages in the DNA backbone or through formation of pyrimidine dimers that led to a permanent base change in the DNA sequence.

- *E.g.: X-rays, gamma rays, UV rays*

3.4. Loss of Immunity (Infectious Agents)

Loss of immunity allows the presence of the infectious agents to result in a dysregulation of cell cycle checkpoints. Infectious agents may integrate their genetic material into human DNA, changed the sequence of the genes involved in the synthesis of proteins controlling the regulation of the normal cell cycle, leading to dysregulation of the cell cycle checkpoints.

- *E.g.: Human papillomavirus (HPV)*

Learning Outcome 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.

4 Important Genes Controlling The Regulation Of Cell Cycle

Proto-oncogenes and tumour suppressor genes are **normal** cellular genes.

- **Proto-oncogenes** are genes coding for proteins that promote normal cell growth and division.
    - The proteins promote the synthesis of organelles etc and are needed for the promotion of normal cell division.
- **Tumour suppressor genes** are genes coding for proteins that normally inhibit cell division.
    - The proteins function in cell cycle arrest, DNA repair, blocking angiogenesis and apoptosis (programmed cell death) etc.

However, mutation to these genes can result in the development of cancer.

- Proto-oncogene mutated to Oncogene → too much cell division (too much gas)
- Tumour suppressor gene mutated to non-functional form → uncontrol cell division (no brakes)

Such mutations lead to uncontrolled cell division, the formation of tumours and subsequently cancer.

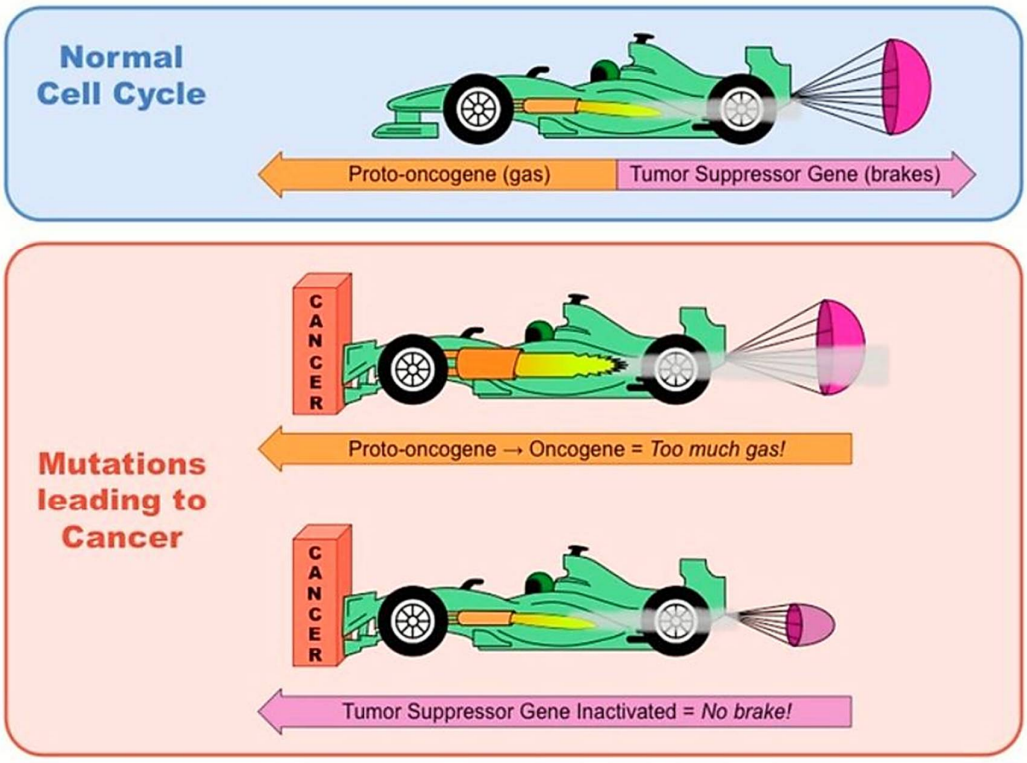


Fig. 4: Relationship between proto-oncogene and tumour suppressor gene

4.1 Proto-oncogenes

4.1.1 Proto-oncogenes mutate to become oncogenes

Proto-oncogenes are genes coding for proteins that promote normal cell growth and division.

Gain-in-function mutation converts a proto-oncogene to an oncogene, a gene coding for proteins that promote excessive cell growth and division.

- **Dominant** phenotype: Only 1 allele of the proto-oncogene needs to mutate to result in a gain-of-function mutation to become an oncogene. Oncogene products resulted in excessive cellular division.

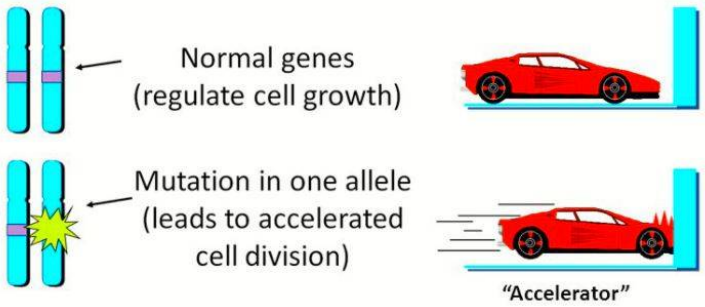


Fig. 4.1.1.1: Gain-in-function mutation of proto-oncogene resulting in oncogene

Gain-in-function mutation occurs via two ways:

1. an increase in amount of normal protein produced
    a. **translocation:** cancer cells are frequently found to contain chromosomes that have broken and rejoined incorrectly, translocating fragments from one chromosome to another. If a translocated proto-oncogene ends up near a highly active promoter, gene expression increases and more proteins are produced, making it an oncogene.

    b. **gene amplification**: gene amplification increases the number of copies of proto-oncogene in the cell

    c. **point mutation in the promoter or an enhancer** that controls a proto-oncogene causes an increase in its expression.

2. the malfunction of the proteins resulting in hyperactivity of the protein.
    a. **point mutation in the coding sequence**, changes the gene's product to a protein that is (i) more active or (ii) more resistant to degradation than the norm

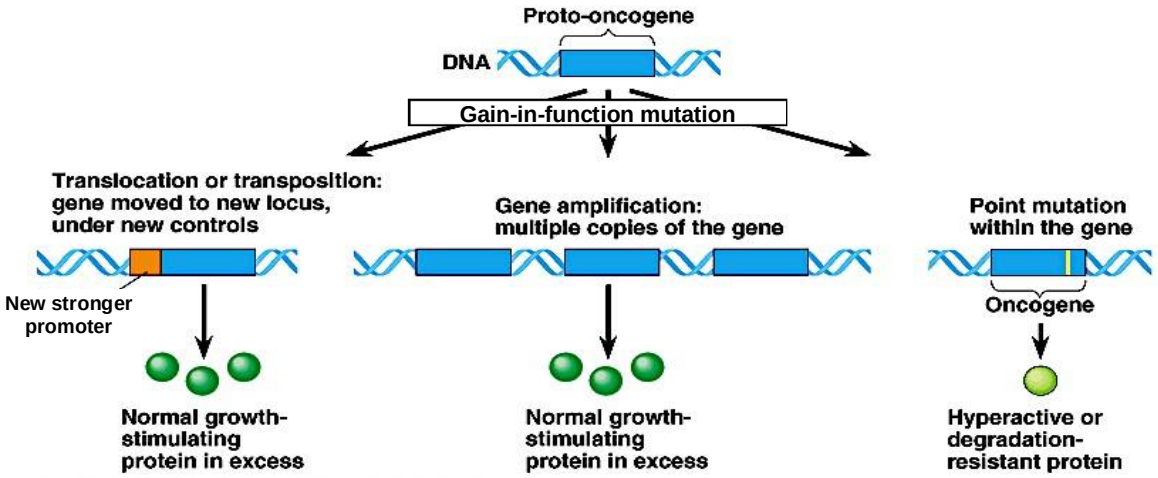


Fig. 4.1.1.2: Oncogene expression results in excessive cell division i.e. tumour formation

4.1.2 An example of a proto-oncogene: *Ras* gene

***Ras* gene codes for Ras proteins.**

Function of Ras protein: Transduces signal from extracellular growth factors to increase transcription of genes. This leads to increased translation of mRNA, and hence increased protein synthesis that promotes normal cell growth and division.

- Normally, such a pathway will not operate unless triggered by the appropriate growth factor.

Gain-in-function mutation: The *Ras* proto-oncogene mutated to a *Ras* oncogene. Expression of the mutated *Ras* gene leads to production of hyperactive Ras protein which activate the gene transcription even in the absence of any growth factors. This results in excessive cell growth and division.

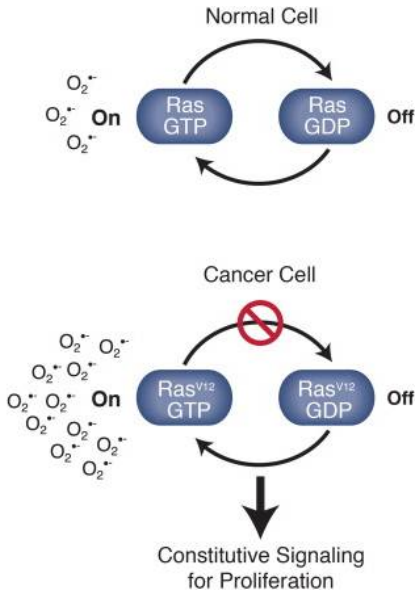


Fig. 4.1.1.1: Role of Ras protein

Checkpoint 3: Proto-oncogenes summary

3.1 What are the definitions of the following?

Proto-oncogenes	Oncogenes

3.2 What kind of mutation converts proto-oncogene to oncogene?

3.3 Summarise the Ras Gene example

Normal Ras protein	Mutated Ras protein

3.4 How do oncogenes increase the amount of protein present?

3.5 How does the mutation in the Ras gene lead to hyper-activity?

4.2 Tumour Suppressor Genes

4.2.1 Tumour suppressor genes normally inhibit cell division

Tumour suppressor genes are genes coding for proteins that normally inhibit cell division.

- The proteins function in cell cycle arrest, DNA repair, blocking angiogenesis and apoptosis (programmed cell death) etc.

Loss-of-function mutation of a tumour suppressor gene results in accumulation of mutations and excessive cell growth and division.

- **Recessive** phenotype: If only one copy of the allele undergoes loss-of-function mutation, the other normal allele can still result in the expression of a normal level of tumour suppressor proteins to suppress tumour formation.
- Both alleles of the tumour suppressor gene need to mutate to result in a loss-in-function mutation to result in excessive cellular division.

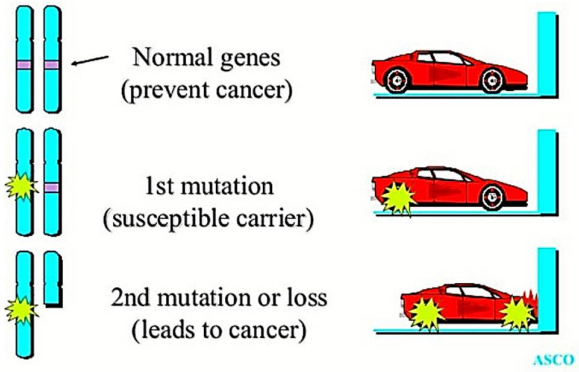


Fig. 4.2.1.1: Loss-of-function mutation of tumour suppressor gene

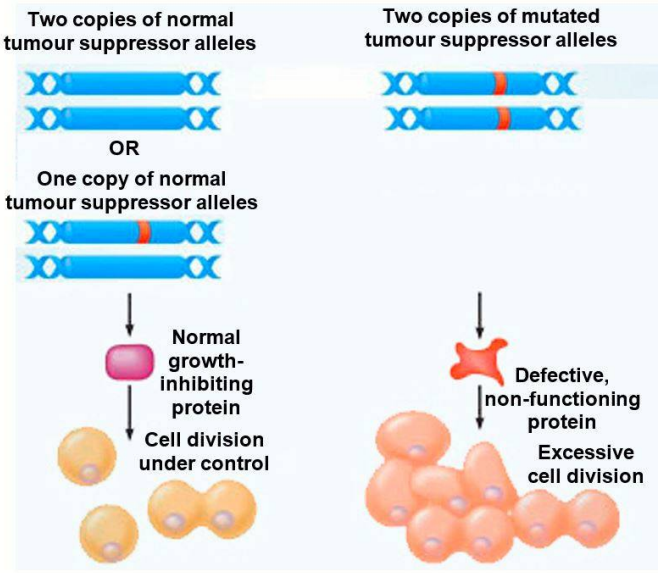


Fig. 4.2.1.2: Loss-of-function of both alleles resulting in tumour formation

4.2.2 An example of a tumour suppressor gene: *p53* gene

***p53* gene codes for p53 protein.**

Function of p53 protein: When triggered by presence of DNA damage or other stress signals, p53 protein is produced. p53 proteins prevent cells from passing on mutations due to DNA damage by activating genes that are involved in:

1. **cell cycle arrest**: halting of cell cycle prevents cells from entering next phase in cell division. This creates time to repair its DNA and prevent formation of mutant daughter cells.

2. **DNA repair**: repairs mutations that may lead to gain-of-function in proto-oncogenes or loss-of-function in tumour suppressor genes.

When the DNA damage is beyond repair,

3. **apoptosis**: initiates programmed cell death to eliminate the cells with irreparable mutations.

Loss-of-function mutation of **both *p53* alleles** produces defective p53 proteins that will not restrict cell growth and division of cells. Mutations are accumulated over time, hence leading to development of cancer cells.

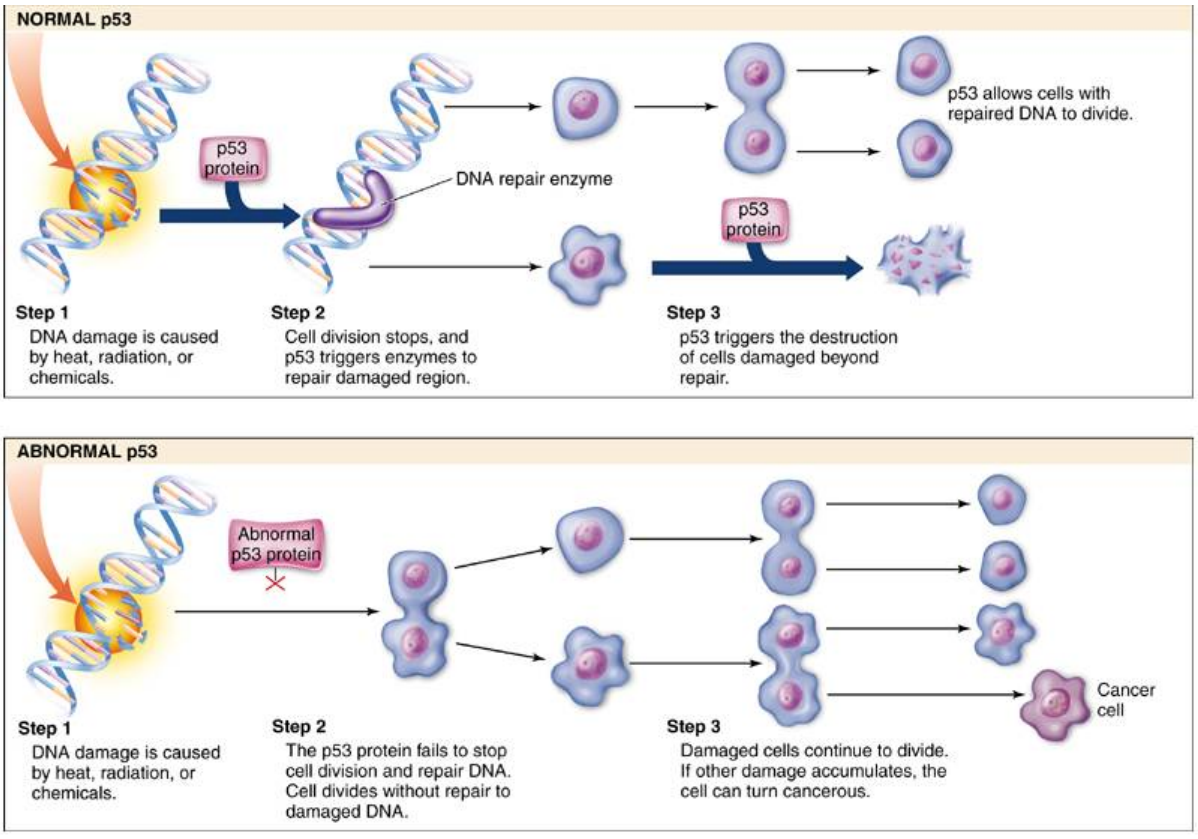


Fig. 4.2.2.1: Defective p53 proteins resulting in cancer cell formation

Learning Outcome 2(r):

Describe the development of cancer as a multi-step process that includes accumulation of mutations, angiogenesis and metastasis.

5 Multistep Model of Cancer Development

5.1 Cancer is an accumulation of mutations

Cancer cells form due to an accumulation of mutations over time in the cell's DNA i.e. cancer development is a multi-step process.

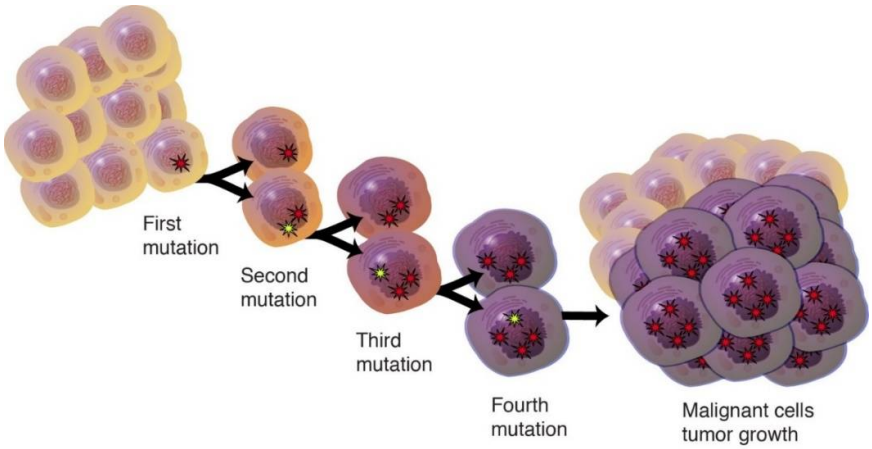


Fig. 5.1: Accumulation of mutations leading to cancer

5.2 Multistep model of Cancer development

It is relatively difficult to develop cancer because:

a. proofreading and DNA repair mechanisms check and repair gene mutations.

b. cells with irreparable mutation undergoes apoptosis activated by p53 proteins.

c. only mutations in the proto-oncogene or tumour suppressor gene will lead to cancer.

d. all the mutations will have to occur in a single cell before cancer cell forms.

Therefore, the probability of cancer occurrence increases steeply with age, as development of cancer requires accumulation of mutation in many genes.

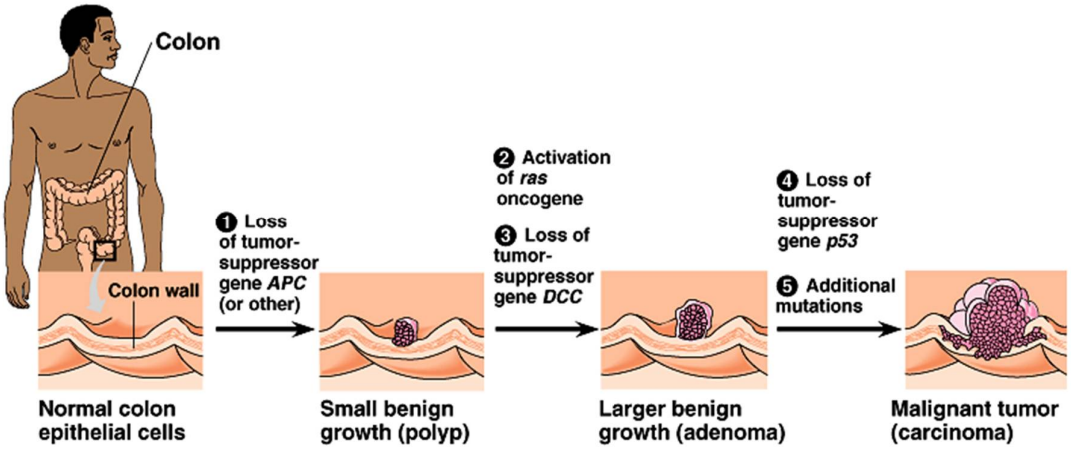


Fig. 5.2: Example of colonic cancer formation via accumulation of mutations

Multi-step model of cancer development involves:

1. Gain-in-Function mutation of proto-oncogene e.g. *Ras* gene (Refer to Section 4.1)

2. Loss-of-Function of tumour suppressor gene e.g. *p53* gene (Refer to Section 4.2)

3. Evasion of Apoptosis

5.3 Apoptosis (programmed cell death)

Apoptosis removes cells that are beyond repair. Mutant cells are continually removed from the body in this way to prevent further accumulation of mutations before they become cancerous.

Therefore, for a tumour to progress, it must inactivate the apoptotic machinery. Evasion of apoptosis is achieved by mutation in the tumour suppressor genes such as the *p53* gene.

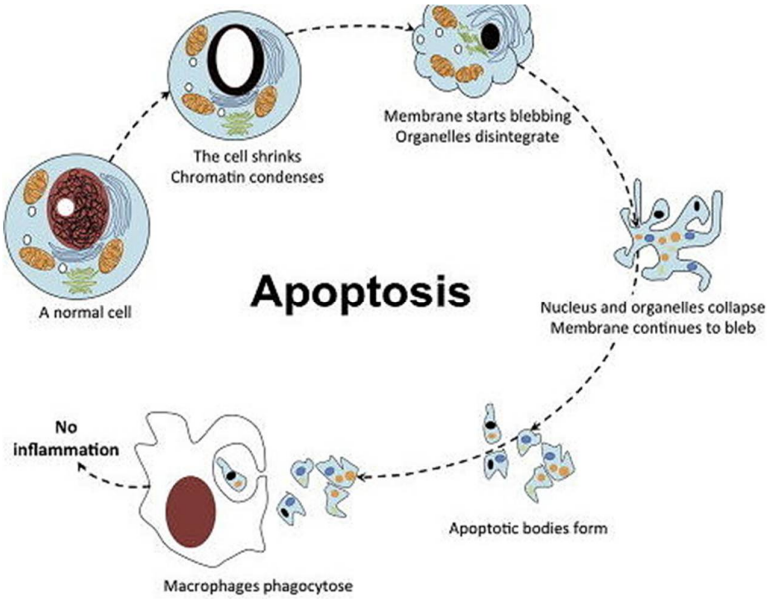


Fig. 5.3: Apoptosis

5.4 Expression of Telomerase Gene

The telomerase genes are found in the chromosomes in the nucleus. These genes code for the components of the enzyme, telomerase. In normal cells, the telomerase gene is no longer actively transcribed to form the enzymes.

However, cancer cells activate the telomerase gene (via mutation) to result in the production of enzyme telomerase to add new copies of telomeric repeat sequence. Their telomeres will be lengthened, hence allowing cells to divide infinitely. Cells with telomerase have limitless replicative potential.

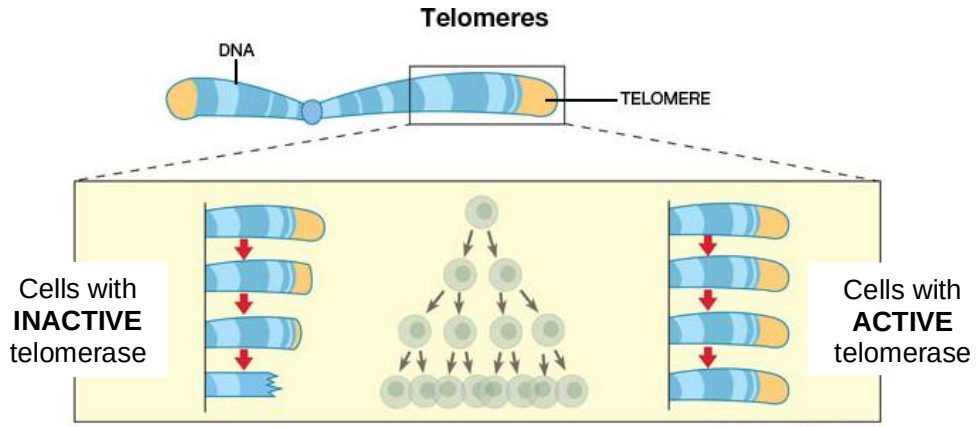


Fig. 5.4: Telomere length in cells with active and inactive telomerase

5.5 Angiogenesis

Most cancers develop gradually:

1. The first sign is often a polyp, a small benign growth in the colon lining.
2. The polyp looks normal, although they divide unusually frequently.
3. The tumour growths, and may eventually become **malignant**, gaining invasive property to extend to surrounding tissue. Such malignant cells displays disorganised pattern of growth with ragged borders.

Angiogenesis refers to the formation of blood vessels. This is a highly regulated process and seldom occurs in adults except in events of injury and wound repair.

In cancer cells, activation of genes involved in angiogenesis results in an increased production of signal molecules for angiogenesis. Cancer cells release these signal molecules to surrounding normal host tissue, which then produces proteins to encourage formation of new blood vessels.

Tumour angiogenesis is the proliferation of a network of blood vessels that penetrates cancerous growths. This supports cancer development by:

1. supplying nutrients and oxygen and removing metabolic waste products.
2. blood vessels also provide a route to the rest of the body, for **metastasis** (refer to Step 6).

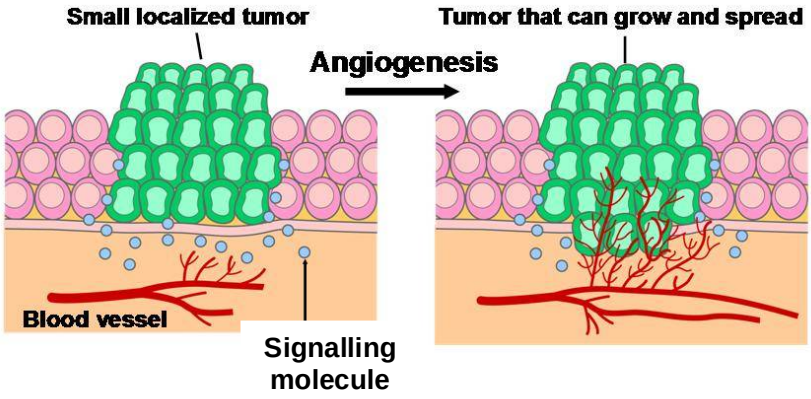


Fig. 5.5: Tumour Angiogenesis

5.6 Metastasis and Tissue Invasion

Metastasis, which occurs after angiogenesis, is the entry of cancer cells into the bloodstream or lymphatic system to travel to distant sites to establish new cancerous cellular colonies at distant sites (e.g. colon tumour cells travel to the brain to form a brain tumour).

- Metastasis is the deadliest aspect of cancer, being responsible for 90% of cancer associated deaths.
- By spreading throughout the body, a cancer becomes almost impossible to eradicate by either surgery or localised irradiation.

Tissue invasion refers to the direct migration and penetration of the cancer cells into neighbouring tissues. Due to the absence of adhesion proteins, cancer cells can detach from the cell mass in the primary tumour and are able to travel in the blood stream or lymph where they establish secondary tumours.

Cancer cells also have upregulated expression of genes encoding extracellular proteases to degrade cytoskeleton and filaments holding surrounding cells and tissue. This opens up paths for metastasis into nearby blood vessels and tissue layers.

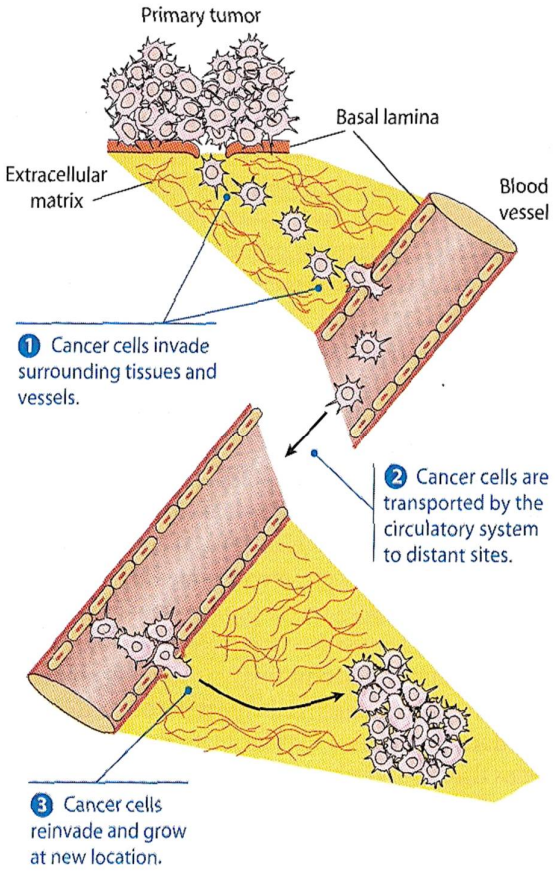


Fig. 5.6: Metastasis and tissue invasion

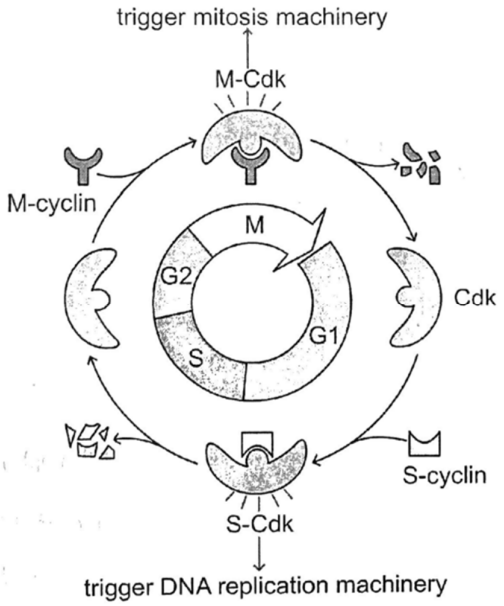
Annex : CYCLIN DEPENDENT KINASES IN CONTROL OF CELL CYCLE

Key components of the cell-cycle control system

Rhythmic fluctuations in the abundance and activity of various cell cycle control molecules pace the sequential events of the cell cycle. These regulatory molecules are mainly proteins of two types – **cyclin-dependent kinases (Cdks),** and **cyclins**.

(Kinases are enzymes that transfer phosphate groups from high energy donor molecules, such as ATP, to specific substrates, thus activating or inactivating these substrates.)

- Many of the Cdks are present at (constant concentration) in the growing cell, but their activities rise and fall as the cell progresses through the cycle.
  - This leads to cyclical changes in the phosphorylation of other intracellular proteins that initiate or regulate major events of the cell cycle.
- Cdks, as their name implies, are dependent on cyclins for their activity. This is because to be **active**, a Cdk must be attached to a cyclin to form a specific **cyclin-Cdk complex**.
- Unlike Cdks, cyclins undergo a cycle of synthesis and degradation in each cell cycle, and thus their concentrations in the cell fluctuate cyclically (*hence their name*).
- Cyclical changes in cyclin protein levels result in the cyclic assembly and activation of the **cyclin-Cdk complexes**. This activation in turn triggers cell-cycle events.



Annex: TYPES OF CANCER

- Sporadic cancer – Occurs by chance. Individuals with sporadic cancer usually do not have relatives with the same type of cancer.
- Hereditary cancer – Mutated gene is passed down from parent to child i.e. inherited. Individuals with hereditary cancers are more likely to have relatives with the same type of cancer. They may also develop the cancers earlier than average age and have more than one cancer.
- Familial cancer – Occurs due to a combination of genetic and environmental risk factors. Individuals with familial cancer may have one or more relatives with the same type of cancer but there is no specific pattern of inheritance.