KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University Established Under Section 3 of UGC Act 1956)
Pollachi Main Road, Eachanari Post, Coimbatore - 641 021. INDIA
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DEPARTMENT OF BIOCHEMISTRY

SYLLABUS

SUBJECT NAME: CORE ELECTIVE I - CANCER BIOLOGY

SUB.CODE: 15BCU505C

SEMESTER: V **CLASS: III B.Sc., BIOCHEMISTRY**

Programme Objective
This paper will initiate and promote the analysis of cancer as a complex biological system. This will encourage the emergence of integrative cancer biology as a distinct field. This paper gives on insight knowledge about the emerging themes and provides, in depth analysis of cancer and its therapeutic approaches.

Programme Learning Outcome
Students after completing this programme they are able to analyze what complex system holds beyond cancer, and this encourages them to learn in depth of emergence of integrative cancer biology and its therapeutic approaches.

UNIT I
Introduction: Cancer- definition, hallmarks of cancer, Distinction between normal cell and cancer cell, cytological changes in cancer cells, Molecular changes in cancer cells, Genetic changes in cancer cells, Types of cancer, development of cancer, causes of cancer, properties of cancer cells.

UNIT II
Mutagens and mutations: Mutagens and mutations, Mechanisms of oncogene activation, Role of growth factors and receptors in carcinogenesis, Retroviral oncogenes, protooncogenes, tumor suppressor genes -P53 and Rb and their functions.

UNIT III
Cell cycle: cell cycle-G1 to S, progression of S phase, G2 to M phase, Anaphase check points and components involved as regulators of check points, role of cyclins and CDKs.

UNIT-IV

Cell death: Types of cell death-apoptosis, necrosis and others. Apoptosis during developmental process and irregular apoptosis and disease. Death causing genes – Ceds, proteins – Caspases, mechanism of programmed cell death (PCD), Pathways of apoptosis-intrinsic and extrinsic.

UNIT V

Treatment of cancer: Early detection of cancer, molecular diagnosis, treatment -radio therapy, chemotherapy, immunotherapy and use of RNAi techniques and stem cells.

TEXT BOOKS

1. Papachristodoulou, D., Snape, A., Elliott, W. H., Elliott, D.C. (2014). *Biochemistry and Molecular Biology* (5th ed.). New York, Oxford University Press.
2. Hayat, M.A. (2010). *Methods of Cancer Diagnosis, Therapy and Prognosis*. Springer Science. ISBN: 978--420-8441-6.

REFERNCES

1. Karp, G. (2013). *Cell and Molecular Biology*. (7th ed.) New York, John Wiley and Sons. Inc
2. Lodish, H., Ber, A., Zipuoskry, L.S., Matsudaira, P., Bahimore, D., Damell J. (2017) *Molecular Biology*. (8th ed.). W.H Freeman G Co.

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LECTURE PLAN

STAFF NAME: Dr. A. MANIMARAN

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S.No	Duration of Period	Topics to be Covered	Books referred with Page No.	Web page referred
UNIT I - INTRODUCTION				
1	1	Introduction to cancer	R1: 694-695	
2	1	Cancer - Definition, Hallmarks of cancer	R1: 694-695	
3	1	Signs and symptoms of cancer	R1: 694-695	
4	1	Distinction between normal cell and cancer cell	R1: 695-696	
5	1	Cytological changes in cancer cells	R1: 696-697	
6	1	Molecular changes in cancer cells	R2: 936-940	
7	1	Genetic changes in cancer cells	R2: 943-946	
8	1	Types of cancer	R2: 936-937	
9	1	Types of cancer - Difference between metastasis and angiogenesis	R2: 936-937	
10	1	Development of cancer	R2: 937-938	
11	1	Causes of cancer	R1: 696-697	
12	1	Major risk factors of cancer	R1: 696-697	
13	1	Properties of cancer cells	R1: 696-697	
14	1	Revision and Possible QP discussion		
15	1	Revision and Possible QP discussion		
Total No. of Hours planned for Unit I is 15 hours				
UNIT II - MUTAGENS AND MUTATIONS				
1	1	Introduction to Mutations	R2: 161-162	
2	1	Mutagens and Mutations	R2: 162-163	
3	1	Different types of mutation	R2: 162-163	

4	1	Mechanisms of oncogene activation	R1: 944-955	
5	1	Oncogene - Ras	R1: 948-950	
6	1	Role of growth factors in carcinogenesis	R2: 961-962	
7	1	Role of receptors in carcinogenesis	R2: 168-175	
8	1	Retroviral oncogenes	R1: 237-239	
9	1	Protooncogenes	R1: 701-702	
10	1	Tumor suppressor genes	R1: 702-705	
11	1	P53 and their functions	R1: 705-716	
12	1	Rb and their functions	R1: 705-716	
13	1	Differences between Oncogene and Tumor suppressor gene and its mechanism	R1: 702-705	
14	1	Revision and Possible QP discussion		
15	1	Revision and Possible QP discussion		
Total No. of Hours planned for Unit II is 15 hours				
UNIT III – CELL CYCLE				
1	1	Introduction to Cell cycle	R1: 602-604	
2	1	Cell cycle - G1 to S phase	R1: 602-604	
3	1	Progression of S phase	R1: 602-604	
4	1	G2 to M phase	R1: 609-610	
5	1	Mitotic phases	R1: 609-610	
6	1	Meiosis	R1: 609-610	
7	1	G0 Phase	R1: 602-604	
8	1	Checkpoints involved in cell cycle	R1: 619-620	
9	1	Anaphase check points	R1: 619-620	
10	1	Components involved as regulators of check points	R2: 886-890	
11	1	Role of Cyclins	R2: 857-886	
12	1	Role of CDKs	R2: 857-886	
13	1	Mechanism of Cyclin and CDK in cell cycle	R2: 857-886	
14	1	Revision and Possible QP discussion		
15	1	Revision and Possible QP discussion		
Total No. of Hours planned for Unit III is 15 hours				
UNIT IV – CELL DEATH				
1	1	Introduction to types of cell death	R2: 924-925	
2	1	Apoptosis	R2: 924-925	
3	1	Necrosis	R2: 930-931	
4	1	Other types of cell death	R2: 930-931	
5	1	Apoptosis during developmental process	R2: 950-960	
6	1	Irregular apoptosis and disease	R1: 950-960	
7	1	Differences between Apoptosis and Necrosis	R2: 932-935	
8	1	Death causing genes - Ceds	T1: 512-514	
9	1	Proteins – Caspases	T1: 515-516	

10	1	Cystine protease in cell cycle	R2: 927-929	
11	1	Mechanism of programmed cell death (PCD)	T1: 513-515	
12	1	Pathways of apoptosis - Intrinsic	T1: 515-516	
13	1	Pathways of apoptosis - Extrinsic	T1: 516-517	
14	1	Revision and Possible QP discussion		
15	1	Revision and Possible QP discussion		
Total No. of Hours planned for Unit IV is 15 hours				
UNIT V – TREATMENT OF CANCER				
1	1	Introduction to early detection of cancer	T2: 17-37	
2	1	Screening test for cancers	T2: 39-48	
3	1	CT Scanning, biopsy, Mammography	T2: 48-53	
4	1	Molecular diagnosis	T2: 75-92	
5	1	Treatment	T2: 93-107	
6	1	Radiotherapy	T2: 109-128	
7	1	Chemotherapy	T2: 193-200	
8	1	Chemotherapeutic agents for treatment of cancer	T2: 193-200	
9	1	Chemoprevention	T2: 166-170	
10	1	Immunotherapy	T2: 129-159	
11	1	Use of RNAi techniques	T2: 333-336	
12	1	sRNAi techniques for single cell targeted therapy	T2: 338-347	
13	1	Use of stem cells	T2: 179-191	
14	1	Revision and Possible QP discussion		
15	1	Revision and Possible QP discussion		
Total No. of Hours planned for Unit V is 15 hours				

TEXT BOOKS

T1. Papachristodoulou, D., Snape, A., Elliott, W. H., Elliott, D.C. (2014). *Biochemistry and Molecular Biology* (5th ed.). New York, Oxford University Press.

T2. Hayat, M.A. (2010). *Methods of Cancer Diagnosis, Therapy and Prognosis*. Springer Science. ISBN: 978--420-8441-6.

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R1. Karp, G. (2013). *Cell and Molecular Biology*. (7th ed.) New York, John Wiley and Sons. Inc

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UNIT I

Introduction: Cancer- definition, hallmarks of cancer, Distinction between normal cell and cancer cell, cytological changes in cancer cells, Molecular changes in cancer cells, Genetic changes in cancer cells, Types of cancer, development of cancer, causes of cancer, properties of cancer cells.

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Cancer - Definition

A term for diseases in which abnormal cells divide without control and can invade nearby tissues. Cancer cells can also spread to other parts of the body through the blood and lymph systems. There are several main types of cancer. Carcinoma is a cancer that begins in the skin or in tissues that line or cover internal organs. Sarcoma is a cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Leukemia is a cancer that starts in blood-forming tissue, such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the blood. Lymphoma and multiple myeloma are cancers that begin in the cells of the immune system. Central nervous system cancers are cancers that begin in the tissues of the brain and spinal cord. Also called malignancy.

Hallmarks of Cancer

Cancer develops over time as mutations and genetic changes accumulate in cells. The traits a normal cell acquires as it slowly transforms into a precancerous one and ultimately into cancer are called the "Hallmarks of Cancer".

1. SELF-SUFFICIENT CELL DIVISION

Cells are organized into tissues and tissues are organized into organs with specific functions, such as the heart, lungs and skin. The cells of each organ must work and communicate as a team to function properly. When growth is necessary, cells collectively send signals to other cells to divide. Cancer cells, on the other hand, do not behave as team members. They control their own proliferation by producing growth signals themselves or by having overactive signal receptors.

2. INSENSITIVITY TO SIGNALS TO STOP CELL DIVISION

Just as there are signals that stimulate cell proliferation, there are signals that put the brakes on cell growth and proliferation. Cancer cells are able to interrupt or ignore these inhibitory messages. Usually this is a result of mutations or alterations to genes known as tumor suppressor genes, which normally control a cell's response to external and internal cues to exit the cell division cycle.

3. RESISTING CELL DEATH

When cells become old or damaged they are programmed to die in a process called apoptosis. This is the body's way of limiting growth and discarding cells with damaged DNA in order to prevent propagation of DNA errors. Cancer cells are dangerous because they avoid the normal cell death cycle and continue to accumulate in the body. Apoptosis signals can be disrupted when tumor suppressor genes suffer mutations or other damage.

4. LIMITLESS REPRODUCTIVE POTENTIAL

The accumulation of the billions of cells it takes to form a tumor requires uncontrolled cell division, avoidance of apoptosis and the ability to replicate an unlimited number of times. In normal cell division, a small portion of the end of each chromosome, in a region called the telomere, is lost every time DNA is copied. Eventually the loss of telomere reaches a critical point and the cell can no longer divide and replicate. In this way, healthy cells self-limit their replication, but activation of an enzyme called telomerase can maintain telomeres and allow the cell to continue to replicate indefinitely. More than 90 percent of "immortalized" cancer cells have activated telomerase, while most normal cells do not.

5. CREATING THEIR OWN BLOOD SUPPLY

In order for a tumor to grow it needs a greater and greater blood supply to provide oxygen and nutrients to the increasing number of cells. A tumor is able to stimulate formation of new blood

vessels, a process known as angiogenesis, to supply it with adequate nutrients and promote its growth.

6. ABILITY TO INVADE OTHER ORGANS

Cancer cells, unlike normal cells, can metastasize – break through tissue barriers and spread from one organ to another. Sometimes they do this by entering the newly formed blood vessels created by the tumor.

7. ABILITY TO SURVIVE WITH LITTLE OXYGEN

Even with an increased blood supply, cells in the interior of a tumor may be oxygen- and nutrient-deprived. This would be detrimental to normal cells, which use oxygen to convert glucose to energy through the process of aerobic metabolism. Cancer cells have the ability to switch from aerobic to anaerobic (oxygen-free) glucose metabolism (glycolysis) to allow oxygen-deprived cells to continue to produce energy and survive.

8. EVADING THE IMMUNE SYSTEM

When functioning properly, the body's immune system detects and destroys foreign and abnormal cells. Although the process is not fully understood, there is evidence that cancer cells are able to evade destruction by the body's immune defenses to some degree, allowing them to proliferate and invade other tissues.

These eight hallmark characteristics that distinguish cancer cells from normal ones are made possible by two final characteristics that enable the alterations necessary for a cell to become cancerous:

9. GENOMIC INSTABILITY

Genes are segments of DNA that provide the instructions for all cellular activity. The accumulation of changes to specific genes that promote cell proliferation (e.g., activating oncogenes) or disrupt control mechanisms (e.g., tumor suppressor genes) can result in normal cells acquiring hallmark characteristics and transforming into cancer cells.

10. INFLAMMATION

Chronic inflammation can result in conditions that promote proliferation, cell survival and angiogenesis. Inflammation can also enhance production of free radicals that can damage DNA.

Distinction between normal cell and cancer cell

All living organisms are composed of cells. These cells grow and divide in a controlled manner in order for the organism to function properly. Changes in normal cells can cause them to grow uncontrollably. This uncontrollable growth is the hallmark of cancer cells.

NORMAL CELL PROPERTIES

Normal cells have certain characteristics that are important for the proper functioning of tissues, organs, and body systems. These cells have the ability to reproduce correctly, stop reproducing when necessary, remain in a specific location, become specialized for specific functions, and self destruct when necessary.

- **Cell Reproduction:** Cell reproduction is needed to replenish the cell population that ages or becomes damaged or destroyed. Normal cells reproduce properly. Except for sex cells, all cells of the body reproduce by mitosis. Sex cells reproduce through a process called meiosis.
- **Cell Communication:** Cells communicate with other cells through chemical signals. These signals help normal cells to know when to reproduce and when to stop reproducing. Cell signals are usually transmitted into a cell by specific proteins.

- **Cell Adhesion:** Cells have adhesion molecules on their surface that allow them to stick to the cell membranes of other cells. This adhesion helps cells to stay in their proper location and also aids in the passage of signals between cells.
- **Cell Specialization:** Normal cells have the ability to differentiate or develop into specialized cells. For example, cells can develop into heart cells, brain cells, lung cells or any other cell of a specific type.
- **Cell Death:** Normal cells have the ability to self destruct when they become damaged or diseased. They undergo a process called apoptosis in which cells break down and are disposed of by white blood cells.

CANCER CELL PROPERTIES

Cancer cells have characteristics that differ from normal cells.

- **Cell Reproduction:** Cancer cells acquire the ability to reproduce uncontrollably. These cells may have gene mutations or chromosome mutationsthat affect the reproductive properties of the cells. Cancer cells gain control of their own growth signals and continue to multiply unchecked. They don't experience biological aging and maintain their ability to replicate and grow.
- **Cell Communication:** Cancer cells lose the ability to communicate with other cells through chemical signals. They also lose sensitivity to anti-growth signals from surrounding cells. These signals normally restrict cellular growth.
- **Cell Adhesion:** Cancer cells lose the adhesion molecules that keep them bonded to neighboring cells. Some cells have the ability to metastasize or spread to other areas of the body through the blood or lymph fluid. Once in the bloodstream, cancer cells release chemical messengers called chemokines that enable them to pass through blood vessels into the surrounding tissues.
- **Cell Specialization:** Cancer cells are unspecialized and do not develop into cells of a specific type. Similar to stem cells, cancer cells proliferate or replicate many times, for long periods of time. Cancer cell proliferation is rapid and excessive as these cells spread throughout the body.
- **Cell Death:** When the genes in a normal cell are damaged beyond repair, certain DNA checking mechanisms signal for cell destruction. Mutations that occur in gene checking mechanisms allow for the damages to go undetected. This results in the loss of the cell's ability to undergo programmed cell death.

Causes of cancer

Cancer results from the development of abnormal properties in normal cells that enable them to grow excessively and spread to other locations. This abnormal development can be caused by mutations that occur from factors such as chemicals, radiation, ultraviolet light, and chromosome replication errors. These mutagens alter DNA by changing nucleotide bases and can even change the shape of DNA. The altered DNA produces errors in DNA replication, as well as errors in protein synthesis. These changes influence cell growth, cell division, and cell aging.

Viruses also have the ability to cause cancer by altering cell genes. Cancer viruses change cells by integrating their genetic material with the host cell's DNA. The infected cell is regulated by the viral genes and gains the ability to undergo abnormal new growth. Several viruses have been

linked to certain types of cancer in humans. The Epstein-Barr virus has been linked to Burkitt's lymphoma, the hepatitis B virus has been linked to liver cancer, and the human papilloma viruses have been linked to cervical cancer

Cytological changes in Cancer Cell

1. The tumor cells are much rounded in shape compared to normal cells.
2. Mitochondrial size is said to be smaller in cancer cells than the normal. In cancer cells, the alternation is extremely frequent mitochondrial swelling and it appears denser. Some granules are smaller than mitochondria, termed as growth granules, which are found only in cancer cells.
3. Yoshida and Ehrlick showed that Golgi apparatus are greatly reduced in size in cancer cell. The mammary carcinoma of mice contains Brittner's milk factor, which is virus like particles in the Golgi bodies.
4. Golgi bodies and centrosome actually determine the polarity of the cells. These are generally lost in cancer cells. Abnormal divisions of centrosome often give rise to multipolar spindle in cancer cells.
5. Some inclusions due to persistence of cellular activity are found in tumor cells, e.g., milk in adenocarcinoma in breast, colloid droplets in cytoplasm of thyroid cancer.
6. The nucleus is larger and nucleocytoplasmic ratio is altered. The nucleolus is also larger in size than normal cells.
7. Chromosomal abnormality is one of the most cytological changes that occurs in cancer cells. The tumor cells have an abnormal number of chromosomes, generally too many (aneuploidy). They often contain translocations. The first discovered Philadelphia chromosome was found in all hematopoietic cells with myelogeneous leukemia, where the chromosome results from a translocation between chromosome 9 and 22. Such translocation between chromosome 8 and 14 has also been observed in Burkitts lymphoma.

Another common chromosomal abnormality in tumor cells is the localized reduplication of DNA to produce as many as 100 copies in a given region. It may be tandemly organized at a single site or it may exist as small independent chromosome like structure. The former case leads to a homogeneously staining region (HSR) that is visible under the microscope.

Molecular and Genetic changes in cancer cells

Only a small number of the approximately 35,000 genes in the human genome have been associated with cancer. Alterations in the same gene often are associated with different forms of cancer. These malfunctioning genes can be broadly classified into three groups. The first group, called proto-oncogenes, produces protein products that normally enhance cell division or inhibit normal cell death. The mutated forms of these genes are called oncogenes. The second group, called tumor suppressors, makes proteins that normally prevent cell division or cause cell death. The third group contains DNA repair genes, which help prevent mutations that lead to cancer. Proto-oncogenes and tumor suppressor genes work much like the accelerator and brakes of a car, respectively. The normal speed of a car can be maintained by controlled use of both the accelerator and the brake. Similarly, controlled cell growth is maintained by regulation of proto-oncogenes, which accelerate growth, and tumor suppressor genes, which slow cell growth. Mutations that produce oncogenes accelerate growth while those that affect tumor suppressors prevent the normal inhibition of growth. In either case, uncontrolled cell growth occurs. Genetic mutations are responsible for the generation of cancer cells and are thus present in all cancers. These mutations alter the quantity or function of protein products that regulate cell growth and

division and DNA repair. Two major categories of mutated genes are oncogenes and tumor suppressor genes.

Oncogenes

These are abnormal forms of normal genes (proto-oncogenes) that regulate various aspects of cell growth. Mutation of these genes may result in direct and continuous stimulation of the pathways (eg, cell surface growth factor receptors, intracellular signal transduction pathways, transcription factors, secreted growth factors) that control cellular growth and division, DNA repair, angiogenesis, and other physiologic processes.

There are > 100 known oncogenes that may contribute to human neoplastic transformation. For example, the RAS gene encodes the ras protein, which carries signals from membrane bound receptors down the RAS-MAPK pathway to the cell nucleus, and thereby regulates cell division. Mutations may result in the inappropriate activation of the ras protein, leading to uncontrolled cell growth. In fact, the ras protein is abnormal in about 25% of human cancers. Other oncogenes have been implicated in specific cancers. These include

HER2-NEU (amplified but not mutated in breast cancer)

BCR-ABL (a translocation of 2 genes that underlies chronic myelocytic leukemia and some B-cell acute lymphocytic leukemia's)

C-MYC (Burkitt lymphoma)

N-MYC (small cell lung cancer, neuroblastoma)

Mutated EGFR (adenocarcinoma of the lung)

EML4-ALK (a translocation that activates the ALK tyrosine kinase and causes a unique form of adenocarcinoma of the lung)

Specific oncogenes may have important implications for diagnosis, therapy, and prognosis (see individual discussions under the specific cancer type).

Oncogenes typically result from acquired somatic cell mutations secondary to point mutations (eg, from chemical carcinogens), gene amplification (eg, an increase in the number of copies of a normal gene), or translocations (in which pieces of different genes merge to form a unique sequence). These changes may either increase the activity of the gene product (protein) or change its function. Occasionally, mutation of genes results in inheritance of a cancer predisposition, as in the inherited cancer syndrome associated with mutation and loss of function of BRCA1, BRCA2, or p53.

Tumor suppressor genes

Genes such as the p53 gene play a role in normal cell division and DNA repair and are critical for detecting inappropriate growth signals or DNA damage in cells. If these genes, as a result of inherited or acquired mutations, become unable to function, the system for monitoring DNA integration becomes inefficient, cells with spontaneous genetic mutations persist and proliferate, and tumors result.

As with most genes, 2 alleles are present that encode for each tumor suppressor gene. A defective copy of one gene may be inherited, leaving only one functional allele for the individual tumor suppressor gene. If a mutation is acquired in the other allele, the normal protective mechanism of the 2nd normal tumor suppressor gene is lost. For example, the retinoblastoma (RB) gene encodes for the protein Rb, which regulates the cell cycle by stopping DNA replication. Mutations in the RB gene family occur in many human cancers, allowing affected cells to divide continuously.

Another important regulatory protein, p53, prevents replication of damaged DNA in normal cells and promotes cell death (apoptosis) in cells with abnormal DNA. Inactive or altered p53 allows cells with abnormal DNA to survive and divide. Mutations are passed to daughter cells, conferring a high probability of replicating error-prone DNA, and neoplastic transformation results. The p53 gene is defective in many human cancers. As with oncogenes, mutation of tumor suppressor genes such as p53 or RB in germ cell lines may result in vertical transmission and a higher incidence of cancer in offspring.

Chromosomal abnormalities

Gross chromosomal abnormalities (see Overview of Chromosomal Anomalies) can occur through deletion, translocation, or duplication. If these alterations activate or inactivate genes that result in a proliferative advantage over normal cells, then a tumor may develop. Chromosomal abnormalities occur in most human cancers. In some congenital diseases (Bloom syndrome, Fanconi anemia, Down syndrome), DNA repair processes are defective and chromosomes breaks are frequent, putting children at high risk of developing acute leukemia and lymphomas.

Other influences

Most epithelial cancers likely result from a sequence of mutations that lead to neoplastic conversion. For example, the development of tumor in familial polyposis takes place through a sequence of genetic events: epithelium hyperproliferation (loss of a suppressor gene on chromosome 5), early adenoma (change in DNA methylation), intermediate adenoma (overactivity of the RAS oncogene), late adenoma (loss of a suppressor gene on chromosome 18), and finally, cancer (loss of a gene on chromosome 17). Further genetic changes may be required for metastasis.

Telomeres are nucleoprotein complexes that cap the ends of chromosomes and maintain their integrity. In normal tissue, telomere shortening (which occurs with aging) results in a finite limit in cell division. The enzyme telomerase, if activated in tumor cells, provides for new telomere synthesis and allows continuous proliferation of tumors.

Types of cancer

Our bodies are made up of billions of cells. The cells are so small that they can only be seen under a microscope. These cells are grouped together to make up the tissues and organs of our bodies. These cells are basically the same, but they vary in some ways. This is because the body organs do very different things. For example, nerves and muscles do very different things. So nerve and muscle cells have different structures.

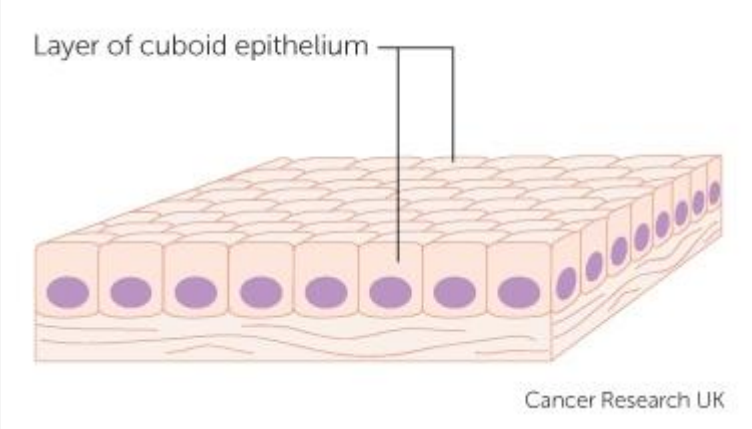
Cancers can be grouped according to the type of cell they start in. There are 5 main categories

- **Carcinoma** – cancer that begins in the skin or in tissues that line or cover internal organs. There are a number of subtypes, including adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, and transitional cell carcinoma
- **Sarcoma** – cancer that begins in the connective or supportive tissues such as bone, cartilage, fat, muscle, or blood vessels
- **Leukaemia** – cancer that starts in blood forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and go into the blood
- **Lymphoma and myeloma** – cancers that begin in the cells of the immune system
- **Brain and spinal cord cancers** – these are known as central nervous system cancers

Cancers can also be classified according to where they start in the body, such as breast cancer or lung cancer.

Carcinomas

Carcinomas start in epithelial tissues. These cover the outside of the body as the skin. They also cover and line all the organs inside the body, such as the organs of the digestive system. And they line the body cavities, such as the inside of the chest cavity and the abdominal cavity.

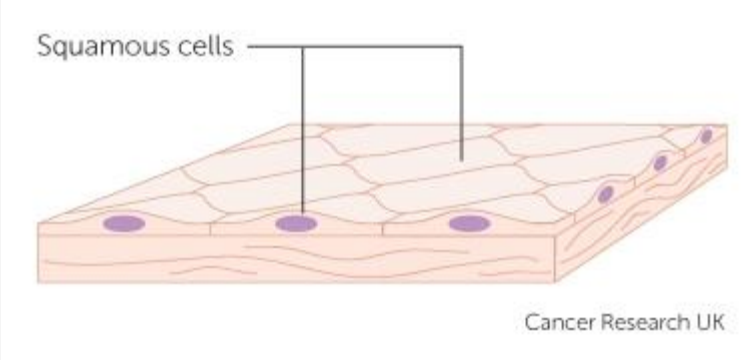


Carcinomas are the most common type of cancer.

There are different types of epithelial cells and these can develop into different types of carcinoma. These include those below.

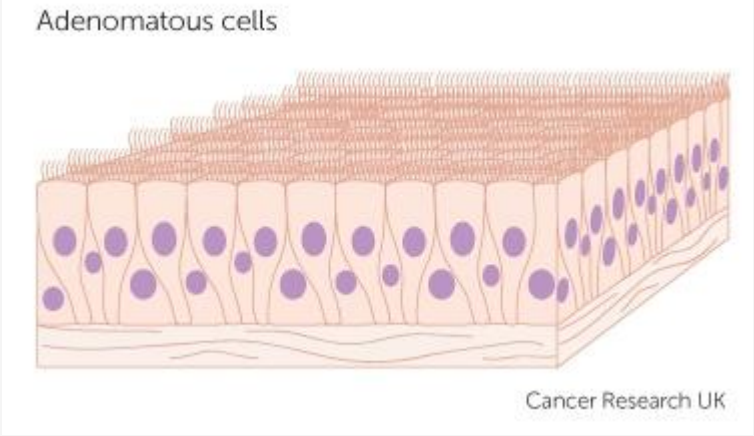
Squamous cell carcinoma

Squamous cell carcinoma starts in squamous cells. These are the flat, surface covering cells found in areas such as the skin or the lining of the throat or food pipe (oesophagus).



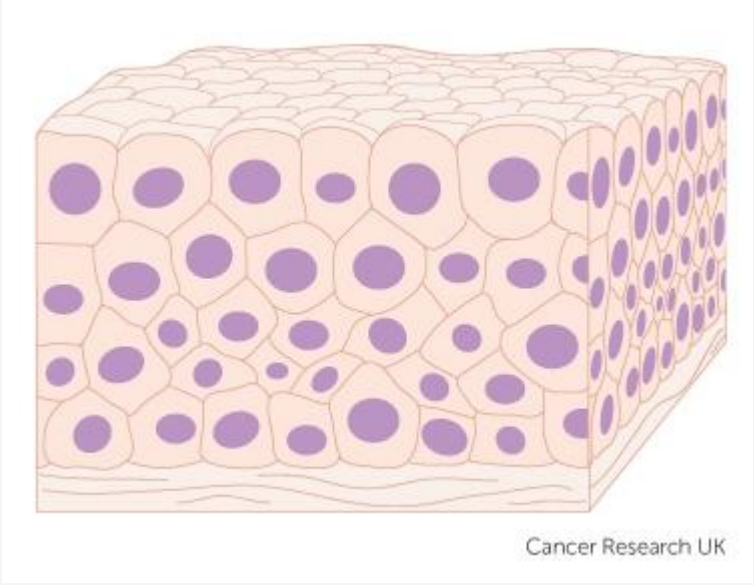
Adenocarcinoma

Adenocarcinomas start in glandular cells called adenomatous cells that produce fluids to keep tissues moist.



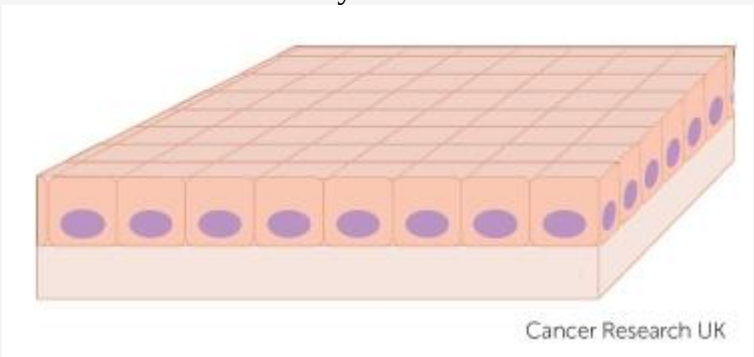
Transitional cell carcinoma

Transitional cells are cells that can stretch as an organ expands, and they make up tissues called transitional epithelium. An example is the lining of the bladder. Cancers that start in these cells are called transitional cell carcinoma.



Basal cell carcinoma

Basal cells are found in the deepest layer of skin cells. Cancers that start in these cells are called basal cell carcinomas. They are common.



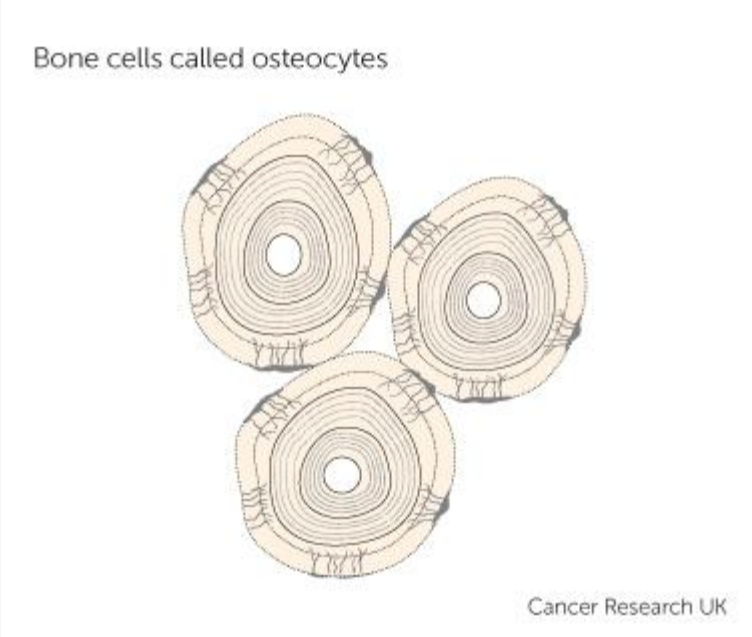
Sarcomas

Sarcomas start in connective tissues, which are the supporting tissues of the body. Connective tissues include the bones, cartilage, tendons and fibrous tissue that support the body organs.

Sarcomas are much less common than carcinomas. They are usually grouped into two main types – bone sarcomas (osteosarcoma) and soft tissue sarcomas. Altogether, these make up less than 1 in every 100 cancers diagnosed (1%).

Bone sarcomas

Sarcomas of bone start from bone cells.



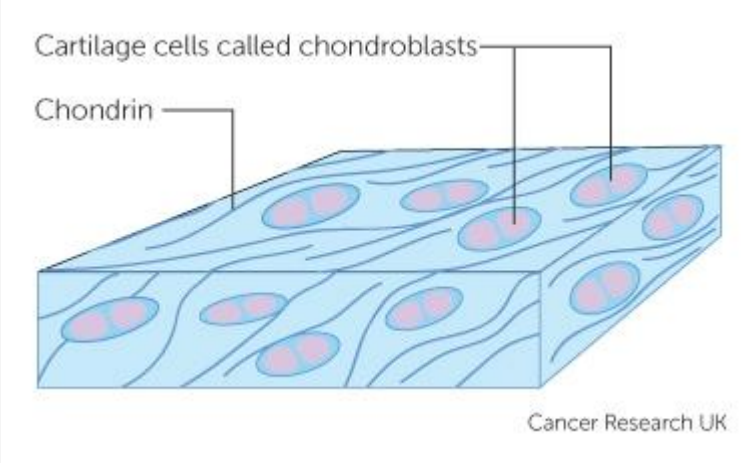
Bone cancers

Soft tissue sarcomas

Soft tissue sarcomas are rare but the most common types start in cartilage or muscle.

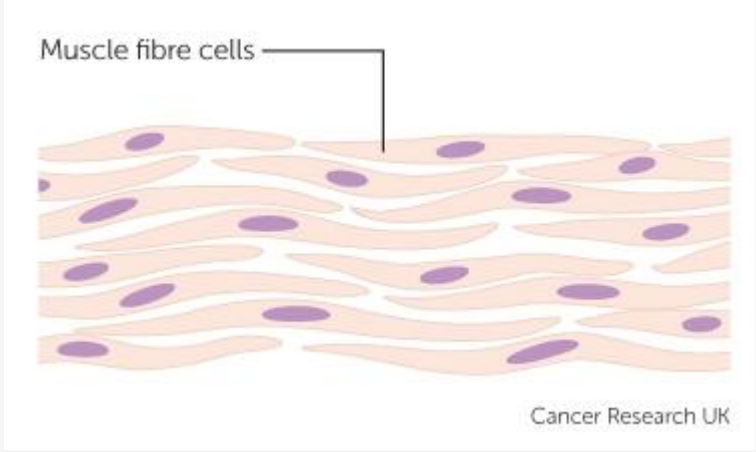
Cartilage

Cancer of the cartilage is called chondrosarcoma.



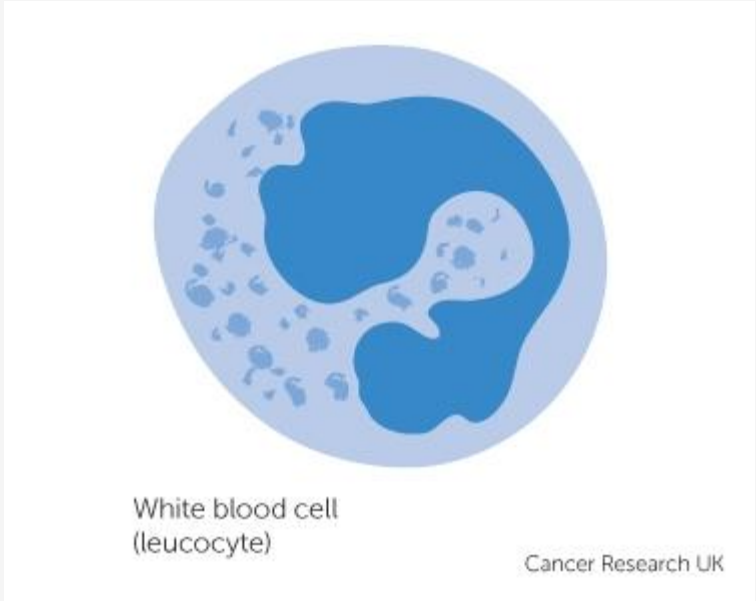
Muscle

Cancer of muscle cells is called rhabdomyosarcoma.



Leukaemias – cancers of blood cells

Leukaemia is a condition in which the bone marrow makes too many white blood cells. The blood cells are not fully formed and so don't work properly to fight infection. The cells build up in the blood.



Leukaemias are uncommon and make up 3 out of 100 of all cancer cases (3%). But they are the most common type of cancer in children.

There are different types of leukaemia.

Lymphomas and myeloma – lymphatic system cancers

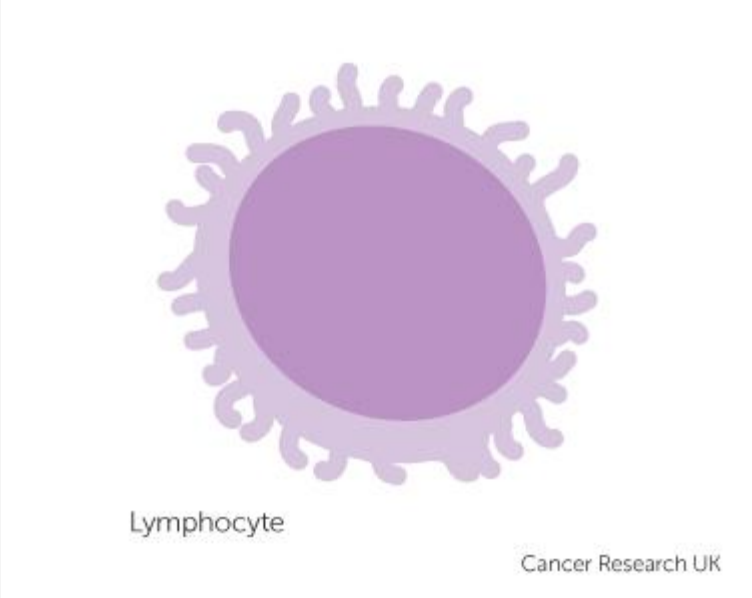
Lymphatic system cancers include lymphomas and myeloma.

Lymphomas

Lymphomas start from cells in the lymphatic system. The lymphatic system is a system of tubes and glands in the body that filters body fluid and fights infection. It is made up of the lymph glands, lymphatic vessels and the spleen.

Because the lymphatic system runs all through the body, lymphoma can start just about anywhere. Some of the white blood cells (lymphocytes) start to divide abnormally. And they

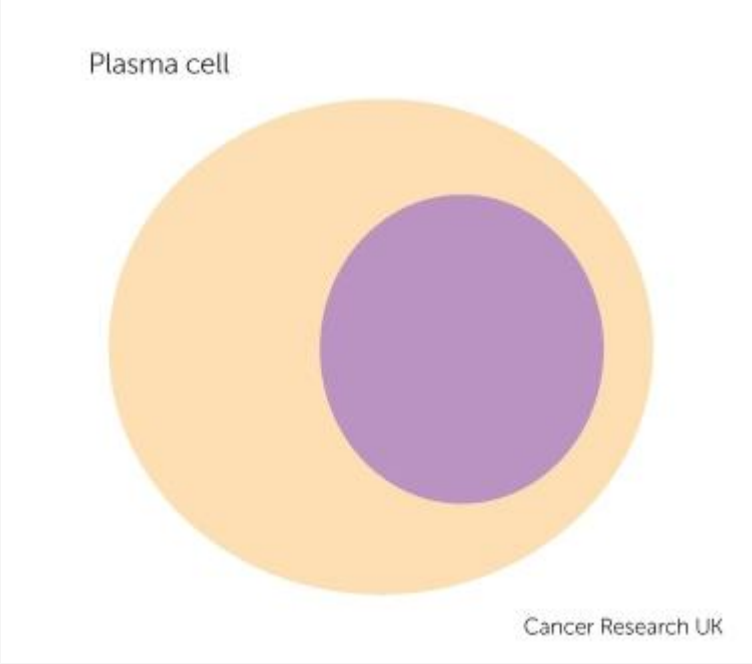
don't naturally die off as they usually do. These cells start to divide before they are fully mature so they can't fight infection.



The abnormal lymphocytes start to collect in the lymph nodes or other places such as the bone marrow or spleen. They can then grow into tumours.

Myeloma

Myeloma is also known as multiple myeloma. It is a cancer that starts in plasma cells. Plasma cells are a type of white blood cell made in the bone marrow. They produce antibodies, also called immunoglobulins, to help fight infection.

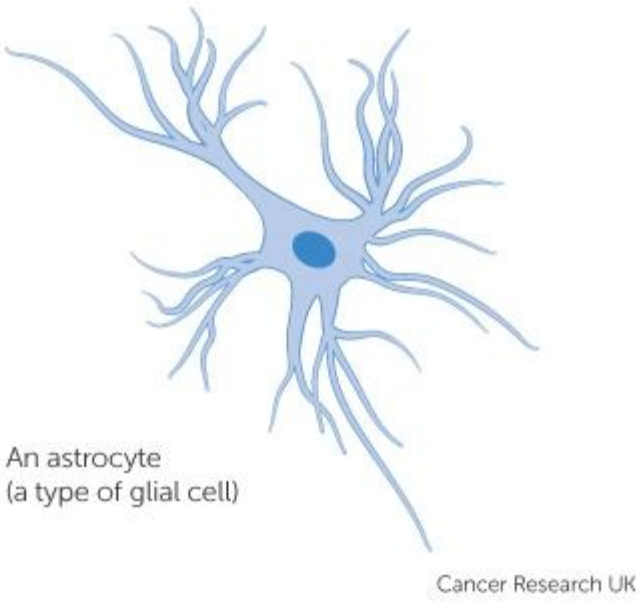


In myeloma, the plasma cells become abnormal, multiply uncontrollably, and make only one type of antibody that does not work properly to fight infection.

Brain and spinal cord cancers

Cancer can start in the cells of the brain or spinal cord. The brain controls the body by sending electrical messages along nerve fibres. The fibres run out of the brain and join together to make the spinal cord, which also takes messages from the body to the brain. Together, the brain and spinal cord form the central nervous system. The brain is made up of billions of nerve cells called neurons. It also contains special connective tissue cells called glial cells that support the nerve cells.

The most common type of brain tumour develops from glial cells and is called glioma. Some tumours that start in the brain or spinal cord are non cancerous (benign) and grow very slowly but others are cancerous and are more likely to grow and spread.



Development of cancer

Cancerous cells develop from healthy cells in a complex process called malignant transformation.

Initiation

Initiation is the first step in the two-stage model of cancer development. Initiators, if not already reactive with DNA, are altered (frequently they are made electrophilic) via drug-metabolizing enzymes in the body and are then able to cause changes in DNA (mutations). Since many initiators must be metabolized before becoming active, initiators are often specific to particular tissue types or species. The effects of initiators are irreversible; once a particular cell has been affected by an initiator it is susceptible to promotion until its death. Since initiation is the result of permanent genetic change, any daughter cells produced from the division of the mutated cell will also carry the mutation. In studies of mouse skin carcinogenesis, a linear relationship has been observed between the dose of initiator and the quantity of tumors that can be produced, thus any exposure to the initiator increases risk and this risk increases indefinitely with higher levels of exposure.

Promotion

Once a cell has been mutated by an initiator, it is susceptible to the effects of promoters. These

compounds promote the proliferation of the cell, giving rise to a large number of daughter cells containing the mutation created by the initiator. Promoters have no effect when the organism in question has not been previously treated with an initiator.

Unlike initiators, promoters do not covalently bind to DNA or macromolecules within the cell. Many bind to receptors on the cell surface in order to affect intracellular pathways that lead to increased cell proliferation. There are two general categories of promoters: specific promoters that interact with receptors on or in target cells of defined tissues and nonspecific promoters that alter gene expression without the presence of a known receptor. Promoters are often specific for a particular tissue or species due to their interaction with receptors that are present in different amounts in different tissue types.

While the risk of tumor growth with promoter application is dose-dependent, there is both a threshold and a maximum effect of promoters. Very low doses of promoters will not lead to tumor development and extremely high doses will not produce more risk than moderate levels of exposure.

Progression

In mice, repeated promoter applications on initiator-exposed skin produces benign papillomas. Most of these papillomas regress after treatment is stopped, but some progress to cancer. The frequency of progression suggests that the papillomas that progress to cancer have acquired an additional, spontaneous, mutation. The term progression, coined by Leslie Foulds, refers to the stepwise transformation of a benign tumor to a neoplasm and to malignancy. Progression is associated with a karyotypic change since virtually all tumors that advance are aneuploid (have the wrong number of chromosomes). This karyotypic change is coupled with an increased growth rate, invasiveness, metastasis and an alteration in biochemistry and morphology.

Stages of Tumor Development

The growth of a tumor from a single genetically altered cell is a stepwise progression. The process described below is applicable for a solid tumor such as a carcinoma or a sarcoma. Blood cell tumors go through a similar process but since the cells float freely, they are not limited to one location in the body.

Hyperplasia- The altered cell divides in an uncontrolled manner leading to an excess of cells in that region of the tissue. The cells have a normal appearance but there are too many of them!

Dysplasia- Additional genetic changes in the hyperplastic cells lead to increasingly abnormal growth. The cells and the the tissue no longer look normal. The cells and the tissue may become disorganized.

Carcinoma *in situ*- Additional changes make the cells and tissues appear even more abnormal. The cells are now spread over a larger area and the region of the tissue involved primarily contains altered cells. The cells often 'regress' or become more primitive in their capabilities. An example would be a liver cell that no longer makes liver-specific proteins. Cells of this type are said to be de-differentiated or anaplastic. A key facet of *in situ* growths is that the cells are contained within the initial location and have not yet crossed the basal lamina to invade other tissues. Cancers of this type are often totally curable by surgery since the abnormal cells are all in one location.

Tumors of this type have not yet invaded neighboring tissue. Based on information about patients with similar growths and microscopic examination, these growths are often considered to have the *potential* to become invasive and are treated as malignant growths.

Cancer (Malignant tumors)- These tumors have the ability to invade surrounding tissues and/or spread (metastasize) to areas outside the local tissue. These metastatic tumors are the most dangerous and account for a large percentage of cancer deaths. The next few sections will go into some detail on the changes and capabilities that allow cancer cells to form large tumors and to metastasize to other parts of the body.

Some tumors do not progress to the point where they invade distant tissues. Such tumors are said to be **benign**. Because they do not spread beyond their initial location, they are not considered to be cancerous. Benign tumors are less often lethal than malignant tumors, but they can still cause serious health problems. Large benign tumors can put pressure on organs and cause other problems. In the case of brain tumors, the limited space within the skull means that a large growth in the brain cavity can be fatal.

Causes of cancer

Mutations and cancer

Cancer development is based on the accumulation of somatic mutations over lifetime. Germ line mutations are typically not involved, but in very rare cases of inherited cancer predisposition, they are contributing to disease progression.

Typically the basal mutation rate is low in humans, but it may be enhanced through one of the three following groups of environmental carcinogens: **chemical mutagens, radiation and tumour viruses**. Exposure to mutagens or radiation greatly increases the mutation rate and thus the probability of developing cancer.

Chemical mutagens comprise a quite disparate group of chemicals that modify DNA through a range of mechanisms, such as alkylation or deamination of DNA bases, or through intercalation between base pairs and formation of DNA adducts (e.g. aromatic hydrocarbons). Oxidative damage may also affect DNA integrity.

X-rays and radioactive radiation tend to induce DNA double-strand breaks, whereas **UV radiation** results in the formation of pyrimidine dimers, by cross-linking of adjacent pyrimidine bases.

Viral causes of cancer

Certain viruses, derived from quite different taxonomic groups (Table 3), are able to induce cancer development. We distinguish the highly **oncogenic viruses**, which contain **viral oncogenes** in their genomes that are in most cases derived from cellular proto-oncogenes, whereas **slowly transforming viruses** do not contain such genes. They tend to use one of the following mechanisms to stimulate proliferation of their host cells:

- Insertion of a strong promoter in the vicinity of a host cell proto-oncogene
- Expression of proteins that neutralise host cell tumour suppressor proteins
- Expression of proteins that prevent or delay apoptosis

Characteristics of viral carcinogenesis include:

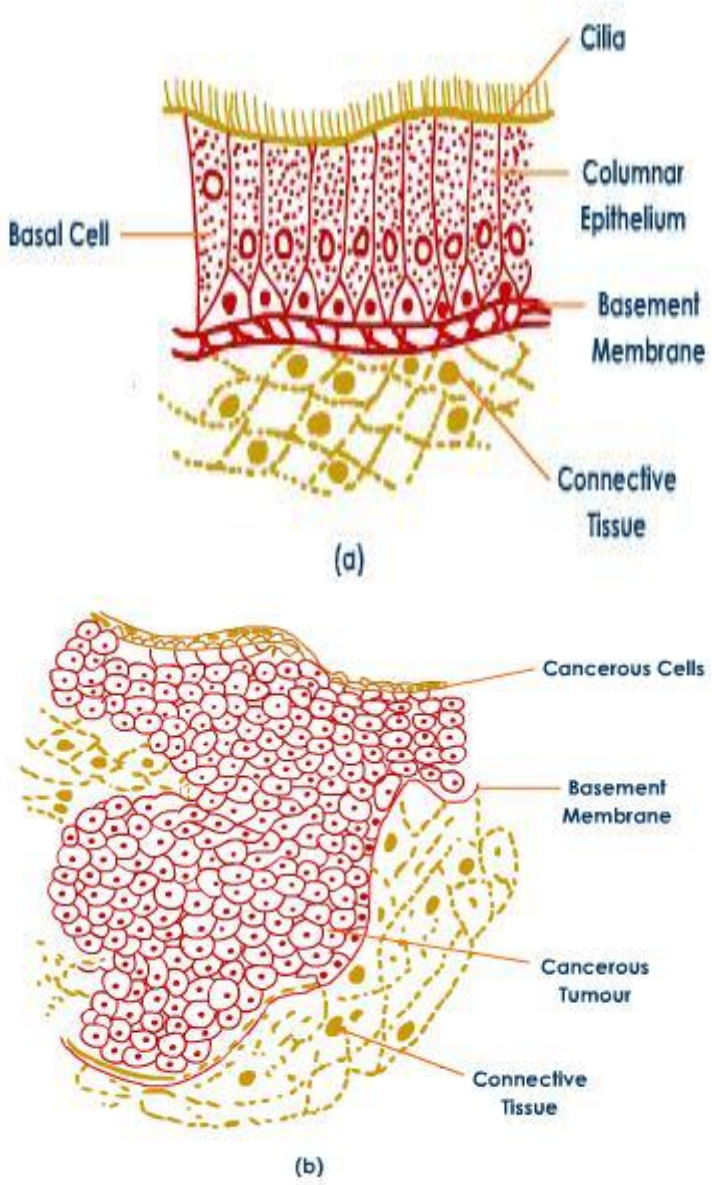
- Tumour viruses often establish persistent infections in the human host
- Host factors are important determinants of virus-induced carcinogenesis
- Viruses are rarely complete carcinogens; they require additional factors to fully activate carcinogenesis.

Table. Human tumour viruses

Virus (Group)	Associated Human Cancer
DNA VIRUSES	
Papilloma virus family Human papilloma virus (HPV) (various subtypes)	Genital tumours, squamous cell carcinoma
Herpes virus family Human herpes virus 8 (HHV8) Epstein-Barr virus (EBV)	Kaposi sarcoma Burkitt's lymphoma, Hodgkin's disease, Nasopharyngeal carcinoma
Hepadnavirus family Hepatitis B virus	Hepatocellular carcinoma
RNA VIRUSES	
Retrovirus family Human T-cell leukaemia virus Human immunodeficiency virus	Adult T-cell leukaemia AIDS-related malignancies
Flavivirus family Hepatitis C virus	Hepatocellular carcinoma

Properties of Cancer Cells

1) Cancer cells show uncontrolled mitotic divisions causing unorganized growth
2) Due to uncontrolled growth and division of cells, a tumour (also called Neoplasm is generally formed)
3) Cancer cells are far less adhesive than the normal cells, so these generally wander through the tissues to cause cancerous growth in different parts of the body.
This ability of cancer cells to invade new sites is termed as Metastasis.
4) Cancer cells exhibit a number of alterations on cell surface, in the cytoplasm and in their genes.
5) Cancer cells do not undergo differentiation



Development of cancer (a) Normal Lung epithelium (b) A cancerous tumour in lung epithelium

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DEPARTMENT OF BIOCHEMISTRY
III-B.Sc., BIOCHEMISTRY
CANCER BIOLOGY (15BCU505C)
MULTIPLE CHOICE QUESTIONS
UNIT I

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	Cancer is caused by	uncontrolled mitosis	uncontrolled meiosis	rupturing of cells	loss of immunity of the cells	**uncontrolled mitosis**
2	Cancer cells are	BHK	Veo	HL-8	Hela cells	**Hela cells**
3	Cancer cells can easily be destroyed by radiations due to	fast mutation	rapid cell division	lack of mutation	lack of oxygen	**rapid cell division**
4	Cancer of β lymphocytes is called	Sarcoma	Melanoma	myeloma	carcinoma	**Myeloma**
5	The basic difference between a cancer cell and a normal cell is	cancer cells divide continuously but normal cells do not divide	normal cell is bigger than cancer cells	normal cells are immortal but cancer cells are mortal	cancer cells divide do not differentiate like normal cells	**cancer cells divide do not differentiate like normal cells**
6	Diethylstibetorol (DES) is a carcinogen. Which organ of the body does it effect?	vagina	heart	lung	kidney	**vagina**
7	Reason of lung cancer is	coal mining	cement factory	calcium fluoride	bauxite mining	**coal mining**
8	Cancer may start when	diploid number of chromosomes become defective	changes occur in genes controlling cell divisions	when loci start to change in chromosomes	All of above	**changes occur in genes controlling cell divisions**
9	Carcinogens	may not occur naturally	are all man made	are all caused due to ionic radiation	may be caused due to tar of tobacco smoke	**may be caused due to tar of tobacco smoke**
10	Any agent that causes cancer is called	mutagen	carcinogen	oncogene	None of above	**carcinogen**
11	Cancer is caused due to	controlled mitosis	uncontrolled mitosis	controlled meiosis	exposure to stress	**uncontrolled mitosis**
12	Naturally occur carcinogens include	asbestos	certain dioxins	aflatoxin	aniline dyes	**aflatoxin**
13	The term cancer means	cell division.	out of control	crab	lobster	**crab**
14	Which of the following is not a characteristic of cancer cells?	loss of cell cycle control	transplantability	loss of contact inhibition	all are characteristic	**all are characteristic**
15	The Philadelphia chromosome is associated with which type of cancer?	breast	thyroid	nerve	leukemia	**leukemia**
16	BRCA-1 is associated with which cancer?	breast	thyroid	nerve	leukemia	**breast**
17	If 85% of lung cancer cases occur in heavy smokers, can lung cancer still have a genetic origin?	yes	no	may	maybe	**yes**
18	An increasing number of women in the rural south die from ____ cancer.	breast	colon	lung	mouth	**mouth**
19	Familial cancer is caused by	a mutation in somatic cells only.	a mutation in germline cells only.	a germline mutation plus a somatic mutation in affected tissue	two germline mutations	**a germline mutation plus a somatic mutation in affected tissue**
20	Which type of cancer in humans is directly caused by a viral infection?	acute T cell leukemia	Wilms' tumor	Burkitt's lymphoma	Rous sarcoma	**acute T cell leukemia**
21	Which type of study compares the incidence of a type of	population	case-control	prospective	empiric	**population**

	cancer among very different groups of people?					
22	Which type of study would compare cancer rates seen in a group of individuals who take herbal supplements versus those in a control group who do not use the supplement?	population	case-control	prospective	empiric	**prospective**
23	Which of the following may contribute to causing cancer?	a mutation in a gene that slows the cell cycle	faulty DNA repair	loss of control over telomere length	all of the above	**all of the above**
24	Enzymes of which type are responsible for converting procarcinogens to ultimate carcinogens	cyt p450 enzyme system	hydrolase	transferases	acetylases	**cyt p450 enzyme system**
25	Which of the following is the most commonly mutated oncogene in cancer?	p53	abl	ras	myc	**ras**
26	What percentage of all breast cancer cases is related to genetic susceptibility?	5%	10%	25%	40%	**25%**
27	Obesity is a risk factor for which of the following types of cancer?	Renal	Endometrial	Esophageal adenocarcinoma	All of the above	**All of the above**
28	In any discussion involving cancer with a patient, there are usually two terms - staging and grade. Which term determines the prognosis of the cancer?	staging	grade	anaplasia	dysplasia	**staging**
29	Cancer has several defining characteristics. Which one of the following would not be a hallmark of cancer?	Limited potential for replication	Evasion of apoptosis	immunity to anti-growth signals	sustained angiogenesis	**Limited potential for replication**
30	Cells are usually capable of undergoing processes which allow them to adapt to a given stress. However, sometimes there are irreversible changes. Which one of the following is an irreversible cellular adaptation?	anaplasia	hyperplasia	metaplasia	dysplasia	**anaplasia**
31	In the development of cancer, there are two main types of genes; oncogenes, which promote cancer, and tumor suppressor genes, which require the loss of both in order for disease to be expressed. Which of the following genes is not a tumor suppressor gene?	Blc-2	p53	rb	BRAC1	**blc-2**
32	*How many types cancers make up over half of all newly diagnosed cases?*	6	8	4	2	**4**
33	A carcinogen is:	Any substance involved in causing cancer.	Another name for cancer.	A gene.	A type of blood disease.	**Any substance involved in causing cancer.**
34	Tumours are classified by:	The person who discovered them.	Their ability to spread.	Their weight	The tissue or cell of origin.	**Their ability to spread.**
35	*TNM stands for:*	*Temperature, Metabolism, Nutrition.*	*Tumour, Nerve, Metastases*	*Tumour, Node, Metastases.*	*Tumour, Nodule, Metastases*	***Tumour, Node, Metastases.***
36	*Tumour markers are:*	*Signs of infection*	*Related to genetics*	*Chemicals that can be detected in the blood*	External growths	***Chemicals that can be detected in the blood***
37	One difference between cancer cells and normal cells is that cancer cells	are unable to synthesize DNA.	are unable to synthesize DNA.	continue to divide even when they are tightly packed together.	cannot function properly because they are affected by density-dependent inhibition	**continue to divide even when they are tightly packed together.**

38	he characteristics of cancer cells include	ability to produce signals to stimulate angiogenesis.	uncontrollable growth.	dedifferentiation.	All of the above are correct.	**All of the above are correct.**
39	The term for a mass of cells growing out of control is _____.	a benign tumor	a malignant tumor	metastasis	All of the above are correct.	**a malignant tumor**
40	What term is used to indicate the ability of a cancer to invade other parts of the body and to produce secondary tumours?	Carcinogenesis	Apoptosis	Metastasis	Mutagenesis	**Metastasis**
41	What is the term used to indicate the growth of new blood vessels?	Biosynthesis	Angiogenesis	Apoptosis	Metastasis	**Angiogenesis**
42	Which molecules are involved in the anchoring of cells to an extracellular matrix?	Integrins	Interleukins	Angiostatin	Cyclins	**Integrins**
43	Cancer causing agents are called	Carcinogens	Mutagens	Teratogens	Tumorgens	**Carcinogens**
44	Most human cancers are caused by:	Cancer viruses	Chromosomal arrangements	Inherited disorders	Environmental factors	**Environmental factors**
45	Cancer cells:	Divide uncontrollably and then die	Are particularly sensitive to extracellular messages	Divide uncontrollably and are immortal	Are impossible to grow in culture	**Divide uncontrollably and are immortal**
46	Cancer cells are not:	Contact inhibited	Transplantable	Invasive	De-differentiated	**Contact inhibited**
47	A cancer cell is said to be _____ if it is shown that the disease will spread when injected into a healthy, susceptible animal.	Contact inhibited	Transplantable	Benign	Invasive	**Transplantable**
48	A cancer that spreads is termed:	Benign	Carcinogenic	Metastatic	Mutagenic	**Metastatic**
49	A cytogenetic diagnosis of chronic myeloid leukemia is made by identification of:	Barr bodies	Viral infection	Promyelocytes	The Philadelphia chromosome	**The Philadelphia chromosome**
50	Which of the following are known to cause cancer?	Viruses	Radiation	Chemicals	All of these	**All of these**
51	_____ cells are commonly used today in research laboratories. They are from a cervical cancer patient who died in 1951.	HeLa	PSA	AML	CML	**HeLa**
52	Cancer cells are considered to be	Heritable	Dedifferentiated	Invasive	All of these	**All of these**
53	All of the following are correct except:	Caretaker genes control mutation rates of gatekeeper genes	Proto-oncogenes normally regulate the cell cycle	Oncogenes are often overexpressed in cancer cells	Tumor suppressor genes are often overexpressed leading to cancer	**Tumor suppressor genes are often overexpressed leading to cancer**
54	Which of the following are thought to lower the risk of developing cancer?	Avoiding excess exposure to the sun	Eating less meat and more whole grains and vegetables	Eating cruciferous vegetables	All of these	**All of these**
55	Cancer occurs due to	signal transduction	transcription	meiosis	none of the above	**none of the above**
56	The spreading of cancer across the body is called	Homeostasis	metastasis	osmosis	angiogenesis	
57	Which of the following are carcinogens?h of	silicon	insecticide	tobacco	all of the above	**all of the above**

58	Melanoma is a type of	skin cancer	lung cancer	tesicular cancer	blood cancer	**skin cancer**
59	The virus implicated in cancer is	Herpes virus	HIV	Influenza virus	Epstein Barr virus	**Epstein Barr virus**
60	Malignant tumors _____.	are surrounded by connective tissue	can be easily removed surgically	remain in one place as a well-defined mass of cells	can travel and begin growing in distant body locations	**can travel and begin growing in distant body locations**

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DEPARTMENT OF BIOCHEMISTRY

SYLLABUS

SUBJECT NAME: CORE ELECTIVE I - CANCER BIOLOGY

SUB.CODE: 15BCU505C

SEMESTER: V **CLASS: III B.Sc., BIOCHEMISTRY**

UNIT II: Mutagens and mutations: Mutagens and mutations, Mechanisms of oncogene activation, Role of growth factors and receptors in carcinogenesis, Retroviral oncogenes, protooncogenes, tumor suppressor genes -P53 and Rb and their functions.

TEXT BOOKS

1. Papachristodoulou, D., Snape, A., Elliott, W. H., Elliott, D.C. (2014). *Biochemistry and Molecular Biology* (5th ed.). New York, Oxford University Press.
2. Hayat, M.A. (2010). *Methods of Cancer Diagnosis, Therapy and Prognosis*. Springer Science. ISBN: 978--420-8441-6.

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Mutagens and Mutations

Mutations, or heritable alterations in the genetic material, may be gross (at the level of the chromosome, which we have already discussed) or point alterations (this technically means mutations not visible as cytological abnormalities and/or those which map to a single "point" in experimental crosses). The latter can involve just a single nucleotide pair in DNA.

Types of Mutations

A. Base pair (nucleotide pair) substitutions

These are of two types: **transitions** (purine to purine or pyrimidine to pyrimidine) and **transversions** (purine to pyrimidine or pyrimidine to purine). We break these down into the two categories because they can occur in different ways.

The consequences of base substitution mutations in protein coding regions of a gene depend on the substitution and its location. They may be *silent*, not resulting in a new amino acid in the protein sequence, eg. GCA or GCG codons in mRNA both mean arginine [this is often true in the third position of a codon, especially with transitions because of "wobble" base pairing]. A base substitution could also result in an amino acid substitution; this is referred to as a *missense* mutation. For example, CTC in the DNA sense strand [GAG in mRNA] will specify a glutamate residue in the protein; this is altered to CAC in the DNA or GUG in the mRNA, resulting in a valine residue in the beta-globin protein chain causing sickle-cell anemia. Missense mutations may have very serious consequences, as in the case of sickle-cell anemia, mild consequences as in the case of hemoglobin C (a different amino acid substitution in position 6 of beta-globin) or no phenotype as in the case of two known amino acid substitutions at position 7 of beta-globin. Finally, base substitutions in a protein coding region may mutate an amino acid codon to a termination codon or vice versa. The former type, which results in a prematurely shortened protein is referred to as a *nonsense* mutation. The effects of nonsense mutations are variable depending upon how much of the truncated protein is present and is required for its function.

Base substitution mutations may also occur in promoters or 5' regulatory regions of genes or in introns and may affect their transcription, translation, or splicing. Many of the beta-thalassemias are the result of these types of non-structural mutations that affect the level of expression of the globin genes. All of the types of mutation described above have been observed in human globin genes. Their consequences depend on what they do to the level of expression of the gene product and/or on what amino acid substitution may have occurred and where it is in the protein.

B. Frameshift mutations

These result from the insertion or deletion of one or more (not in multiples of three) nucleotides in the coding region of a gene. This causes an alteration of the reading frame: since codons are groups of three nucleotides, there are three possible reading frames for each gene although only one is used. eg. mRNA with sequence AUG CAG AUA AAC GCU GCA UAA amino acid sequence from the first reading frame: met gln ile asn ala ala **stop** the second reading frame gives: cys arg **stop**

A mutation of this sort changes all the amino acids downstream and is very likely to create a nonfunctional product since it may differ greatly from the normal protein. Further, reading frames other than the correct one often contain stop codons which will truncate the mutant protein prematurely.

III. Origins of spontaneous mutation

A. Definition and sources

A spontaneous mutation is one that occurs as a result of natural processes in cells. We can distinguish these from induced mutations; those that occur as a result of interaction of DNA with an outside agent or mutagen. Since some of the same mechanisms are involved in producing spontaneous and induced mutations, we will consider them together. Some so-called "spontaneous mutations" probably are the result of naturally occurring mutagens in the environment; nevertheless there are others that definitely arise spontaneously, for example, DNA replication errors.

B. DNA replication errors and polymerase accuracy

Mistakes in DNA replication where an incorrect nucleotide is added will lead to a mutation in the next round of DNA replication of the strand with the incorrect nucleotide. The frequency at which a DNA polymerase makes mistakes (inserts an incorrect base) will influence the spontaneous mutation frequency and it has been observed that different polymerases vary in their accuracy. One major factor affecting polymerase accuracy is the presence of a "proofreading" 3'-5' exonuclease which will remove incorrectly paired bases inserted by the polymerase. This was shown *in vitro* with purified DNA polymerases (those with 3'-5' exonucleases make fewer mistakes) and genetically by Drake with bacteriophage T4 mutants: T4 has its own polymerase with a 3'-5' exo. Drake isolated *mutator* mutants (which had a higher spontaneous mutation rate than normal) and *antimutator* mutants (lower mutation rate than normal) in the polymerase gene and showed that the mutators had a higher ratio of polymerizing to exonuclease activity than normal and that the antimutators had a lower ratio. These studies showed that the function of the 3'-5' exonuclease is to prevent misincorporation during DNA replication and to prevent mutations. Mutator mutants have since been isolated in other organisms and have been shown to affect various components of the DNA replication complex; alterations in a number of these proteins are likely to affect the accuracy of the system.

C. Base alterations and base damage

The bases of DNA are subject to spontaneous structural alterations called **tautomerization**: they are capable of existing in two forms between which they interconvert. For example, guanine can exist in keto or enol forms. The keto form is favored but the enol form can occur by shifting a proton and some electrons; these forms are called tautomers or structural isomers. The various tautomer forms of the bases have different pairing properties. Thymine can also have an enol form; adenine and cytosine exist in amino or imino forms. If during DNA replication, G is in the enol form, the polymerase will add a T across from it instead of the normal C because the base pairing rules are changed (not a polymerase error). The result is a G:C to A:T transition; tautomerization causes transition mutations only.

Another mutatgenic process occurring in cells is spontaneous base degradation. The **deamination** of cytosine to uracil happens at a significant rate in cells.

Deamination can be repaired by a specific repair process which detects uracil, not normally present in DNA; otherwise the U will cause A to be inserted opposite it and cause a C:G to T:A transition when the DNA is replicated.

Deamination of methylcytosine to thymine can also occur. Methylcytosine occurs in the human genome at the sequence 5'CpG3', which is normally avoided in the coding regions of genes. If the meC is deaminated to T, there is no repair system which can recognize and remove it

(because T is a normal base in DNA). This means that wherever CpG occurs in genes it is a "hot spot" for mutation. Such a hot spot has recently been found in the achondroplasia gene.

A third type of spontaneous DNA damage that occurs frequently is damage to the bases by free radicals of oxygen. These arise in cells as a result of oxidative metabolism and also are formed by physical agents such as radiation. An important **oxidation** product is 8-hydroxyguanine, which mispairs with adenine, resulting in G:C to T:A transversions.

Still another type of spontaneous DNA damage is **alkylation**, the addition of alkyl (methyl, ethyl, occasionally propyl) groups to the bases or backbone of DNA. Alkylation can occur through reaction of compounds such as S-adenosyl methionine with DNA. Alkylated bases may be subject to spontaneous breakdown or mispairing.

D. Spontaneous frameshift mutations

Streisinger observed in the 1960's that frameshift mutations in bacteriophages tended to occur in areas with "runs" of repeats of one nucleotide.

Example:

5' AGTCAATCCATGAAAAAATCAG 3'

3' TCAGTTAGGTACTTTTTTAGTC 5'

He proposed that these frameshifts are the result of "slipped mispairing" between the template DNA strand and the newly synthesized strand during DNA replication. In the sequence above, a likely spot for frameshift mutations to occur would be in the stretch of 6 A:T base pairs. Subsequent studies with genes from other organisms, including humans, have shown that runs of repeated nucleotides are indeed hotspots for frameshift mutations.

IV. Mutagens

A mutagen is a natural or human-made agent (physical or chemical) which can alter the structure or sequence of DNA.

A. Chemical mutagens

The first report of mutagenic action of a chemical was in 1942 by Charlotte Auerbach, who showed that nitrogen mustard (component of poisonous mustard gas used in World Wars I and II) could cause mutations in cells. Since that time, many other mutagenic chemicals have been identified and there is a huge industry and government bureaucracy dedicated to finding them in food additives, industrial wastes, etc.

It is possible to distinguish chemical mutagens by their modes of action; some of these cause mutations by mechanisms similar to those which arise spontaneously while others are more like radiation (to be considered next) in their effects.

1. Base analogs

These chemicals structurally resemble purines and pyrimidines and may be incorporated into DNA in place of the normal bases during DNA replication:

- **bromouracil** (BU)--artificially created compound extensively used in research. Resembles thymine (has Br atom instead of methyl group) and will be incorporated into DNA and pair with A like thymine. It has a higher likelihood for tautomerization to the enol form (BU*)
- **aminopurine** --adenine analog which can pair with T or (less well) with C; causes A:T to G:C or G:C to A:T transitions. Base analogs cause transitions, as do spontaneous tautomerization events.

2. Chemicals which alter structure and pairing properties of bases

There are many such mutagens; some well-known examples are:

- **nitrous acid**--formed by digestion of nitrites (preservatives) in foods. It causes C to U, meC to T, and A to hypoxanthine deaminations. [See above for the consequences of the first two events; hypoxanthine in DNA pairs with C and causes transitions. Deamination by nitrous acid, like spontaneous deamination, causes transitions.
- **nitrosoguanidine, methyl methanesulfonate, ethyl methanesulfonate**--chemical mutagens that react with bases and add methyl or ethyl groups. Depending on the affected atom, the alkylated base may then degrade to yield a baseless site, which is mutagenic and recombinogenic, or mispair to result in mutations upon DNA replication.

3. Intercalating agents

acridine orange, proflavin, ethidium bromide (used in labs as dyes and mutagens)

All are flat, multiple ring molecules which interact with bases of DNA and insert between them. This insertion causes a "stretching" of the DNA duplex and the DNA polymerase is "fooled" into inserting an extra base opposite an intercalated molecule. The result is that intercalating agents cause frameshifts.

4. Agents altering DNA structure

We are using this as a "catch-all" category which includes a variety of different kinds of agents. These may be:

- --large molecules which bind to bases in DNA and cause them to be noncoding--we refer to these as "bulky" lesions (eg. **NAAAF**)
- --agents causing intra- and inter-strand crosslinks (eg. **psoralens**--found in some vegetables and used in treatments of some skin conditions)
- --chemicals causing DNA strand breaks (eg. **peroxides**)

B. Radiation

Radiation was the first mutagenic agent known; its effects on genes were first reported in the 1920's. Radiation itself was discovered in 1890's: Roentgen discovered X-rays in 1895, Becquerel discovered radioactivity in 1896, and Marie and Pierre Curie discovered radioactive elements in 1898. These three discoveries and others led to the birth of atomic physics and our understanding of electromagnetic radiation.

1. EM spectrum

Visible light and other forms of radiation are all types of electromagnetic radiation (consists of electric and magnetic waves). The length of EM waves (wavelength) varies widely and is inversely proportional to the energy they contain: this is the basis of the so-called EM spectrum.

The longest waves (AM radio) have the least energy while successively shorter waves and increasing energy are seen with FM radio, TV, microwaves, infrared, visible, ultraviolet (UV), X and gamma radiation. The portion which is biologically significant is UV and higher energy radiation.

2. Ionizing radiation

X- and gamma-rays are energetic enough that they produce reactive ions (charged atoms or molecules) when they react with biological molecules; thus they are referred to as ionizing radiation. This term also includes corpuscular radiation--streams of atomic and subatomic particles emitted by radioactive elements: these are of two types, alpha- and beta-particles [alpha are helium nuclei, 2 protons and 2 neutrons; beta are electrons].

UV radiation is not ionizing but can react with DNA and other biological molecules and is also important as a mutagen.

The units now used for ionizing radiation of all types are rems (roentgen equivalent man): 1 rem of any ionizing radiation produces similar biological effects. The unit used previously was the rad (radiation absorbed dose). However, the effects of different types of radiation differ for one rad unit: one rad of alpha particles has a much greater damaging effect than one rad of gamma rays; alpha particles have a greater RBE (relative biological effectiveness) than gamma rays. The relationship between these units is that:

rads x RBE = # rems

In addition to the energy type and total dose of radiation the dose rate should be considered: the same number of rems given in a brief, intense exposure (high dose rate) causes burns and skin damage versus a long-term weak exposure (low dose rate) which would only increase risk of mutation and cancer.

3. Sources of radiation

Natural sources of radiation produce so-called background radiation. These include cosmic rays from the sun and outer space, radioactive elements in soil and terrestrial products (wood, stone) and in the atmosphere (radon). One's exposure due to background radiation varies with geographic location.

In addition, humans have created artificial sources of radiation which contribute to our radiation exposure. Among these are medical testing (diagnostic X-rays and other procedures), nuclear testing and power plants, and various other products (TV's, smoke detectors, airport X-rays).

Taken together, our overall total average exposure from all sources is about 350 mrem/year; the major contributor of which is from radon exposure. See the graph on page 281 of your text for the breakdown.

4. Biological effects of radiation

Ionizing radiation produces a range of damage to cells and organisms primarily due to the production of free radicals of water (the hydroxyl or OH radical). Free radicals possess unpaired electrons and are chemically very reactive and will interact with DNA, proteins, lipids in cell membranes, etc. Thus X-rays can cause DNA and protein damage which may result in organelle failure, block cell division, or cause cell death. The rapidly dividing cell types (blood cell-forming areas of bone marrow, gastrointestinal tract lining) are the most affected by ionizing radiation and the severity of the effects depends upon the dose received. The information below is based upon accidental exposures of nuclear plant workers and victims of atomic bomb explosions such as those in Hiroshima and Nagasaki:

sublethal dose (100-250 rems): nausea and vomiting early; 1-2 wk. latent period followed by malaise, anorexia, diarrhea, hair loss, recovery (latency due to time it takes hematopoetic or other damage to show up)

lethal dose (350-450 rems): nausea and vomiting early; 1 wk. latent period followed by above with more severe symptoms including internal bleeding; a 50% chance of death [LD50 : dose at which half of exposed individuals will die; ca. 400 rems for humans]. Death is due to blood cell or gastrointestinal failure.

supralethal dose (>650 rems): nausea and vomiting early, followed by shock, abdominal pain, diarrhea, fever and death within hours or days. Death is due to heart or CNS damage.

For the affected tissues and organs, the number of destroyed cells and the likelihood of their replacement determines the survival chances. The long term effects include increased cancer risk and increased risk of mutations in one's offspring.

5. Genetic effects of radiation

Ionizing radiation produces a range of effects on DNA both through free radical effects and direct action:

- -breaks in one or both strands (can lead to rearrangements, deletions, chromosome loss, death if unrepaired; this is from stimulation of recombination)
- -damage to/loss of bases (mutations)
- -crosslinking of DNA to itself or proteins

The genetic effects of radiation were reported in 1927 in Drosophila by Muller and in 1928 in plants (barley) by Stadler; both showed that the frequency of induced mutations is a function of X-ray dose. Their experiments revealed that there was a linear relationship between X-ray dose and induced mutation level, that there was no threshold or "safe" dose of radiation and that all doses are significant, and finally, that "split dose" experiments showed that the genetic effects of radiation are cumulative.

6. UV (ultraviolet)

UV radiation is less energetic, and therefore non-ionizing, but its wavelengths are preferentially absorbed by bases of DNA and by aromatic amino acids of proteins, so it, too, has important biological and genetic effects.

UV is normally classified in terms of its wavelength: **UV-C** (180-290 nm)--"germicidal"--most energetic and lethal, it is not found in sunlight because it is absorbed by the ozone layer; **UV-B** (290-320 nm)--major lethal/mutagenic fraction of sunlight; **UV-A** (320 nm--visible)--"near UV"--also has deleterious effects (primarily because it creates oxygen radicals) but it produces very few pyrimidine dimers. Tanning beds will have UV-A and UV-B. To see a graphic representation of the wavelengths of UV and ozone absorption, click here.

The major lethal lesions are pyrimidine dimers in DNA (produced by UV-B and UV-C)--these are the result of a covalent attachment between adjacent pyrimidines in one strand. This is shown here for a thymine-thymine dimer and here for a thymine-cytosine dimer. These dimers, like bulky lesions from chemicals, block transcription and DNA replication and are lethal if unrepaired. They can stimulate mutation and chromosome rearrangement as well.

V. DNA repair systems

Because DNA damage occurs spontaneously and as a result to ubiquitous environmental agents, most organisms possess some capacity to repair their DNA and DNA is the only macromolecule which IS repaired by cells. We can divide "repair" mechanisms into 3 categories:

damage reversal--simplest; enzymatic action restores normal structure without breaking backbone

damage removal--involves cutting out and replacing a damaged or inappropriate base or section of nucleotides

damage tolerance--not truly repair but a way of coping with damage.

A. Damage reversal

1. Photoreactivation

This is one of the simplest and perhaps oldest repair systems: it consists of a single enzyme which can split pyrimidine dimers (break the covalent bond) in presence of light. Click here to see the photoreactivation reaction.

The photolyase enzyme catalyzes this reaction; it is found in many bacteria, lower eukaryotes, insects, and plants. It seems to be absent in mammals (including humans). The gene is present in mammals but may code for a protein with an accessory function in another type of repair.

2. Ligation of single strand breaks

X-rays and some chemicals like peroxides can cause breaks in backbone of DNA. Simple breaks in one strand are rapidly repaired by DNA ligase. Microbial mutants lacking ligase tend to have high levels of recombination since DNA ends are recombinogenic (very reactive). A human known only by the code name of 46BR was found to have mutations in both of her DNA ligase I genes; she had poor growth, immunodeficiency, and sun sensitivity and died at a young age of lymphoma. Fibroblast cells from 46BR are sensitive to killing by DNA damaging agents including ionizing radiation. In addition, the rare hereditary disease **Bloom syndrome** also somehow is involved with DNA ligase deficiency (although the Bloom syndrome protein is a DNA helicase); patients' cultured cells have high levels of chromosome aberrations and spontaneous mutation.

B. Damage removal

1. Base excision repair

The damaged or inappropriate base is removed from its sugar linkage and replaced. These are glycosylase enzymes which cut the base-sugar bond. example: **uracil glycosylase**--enzyme which removes uracil from DNA. Uracil is not supposed to be in DNA--can occur if RNA primers not removed in DNA replication or (more likely) if cytosine is deaminated (this is potentially mutagenic). The enzyme recognizes uracil and cuts the glyscosyl linkage to deoxyribose. The sugar is then cleaved and a new base put in by DNA polymerase using the other strand as a template. Mutants lacking uracil glycosylase have elevated spontaneous mutation levels (C to U is not fixed, which leads to transitions) and are hyper-sensitive to killing and mutation by nitrous acid (which causes C to U deamination).

There are other specific glycosylases for particular types of DNA damage caused by radiation and chemicals.

2. Mismatch repair

This process occurs after DNA replication as a last "spell check" on its accuracy. In E. coli, it adds another 100-1000-fold accuracy to replication. It is carried out by a group of proteins which can scan DNA and look for incorrectly paired bases (or unpaired bases) which will have aberrant dimensions in the double helix. The incorrect nucleotide is removed as part of a short stretch and then the DNA polymerase gets a second try to get the right sequence.

Human mismatch repair proteins have recently been identified and are very similar to those of the prokaryote *E. coli* and the simple eukaryote yeast (this is an old invention of cells); mutations are found to be passed in the germline of families with some types of inherited colon cancer (**HPNCC**).

3. Nucleotide excision repair

This system works on DNA damage which is "bulky" and creates a block to DNA replication and transcription (so--UV-induced dimers and some kinds of chemical adducts). It probably recognizes not a specific structure but a distortion in the double helix. The mechanism consists of cleavage of the DNA strand containing the damage by endonucleases on either side of damage followed by exonuclease removal of a short segment containing the damaged region. DNA polymerase can fill in the gap that results. Excision repair is shown here .

Mutants that are defective in NER have been isolated in many organisms and are sensitive to killing and mutagenesis by UV and chemicals which act like UV. Humans with the hereditary

disease **xeroderma pigmentosum** are sunlight-sensitive, they have very high risks of skin cancers on sun-exposed areas of the body and have defects in genes homologous to those required for NER in simple eukaryotes. NER mutants in lower organisms are UV-sensitive and have elevated levels of mutation and recombination induced by UV (because they are unable to use the accurate NER method to remove pyrimidine dimers and must use mutagenic or recombinogenic systems).

C. DNA damage tolerance

Not all DNA damage is or can be removed immediately; some of it may persist for a while. If a DNA replication fork encounters DNA damage such as a pyrimidine dimer it will normally act as a block to further replication.

However, in eukaryotes, DNA replication initiates at multiple sites and it may be able to resume downstream of a dimer, leaving a "gap" of single-stranded unreplicated DNA. The gap is potentially just as dangerous if not more so than the dimer if the cell divides. So there is a way to repair the gap by recombination with either the other homolog or the sister chromatid--this yields two intact daughter molecules, one of which still contains the dimer.

1. Recombinational (daughter-strand gap) repair

This is a repair mechanism which promotes recombination to fix the daughter-strand gap--not the dimer--and is a way to cope with the problems of a non-coding lesion persisting in DNA. The events of recombinational repair are shown here . This type of recombinational repair is generally accurate (although it can cause homozygosis of deleterious recessive alleles) and requires a homolog or sister chromatid. The products of the human breast cancer susceptibility genes *BRCA1* and *BRCA2* may be involved in recombinational repair together with homologs of the yeast *RAD51* and *RAD52* genes.

A second type of recombinational repair which is used primarily to repair broken DNA ends such as are caused by ionizing radiation and chemical mutagens with similar action is the non-homologous end-joining reaction. This repair system is also employed by B and T cells of the immune system for genetic rearrangements needed for their function. The Ku70, Ku80, and DNA-dependent protein kinase proteins are needed for non-homologous end-joining. Rodent cell lines with mutations in these genes are very sensitive to killing by ionizing radiation and defective in immune system rearrangement.

2. Mutagenic repair (trans-lesion synthesis)

An alternative scenario for a DNA polymerase blocked at a dimer is to change its specificity so that it can insert any nucleotide opposite the dimer and continue replication ("mutate or die" scenario). See thefigure . We know that this can happen in bacteria and think that it probably happens in eukaryotes, though the mechanism is not well understood. This is a reason why repair may sometimes cause mutations.

VI. Checkpoints

Ataxia telangiectasia is a human autosomal recessive hereditary disease which causes several defects including about a hundred-fold increase in cancer susceptibility. AT patients' cells in culture show abnormalities including spontaneous and radiation-induced chromosome breaks and sensitivity to killing by X-rays. (Ironically, the patients also show extreme sensitivity to killing by X-ray doses intended to be therapeutic for their cancers.) However, AT cultured cells do not show a defect in repair of X-ray damage to their DNA; instead, unlike normal cells, they continue to replicate their DNA even when it has been damaged by X-rays. It is the failure to

recognize DNA damage and respond appropriately by halting the cell cycle until repair can occur that leads to chromosome aberrations and death after X-ray in the AT patients.

The defect in AT is one in a cell cycle checkpoint, a decision point that governs progression through the next phase of the cell cycle. There are genetically controlled checkpoints that decide entry into a new cell cycle (G0 to G1 point), the decision to replicate the DNA (G1 to S point), and the decision to divide (G2 to M point). Mutations in the checkpoint genes can lead to uncontrolled cell growth, ie. cancer.

Although AT itself is a rare condition, it has been estimated that the frequency of heterozygotes with one AT mutation is about 1% in the population. These individuals also have a higher cancer risk and intermediate radiation sensitivity. Thus, screening by X-ray methods (eg. mammography) may increase the chances of an AT heterozygote developing cancer.

Oncogenes

Proto-oncogenes are genes that normally help cells grow. When a proto-oncogene mutates (changes) or there are too many copies of it, it becomes a "bad" gene that can become permanently turned on or activated when it is not supposed to be. When this happens, the cell grows out of control, which can lead to cancer. This bad gene is called an oncogene.

It may be helpful to think of a cell as a car. For it to work properly, there need to be ways to control how fast it goes. A proto-oncogene normally functions in a way that is much like a gas pedal. It helps the cell grow and divide. An oncogene could be compared with a gas pedal that is stuck down, which causes the cell to divide out of control.

A few cancer syndromes are caused by inherited mutations of proto-oncogenes that cause the oncogene to be turned on (activated). But most cancer-causing mutations involving oncogenes are acquired, not inherited. They generally activate oncogenes by:

- Chromosome rearrangements: Changes in chromosomes that put one gene next to another, which allows one gene to activate the other
- Gene duplication: Having extra copies of a gene, which can lead to it making too much of a certain protein

Tumor suppressor genes

Tumor suppressor genes are normal genes that slow down cell division, repair DNA mistakes, or tell cells when to die (a process known as *apoptosis* or *programmed cell death*). When tumor suppressor genes don't work properly, cells can grow out of control, which can lead to cancer.

A tumor suppressor gene is like the brake pedal on a car. It normally keeps the cell from dividing too quickly, just as a brake keeps a car from going too fast. When something goes wrong with the gene, such as a mutation, cell division can get out of control.

An important difference between oncogenes and tumor suppressor genes is that oncogenes result from the *activation* (turning on) of proto-oncogenes, but tumor suppressor genes cause cancer when they are *inactivated* (turned off).

Inherited abnormalities of tumor suppressor genes have been found in some family cancer syndromes. They cause certain types of cancer to run in families. But most tumor suppressor gene mutations are acquired, not inherited.

For example, abnormalities of the *TP53* gene (which codes for the p53 protein) have been found in more than half of human cancers. Acquired mutations of this gene appear in a wide range of cancers.

Mechanisms of oncogene activation

Oncogenes arise from normal genes within the genome (proto-oncogenes) that have been altered in a way that leads to transformation of the cell. This change from a normal proto-oncogene to a cancer-causing oncogene is called **oncogene activation**. Virus could activate oncogenes by insertional mutagenesis, or by incorporating active versions of oncogenes into the genome. In the absence of viral influences, oncogenes are primarily activated in one of three ways: chromosomal rearrangements, gene mutations, and gene amplification.

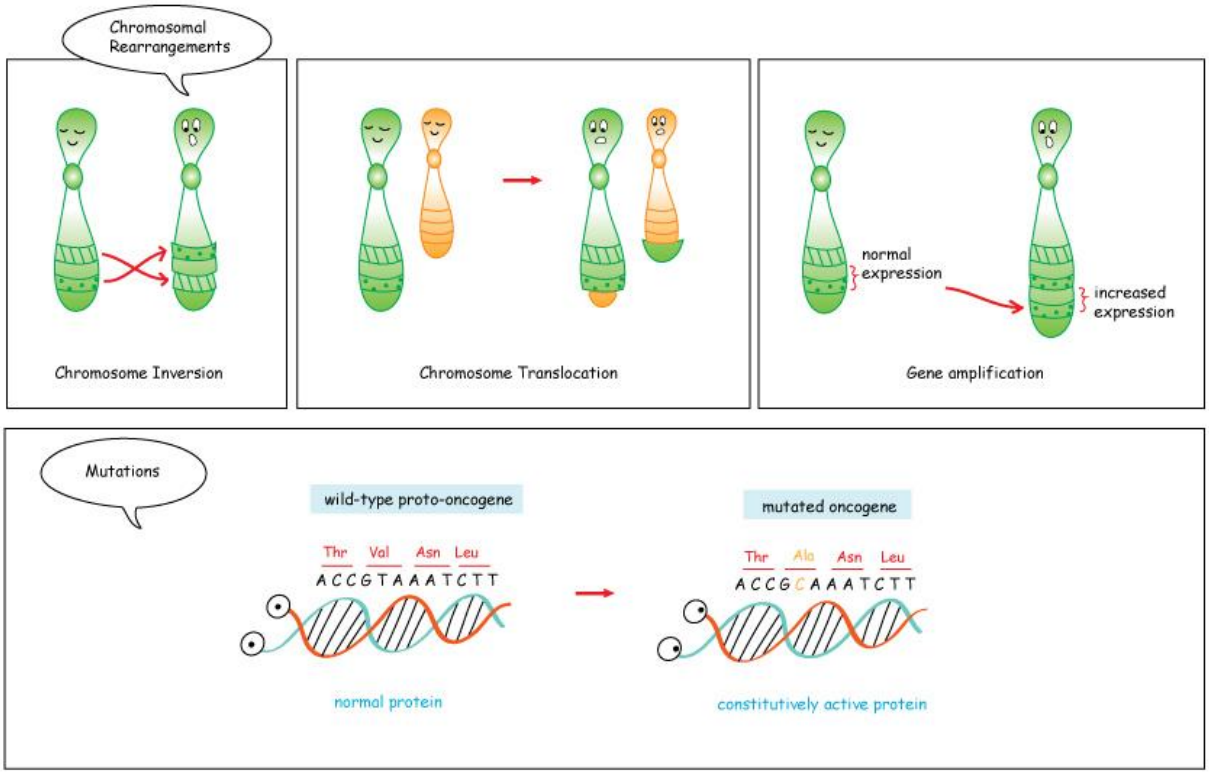


Figure - Oncogenes can be activated by chromosomal rearrangements (inversion and translocation), mutations, and gene amplification.

Chromosomal rearrangements

Chromosomal rearrangements (including **chromosome inversion** and **translocation**) occur when segments of a chromosome are moved and recombine in a novel location, either within the same chromosome or to a non-homologous one. These rearrangements can result in either the fusion of a proto-oncogene to regulatory regions that increase its expression, or the fusion of two genes that now code a novel protein with oncogenic function.

One example of a chromosome translocation event occurs in **Chronic Myelogenous Leukemia (CML)** and results in the production of a fusion protein known as the Philadelphia chromosome. This rearranged chromosome is found in over 90% of CML cases. It is created by a reciprocal translocation between a segment from chromosome 9, which contains the *ABL* gene and a segment on chromosome 22 containing the *BCR* gene. The outcome is a shortened chromosome 22, also known as the Philadelphia chromosome, that contains a *BCR-ABL* fusion gene encoding

the fusion protein. Abl is a member of the Src family of kinases that normally function in signal transduction pathways which are under tight regulatory control. Bcr-Abl performs similar functions to the wildtype Abl protein but is constitutively active, and thus drives cell growth and transformation.

Oncogenic fusion genes produced by chromosomal rearrangements are common in many cancers, especially haematological cancers, sarcomas and prostate cancer. While some chromosomal rearrangements are caused by translocations (like the Philadelphia chromosome), others are caused by inversion events. One such example is the **tropomyosin receptor kinases (***trk***)** oncoprotein. The neurotrophic tyrosine receptor kinase (NTRK) family are receptors that regulate cytoskeleton assembly, axonal and dendritic growth, synaptic and protein channel functions, cell survival and proliferation, retrograde signaling and receptor communication. The second component of this fusion gene, Tropomyosin 3 (TPM3), is a non-muscle, actin binding protein with a coiled-coil structure. A chromosomal inversion in chromsome 1 between the NTRK gene and the TPM3 gene results in a chimeric protein, *trk*, in which 7 out of 8 exons from TPM3 have replaced the transmembrane and cytoplasmic domains of the NTRK protein. Though not well understood, this chimeric protein is predicted to be constitutively expressed by the transphosphroylation of tropomyosin kinase domains that are exposed cytoplasmically, or dimerization caused by the added tropomyosin coiled-coil structure. Figure shows the inversion between NTRK and TPM3 which causes formation of the trk oncogene.

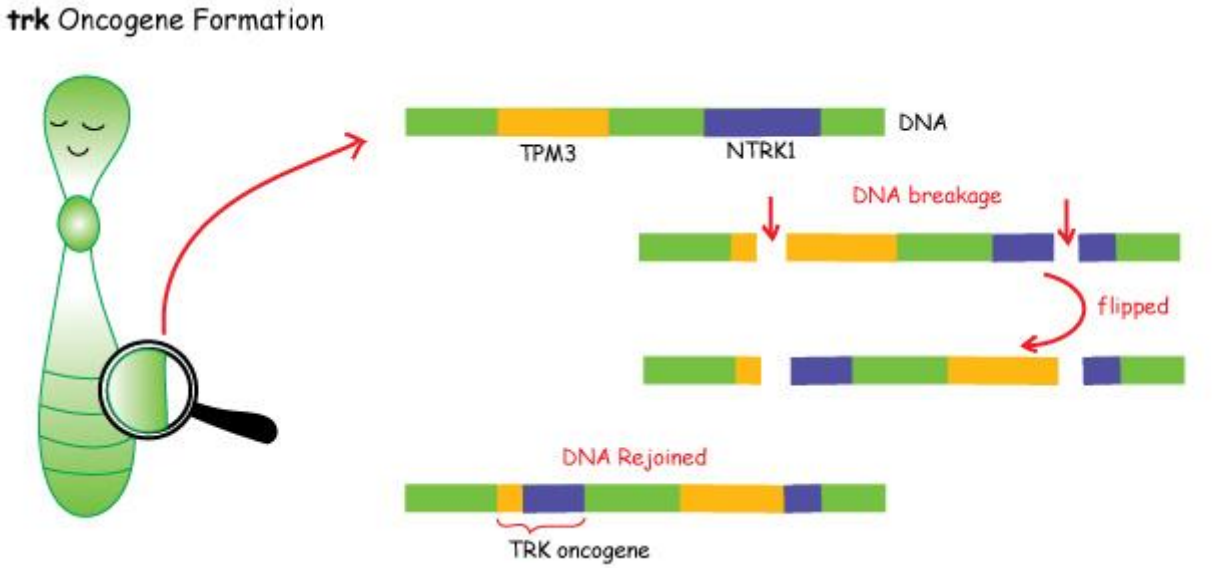


Figure - Formation of the trk oncogene through chromosomal inversion

Aside from the creation of fusion proteins, chromosomal rearrangements can also activate oncogenes by driving over-expression of a normal protein. An example of this can be found in Burkitt's lymphoma, which was previously discussed in this chapter. A segment from chromosome 8 carrying the **c-***MYC* gene is translocated to a region on chromosome 14 encoding immunoglobulin lymphocyte-specific genes and is positioned near an Ig regulating enhancer which drives the expression of c-MYC. This results in increased expression of c-*MYC*, a transcription factor that drives cell proliferation pathways, thus causing excessive growth of B lymphocytes.

Point Mutations

Point mutations (the alteration of a single base pair) can be caused by environmental carcinogens or occur spontaneously, and these small genetic changes can alter the conformation of encoded proteins enough to confer oncogenic activity. One example of this is seen in the *RAS* family of oncogenes. Ras proteins have two states: the inactive form which is bound to the guanine nucleotide GDP, and the active form which is bound to GTP. The active GTP-bound form is normally inactivated relatively quickly by GTPase-activating proteins (GAPs) which cause the hydrolysis of GTP to GDP. Many Ras mutations interfere with this process, producing a constitutively active oncoprotein that triggers signaling pathways for continuous cell growth. Point mutations at codons 12, 13 or 61 within the *RAS* gene are found in many cancers, the highest incidence being 90% in pancreatic cancer cases. Another example of an oncogene which can be activated by point mutations is the epidermal growth factor (EGF) receptor, which is commonly mutated in lung cancers. When this cell surface receptor binds its ligand, it homodimerizes and triggers downstream signaling. Multiple point mutations can produce a constitutively active version of this protein, often by deleting the extracellular domain. Other types of local DNA rearrangements, such as insertions, deletions, and transpositions may also activate oncogenes. However, these types of mutations usually disable a gene and thus are found more frequently in the activation of tumor suppressor genes.

Gene amplification

Gene amplification is the third main way that oncogenes can be activated. Gene amplification occurs when a portion of the genome is duplicated, resulting in a higher than normal copy number of a certain gene. This often occurs during tumor progression and can involve hundreds of kilobases of DNA containing many genes. This leads to increased expression of the amplified genes and, if they are involved in cell proliferation and survival, can drive transformation of the cell. Commonly amplified oncogenes include some that we have already discussed such as *RAS* proteins and *EGF* receptors. Rather than finding mutated versions of these proteins in the cell, the normal protein can be expressed at high levels and achieve similar oncogenicity. The *MYC* gene family is also often amplified in many cancers. Earlier we discussed c-*MYC* in the context of Burkitt's lymphoma, where chromosomal translocation causes increased expression of this gene. Gene amplification of c-*MYC* is common in other cancers including lung, breast, cervical, and ovarian cancer, and has similar oncogenic effects, though the protein is unregulated by duplication of the gene rather than a translocation event.

Another type of commonly amplified proto-oncogene is **ERBB2**, which is implicated in breast and ovarian cancers. This gene encodes a tyrosine kinase, the ERBB2 protein which is a transmembrane receptor, and is similar to EGF receptors. There has been debate as to whether gene amplification is a spontaneous or induced process. However, experimental evidence indicates that gene amplification is not a direct result of exposure to cytotoxic or exogenous agents. Rather, amplification occurs in the replication and repair stage, after the cell's DNA has been damaged by internal or external agents. Gene amplifications give an impression of how genetically unstable tumor cells can be due to genome alteration.

Local DNA Rearrangement

There are also other mechanisms by which oncogenes arise. Similar to the mechanism of chromosomal rearrangements, local DNA rearrangements can also induce activation of oncogenes. However, rearrangements of local DNA involve deletion, insertion, transposition and inversion of smaller pieces of DNA on a local scale. For example, the tal-1 gene undergoes a chromosomal translocation to cause T cell acute lymphoblastic leukemia (T-ALL) in 3% of patient. However, there are 25% of T-ALL patients that have gene rearrangement but cannot be detected by karyotype analysis, which measure chromosomal appearance to detect translocations. It was found that these patients have a small deletion (90kb). These deletion events arise independently in patients through site-specific DNA rearrangements.

Insertional Mutagenesis

Insertional mutagenesis is another mechanism that can activate oncogenes. This type of activation involves the infection of host cell with viruses or transposons. The viral or transposable element must be inserted into the proper location to trigger activation of an oncogene. For example, a proto-oncogene involved in cell proliferation can acquire increased expression due to an insertion of an enhancer. This gain-of-function mutation can occur through recombination, where an enhancer is inserted either upstream or downstream of the gene and can cause the gene to become an oncogene, promoting tumor formation as cell proliferation occurs at higher than normal levels.

Viral Oncogenes

The parasitic nature of viruses requires the need for cellular machinery for its own replication. Increasing the host cell proliferation would also be beneficial for the virus to increase its own survival. Therefore, viruses have acquired the ability to over express genes to drive host cell proliferation.

Activation By Epigenetic Derepression

Unlike the cases of epigenetic silencing of tumor suppressor genes in cancer cells, the epigenetic events that lead to oncogene overexpression are far less well characterized. Much like promoter DNA hypermethylation functions in the silencing of tumor suppressor genes, promoter hypomethylation is thought to contribute to the activation of genes that are normally repressed or at low transcription levels in normal cell types. The correlation between upregulation and promoter hypomethylation has been reported within several oncogenes, including the signaling cascade activator SNGG, MCJ, MAL, HOXA10, and TUBB3.
Evidence linking the epigenetic modification of chromatin to oncogene activation has also been reported, but remains under established as well. The upregulation of claudin-3 and claudin-4, proteins which aid in ovarian cell invasion, was shown to be associated with a loss of the repressive histone modification H3K27me3 along the gene body in ovarian cancer cells. Such findings open the field to the suggestion that further chromatin modification could influence the activation of cancer-promoting genes, independent of DNA methylation.

Role of Growth factors and receptors in carcinogenesis

Growth factors, which generally considered as a subset of cytokines, refer to the diffusible signaling proteins that stimulate cell growth, differentiation, survival, inflammation, and tissue repair. They can be secreted by neighboring cells, distant tissues and glands, or even tumor cells themselves. Normal cells show a requirement for several growth factors to maintain proliferation and viability. Growth advantage is often found for the cells which secrete a growth factor.

Growth factor can exert their stimulation though endocrine, paracrine or autocrine mechanisms. Due to their short half-lives and slow diffusion in intercellular spaces, growth factors usually act locally. Typically, the signal transduction of growth factors is initiated by binding to their receptors on the surface of target cells. The specific instruction conveyed by a growth factor to a particular subpopulation of cells depends on the type of receptors, number of target cell, and the intracellular signal transduction subsequent to factor binding. Moreover, external factors such as the binding ability of a growth factor to extracellular matrices (ECM), ECM degradation, and concentration of the growth factor may have an effect on the ultimate response of a target cell to a specific growth factor.

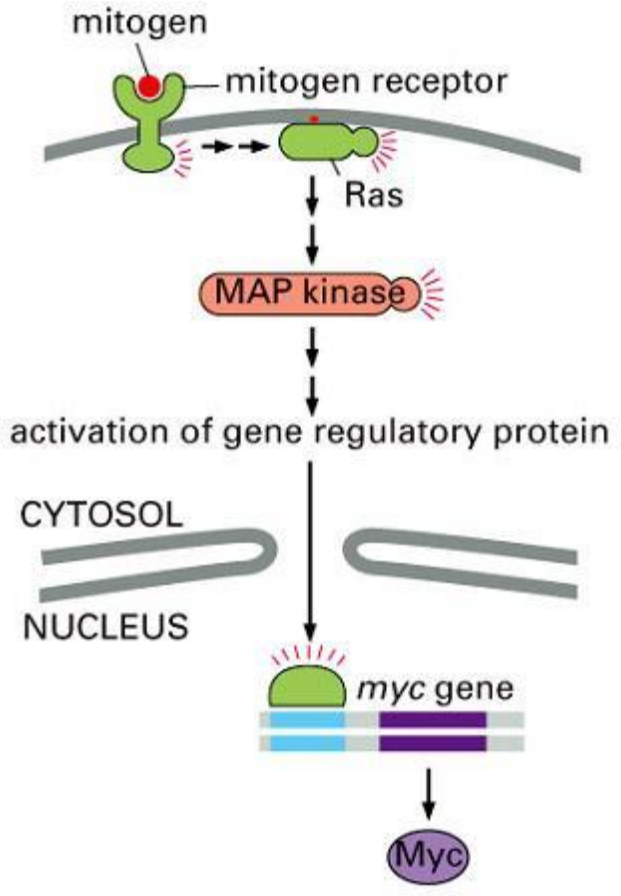


Figure: The MAP kinase pathway as an example of a growth signalling pathway.

The mitogen (or growth factor) binds to its receptor, a receptor tyrosine kinase. Tyrosine phosphorylation of the receptor leads to activation of several docking proteins, and eventually to the activation Ras, bound to the inside of the cell membrane. Active Ras in turn activates the MAP kinase signaling cascade, beginning with Raf (not shown here). The final MAP kinase in this sequence activates several target proteins, for example a transcription factor that activates expression of the Myc gene. Myc itself is a transcription factor that activates the expression of cell cycle regulatory genes.

Steps that characterize normal cell proliferation include:

- the binding of a GF to its specific receptor on the cell membrane
- transient and limited activation of the GFR, which, activates several signal-transducing proteins (e.g. Ras) on the inner leaflet of the plasma membrane
- transmission of the signal by signal transduction molecules, either to cytosolic targets or to the nucleus where they activate transcription of specific genes
- entry of the cell into the cell cycle, ultimately resulting in cell division.

This pathway is often derailed in cancer and allows wayward cells to generate their own internal signals that stimulate proliferation and become independent of their environments. Cancer cells are able to induce their own growth stimulatory signals when mutations in the GFR gene occur, which facilitates activation in the absence of GFs or when overproduction of GFs results in an autocrine signaling loop.

Other elements of cell signaling

An alternative strategy by which cancer cells can become GF independent involves constitutive activation of internal signaling components. For example, the Ras protein in normal cells is switched off and does not signal unless a GFR becomes activated, which through a series of intermediaries, is able to activate the Ras protein, converting it from its quiescent state to an active, signal-emitting state. Thereafter, the Ras protein is able to release further downstream signals that are capable of inducing proliferation. In cancer cells, this signalling pathway is deregulated because structurally altered Ras proteins are able to continuously send growth stimulatory signals into the interior of the cell in the absence of GFs.

The epidermal growth factor (EGF) family include EGF, Amphiregulin (AREG), Betacellulin (BTC), Epiregulin (EPR), HB-EGF, Neuregulins, and others. Members of epidermal growth factor family have highly similar structural and functional characteristics. The activity of epidermal growth factor family members is mediated by the epidermal growth factor (EGFR/ErbB) receptor tyrosine kinases. Members of epidermal growth factor family are known to be involved in tumor formation. The mediations of EGF therapy are so far mainly based on inhibiting the EGF receptor.

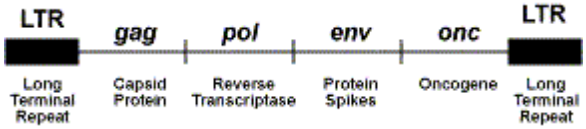
EGF (epidermal growth factor) is the founding member of the EGF family of proteins, which also include Amphiregulin (AREG), Betacellulin (BTC), Epiregulin (EPR), HB-EGF, Neuregulins, and others. Members of epidermal growth factor family have highly similar structural and functional characteristics. They have at least one common structural motif, the EGF domain, which consists of six conserved cysteine residues forming three disulfide bonds. The main structure of EGF domain is a two-stranded beta-sheet followed by a loop to a C-terminal short two-stranded sheet. In addition to their EGF domain, the epidermal growth factor family members are characterized by two features: Production of mitogenic responses in EGF-sensitive cells, and high affinity binding to the EGF receptor.

The activity of epidermal growth factor family members is mediated by the epidermal growth factor receptor tyrosine kinases (EGFR/ErbB). Members of the EGFR/ErbB family are made up of an extracellular region or ectodomain that contains approximately 620 amino acids, a single transmembrane spanning region and a cytoplasmic tyrosine kinase domain. The extracellular domain of the EGF receptor is characterized by its capacity to bind EGF and EGF-like ligands with high affinity. Chemically this portion of the receptor contains 10-11 N-linked oligosaccharide chains, high content of half-cystine residues (10%) that could give rise to as many as 25 disulfides. The region between the two half-cystine-rich clusters is involved in ligand binding. The hallmark of the cytoplasmic portion of epidermal growth factor receptor is the sequence defining the tyrosine kinase domain. Near the carboxyl terminus of the receptor are four sites of EGF-dependent autophosphorylation.

Epidermal growth factor plays an important role in the regulation of cell growth, proliferation, and differentiation. EGF acts by binding to EGF receptor (EGFR) on the cell surface and stimulating the intrinsic protein-tyrosine kinase activity of the receptor, and initiates a signal transduction cascade. As a result a variety of biochemical changes take place within the cell, including increased intracellular calcium levels, glycolysis and protein synthesis and transcription of certain genes, which ultimately lead to DNA synthesis and cell proliferation. Members of epidermal growth factor family are known to be involved in tumor formation. The mediations of EGF therapy are so far mainly based on inhibiting the EGF receptor.

Retroviral Oncogenes

We have already discussed the structure of the retroviruses in relation to retrotransposons. It was mentioned that the oncogene of those viruses transform a cell to unproliferated growth carry an oncogene in addition to the three primary genes required of all retroviruses. The figure below is the generalized structure of a retrovirus.



One of the best studied retroviruses infects chicken and is called **Rous Sarcoma Virus**. The oncogene found in this retrovirus is ***src***. The product of this gene is a protein kinase that phosphorylates tyrosine residues in other proteins. The protein products of other retroviral oncogenes also regulate cell division processes and contain one of four functions.

- **Growth factor receptors** - One example is epidermal growth factor receptor which promotes wound healing by stimulating cell growth. Some factors function as transmembrane protein kinases that are activated by an extracellular signal. An example is ***v-erbB*** found in the Avian erythroblastosis virus that infects chicken.
- **Protein kinases** - These proteins alter the function of other proteins by phosphorylating specific amino acid residues. The ***v-src*** from the Rous Sarcoma virus which infects chickens is an example.
- **G-proteins** - These proteins bind the nucleotide GTP, and also exhibit GTPase activity. The ***v-H-ras*** oncogene of the Harvey murine sarcoma virus which infects rats is an example.

- **Transcription factors** - These proteins function by binding to DNA and activating transcription. An example is the ***v-jun*** oncogene of the Avian sarcoma virus that infects chickens.
- **The RAS oncogene** is the most frequently mutated oncogene in human cancer. It encodes a GTP-binding protein Ras that functions as an on-off 'switch' for a number of key signaling pathways controlling cellular proliferation. In a normal cell, Ras is transiently activated and recruits Raf, to activate the MAP-kinase pathway to transmit growth-promoting signals to the nucleus. The mutant Ras protein is permanently activated leading to continuous stimulation of cells without any external trigger. Other oncogenes frequently mutated in cancer are listed in Table 1.

- **Table 1. Selected oncogenes and associated cancers**

Category / Protein Function	**Proto-oncogene**	**Mode of Activation**	**Associated Cancer**
Growth Factors			
PDGF (β chain)	SIS	Overexpression	Astrocytoma, osteosarcoma
Fibroblast growth factors	HST-1 INT-2	Overexpression Amplification	Stomach cancer Bladder & breast cancer
Transforming growth factor α	TGFα	Overexpression	Melanoma Astrocytomas Hepatocellular carcinomas
Growth Factors Receptors			
EGF-receptor family	ERB-B1 ERB-B2	Overexpression Amplification	SCC of the lung, gliomas Breast and ovarian cancers
PDGF receptor	PDGF-R	Overexpression	Gliomas
Receptor for stem cell (steel) factor	KIT	Point Mutation	Gastrointestinal stromal tumours
Proteins Involved in Signal Transduction			
GTP-binding	K-RAS	Point mutation	Colon, lung, pancreatic tumours
	H-RAS	Point mutation	Bladder & kidney tumours
	N-RAS	Point mutation	Melanoma, leukaemia, lymphoma
Non-receptor tyrosine kinase	ABL	Translocation	CML, ALL
	BRAF	Point mutation	
	β-catenin	Point	

RAS signal transduction WNT signal transduction		mutation/Overexpression	Melanomas Hepatoblastomas & HCC
Nuclear Regulatory Proteins Transcriptional activators	C-MYC N-MYC L-MYC	Translocation Amplification Amplification	Burkitt lymphoma Neuroblastoma, small cell carcinoma of lung SCC of the lung
Cell-Cycle Regulators Cyclins Cyclin-dependent kinase	CYCLIN D CYCLIN E CDK4	Translocation Amplification Overexpression Amplification or Point mutation	Mantle cell lymphoma Breast & oesophageal cancers Breast cancer Glioblastoma, melanoma, sarcoma

Proto-Oncogenes

Genes that promote autonomous cell growth in cancer cells are called **oncogenes**, and their normal cellular counterparts are called **proto-oncogenes**. Proto-oncogenes are physiologic regulators of cell proliferation and differentiation while oncogenes are characterized by the ability to promote cell growth in the absence of normal mitogenic signals. Their products, oncoproteins, resemble the normal products of proto-oncogenes with the exception that oncoproteins are devoid of important regulatory elements. Their production in the transformed cells becomes constitutive, that is, not dependent on growth factors or other external signals. Proto-oncogenes can be converted to oncogenes by several mechanisms including point mutation and gene amplification resulting in:

- Overproduction of growth factors
- Flooding of the cell with replication signals
- Uncontrolled stimulation in the intermediary pathways
- Cell growth by elevated levels of transcription factors

Tumour suppressor genes

Tumour suppressor genes (Table 2) encode proteins that are:

- receptors for secreted hormones that function to inhibit cell proliferation
- negative regulators of cell cycle entry or progression
- negative regulators of growth signaling pathways (e.g. APC or PTEN)
- checkpoint-control proteins that arrest the cell cycle if DNA is damaged or chromosomes are abnormal
- proteins that promote apoptosis DNA repair enzymes.

The transformation of a normal cell to a cancer cell is accompanied by the loss of function of one or more tumour suppressor genes and both gene copies must be defective in order to promote tumour development

Table 2. Examples of tumour suppressor genes

Gene	Protein function	Inherited Disease	Spontaneous Tumours
APC	Negative regulator of the signalling pathway	Adenomatous polyposis coli (APC)	Most colon cancers
BRCA1, BRCA2	Components of DNA repair systems	Familial breast and ovarian cancer	Spontaneous breast cancers
CDH1	E-cadherin, a cell adhesion molecule	Hereditary diffuse gastric cancer	Many epithelial cancers
CDKN2A	INK4a, inhibitor of cyclin-dependent kinase Cdk4	Some familial melanomas	Some esophageal and pancreatic cancers
MEN1	Transcription factor and protein kinase	Multiple endocrine neoplasia	Many metastatic cancers
NF1	Neurofibromin, Ras-GTPase activation	Neurofibromatosis type 1	Some tumours of neural crest origin
PTEN	Negative regulator of PI3K growth signalling pathway	Cowden disease	30%-50% of spontaneous cancers
RB	Repression of transcription factor E2F	Retinoblastoma, osteosarcoma	Retinoblastoma, sarcomas, several carcinomas
SMAD4	Signal transducer in TGF- signalling	Juvenile polyposis	Colon and pancreatic cancers
TP53	Transcription factor; guardian of the genome'	Li-Fraumeni syndrome	Most frequently mutated in human cancers
TSC1, TSC2	Inhibitor of mTOR	Tuberous sclerosis	Rare
VHL	Ubiquitin ligase	von Hippel-Lindau disease	Many renal cell carcinomas
WT1	Transcription factor	Wilms tumour	Some leukaemias

p53 and its functions

INTRODUCTION

p53, also known as **TP53** or **tumor protein** (EC :2.7.1.37) is a gene that codes for a protein that regulates the cell cycle and hence functions as a tumor suppression. It is very important for cells in multicellular organisms to suppress cancer. P53 has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation. The name is due to its molecular mass: it is in the 53 kilo Dalton fraction of cell proteins. p53 was identified in 1979 by Arnold Levine, David Lane and William Old, working at Princeton University, Dundee University (UK) and Sloan-Kettering Memorial Hospital, respectively. It had been hypothesized to exist before as the target of the SV40 virus, a strain that induced development of tumors. Although it was initially presumed to be an oncogene, its character as a tumor suppressor gene was revealed in 1989.

The human p53 gene is located on the seventeenth chromosome (17p13.1).The p53 protein is a phosphoprotein made of 393 amino acids. It consists of four units (or domains):

- A domain that activates transcription factors.
- A domain that recognizes specific DNA sequences (core domain).
- A domain that is responsible for the tetramerization of the protein.
- A domain that recognized damaged DNA, such as misaligned base pairs or single-stranded DNA.

Wild-type p53 is a labile protein, comprising folded and unstructured regions which function in a synergistic manner. p53 protein has been voted molecule of the year.

MECHANISM

It plays an important role in cell cycle control and apoptosis. Defective p53 could allow abnormal cells to proliferate, resulting in cancer. As many as 50% of all human tumors contain p53 mutants. In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: **growth arrest**, **DNA repair** and **apoptosis** (cell death). The growth arrest stops the progression of cell cycle, preventing replication of damaged DNA. During the growth arrest, p53 may activate the transcription of proteins involved in DNA repair. Apoptosis is the "last resort" to avoid proliferation of cells containing abnormal DNA.

The cellular concentration of p53 must be tightly regulated. While it can suppress tumors, high level of p53 may accelerate the aging process by excessive apoptosis. The major regulator of p53 is **Mdm2**, which can trigger the degradation of p53 by the ubiquitin system.

Target Genes

p53 is a transcriptional activator, regulating the expression of **Mdm2** (for its own regulation) and the genes involved in growth arrest, DNA repair and apoptosis. Some important examples are listed below.

1. **Growth arrest:** p21, Gadd45
2. **DNA repair:** p53R2.
3. **Apoptosis:** Bax, Apaf-1, PUMA and NoxA.

Regulation of p53

As mentioned above, p53 is mainly regulated by Mdm2. The regulation mechanism is illustrated in the following figure.

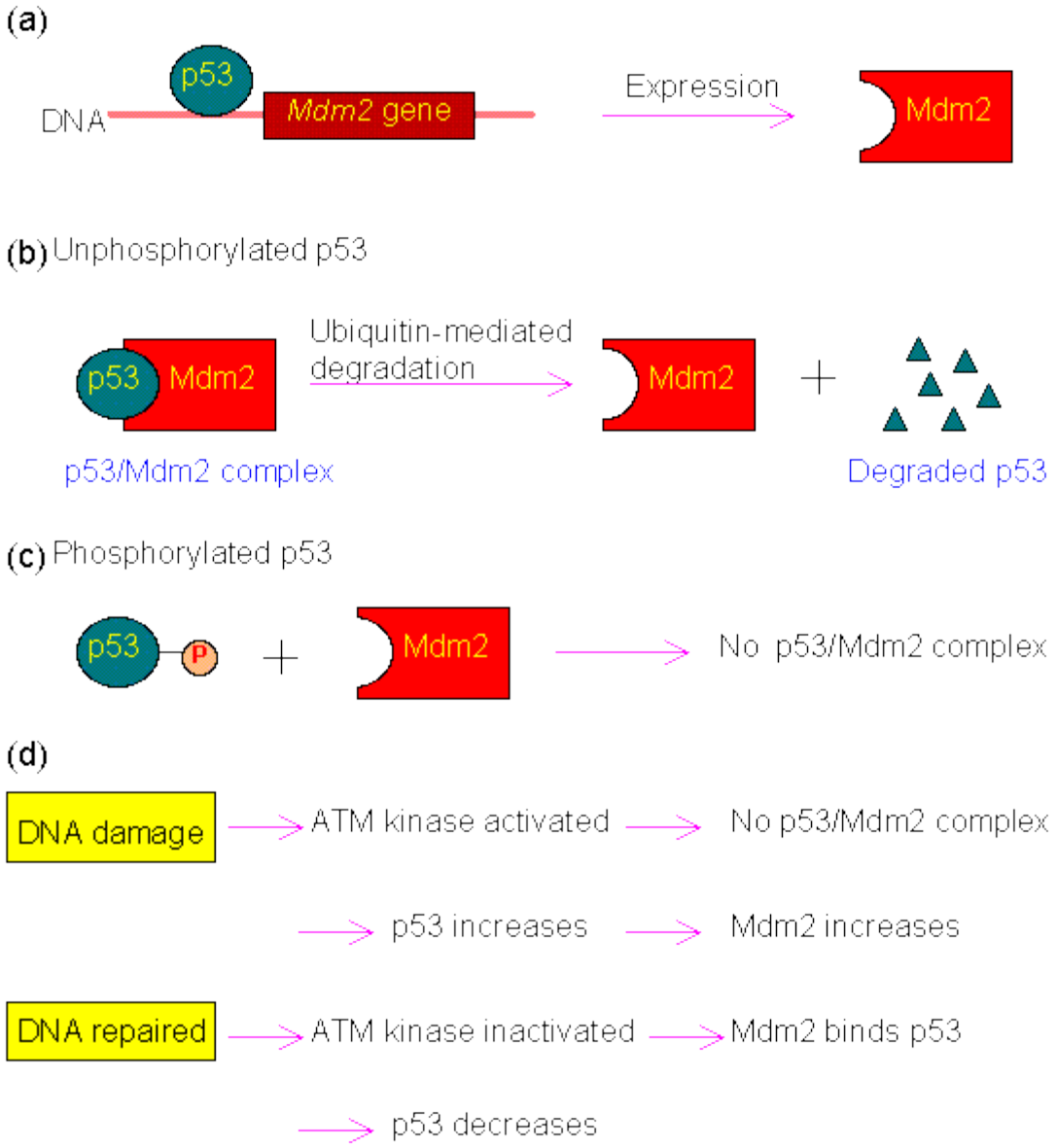


Figure. Regulation of p53.

(a) Expression of Mdm2 is activated by p53.

(b) Binding of p53 by Mdm2 can trigger the degradation of p53 via the ubiquitin system.

(c) Phosphorylation of p53 at Ser15, Thr18 or Ser20 will disrupt its binding with Mdm2. In normal cells, these three residues are not phosphorylated, and p53 is maintained at low level by Mdm2.

(d) DNA damage may activate protein kinase (such as ATM, DNA-PK, or CHK2) to phosphorylate p53 at one of these three residues, thereby increasing p53 level. Since Mdm2

expression is activated by p53, the increase of p53 also increases Mdm2, but they have no effect while p53 is phosphorylated. After the DNA damage is repaired, the ATM kinase is no longer active. p53 will be quickly dephosphorylated and destroyed by the accumulated Mdm2.

Roles of p53

The roles of p53 in growth arrest and apoptosis are illustrated in Figure 4-H-6. p53 is also directly involved in DNA repair. One of its transcriptional target gene, p53R2, encodes ribonucleotide reductase, which is important for both DNA replication and repair. p53 also interacts directly with AP endonuclease and DNA polymerase which are involved in base excision repair.

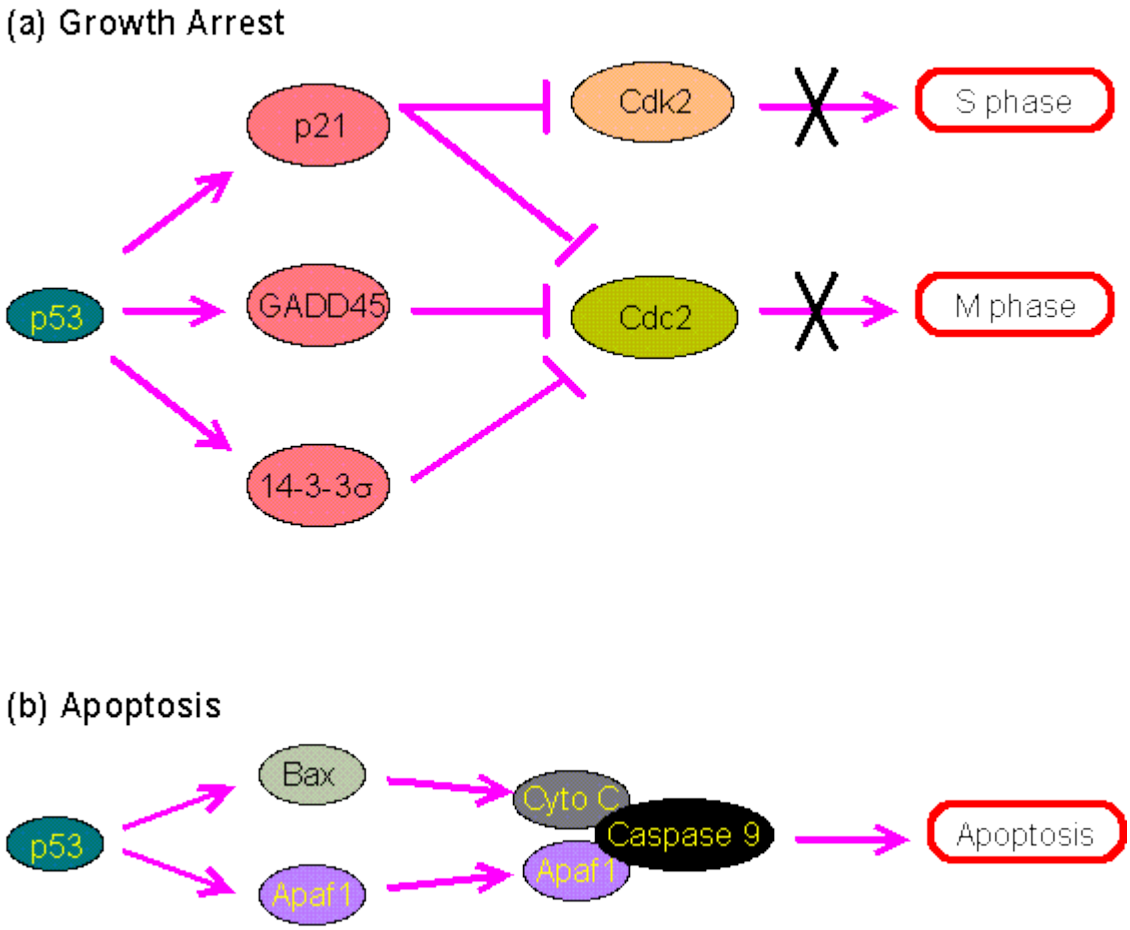


Figure. The roles of p53 in growth arrest and apoptosis.
(a) The cell cycle progression into the S phase requires the enzyme Cdk2, which can be inhibited by p21. The progression into the M phase requires Cdc2 which can be inhibited by p21, GADD45 or 14-3-3. p53 regulates the expression of these inhibitory proteins to induce growth arrest.
(b) Apoptosis can be induced by the binding of Caspase 9 to cytochrome c and Apaf1. p53 may activate the expression of Apaf1 and Bax. The latter can then stimulate the release of cytochrome c from mitochondria (see Mitochondria, Apoptosis and Aging).

ROLE IN DISEASE

If the p53 gene is damaged, tumor suppression is severely reduced. People who inherit only one functional copy of p53 will most likely develop tumors in early adulthood, a disease known as Li-Fraumeni syndrome. p53 can also be damaged in cells by mutagens (chemicals, radiation or viruses), increasing the likelihood that the cell will begin uncontrolled division. More than 50 percent of human tumors contain a mutation or deletion of the p53 gene.

In health p53 is continually produced and degraded in the cell. The degradation of p53 is, as mentioned, associated with MDM-2 binding. In a negative feedback loop MDM-2 is itself induced by p53. However mutant p53s often don't induce MDM-2, and are thus able to accumulate at very high concentrations. Worse, mutant p53 protein itself can inhibit normal p53.

POTENTIAL THERAPEUTIC USE

In-vitro introduction of p53 in to p53-deficient cells has been shown to cause rapid death of cancer cells or prevention of further division. It is more these acute effects which hopes rest upon therapeutically. The rationale for developing therapeutics targeting p53 is that "the most effective way of destroying a network is to attack its most connected nodes". P53 is extremely well connected (in network terminology it is a hub) and knocking it out cripples the normal functioning of the cell. This can be seen as 50% of cancers have missense point mutations in the p53 gene, these mutations impair its anti-cancer gene inducing effects. Restoring its function would be a major step in curing many cancers.

Various strategies have been proposed to restore p53 function in cancer cells. A number of groups have found molecules which appear to restore proper tumour suppressor activity of p53 in vitro. These work by altering the conformation of mutant conformation of p53 back to an active form. So far, no molecules have shown to induce biological responses, but some may be lead compounds for more biologically active agents. A promising target for anti-cancer drugs is the molecular chaperone Hsp90, which interacts with p53 in vivo.

Adenoviruses rely on their host cells to replicate, they do this by secreting proteins which compel the host to replicate the viral DNA. Adenoviruses have been implicated in cancer-causing diseases, but in a twist it is now modified viruses which are being used in cancer therapy. ONYX-015 (dl1520, CI-1042) is a modified adenovirus which selectively replicates in p53-deficient cancer cells but not normal cells. It is modified from a virus that expresses the early region protein, E1B, which binds to and inactivates p53. P53 suppression is necessary for the virus to replicate. In the modified version of the virus E1B has been deleted. It was hoped that the viruses would select tumour cells, replicate and spread to other surrounding malignant tissue thus increasing distribution and efficacy. The cells which the adenovirus replicates in are lysed and so the tumour dies. Preclinical trials using the ONYX-015 virus on mice were promising however clinical trials have been less so. No objective responses have been seen except when the virus was used in combination with chemotherapy. This may be due to the discovery that E1B has been found to have other functions vital to the virus. Additionally its specificity has been undermined by findings showing that the virus is able replicate in some cells with wild-type p53. The failure of the virus to produce clinical benefits may in large part be due to extensive fibrotic tissue hindering virus distribution around the tumour.

p53 is a nuclear DNA binding phosphoprotein that normally exists as homotetramer or complex of tetramers. It is transcriptional activator of a specific set of target genes, and can exert transcriptional repression probably by interaction with transcription factors or the general

transcription machinery. It interacts directly with cellular proteins. Loss of p53 function can contribute to genomic instability within cells. p53 is important in preventing cancers because of its unique functional capabilities. It regulates gene expression and control several key genes involved in growth regulation. It facilitates DNA repair. If the damage is beyond repair, p53 triggers apoptosis of these cells, besides DNA damages, p53 dependent apoptosis is also induced by inappropriate oncogene activation, certain cytokines, hypoxia, heat shock, telomerase erosion etc. DNA damage can be due to exposure to radiation or drugs, causing damage to signals, the activation of cellular checkpoint kinase, such as ATM (Ataxia telangiactesia mutated) and ATR (ATM and Rad-3 related), which then leads to phosphorylation of p53. p53 is normally sequestrated by Mdm2. Phosphorylation of p53 disrupts p53"s interaction with Mdm2 and ushers its activation. p53 then holds the cell at check point until the damage is repaired. If the damage is irreversible, apoptosis is triggered. Oncogene such as myc, Ras V12 and E2F-1 have also been shown to induce apoptosis by indirectly activating p53 via activation of ARF (Alternating Radiation Frame). ARF acts by sequestering Mdm2 thus releasing p53. P53 has been shown to regulate apoptosis in both, a transcription dependent and independent manner. In the transcription dependent pathway p53 activates the expression of several proapoptotic proteins such as PUMA, Bax and BID, which is involved in the regulation of the intrinsic cell death pathway as well as upgrade CD95 (Fas/Apo1) and DR5 receptors which mediate the extrinsic cell death signals. In addition, transcriptional activation of proapoptotic protein p53 has also been shown to suppress anti-apoptotic proteins such as surviving.
II. P53 Structure And Function The human TP53 gene spans 20kb on chromosome band 17p13.1. The gene is composed of 11 exons, the first of which is noncoding. Its promoter does not contain TATA box but harbours a number of consensus binding sites. For common, transcription factors such as Spl, NF-kappaB or C-Jun. Despite these potential sites for transcriptional regulation, the expression of TP53 is constitutive and ubiquitous, most of the protein regulation taking place at the post translational level.

The p53 protein is a nuclear phosphoprotein, composed of 393 amino acids in human. It has five structural and functional domains i.e. an N-terminal transactivation domain, a protein rich regulatory domain, an oligomerization domain and a C-terminal domain involved in the regulation of DNA binding. The most common mutation that occurs in cancer alters this structure either by abrogating protein-DNA contacts or by disrupting protein folding. Various types of genotoxic and non genotoxic stresses can lead to p53 activation, like;- ¬ Gamma or UV radiation, free radical damage, inhibition of topiosomarase, which causes single or double strand break in DNA. ¬ Mutagens that form bulky DNA adducts like Aflotoxins, benzopyrines, alkylating agents, etc. ¬ Agents that block elongation by RNA polymerization. ¬ Agents that cause damage to mitotic spindle, ribonucleotide depletion, hypoxia, heat stroke, exposure to nitric acid etc. ¬ Once activated, p53 can trigger several cellular events, via two distinct parallel pathways i.e. transcription dependent and/or transcription independent pathways. However the response of p53 activation e.g. cell cycle arrest or apoptosis depends upon the nature and amplitude of inducing signals and also the tissue and cell type. It is also important to understand that the two biological responses may co-exist within the same tissue. Its role in carcinogenesis: p53 and its downstream pathways play a critical role in preventing tumour formation. Its role in oncogenesis may be described under three main actions i.e.

Loss of function

More than 50% of cancer patients‟ harbour somatic mutation on p53 gene and about 80% mutations are missense mutations. The germ line p53 mutation causes a rare type of cancer predisposition disorder called LiFraumeni syndrome (LFS). Both somatic and germ line mutations are usually followed by loss of heterozygosity (LOH) during tumour progression, which results in the inactivation of the remaining wild type alleles of p53. So the loss of function of p53 causes genomic instability, metastasis, resistance to chemotherapy and radiotherapy, poor patient survival and tumour progression.

Dominant negative activity of mutant p53.

The mutated p53 is over expressed in human tumours. This mutant p53 not only loses its tumour suppressive function but also has dominant negative activity on remaining wild type p53. It leads to accelerated tumour development and its growth.

Oncogenic properly of mutant p53.

Mutant p53 acquires oncogenic properties that lead to "gain-of-function". Several mechanisms are attributed to this "gain-of-function" phenomenon. e.g. 2.3.1. Mutant p53 can inhibit the function of the p53 family proteins i.e. p63 and p73 by protein-to-protein interaction. It is found that mutant p53 inhibits p73 and p63 only when it is present in excess. (of p63, and p73). This situation is common in tumours.

Regulation of gene transcription by mutant p53 is an important "gain-of-function" mechanism. Mutant p53 have the ability to activate the transcription of multi drug resistance 1 (MDR1) gene, which causes drug resistance in mutant p53 expressing cancer cells. Besides MDR1 mutant, p53 has been implicated in the transcriptional regulation of several genes including PCNA, c-myc, FAS, bcl-x1 and VEGF. The transcriptional regulation of these specific genes by mutant p53 may be modulated through preferential binding to structural DNA motif such as non-B DNA structures. It can do so, through interaction of mutant p53 with sequence specific transcription factor. 2.3.3. Mutant p53 inhibits the DNA repair pathway and thus have gain-of-function and genetic instability.

Rb and its Functions

Retinoblastoma is caused by mutations (changes) in certain genes. The most important gene in retinoblastoma is the *RB1* tumor suppressor gene. This gene makes a protein (pRb) that helps stop cells from growing too quickly. Each cell normally has 2 *RB1* genes. As long as a retinal cell has at least one *RB1* gene that works as it should, it will not form a retinoblastoma. But when both of the *RB1* genes are mutated or missing, a cell can grow unchecked. This can lead to further gene changes, which in turn may cause cells to become cancerous. In other words, pRB protein acts as a tumor suppressor, which means that it regulates cell growth and keeps cells from dividing too fast or in an uncontrolled way. Under certain conditions, pRB stops other proteins from triggering DNA replication, the process by which DNA makes a copy of itself. Because DNA replication must occur before a cell can divide, tight regulation of this process controls cell division and helps prevent the growth of tumors. Additionally, pRB interacts with other proteins to influence cell survival, the self-destruction of cells (apoptosis), and the process by which cells mature to carry out special functions (differentiation).

The *Rb* gene product interacts with a protein called E2F, nuclear transcription factor involved in cellular replication functions during the S phase of the cell cycle. By interacting with E2F it prevents it this function. The *Rb* gene product is only active when it is not phosphorylated by a

kinase. It cannot interact with E2F when it is phosphorylated. The mutant *Rb* gene product is always phosphorylated and cannot regulate E2F, control of cell division at the S phase does not occur, and normal cells become cancerous.

Hundreds of mutations in the *RB1* gene have been identified in people with retinoblastoma, a rare type of eye cancer that typically affects young children. This cancer develops in the retina, which is the specialized light-sensitive tissue at the back of the eye that detects light and color. Researchers estimate that 40 percent of all retinoblastomas are germinal, which means that *RB1* mutations occur in all of the body's cells and can be passed to the next generation. The other 60 percent are non-germinal, which means that *RB1* mutations occur only in the eye and cannot be passed to the next generation.

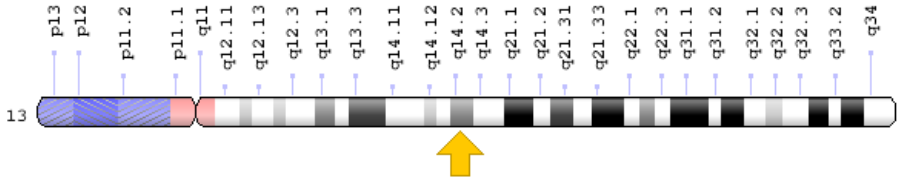
In germinal retinoblastoma, an *RB1* mutation is present in all of the body's cells. For retinoblastoma to develop, the other copy of the *RB1* gene also must be mutated or lost. This second mutation typically occurs early in life in retinal cells. Cells with two altered copies of the *RB1* gene produce no functional pRB and are unable to regulate cell division effectively. As a result, retinal cells lacking functional pRB can divide uncontrollably to form cancerous tumors. Some studies suggest that additional genetic changes can influence the development of retinoblastoma; these changes may help explain variations in the development and growth of tumors in different people.

In people with germinal retinoblastoma, *RB1* mutations increase the risk of several other cancers outside the eye. Specifically, these people are more likely to develop a cancer of the pineal gland in the brain (pinealoma), a type of bone cancer known as osteosarcoma, cancers of soft tissues such as muscle, and an aggressive form of skin cancer called melanoma.

Non-germinal retinoblastoma occurs in people with no history of the disorder in their family. Affected individuals are born with two normal copies of the *RB1* gene. Then, usually in early childhood, both copies of the gene in retinal cells acquire mutations or are lost. These genetic changes prevent the cells from producing any functional pRB. The loss of this protein allows retinal cells to grow and divide without control or order, leading to the development of a cancerous tumor.

Some gene mutations are acquired during a person's lifetime and are present only in certain cells. These changes, which are called somatic mutations, are not inherited. Somatic mutations that turn off (inactivate) the *RB1* gene have been reported in some cases of bladder cancer. Mutations in *RB1* are thought to contribute to the development of bladder cancer, and these genetic changes may help predict whether tumors will grow rapidly and spread to other tissues. In addition to bladder cancer, somatic mutations in the *RB1* gene are associated with many other types of cancer. For example, changes in the *RB1* gene have been reported in some cases of lung cancer, breast cancer, a bone cancer known as osteosarcoma, and an aggressive form of skin cancer called melanoma. Somatic *RB1* mutations have also been identified in some leukemias, which are cancers of blood-forming cells. Somatic *RB1* mutations in cancer cells inactivate pRB so it can no longer regulate cell division effectively.

Cytogenetic Location: 13q14.2, which is the long (q) arm of chromosome 13 at position 14.2
Molecular Location: base pairs 48,303,747 to 48,481,890 on chromosome 13 (Homo sapiens Annotation Release 108, GRCh38.p7) (NCBI)



KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
III-B.Sc., BIOCHEMISTRY
CANCER BIOLOGY (15BCU505C)
MULTIPLE CHOICE QUESTIONS
UNIT II

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	Oncogenes are the cancer causing genes in the cells but they do not express usually. This is because of the presence of	proto oncogenes	tumour promoters	tumour suppressor genes	transposons or jumping genes	**tumour suppressor genes**
2	Which one of the following genes is involved in the conversion of proto-oncogenes into oncogenes causing cancer?	metastasis genes	angiogenesis genes	transposons	tumour suppressor genes	**tumour suppressor genes**
3	Cancer is often the result of activation of ____ to ____ and the inactivation of ____ genes.	oncogenes, tumor-suppressor genes, proto-oncogenes	proto-oncogenes, oncogenes, tumor-suppressor genes	oncogenes, proto-oncogenes, tumor-suppressor genes	proto-suppressor genes, suppressors, oncogenes	**proto-oncogenes, oncogenes, tumor-suppressor genes**
4	About 50% of all human cancers may involve an abnormal or missing	oncogene	proto-oncogene.	p53 gene.	BRCA-1 gene	**oncogene**
5	Inherited retinoblastoma requires ____ mutation(s) or deletion(s)	one	two	three	four	**two**
6	_____ cancer in humans is directly caused by a viral infection	acute T cell leukemia	Wilms' tumor	Burkitt's lymphoma	Rous sarcoma	**acute T cell leukemia**
7	An oncogene transcribed and translated with another gene produces a	transcribed protein.	fusion protein	fusion cell	cancer protein	**fusion protein**
8	The P53 protein normally promotes	DNA repair.	tumor formation	cell division	apoptosis	**apoptosis**
9	The P53 gene is especially prone to	point mutation	chromosomal rearrangement	loss.	none of the above	**point mutation**
10	FAP colon cancer results from ____ mutation(s).	one	two	three	four or more	**four or more**
11	Which of the following cancers develops from loss of tumor suppression?	acute T cell leukemia	Wilms' tumor	Burkitt's lymphoma	Rous sarcoma	**acute T cell leukemia**
12	A mutation in which gene makes nearby DNA more susceptible to replication errors?	APC	BRCA 1	p53	RB	**APC**
13	Which of the following is the most commonly mutated oncogene in cancer?	p53	abl	ras	myc	**ras**
14	Inactivation of _____ is one of the steps leading to the development of cancer.	tumor suppressor genes	oncogenes	growth factors	stem cells	**tumor suppressor genes**
15	What is acquired during the progression of ALL tumors?	loss of the RB gene	mutations	the ability to synthesize mitogens	activation of tumor suppressors	**mutations**
16	A proto-oncogene can become an oncogene when:	It is shut off	It is translocated next to a highly expressed gene	Growth factors decrease cell division rate	A person is exposed to pesticides	**It is translocated next to a highly expressed gene**

17	A(n) _____ is a type of cancer-causing gene that promotes cancer by activating cell division at an inappropriate time or place.	Mutated DNA repair gene	Tumor suppressor gene	Oncogene	Proto-oncogene	**Oncogene**
18	The oncogene that causes Burkitt's lymphoma results from:	A translocation that moves a proto-oncogene next to an antibody gene	An inversion that places a proto-oncogene next to a transcription factor gene	A point mutation in a proto-oncogene	A virus that inserts next to a proto-oncogene	**A translocation that moves a proto-oncogene next to an antibody gene**
19	The Philadelphia translocation involves:	An exchange between chromosomes 9 and 22	An exchange between chromosomes 8 to 14	Translocation between chromosome 15 and 17	A fusion between chromosomes 14 and 21	**An exchange between chromosomes 9 and 22**
20	Genes that normally prevent cell division are:	Tumor suppressors	Transcription factors	Proto-oncogenes	Growth factors	**Tumor suppressors**
21	Chronic myeloid leukemia is caused by a translocation that creates:	A proto-oncogene	A fusion protein that acts like a transcription factor	A protein that increases growth factor production	A fusion protein that deregulates the cell cycle of myeloid white blood cells	**A fusion protein that deregulates the cell cycle of myeloid white blood cells**
22	Genes that normally prevent cell division are:	Tumor suppressors	Transcription factors	Proto-oncogenes	Growth factors	**Tumor suppressors**
23	Loss of tumor suppression in a cell usually results from:	Cytokine activation of a tumor suppressor gene	A translocation of a tumor suppressor gene	An inversion involving a tumor suppressor gene	A deletion of a tumor suppressor gene	**A deletion of a tumor suppressor gene**
24	The childhood kidney cancer Wilms' tumor is caused by:	Activation of an oncogene	Translocation of an oncogene	Loss of a tumor suppressor gene	A transposon	**Loss of a tumor suppressor gene**
25	With a germ-line mutation:	An oncogene is activated in some cells and a tumor suppressor deleted in others	Only some cells are affected	Two somatic mutations occur	All cells are affected	**All cells are affected**
26	Which of the following is a tumor suppressor gene instead of an oncogene?	Ras	Myc	P53	Abl	**P53**
27	Which statement is true?	All people who inherit proto-oncogenes develop cancer	All people who inherit the p53 gene develop cancer	Most cancers are caused by a series of genetic changes	Oncogenes and tumor suppressors act by the same mechanism	**Most cancers are caused by a series of genetic changes**
28	Growth of new blood vessels in and around tumors is called:	Invasiveness	Angiogenesis	Metastasis	Dedifferentiation	**Angiogenesis**
29	*BRCA*1 and *BRCA*2 mutations:	Are X-linked	Are incompletely penetrant	Result from translocations	Occur only in malignant breast tumors	**Are incompletely penetrant**
30	The BRAC1 gene is involved in regulating:	Cell division	Cell death	DNA repair	DNA replication	**DNA repair**

31	Metastasis is the process of _____.	changes accumulating in the structure of a cancer cell	cancer cells breaking free of the main tumor and spreading to other locations	naturally limiting cancer spread with the body's immune system.	destroying cancer cells with radiation	**cancer cells breaking free of the main tumor and spreading to other locations**
32	Any gene, mutated or not, that is abnormally expressed and associated with the development of cancer is called a(n) _____.	Oncogene	growth factor	tumor suppressor gene	proto-oncogene	**oncogene**
33	Cancer in situ _____.	invades normal tissue	is found in one place	dies quickly	spreads quickly	**is found in one place**
34	Cancer cells produce _____ enzymes that degrade membranes which allows movement into other tissues.	Suppressor	proteinase	inhibitor	metastase	**metastase**
35	which gene encode for proteins that promote the cell cycle and prevent apoptosis.	Tumor-suppressor genes	Proto-oncogenes	both a and b	none of the above	**Proto-oncogenes**
36	A(n) _____ is a cancer-causing gene.	Oncogene	mutagen	antibody	antigen	**oncogene**
37	The oncogenes most frequently involved in human cancers belong to the _____ gene family.	GPA	HIV	AIDS	ras	**ras**
38	umors that fail to have cells undergoing apoptosis lack an active _____ gene.	p53	BRAC-1	ras	RB	**ras**
39	In cancer cells, oncogenes cause:	an excess of Cyclin D which gives an uncontrollable cell division.	an excess of p53 inhibitors so apoptosis does not occur.	Both a and b are correct	Neither a nor b are correct	**Both a and b are correct**
40	Mutated tumor-suppressor genes cause:	no inhibitors of cyclin giving uncontrolled growth.	an increase of ras proteins.	no promotors of p53 giving no apoptosis.	Both a and c are correct.	**Both a and c are correct.**
41	___ is the study of cancer.	Oncology	Cytology	Histology	Tumorology	**Oncology**
42	An agent that causes mutation is a/an:	Carcinogen	mutagen.	oncogene	none of the above	**oncogene**
43	Which of the following is NOT an environmental carcinogen?	Radiation	high fiber food	viruses	certain organic chemicals	**high fiber food**

44	Each of the following have been observed as mechanisms resulting in the activation of a proto-oncogene except:	a point mutation altering the function of the oncogene protein product	inactivation of an oncogene by telomerase activity	amplification of an oncogene as small, sub-chromosomal fragments (double minutes)	a chromosome translocation fusing portions of the oncogene and another cellular gene	**inactivation of an oncogene by telomerase activity**
45	The functions which have been identified for the proteins expressed by cellular proto-oncogenes include all of the following except:	growth factor	transcription factor	growth factor receptor	enzyme involved in DNA mismatch repair	**enzyme involved in DNA mismatch repair**
46	Which of the following gene defect is associated with development of medullary carcinoma of thyroid?	RET protooncogene	FAP gene	Rb gene	BRCA 1 gene	**RET protooncogene**
47	Patients with familial retinoblastoma carry a germline mutation in one copy of the Rb gene. Potential mechanisms for inactivation of the other allele in a retinoblastoma tumor arising in one of these patients include:	loss of the normal chromosome 13	Mitotic crossing over	an independent point mutation	all of the above	**all of the above**
48	Which is NOT a typical mechanism by which a proto-oncogene is converted to an oncogene?	Amplification of the proto-oncogene	A chromosomal translocation resulting in the up-regulation of the proto-oncogene	Complete deletion of the proto-oncogene	A point mutation in the proto-oncogene	**Complete deletion of the proto-oncogene**
49	Which of the following is an example of a condition caused by a mutation in a single gene?	Colon cancer	Heart disease	AIDS	Cystic fibrosis	**Cystic fibrosis**
50	A man presents with a tumor that is found to carry a translocation of chromosomes 9 and 22. This is most characteristic of which one of the following?	Retinoblastoma	Li–Fraumeni syndrome	Chronic myelogenous leukemia	Soft tissue sarcoma	**Chronic myelogenous leukemia**
51	Chromosomal analysis of a tumor reveals the presence of double-minute chromatin bodies. This finding is indicative of which one of the following?	Translocation involving an oncogene	Inactivation of a tumor suppressor gene	Apoptosis	Amplification of an oncogene	**Amplification of an oncogene**
52	One of the following is an apoptosis inhibitor gene	p53′	BcL-2	Rb	c-Myc	**BcL-2**
53	The proteins expressed by cellular proto-oncogenes include all of the following except:	growth factor	transcription factor	growth factor receptor	enzyme involved in DNA mismatch repair	**enzyme involved in DNA mismatch repair**
54	In a cell loss of tumor suppression usually results from:	Cytokine activation of a tumor suppressor	A translocation of a tumor suppressor gene	An inversion involving a tumor	A deletion of a tumor suppressor	**A deletion of a tumor suppressor gene**

		gene		suppressor gene	gene	
55	The study of cancer is called	Oncology	Cytology	Histology	Tumorology	**Oncology**
56	P53 is _____	Oncogene	Tumor suppressor gene	Tumor activator gene	Protooncogene	**Oncogene**
57	Rb is _____.	Oncogene	Tumor suppressor gene	Tumor activator gene	Protooncogene	**Oncogene**
58	Acute T cell leukemia cancer in humans is directly caused by a _____infection	viral	Bacterial	Fungal	Retroviral	Viral
59	In cell division, the gene which prevents the cycle are:	Tumor suppressors	Transcription factors	Proto-oncogenes	Growth factors	**Tumor suppressors**
60	The gene that involved in regulating DNA repair	BRAC1	BRAC2	BRAC3	BRAC4	**BRAC1**

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DEPARTMENT OF BIOCHEMISTRY

SYLLABUS

SUBJECT NAME: CORE ELECTIVE I - CANCER BIOLOGY

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SEMESTER: V **CLASS: III B.Sc., BIOCHEMISTRY**

UNIT III

Cell cycle: cell cycle-G1 to S, progression of S phase, G2 to M phase, Anaphase check points and components involved as regulators of check points, role of cyclins and CDKs.

TEXT BOOKS

1. Papachristodoulou, D., Snape, A., Elliott, W. H., Elliott, D.C. (2014). *Biochemistry and Molecular Biology* (5th ed.). New York, Oxford University Press.
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1. Karp, G. (2013). *Cell and Molecular Biology*. (7th ed.) New York, John Wiley and Sons. Inc
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Cell cycle

The **cell cycle** can be thought of as the life cycle of a cell. In other words, it is the series of growth and development steps a cell undergoes between its "birth"—formation by the division of a mother cell—and reproduction—division to make two new daughter cells.

Stages of the cell cycle

To divide, a cell must complete several important tasks: it must grow, copy its genetic material (DNA), and physically split into two daughter cells. Cells perform these tasks in an organized, predictable series of steps that make up the cell cycle. The cell cycle is a cycle, rather than a linear pathway, because at the end of each go-round, the two daughter cells can start the exact same process over again from the beginning.

In eukaryotic cells, or cells with a nucleus, the stages of the cell cycle are divided into two major phases: **interphase** and the **mitotic (M) phase**.

- During *interphase*, the cell grows and makes a copy of its DNA.
- During the *mitotic (M) phase*, the cell separates its DNA into two sets and divides its cytoplasm, forming two new cells.

G1 to S phase

Interphase

- **G1 phase.** Metabolic changes prepare the cell for division. At a certain point - the restriction point - the cell is committed to division and moves into the S phase. The G1/S transition is a stage in the cell cycle at the boundary between the G1 phase, in which the cell grows, and the S phase, during which DNA is replicated. It is a cell cycle check point where DNA integrity is assessed and the cell cycle can pause in response to improperly or partially replicated DNA. During this transition the cell makes decisions to become quiescent (enter G0), differentiate, make DNA repairs, or proliferate based on environmental cues and molecular signaling inputs. The G1/S transition occurs late in G1 and the absence or improper application of this highly regulated check point can lead to cellular transformation and disease states such as cancer.

  During this transition, G1 cyclin D-Cdk4/6 dimer phosphorylates retinoblastoma releasing transcription factor E2F, which then drives the transition from G1 to S phase. The G1/S transition is highly regulated by transcription factor p53 in order to halt the cell cycle when DNA is damaged. It is a "point of no return" beyond which the cell is committed to dividing; in yeast this is called START and in multicellular eukaryotes it is termed the restriction point (R-Point). If a cell passes through the G1/S transition the cell will continue through the cell cycle regardless of incoming mitogenic factors due to the positive feed-back loop of G1-S transcription. Positive feed-back loops include G1 cyclins and accumulation of E2F.

  In mid to late G1 phase, cyclin D bound to Cdk4/6 activates the expression of the S phase cyclin-Cdk components; however, the cell does not want S phase cyclins to become active in G1. Therefore, an inhibitor, protein Slc-1, is present that interacts with the dimer so that the S phase cyclin-Cdk dimer remains inactive until the cell is ready to move into S phase. After the cell has grown and is ready to synthesize DNA, G1 cyclin-Cdks phosphorylate the S phase cyclin inhibitor signaling ubiquitination, resulting in the addition of groups to the inhibitor. Ubiquitination of the inhibitor signals the SCF/proteasome to degrade the inhibitor releasing and allowing the S phase cyclin-Cdk to become activated and the cell moves into S phase. Once in S phase, cyclin-Cdks phosphorylate several factors on the replication complex promoting DNA replication by causing inhibitory proteins to fall off of replication complexes or through activation of components on the replication complex to induce DNA replication initiation.

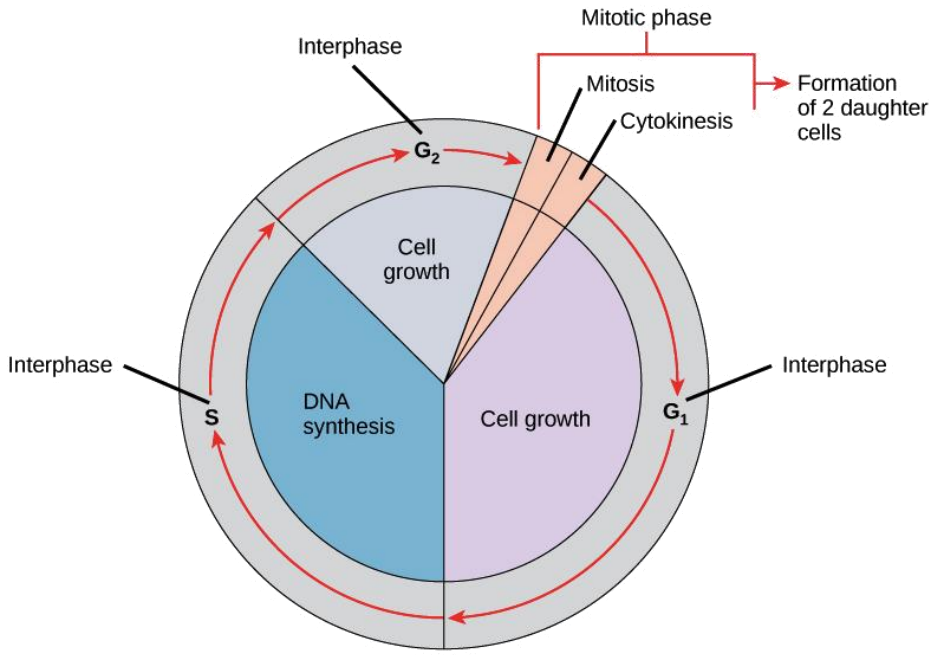
Progression of S phase

- **S phase**. DNA synthesis replicates the genetic material. Each chromosome now consists of two sister chromatids.

G2 to M phase

- **G2 phase.** Metabolic changes assemble the cytoplasmic materials necessary for mitosis and cytokinesis.
- **M phase.** A nuclear division (mitosis) followed by a cell division (cytokinesis).

The period between mitotic divisions - that is, G1, S and G2 - is known as interphase.



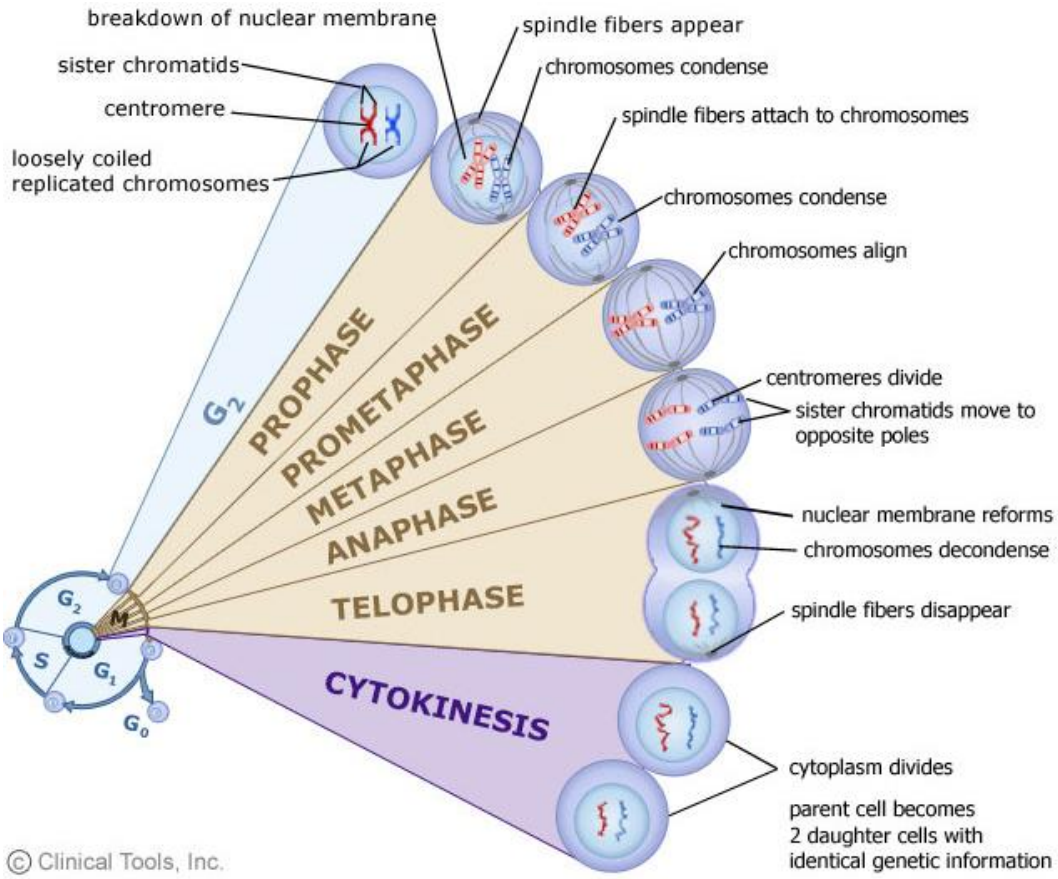
Mitosis

Mitosis is a form of eukaryotic cell division that produces two daughter cells with the same genetic component as the parent cell. Chromosomes replicated during the S phase are divided in such a way as to ensure that each daughter cell receives a copy of every chromosome. In actively dividing animal cells, the whole process takes about one hour.

The replicated chromosomes are attached to a 'mitotic apparatus' that aligns them and then separates the sister chromatids to produce an even partitioning of the genetic material. This separation of the genetic material in a mitotic nuclear division (or **karyokinesis)** is followed by a separation of the cell cytoplasm in a cellular division (or **cytokinesis**) to produce two daughter cells.

In some single-celled organisms mitosis forms the basis of asexual reproduction. In diploid multicellular organisms sexual reproduction involves the fusion of two haploid gametes to produce a diploid zygote. Mitotic divisions of the zygote and daughter cells are then responsible for the subsequent growth and development of the organism. In the adult organism, mitosis plays a role in cell replacement, wound healing and tumour formation.

Mitosis, although a continuous process, is conventionally divided into five stages: prophase, prometaphase, metaphase, anaphase and telophase.



The phases of mitosis

Prophase

Prophase occupies over half of mitosis. The nuclear membrane breaks down to form a number of small vesicles and the nucleolus disintegrates. A structure known as the **centrosome** duplicates itself to form two daughter centrosomes that migrate to opposite ends of the cell. The centrosomes organize the production of microtubules that form the spindle fibres that constitute the **mitotic spindle**. The chromosomes condense into compact structures. Each replicated chromosome can now be seen to consist of two identical **chromatids** (or **sister chromatids**) held together by a structure known as the **centromere**.

Prometaphase

The chromosomes, led by their centromeres, migrate to the equatorial plane in the mid-line of the cell - at right-angles to the axis formed by the centrosomes. This region of the mitotic spindle is known as the **metaphase plate**. The spindle fibres bind to a structure associated with the centromere of each chromosome called a kinetochore. Individual spindle fibres bind to a **kinetochore** structure on each side of the centromere. The chromosomes continue to condense.

Metaphase

The chromosomes align themselves along the metaphase plate of the spindle apparatus.

Anaphase

The shortest stage of mitosis. The centromeres divide, and the sister chromatids of each chromosome are pulled apart - or 'disjoin' - and move to the opposite ends of the cell, pulled by spindle fibres attached to the kinetochore regions. The separated sister chromatids are now

referred to as **daughter chromosomes**. (It is the alignment and separation in metaphase and anaphase that is important in ensuring that each daughter cell receives a copy of every chromosome.)

Telophase

The final stage of mitosis, and a reversal of many of the processes observed during prophase. The nuclear membrane reforms around the chromosomes grouped at either pole of the cell, the chromosomes uncoil and become diffuse, and the spindle fibres disappear.

Cytokinesis

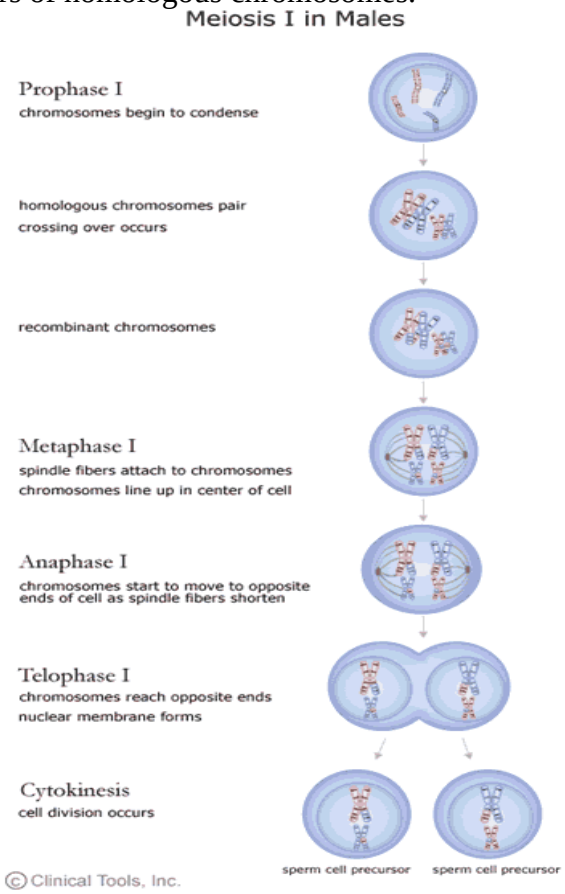
The final cellular division to form two new cells. In plants a cell plate forms along the line of the metaphase plate; in animals there is a constriction of the cytoplasm. The cell then enters interphase - the interval between mitotic divisions.

Meiosis

Meiosis is the form of eukaryotic cell division that produces **haploid** sex cells or gametes (which contain a single copy of each chromosome) from **diploid** cells (which contain two copies of each chromosome). The process takes the form of one DNA replication followed by two successive nuclear and cellular divisions (Meiosis I and Meiosis II). As in mitosis, meiosis is preceded by a process of DNA replication that converts each chromosome into two sister chromatids.

Meiosis I

Meiosis I separates the pairs of homologous chromosomes.



In Meiosis I a special cell division reduces the cell from diploid to haploid.

Prophase I

The homologous chromosomes pair and exchange DNA to form recombinant chromosomes. Prophase I is divided into five phases:

- **Leptotene**: chromosomes start to condense.
- **Zygotene**: homologous chromosomes become closely associated (synapsis) to form pairs of chromosomes (bivalents) consisting of four chromatids (tetrads).
- **Pachytene**: crossing over between pairs of homologous chromosomes to form chiasmata (sing. chiasma).
- **Diplotene**: homologous chromosomes start to separate but remain attached by chiasmata.
- **Diakinesis**: homologous chromosomes continue to separate, and chiasmata move to the ends of the chromosomes.

Prometaphase I

Spindle apparatus formed, and chromosomes attached to spindle fibres by kinetochores.

Metaphase I

Homologous pairs of chromosomes (bivalents) arranged as a double row along the metaphase plate. The arrangement of the paired chromosomes with respect to the poles of the spindle apparatus is random along the metaphase plate. (This is a source of genetic variation through random assortment, as the paternal and maternal chromosomes in a homologous pair are similar but not identical. The number of possible arrangements is 2^n, where n is the number of chromosomes in a haploid set. Human beings have 23 different chromosomes, so the number of possible combinations is 2^{23}, which is over 8 million.)

Anaphase I

The homologous chromosomes in each bivalent are separated and move to the opposite poles of the cell

Telophase I

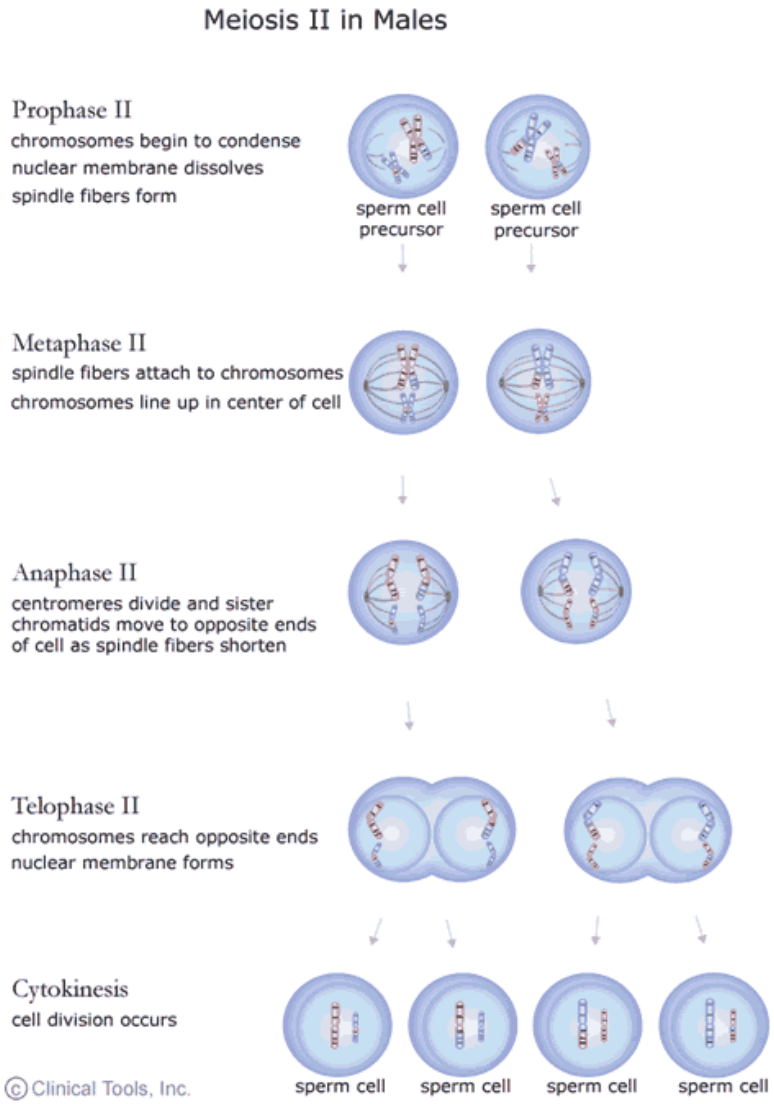
The chromosomes become diffuse and the nuclear membrane reforms.

Cytokinesis

The final cellular division to form two new cells, followed by Meiosis II. Meiosis I is a reduction division: the original diploid cell had two copies of each chromosome; the newly formed haploid cells have one copy of each chromosome.

Meiosis II

Meiosis II separates each chromosome into two chromatids.

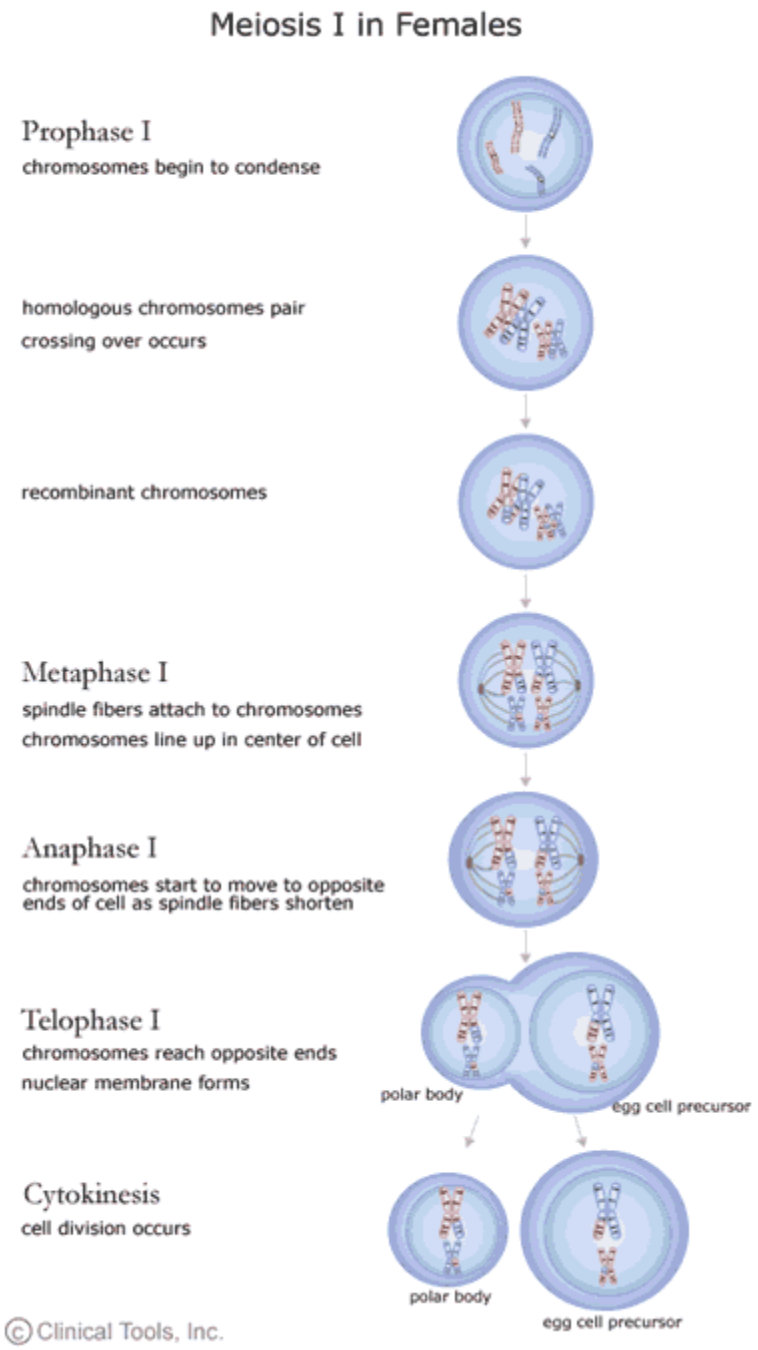


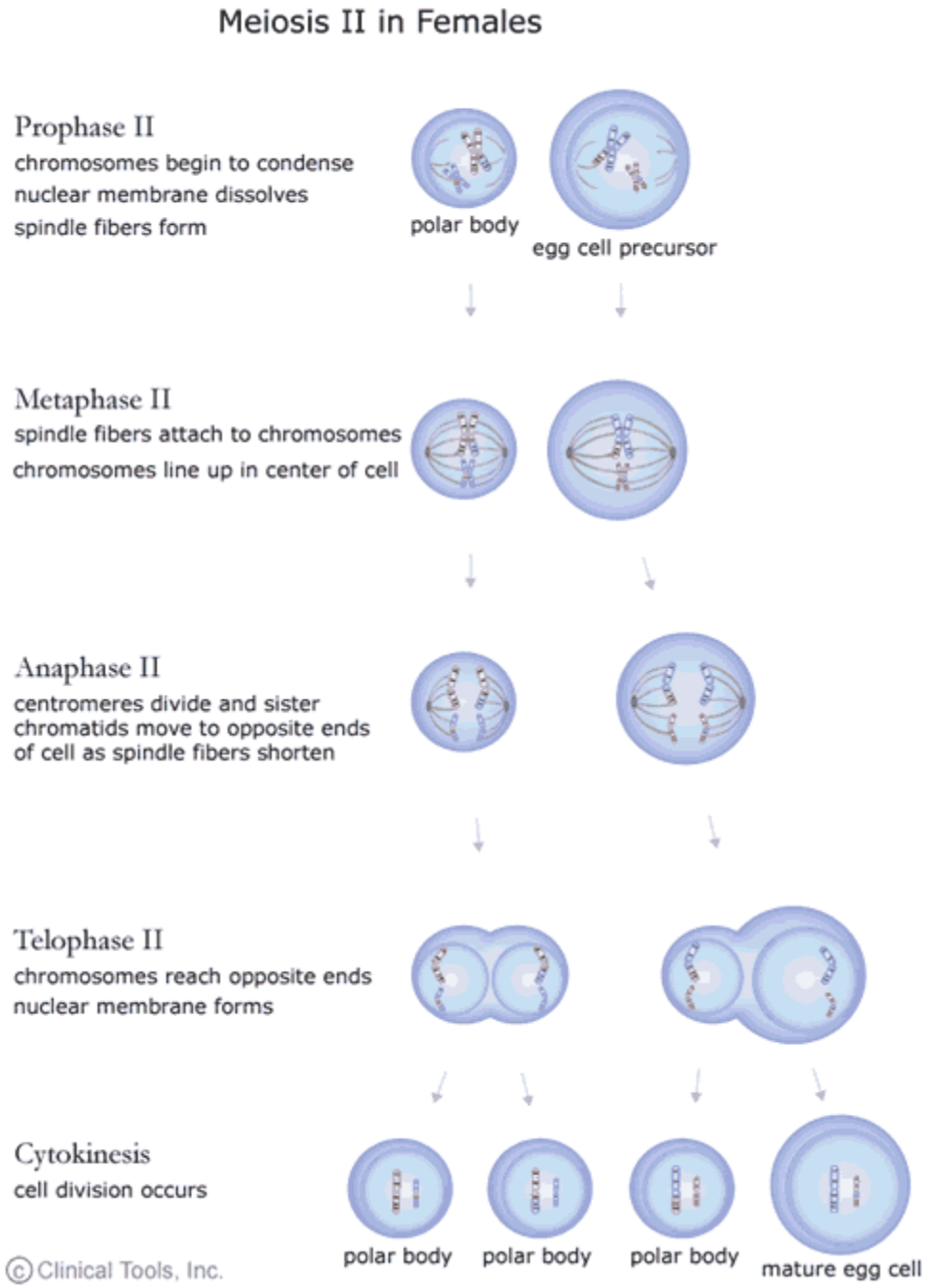
The events of Meiosis II are analogous to those of a mitotic division, although the number of chromosomes involved has been halved.

Meiosis generates **genetic diversity** through:

- the exchange of genetic material between homologous chromosomes during Meiosis I
- the random alignment of maternal and paternal chromosomes in Meiosis I
- the random alignment of the sister chromatids at Meiosis II

Meiosis in females





Anaphase check points and components involved as regulators of check points

A **checkpoint** is a stage in the eukaryotic cell cycle at which at which the cell examines internal and external cues and "decides" whether or not to move forward with division.

There are a number of checkpoints, but the three most important ones are:

- The G_11start subscript, 1, end subscript checkpoint, at the G_11start subscript, 1, end subscript/S transition.

- The G_22start subscript, 2, end subscript checkpoint, at the G_22start subscript, 2, end subscript/M transition.
- The spindle checkpoint, at the transition from metaphase to anaphase.

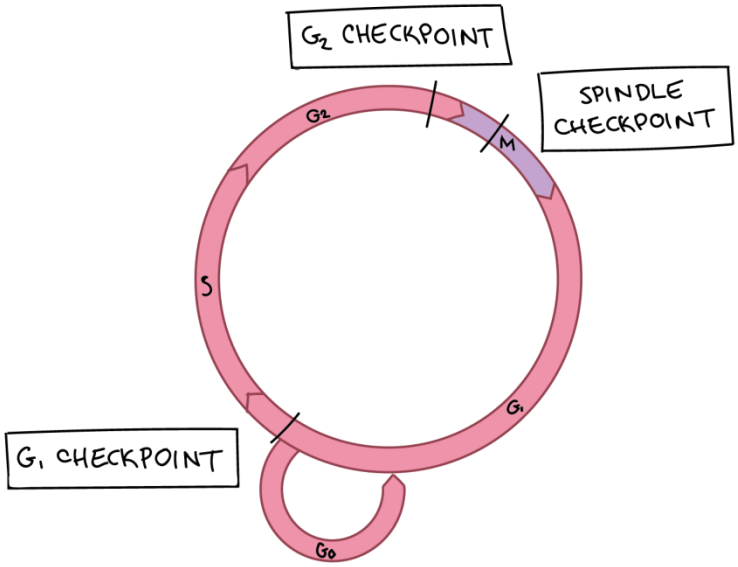
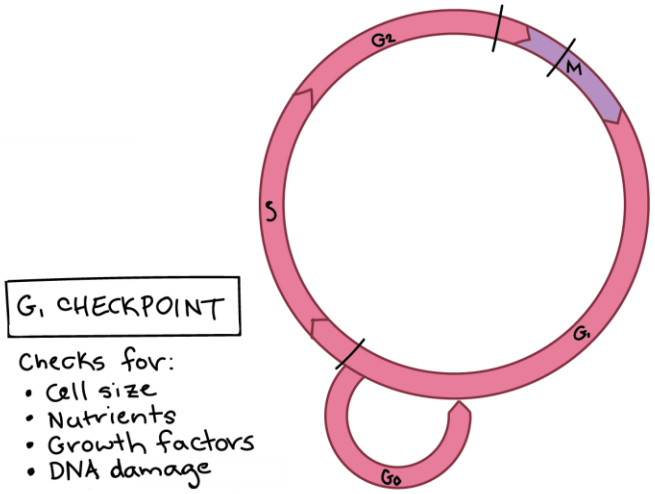


Diagram of cell cycle with checkpoints marked. G1 checkpoint is near the end of G1 (close to the G1/S transition). G2 checkpoint is near the end of G2 (close to the G2/M transition). Spindle checkpoint is partway through M phase, and more specifically, at the metaphase/anaphase transition.

The G_11start subscript, 1, end subscript checkpoint

The **G_11start subscript, 1, end subscript checkpoint** is the main decision point for a cell – that is, the primary point at which it must choose whether or not to divide. Once the cell passes the G_11start subscript, 1, end subscript checkpoint and enters S phase, it becomes irreversibly committed to division. That is, barring unexpected problems, such as DNA damage or replication errors, a cell that passes the G_11start subscript, 1, end subscript checkpoint will continue the rest of the way through the cell cycle and produce two daughter cells.



The G1 checkpoint. The G1 checkpoint is located at the end of G1 phase, before the transition to S phase. If cells don't pass the G1 checkpoint, they may "loop out" of the cell cycle and into a resting state called G0, from which they may subsequently re-enter G1 under the appropriate conditions.

At the G1 checkpoint, cells decide whether or not to proceed with division based on factors such as:

- Cell size
- Nutrients
- Growth factors
- DNA damage

At the G_11start subscript, 1, end subscript checkpoint, a cell checks whether internal and external conditions are right for division. Here are some of the factors a cell might assess:

- **Size**
- **Nutrients**
- **Molecular signals**
- **DNA integrity**

These are not the only factors that can affect progression through the G_11start subscript, 1, end subscript checkpoint, and which factors are most important depend on the type of cell. For instance, some cells also need mechanical cues (such as being attached to a supportive network called the extracellular matrix) in order to divide^11start superscript, 1, end superscript.

If a cell doesn't get the go-ahead cues it needs at the G_11start subscript, 1, end subscript checkpoint, it may leave the cell cycle and enter a resting state called **G_00start subscript, 0, end subscript phase**. Some cells stay permanently in G_00start subscript, 0, end subscript, while others resume dividing if conditions improve.

The G_22start subscript, 2, end subscript checkpoint

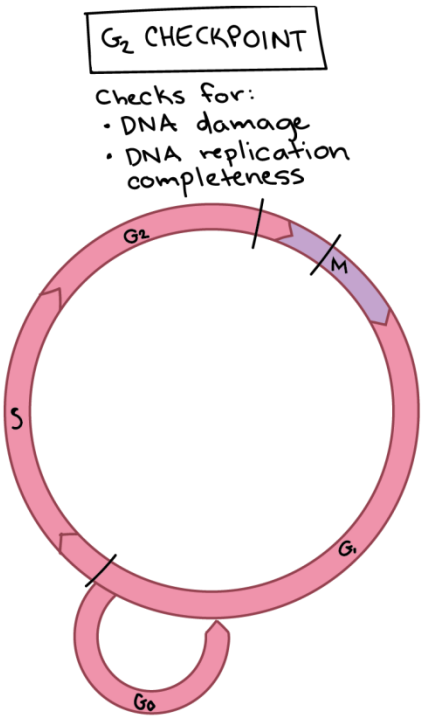


Image of the cell cycle with the G2 checkpoint marked. At the G2 checkpoint, the cell checks for:

- DNA damage
- DNA replication completeness

To make sure that cell division goes smoothly (produces healthy daughter cells with complete, undamaged DNA), the cell has an additional checkpoint before M phase, called the **G_22start subscript, 2, end subscript checkpoint**. At this stage, the cell will check:

- **DNA integrity**
- **DNA replication**

If errors or damage are detected, the cell will pause at the G_22start subscript, 2, end subscript checkpoint to allow for repairs. If the checkpoint mechanisms detect problems with the DNA, the cell cycle is halted, and the cell attempts to either complete DNA replication or repair the damaged DNA.

If the damage is irreparable, the cell may undergo apoptosis, or programmed cell death^22start superscript, 2, end superscript. This self-destruction mechanism ensures that damaged DNA is not passed on to daughter cells and is important in preventing cancer.

The spindle checkpoint

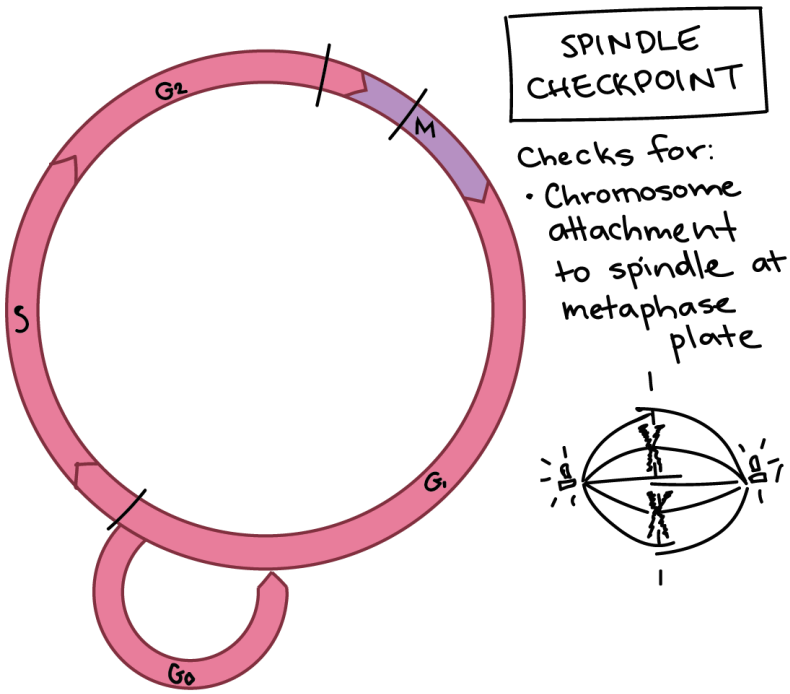


Image of the cell cycle with the spindle checkpoint marked. At the spindle checkpoint, the cell checks for:

- Chromosome attachment to spindle at the metaphase plate

The M checkpoint is also known as the **spindle checkpoint**: here, the cell examines whether all the sister chromatids are correctly attached to the spindle microtubules. Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until all the chromosomes are firmly attached to at least two spindle fibers from opposite poles of the cell.

The cells don't actually scan the metaphase plate to confirm that all of the chromosomes are there. Instead, they look for "straggler" chromosomes that are in the wrong place (e.g., floating around in the cytoplasm)^33start superscript, 3, end superscript. If a chromosome is misplaced, the cell will pause mitosis, allowing time for the spindle to capture the stray chromosome.

The factors that influence a cell's decision to pause or progress at each checkpoint. However, you may be wondering what these factors actually do to the cell, or change inside of it, to cause (or block) progression from one phase of the cell cycle to the next.

The general answer is that internal and external cues trigger signaling pathways inside the cell that activate, or inactivate, a set of core proteins that move the cell cycle forward. You can learn more about these proteins, and see examples of how they are affected by cues such as DNA damage, in the article on cell cycle regulators.

Multiple checkpoints in the eukaryotic cell cycle ensure that division occurs only after sufficient growth and faithful DNA replication, and only when favorable conditions exist. At each checkpoint, numerous proteins engage in a series of carefully coordinated biochemical reactions. This complexity allows for precise regulation of all steps in the cell cycle — and it is essential to preventing the devastating consequences of cell division gone awry (Figure).

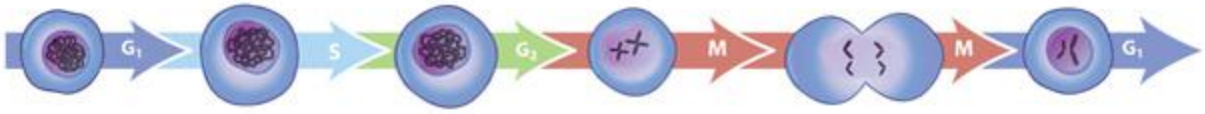


Figure: The sequence of eukaryotic cell cycle phases
Between each arrow, the cell passes through a particular cell cycle checkpoint.

Role of Cyclin and cyclin-dependent kinases (CDKs)

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 specific stretches of amino acids in the substrates. Different types of eukaryotic cells contain different types and numbers of CDKs. For example, yeast have only a single CDK, whereas vertebrates have four different ones.

As their name suggests, CDKs require the presence of **cyclins** to become active. Cyclins are a family of proteins that have no enzymatic activity of their own but activate CDKs by binding to them. CDKs must also be in a particular phosphorylation state — with some sites phosphorylated and others dephosphorylated — in order for activation to occur. Correct phosphorylation depends on the action of other kinases and a second class of enzymes called phosphatases that are responsible for removing phosphate groups from proteins.

How Do CDKs Control the Cell Cycle?

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.) All cyclins are named according to the stage at which they assemble with CDKs. Common classes of cyclins include G_1-phase cyclins, G_1/S-phase cyclins, S-phase cyclins, and M-phase cyclins. M-phase cyclins form M-CDK complexes and drive the cell's entry into mitosis; G_1 cyclins form G_1-CDK complexes and guide the cell's progress through the G_1phase; and so on.

All CDKs exist in similar amounts throughout the entire cell cycle. In contrast, cyclin manufacture and breakdown varies by stage — with cell cycle progression dependent on the synthesis of new cyclin molecules. Accordingly, cells synthesize G_1- and G_1/S-cyclins at different times during the G_1 phase, and they produce M-cyclin molecules during the G_2phase (Figure 2). Cyclin degradation is equally important for progression through the cell cycle. Specific enzymes break down cyclins at defined times in the cell cycle. When cyclin levels decrease, the corresponding CDKs become inactive. Cell cycle arrest can occur if cyclins fail to degrade.

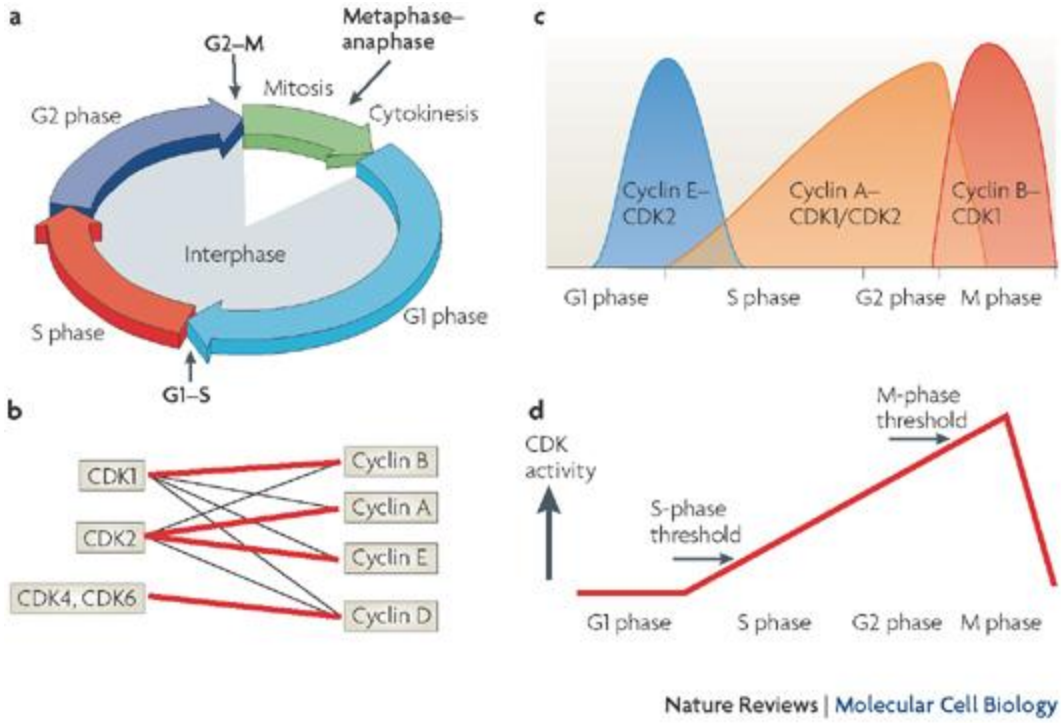


Figure 2: The classical and minimal models of cell cycle control

Where and when do cyclins act on the cell cycle?

(A) Cycling cells undergo three major transitions during their cell cycle. The beginning of S phase is marked by the onset of DNA replication, the start of mitosis (M) is accompanied by breakdown of the nuclear envelope and chromosome condensation, whereas segregation of the sister chromatids marks the metaphase-to-anaphase transition. Cyclin-dependent kinases (CDKs) trigger the transition from G1 to S phase and from G2 to M phase by phosphorylating distinct sets of substrates.

(B) CDK1 and CDK2 bind to multiple cyclins (cyclin types A, B, D and E), whereas CDK4 and CDK6 only partner D-type cyclins. Thick lines represent the preferred pairing for each kinase.

(C) According to the classical model of cell cycle control, D-type cyclins and CDK4 or CDK6 regulate events in early G1 phase (not shown), cyclin E-CDK2 triggers S phase, cyclin A-CDK2 and cyclin A-CDK1 regulate the completion of S phase, and CDK1-cyclin B is responsible for mitosis.

(D) Based on the results of cyclin and CDK-knockout studies, scientists have constructed a new threshold model of cell cycle control. Accordingly, either CDK1 or CDK2 bound to cyclin A is sufficient to control interphase, whereas cyclin B-CDK1 is essential to take cells into mitosis. The differences between interphase and mitotic CDKs are not necessarily due to substrate specificity, but are more likely a result of different localization and a higher activity threshold for mitosis than interphase.

Which Proteins Do CDKs Modify?

Each of the cyclin-CDK complexes in a cell modifies a specific group of protein substrates. Proper phosphorylation of these substrates must occur at particular times in order for the cell cycle to continue. Because cyclin-CDK complexes recognize multiple substrates, they are able to

coordinate the multiple events that occur during each phase of the cell cycle. For example, at the beginning of S phase, S-CDK catalyzes phosphorylation of the proteins that initiate DNA replication by allowing DNA replication complexes to form. Later, during mitosis, M-CDKs phosphorylate a wide range of proteins. These include condensin proteins, which are essential for the extensive condensation of mitotic chromosomes, and lamin proteins, which form a stabilizing network under the nuclear membrane that dissembles during mitosis. M-CDKs also influence the assembly of the mitotic spindle by phosphorylating proteins that regulate microtubule behavior. The net effect of these coordinated phosphorylation reactions is the accurate separation of chromosomes during mitosis.

The life cycle of a cell is a carefully regulated series of events orchestrated by a suite of enzymes and other proteins. The main regulatory components of cell cycle control are cyclins and CDKs. Depending on the presence and action of these proteins, the cell cycle can be speedy or slow, and it may even halt altogether.

CDKs are important master regulators of the cell cycle. Their role is to phosphorylate proteins on either S or T amino acids and thereby regulate the activity of those proteins. Yeast have just one CDK (Cdk1), while 'metazoans' (animals) like us have nine, of which four are really critical to the cell cycle and will be introduced today.

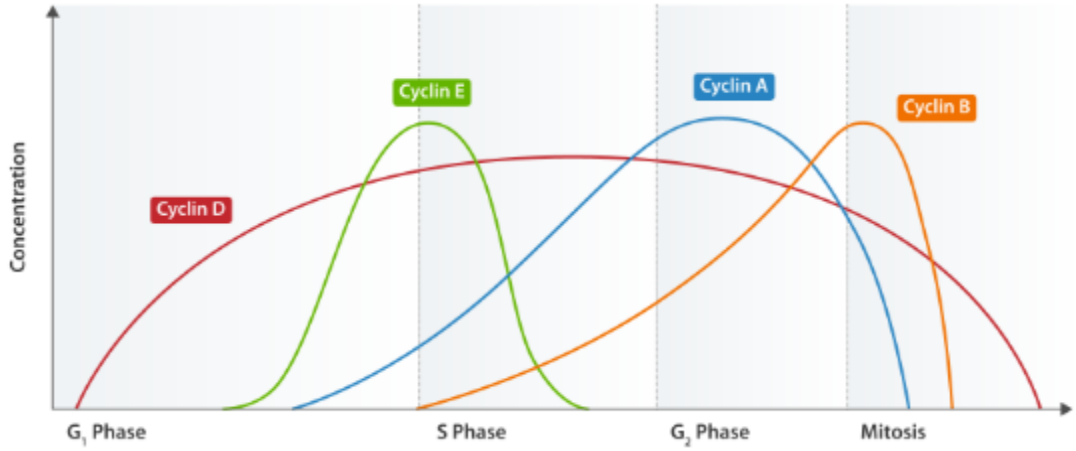
How are the CDKs themselves regulated? The levels of these proteins remain pretty constant throughout the cell cycle, yet their levels of *activity* rise and fall cyclically. CDKs need to hydrolize ATP for energy in order to perform phosphorylation. They have an ATP binding cleft whose ability to bind ATP is regulated by two mechanisms. First, CDKs have a 'flexible T loop' which contains a threonine (T) residue which normally blocks the ATP binding cleft, but not when the T is phosphorylated. Second, cyclins bind CDKs and induce a conformational change that also helps to expose the ATP binding cleft. Therefore a fully active CDK is one which is both phosphorylated at the T on the T loop *and* is bound to a cyclin.

The various activities of the cell cycle, then, are determined by the combination of cyclins and CDKs that are active at each stage, as shown in the following table:

cell cycle stage	**cyclins**	**CDKs**	**comments**
G1	Cyclin D	CDK4&6	Can react to outside signals such as growth factors or mitogens.
G1/S	Cyclins E & A	CDK2	Regulate centrosome duplication; important for reaching START
S	Cyclins E & A	CDK2	Targets are helicases and polymerases

cell cycle stage	cyclins	CDKs	comments
M	Cyclins A & B	CDK1	Regulate G2/M checkpoint. The cyclins are synthesized during S but not active until synthesis is complete. Phosphorylate lots of downstream targets.

All cyclins contain a conserved 100 amino acid 'cyclin box.' Cyclin/CDK complexes regulate the cell cycle both by promoting activates for their respective stages, and by inhibiting activates for future cell cycle stages that must not yet be reached. Therefore cyclins must be able to be both generated *and* degraded in order for the cell cycle to proceed.



Antibodies against Cyclin D inhibit entry into the S phase, which can be measured by whether BrdU (a fluorescent nucleotide analog) gets incorporated into DNA (which only happens during synthesis).

DNA replication starts at 'pre-replication complexes' that get assembled at *origins of replication* during early G1 phase. The S-phase cyclin/CDK complexes phosphorylate these complexes and thereby trigger replication starting from these 'origins'.

Here is a bit more about how the CDKs are regulated through phosphorylation. A CDK-activating kinase (CAK; a trimeric complex composed of CDK-7, Cyclin H and Mat1) phosphorylates amino acid T160 in CDK, located at the T loop, thereby *activating* CDK. CDKs are also regulated by CDK inhibitors p27 (CDKN1B gene), p21 (CDKN1A gene) and p57 (CDKN1C gene), which bind to and inhibit both of the G1 CDKs (CDK4 & CDK6). p27 does this by physically blocking the cyclin/CDK complex's interaction with its targets. p15 (CDKN2B gene) and p16 (CDKN2A gene) are both Ink4s ("inhibitors of kinase 4", though they also inhibit CDK6) control the mid G1 phase by binding to CDK4 and CDK6 and blocking their binding to cyclin D. This results in decreased phosphorylation of target proteins. Overexpression of p16 arrests the cell cycle by inhibiting CDK4/Cyclin D during early G1.

Another inhibitor called Rb prevents entry into the S phase by binding to E2F transcription factors. E2Fs are transcriptional activators when they act alone but repressors when bound to Rb. The mid-G1 cyclin/CDK complexes partially phosphorylate Rb, reducing its binding to

E2Fs; the late G1 complex of CDK2/Cyclin E completely phsophorylates it, preventing its binding to E2F. E2Fs can then act as transcriptional activators for genes needed in the S phase.
S-phase cyclin/CDK complexes accumulate in late G1, but are still bound to an inhibitor called Sic1. But G1 CDK/cyclin complexes polyphosphorylate Sic1 at six sites. When and only when all six sites are phosphorylated (which takes a while), Sic1 is released, whereupon it gets polyubiquitinated and therefore degraded by the proteosome. The S-phase cyclin/CDK complexes are then free to induce DNA replication. This mechanism allows the cell to accumulate many of these complexes 'ahead of time' (starting in G1) but then post-translation activate them all at once in order to suddenly start massive-scale DNA replication in the S phase.
Targeting of proteins such as Sic1 to the proteasome is mediated by two complexes SCF (Skp, Cullin & F-Box) and APC/C (anaphase promoting complex / cyclosome), each composed of ubiquitin and a protein ligase. These two control three major transitions in the cell cycle:

1. the onset of S phase by degradation of Sic1
2. beginning of anaphase by degradation of securin
3. exit from mitosis by degradation of Cyclin B.

APC/C is composed of several proteins including but not limited to cullin (APC2), Ring (APC11) and SCF-like protein. APC/C's role is to ubiquitinate (add ubiquitin tags to) proteins, thus flagging them for degradation by the proteasome. Cdc20 casues APC/C to polyubiquitinate the anaphase inhibitor securin in point (2) above. Cdh1 causes APC/C to polyubiquitinate Cyclin B in point (3) above. Cdh1 is itself regulated by phosphorylation – the G1/S cyclin/CDK complexes phosphorylate it, preventing it from acting too soon; Cdc14 then later activates Cdh1.
SCF has no binding partner – rather, its ability to ubiquitinate targets (substrates) is determined by the latter's phosphorylation state. SCF is active throughout the cell cycle; the cell regulates its activity by regulating the phosphorylation of SCF's targets. An example is Sic1 (above).
MPF (Maturation or Mitosis Promoting Factor depending on who you ask) is a fancy word for the cyclin B / CDK1 complex, which is active in the M phase – actually, required in order for entry to the M phase. Here's how MPF itself is regulated. CDK1 has two phosphorylation sites (in vertebrates; only one in yeast) at amino acids Y15 and T161. In animals, phosphorylation of Y15 is inhibitory, while phosphorylation of T161 is activating. The cyclin/CDK2 complex begins its life wholly unphosphorylated (thus inactive), then Wee1 phosphorylates Y15, CAK then phosphorylates T161 too, then finally Cdc25 removes the Y15 phosphorylation, allowing the complex to be active (about 100x more activity than with neither site phosphorylated). Cdc25 overexpression results in premature removal of this Y15 phosphorylation so that the cell enters the M phase before it has had enough time to grow during the G2 phase. This results in 'wee cells' which are literally small because the cell growth was inhibited, so that the daughter cells after mitosis are smaller than the mother cell was. (Hence the name Wee1, which is of course backwards since Wee1 acts *against* the 'wee cell' phenotype). Once a cell passes the restriction point (R) in late G1, it is committed to passing through the S phase.
Many cancers involve a loss of p16 leading to cyclin D1 overexpression. Other cancers (esp. late onset cancers – breast, lung, bladder) often have hyperphosphorylation of Rb leading to release of E2F to promote the cell cycle. This hyperphosphorylation can be caused by loss or 'misexpression' of one or more things .

Regulatory steps in the cell cycle

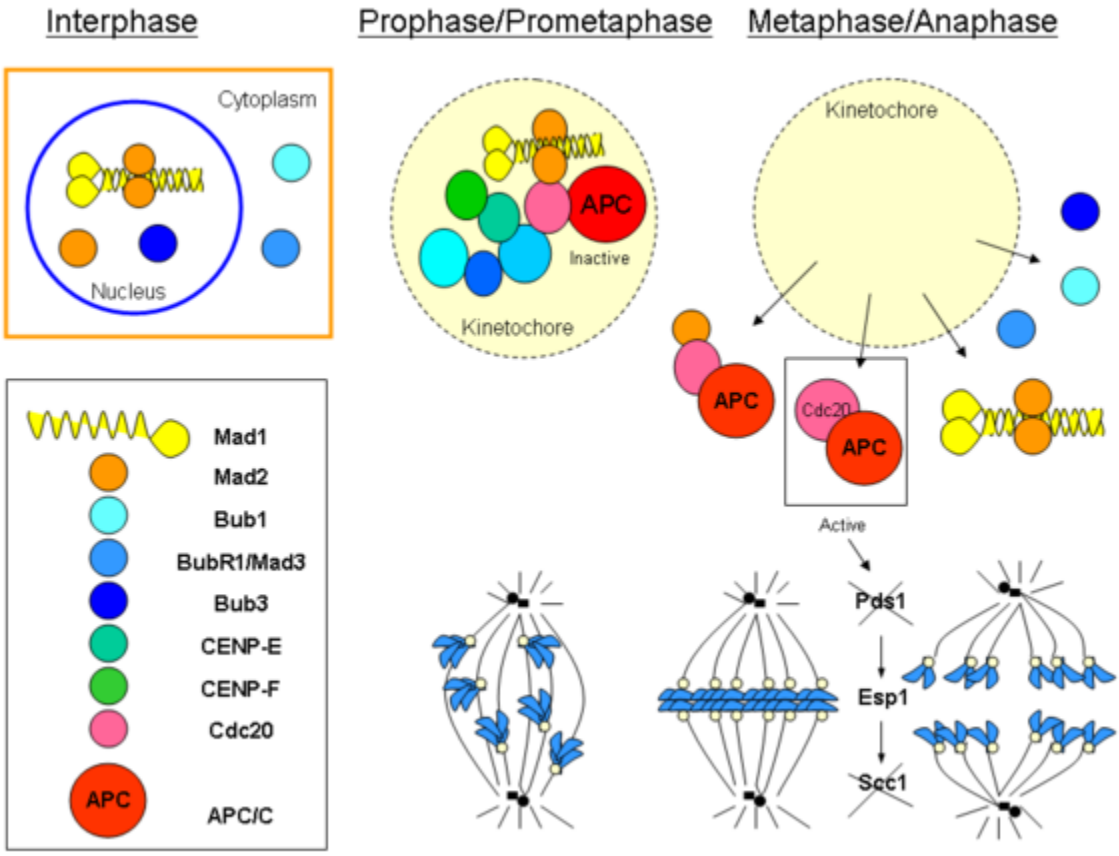
Here is an overview of what are considered to be the 9 fundamental steps of cell cycle regulation.

Cell starts in phase G0 or G1.

1. DNA replication machinery begins to assemble at *origins of replication*
2. G1 cyclin-CDK complexes inactivate Cdh1
3. G1 cyclin-CDK complexes activate the S-phase cyclin-CDK expression
4. G1 cyclin-CDK complexes phosphorylate and thereby inactivate S-phase inhibitor(s?)
5. SCF polyubiquitinates the phosphorylated S-phase inhibitor, targeting it for proteasome degradation. ***Cell enters S phase.***
6. S-phase cyclin/CDK activates the pre-replication complexes that began to assemble in step 1. ***S phase and G2 phase happen.***
7. Cdc25 phosphatase activates M-phase cyclin/CDKs. ***Cell enters M phase and gets to metaphase.***
8. APC/C and Cdc20 target securin for proteosomal degradation. ***Cell advances from metaphase to anaphase.***
9. CdcA phosphatase activates Cdh1, making it possible for APC/C and Cdh1 to target the M-phase cyclins for proteasomal degradation. ***Cell returns from M phase to G0 or G1 phase.***

Step 7 relies on a mechanism to recognize unreplicated DNA and stalled replication forks in order to ensure that mitosis does not proceed before all DNA has been replicated. Two proteins are involved in this mechanism: ATR and Chk1 (pronounced 'check one'). Both are protein kinases that 'sense' the state of DNA. ATR is located at the replication fork and activates other protein kinases, leading to phosphorylation and therefore activation of Chk1 which is also a kinase. Chk1 then phosphorylates and *in*activates Cdc25, which would otherwise activate MPF. ATR thus continues to prevent M phase entry until replication is complete (= when replication forks are gone?).

Step 8 involves an even more complicated mechanism called the mitotic spindle assembly checkpoint which prevents entry into anaphase until each and every kinetochore is attached to a microtubule. Sister chromatids are held together at the centromeres by a multi-protein complex called cohesin (composed of smc1, smc3 and kleisin). Separase is an enzyme that cleaves **kleisin**, physically releasing the chromatids. Securin inhibits separase to prevent separation of the chromatids. Once all kinetochores are attached, securin is targeted for degradation, thus freeing separase from inhibition and causing entry into anaphase. The mechanism for 'knowing' when to target securin for degradation is the really complicated part. It involves Mad1, Mad2, Cdc20, APC/C. Mad2 can have an 'open' or 'closed' conformation. 'closed' Mad2 inactivates Cdc20, thus preventing its association with APC/C. This pretty Wikimedia commons graphic by Dawn08 gives some sense of how complicated it all is:



Proper segregation of the daughter chromosomes is monitored by the 'mitotic exit network'. At this stage, MPF is inactivated, Cdh1 is dephosphorylated, and the cell can enter telophase.
There is also a 'spindle position checkpoint'. In yeast, Tem1 becomes associated with the spindle pole body at the centrosome.
'DNA damage checkpoints' detect whether DNA has been damaged (e.g. by UV light, chemicals, etc.). The cell cycle arrests in G1 or S, preventing the copying of damaged bases until they can be repaired. Arrest in G2 allows double-stranded breaks to be repaired before mitosis. Tumor suppressor genes are important in preventing replication of damaged DNA. Cells can sense UV or gamma radiation damage through a protein called ATM/R. ATM/R phosphorylates and activates Chk2, which then phosphorylates Cdc25, marking it for degradation. ATM/R also stabilizes the tumor suppressor p53 (gene: TP53), which is a transcription factor that activates p21, which (inhibits the G1 CDKs? and) arrests the cell cycle in G1. p53 undergoes loss-of-function mutations in perhaps 50% of cancers, and many of the mutations are dominant negative so only one allele need be mutated. Mdm2 also regulates p53, and ATM/R prevents Mdm2′s binding to p53. p53 is regulated by multiple means, and also has multiple roles in promoting DNA repair, cell cycle arrest, and apoptosis.

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
III-B.Sc., BIOCHEMISTRY
CANCER BIOLOGY (15BCU505C)
MULTIPLE CHOICE QUESTIONS
UNIT III

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	In which of the human cells listed below is telomerase active?	blood	bone	muscle	sperm	**sperm**
2	Which of the following statements about telomerase is incorrect?	It is an enzyme that adds DNA to telomeres.	It serves as the template for telomeres lengthening.	It is not activated in cancer cells.	Its activity continually resets the cellular clock	**It is not activated in cancer cells.**
3	The cell cycle consists of how many phases?	2	4	8	6	**4**
4	In order to enter the cell cycle a cell must be stimulated from outside. What type of molecule provides this stimulation?	Cyclins	Cyclin-dependent kinases	Cytokines and growth factors	Tyrosine kinases	**Tyrosine kinases**
5	In which phase of the cell cycle is DNA replicated?	G1 phase	S phase	G2 phase	M phase	**S phase**
6	The passage of a cell through the stages of the cell cycle is controlled by protein kinases that phosphorylate many different proteins at appropriate times. What are these protein kinases called?	Cdk activating kinases	Cyclin-dependent kinases	Cyclins	Tyrosine kinases	**Cyclin-dependent kinases**
7	At which cell cycle checkpoint is the cell cycle halted if the cell's DNA is damaged?	G1 - S	S - G2	G2 - M	G0 - G1	**G1 - S**
8	Which of the following occurs in meiosis but not in mitosis?	Attachment of spindle fibres to the kinetochore.	Pairing of homologous chromosomes at the metaphase plate.	Replication of DNA prior to the start of cell division	Separation of sister chromatids at anaphase.	**Pairing of homologous chromosomes at the metaphase plate.**
9	In which phase of the cell cycle are the chromosomes inactive, condensed, and not transcribed to messenger RNA?	G1 phase	S phase	G2 phase	M phase	**M phase**
10	Cyclin dependent kinases which control progression through cell cycle checkpoints are fully activated by which of the following:	binding to cyclins.	phosphorylation by Cdk activating protein kinase	binding to cyclin, plus phosphorylation by a Cdk activating protein kinase	phosphorylation by a tyrosine kinase.	**binding to cyclin, plus phosphorylation by a Cdk activating protein kinase**
11	Passage through which checkpoint is the step which commits the cell to proceed through to mitosis and cell division?	G1 to S	S to G2	G2 to M	M to G1	**G1 to S**

12	The cell is not allowed to pass the cell cycle restriction point if DNA damage is detected. Which of the following proteins are involved in detection of DNA damage and inhibition of the cycle at the restriction point? Please select all that apply.	Replication protein A (RPA).	ATM (ataxia telangiectasia mutated) protein	p53	all the above	**all the above**
13	At the end of each phase of the cell cycle cyclins activating Cdks in that phase are inactivated irreversibly by which of the following mechanisms?	Multiple phosphorylations	Dephosphorylation	Ubiquitinylation	Destruction by proteolysis in a proteasome.	**Destruction by proteolysis in a proteasome.**
14	The centromere is a region in which	chromatids remain attached to one another until anaphase	metaphase chromosomes become aligned at the metaphase plate	chromosomes are grouped during telophase.	the nucleus is located prior to mitosis.	**chromatids remain attached to one another until anaphase**
15	) What is a chromatid?	a chromosome in G1 of the cell cycle	a replicate chromosome	a chromosome found outside the nucleus	a special region that holds two centromeres together	**a replicate chromosome**
16	Starting with a fertilized egg (zygote), a series of five cell divisions would produce an early embryo with how many cells?	4	8	16	32	**32**
17	If there are 20 chromatids in a cell, how many centromeres are there?	10	20	30	40	**10**
18	How do the daughter cells at the end of mitosis and cytokinesis compare with their parent cell when it was in G1 of the cell cycle?	The daughter cells have half the amount of cytoplasm and half the amount of DNA.	The daughter cells have half the number of chromosomes and half the amount of DNA.	The daughter cells have the same number of chromosomes and half the amount of DNA.	The daughter cells have the same number of chromosomes and the same amount of DNA.	**The daughter cells have the same number of chromosomes and the same amount of DNA.**
19	Which term describes two centrosomes arranged at opposite poles of the cell?	telophase	anaphase	prometaphase	metaphase	**prometaphase**
20	Which term describes centrioles beginning to move apart in animal cells?	telophase	anaphase	prometaphase	prophase	**prophase**
21	Which is the longest of the mitotic stages?	telophase	anaphase	prometaphase	metaphase	**metaphase**
22	Which term describes centromeres uncoupling, sister chromatids separating, and the two new chromosomes moving to opposite poles of the cell?	telophase	anaphase	prometaphase	metaphase	**anaphase**
23	) If cells in the process of dividing are subjected to colchicine, a drug that interferes with the functioning of the spindle apparatus, at which stage will mitosis be arrested?	telophase	anaphase	prometaphase	metaphase	**metaphase**

24	If there are 20 centromeres in a cell at anaphase, how many chromosomes are there in each daughter cell following cytokinesis?	10	20	30	40	**20**
25	If there are 20 chromatids in a cell at metaphase, how many chromosomes are there in each daughter cell following cytokinesis?	10	20	30	40	**10**
26	Where do the microtubules of the spindle originate during mitosis in both plant and animal cells?	centromere	centrosome	centriole	chromatid	**centrosome**
27	If a cell has 8 chromosomes at metaphase of mitosis, how many chromosomes will it have during anaphase?	4	8	16	32	**16**
28	Cytokinesis usually, but not always, follows mitosis. If a cell completed mitosis but not cytokinesis, the result would be a cell with	a single large nucleus.	high concentrations of actin and myosin.	two abnormally small nuclei.	two nuclei.	**two nuclei.**
29	Regarding mitosis and cytokinesis, one difference between higher plants and animals is that in plants	the spindles contain microfibrils in addition to microtubules, whereas animal spindles do not contain microfibrils.	sister chromatids are identical, but they differ from one another in animals.	a cell plate begins to form at telophase, whereas in animals a cleavage furrow is initiated at that stage.	chromosomes become attached to the spindle at prophase, whereas in animals chromosomes do not become attached until anaphase.	**a cell plate begins to form at telophase, whereas in animals a cleavage furrow is initiated at that stage.**
30	The formation of a cell plate is beginning across the middle of a cell and nuclei are re -forming at opposite ends of the cell. What kind of cell is this?	an animal cell in metaphase	an animal cell in telophase	a plant cell in metaphase	a plant cell undergoing cytokinesis	**a plant cell undergoing cytokinesis**
31	Chromosomes first become visible during which phase of mitosis?	prometaphase	telophase	prophase	metaphase	**prophase**
32	During which phases of mitosis are chromosomes composed of two chromatids?	from G1 of interphase through metaphase	from metaphase through telophase	from anaphase through telophase	from G2 of interphase through metaphase	**from G2 of interphase through metaphase**
33	The somatic cells derived from a single-celled zygote divide by which process?	meiosis	mitosis	replication	cytokinesis alone	**mitosis**
34	In order for anaphase to begin, which of the following must occur?	Chromatids must lose their kinetochores.	Cohesin must attach the sister chromatids to each other.	Cohesin must be cleaved enzymatically	Kinetochores must attach to the metaphase plate	**Cohesin must be cleaved enzymatically**

35	Why do chromosomes coil during mitosis?	to increase their potential energy	to allow the chromosomes to move without becoming entangled and breaking	to allow the chromosomes to fit within the nuclear envelope	to allow the sister chromatids to remain attached	**to allow the chromosomes to move without becoming entangled and breaking**
36	A particular cell has half as much DNA as some other cells in a mitotically active tissue. The cell in question is most likely in	G1	G2.	prophase.	metaphase.	**G1**
37	The decline of MPF activity at the end of mitosis is due to	the destruction of the protein kinase Cdk.	decreased synthesis of cyclin.	the degradation of cyclin	synthesis of DNA.	**the degradation of cyclin**
38	In the cells of some organisms, mitosis occurs without cytokinesis. This will result in	cells with more than one nucleus.	cells that are unusually small.	cells lacking nuclei.	destruction of chromosomes	**cells with more than one nucleus.**
39	Which of the following does not occur during mitosis?	condensation of the chromosomes	replication of the DNA	separation of sister chromatids	spindle formation	**replication of the DNA**
40	Which of the following describe(s) cyclin-dependent kinase (Cdk)?	Cdk is an enzyme that attaches phosphate groups to other proteins.	Cdk is present throughout the cell cycle.	Both A and B are true.	Both A and B are false.	**Both A and B are true.**
41	The MPF protein complex turns itself off by	activating a process that destroys cyclin component.	activating an enzyme that stimulates cyclin.	binding to chromatin.	exiting the cell.	**activating a process that destroys cyclin component.**
42	Proteins that are involved in the regulation of the cell cycle, and that show fluctuations in concentration during the cell cycle, are called	ATPases	centrioles	kinetochores	cyclins	**cyclins**
43	An enzyme that attaches a phosphate group to another molecule is called a	phosphatase.	phosphorylase	kinase	cyclase	**kinase**
44	Which of the following triggers the cell′s passage past the G2 checkpoint into mitosis?	PDGF	MPF	protein kinase	cyclin	**MPF**
45	Which is a general term for enzymes that activate or inactivate other proteins by phosphorylating them?	PDGF	MPF	protein kinase	cyclin	**protein kinase**
46	Fibroblasts have receptors for this substance on their plasma membranes:	PDGF	MPF	protein kinase	cyclin	**PDGF**
47	Which of the following is a protein synthesized at specific times during the cell cycle that associates with a kinase to form a catalytically active complex?	PDGF	MPF	protein kinase	cyclin	**cyclin**
48	Which of the following is a protein maintained at constant levels throughout the cell cycle that requires cyclin to become catalytically active?	PDGF	MPF	protein kinase	CDK	**CDK**
49	Cells that are in a nondividing state are in which phase?	G0	G2	G1	S	**G0**

50	Chromosomes can be counted best at the stage of	Metaphase	Late anaphase	Telophase	Late prophase	**Metaphase**
51	The stage in which daughter chromosomes move toward the poles of the spindle is	Anaphase	Metaphase	Prophase	Telophase	**Anaphase**
52	Protein subunit found within microtubules is	Collagen	Tubulin	Myosin	DNA	**Tubulin**
53	Which of the following cellular structures always disappears during mitosis and meiosis?	Plasma membrane	Nucleolus and nuclear envelope	Plastids	none of these	**Nucleolus and nuclear envelope**
54	Centromere is a constituent of	Ribosome	ER	Chromosome	Mitochondrion	**Chromosome**
55	Cytoplasmic division of a cell is called	Cell plate formation	Cytokinesis	Mitosis	Synapsis	**Cytokinesis**
56	Cdks bind with ____, enabling the Cdks to function as enzymes.	MPF	Cyclins	histones	p53	**Cyclins**
57	Mitosis is controlled at the _____ checkpoint.	C	G1	G2	M	**M**
58	The spindle forms in the _____ phase.	C	G1	G2	M	**M**
59	_____ begins when pairs of sister chromatids align in the center of the cell.	Anaphase	Metaphase	Prophase	Telophase	**Metaphase**
60	The primary growth phase of a cell is the	G0	G1	G2	M	**G1**

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DEPARTMENT OF BIOCHEMISTRY

SYLLABUS

SUBJECT NAME: CORE ELECTIVE I - CANCER BIOLOGY

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SEMESTER: V **CLASS: III B.Sc., BIOCHEMISTRY**

UNIT-IV

Cell death: Types of cell death-apoptosis, necrosis and others. Apoptosis during developmental process and irregular apoptosis and disease. Death causing genes – Ceds, proteins – Caspases, mechanism of programmed cell death (PCD), Pathways of apoptosis-intrinsic and extrinsic.

TEXT BOOKS

1. Papachristodoulou, D., Snape, A., Elliott, W. H., Elliott, D.C. (2014). *Biochemistry and Molecular Biology* (5th ed.). New York, Oxford University Press.
2. Hayat, M.A. (2010). *Methods of Cancer Diagnosis, Therapy and Prognosis*. Springer Science. ISBN: 978--420-8441-6.

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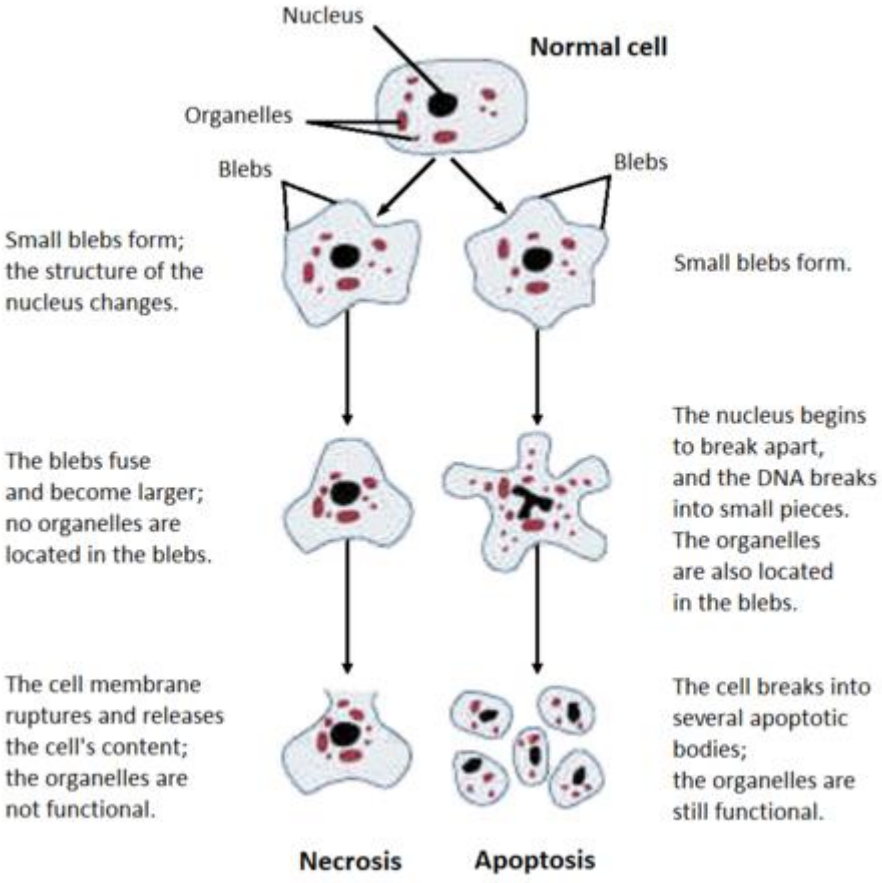
Types of cell death
Apoptosis and Necrosis



Apoptosis

Apoptosis can constitute cell suicide or cell murder. Cells will commit suicide when they lack any incoming survival signal in the form of trophic factors, or when they detect extensive DNA damage in their own nucleus. Cells will murder other cells to clear out unneeded cells or to eliminate potentially self-attacking immune cells.

Either of these processes constitutes programmed cell death. During embryonic development, people have webbed hands and feet and tails; the cells that constitute those parts later apoptize. Apoptosis also goes on constantly in many tissues including the intestines.

Here's a stunning Wikimedia Commons image of apoptosis (read left to right, top to bottom) thanks to Egelberg:

Major steps of apoptosis:

- Cell shrinks
- Cell fragments
- Cytoskeleton collapses
- Nuclear envelope disassembles
- Cells release apoptotic bodies

Notably *absent* from this list is 'send out a signal.' Apoptotic cells do *not* send out any signal, with one exception: they release apoptotic bodies and 'engulfment proteins' to induce other cells ('phagocytic' cells) to engulf the apoptotic bodies and and break them down in their lysosomes, but this is not much of an immune response.

Proteins important in apoptosis:

- 'killer proteins': the caspases (discussed in detail below).
- 'destruction proteins' that digest DNA, fragment the cell and break down the cytoskeleton
- 'engulfment proteins' that elicit and promote phagocytosis by other cells

Necrosis

- Necrosis is when cells die accidentally due to, say, trauma (ex. a poisonous spider bite), or lack of nutrients (ex. lack of blood supply). Necrosis begins with cell swelling, the chromatin gets digested, the plasma and organelle membranes are disrupted, the ER vacuolizes, the organelles break down completely and finally the cell lyses, spewing its intracellular content and eliciting an immune response (inflammation).

Others: Autophagy

Apoptotic bodies and the cellular debris released during lysis of oncotic cells can both be phagocytized and degraded by neighboring viable cells in vivo. Another form of cell death, autophagy or type II cell death, features degradation of cellular components within the dying cell in autophagic vacuoles. The morphological characteristics of autophagy include vacuolization, degradation of cytoplasmic contents, and slight chromatin condensation. Autophagy has been well described during vertebrate development and may be a phylogenetically old process.

Studies on autophagy suggest that it proceeds through a sequence of morphological changes in a highly regulated process. Briefly, the autophagic pathway begins with the sequestration of cytoplasmic material in double-membrane vesicles known as autophagosomes. The sequestration process is under the control of GTPases and phosphatidylinositol kinases and involves novel ubiquitin-like conjugation systems. Autophagosomes then fuse with lysosomes in a process depending on microtubules, and the contents are degraded. In vivo, cells undergoing autophagy can be phagocytized by neighboring cells.

Oncosis

The term oncosis has been accepted by many investigators of cell death as a counterpoint to apoptosis. Oncosis is defined as a prelethal pathway leading to cell death accompanied by cellular swelling, organelle swelling, blebbing, and increased membrane permeability. The process of oncosis ultimately leads to depletion of cellular energy stores and failure of the ionic pumps in the plasma membrane. Oncosis may result from toxic agents that interfere with ATP generation or processes that cause uncontrolled cellular energy consumption. It is now being recognized that the changes accompanying oncosis may result from active enzyme-catalyzed biochemical processes.

Altered intracellular calcium levels may also regulate oncotic cell death. Elevated cytoplasmic calcium concentrations can activate cysteine proteases of the calpain family that mediate plasma membrane breakdown through the proteolysis of cytoskeletal and plasma membrane proteins. Increased intracellular calcium also initiates translocation of cytosolic phospholipase A_2s to

cellular membranes, where the hydrolysis of membrane phospholipids decreases membrane integrity.
Oncosis induced by pathogen infection has been suggested in a number of experimental models. Rotavirus infection of MA104 cells induces cell death morphologically consistent with oncosis, which also requires increased intracellular calcium. In addition, *Pseudomonas aeruginosa* infection induces oncosis in infected macrophages and neutrophils. These cells demonstrate swelling, rapid plasma membrane breakdown, and swollen nuclei without internucleosomal DNA fragmentation.

Pyroptosis

Caspase-1 activation in macrophages infected with *Salmonella* or *Shigella*results in processing of these cytokines and death of the host cell. The mechanism and outcome of this form of cell death are distinctly different from these aspects of apoptosis, which actively inhibits inflammation. We have proposed the term pyroptosis from the Greek roots "pyro," relating to fire or fever, and "ptosis" (pronounced "to-sis"), denoting falling, to describe proinflammatory programmed cell death. The observed caspase-1 activation or dependence during cell death in the immune, central nervous, and cardiovascular systems indicates that pyroptosis plays a significant role in a variety of biological systems.

Apoptosis during developmental process

Every normal living cell of animals, plants and even bacteria are mortal. I.e., they must die after some time. Cell death is a finely tuned programme inherent in the cells genetic machinery. This normal cell death which is the part of normal development and maintenance of homeostasis is called apoptosis or programmed cell death (PCD).
This phenomenon is very much different from death of a cell due to pathological cause or necrosis. This process is highly regulated and any defect in apoptotic machinery will lead to extended survival of cells which may result in neoplastic cell expansion, leading to genetic instability and accumulation of mutations.

Cellular Events of Apoptosis:

It is a normal physiological response to specific suicide signals or lack of survival signals. During this process at first the nucleus and cytoplasm condense, i.e., chromatin material condenses and migrates to nuclear membrane, the cytoplasm undergoes shrinkage without any damage to plasma membrane.
The cell contents are packaged in membrane bound bodies and the cell is broken down into pieces called apoptotic pieces, though still functioning, are engulfed or phagocytosed or digested by macrophages or by neighbouring cells (Fig. 5.33).

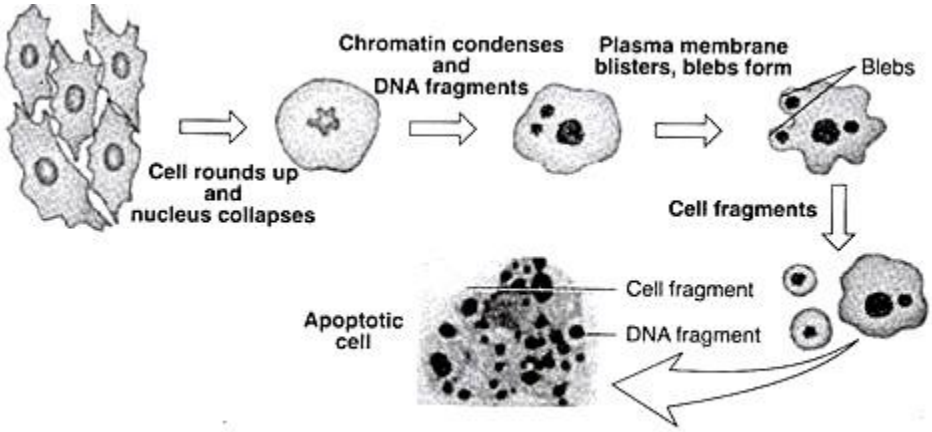


Fig. 5.33A: Sequence of cellular events during apoptosis

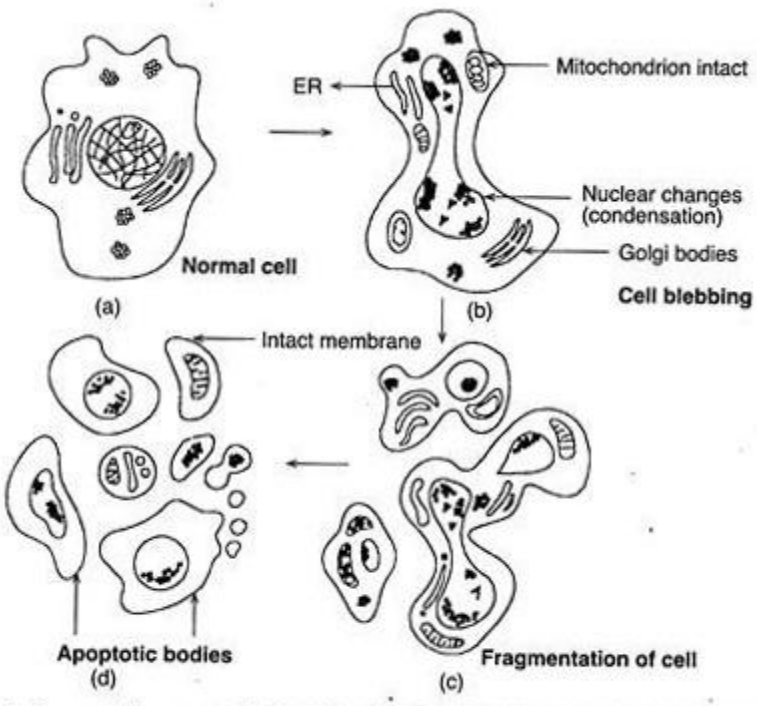


Fig. 5.33B: Cytological changes during apoptosis. Normal cell undergoes shrinkage showing condensation of chromatin and cell blebbing. Thereafter the cell is fragmented to produce apoptotic bodies, but the membranes remain intact (from Rastogi)

Mechanism of Apoptosis:

There are three major pathways for activation of caspase which causes cleavage of substrates leading to apoptosis.

i. Mitochondrial/Cytochrome pathway:

It is mediated through activation of Bcl-2 (gene) which results in production of Apaf-1, caspase-9 and caspase-3 enzyme synthesis which leads to the phenomenon of apoptosis (Fig. 5.34).

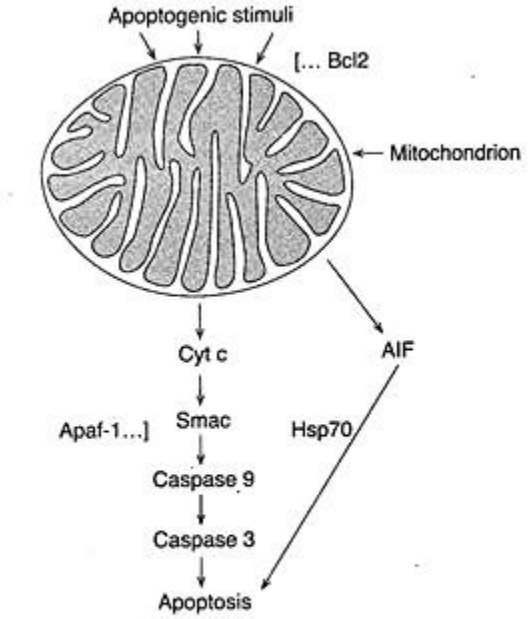


Fig. 5.34: Apoptotic cascade: apoptotic proteins such as Cyt c, Smac, AIF, etc. are released due to several stimuli, triggering caspase activation. Hsp 70 is a negative regulator of Apaf-1. AIF causes condensation of chromatin and cell fragmentation

ii. Tumour-necrosis factor-receptor (TNF) pathway:

In this pathway the ligation of members of the TNF-receptors takes place, activating caspase-8 and then caspase-3 which leads to apoptosis.

iii. Granazyme B pathway:

Granazyme B, a cytosolic T cell product, directly cleaves and activates several caspases, resulting in apoptosis. A number of genes have been identified which play role in the regulation and accomplishment of apoptosis, such as egl-1, Ced-1-10. Studies on these genes indicated that Ced-9 acts upstream of Ced-3 and Ced-4 (Fig. 5.35):

Ced-9 → Ced-3→Ced-4→Cell death

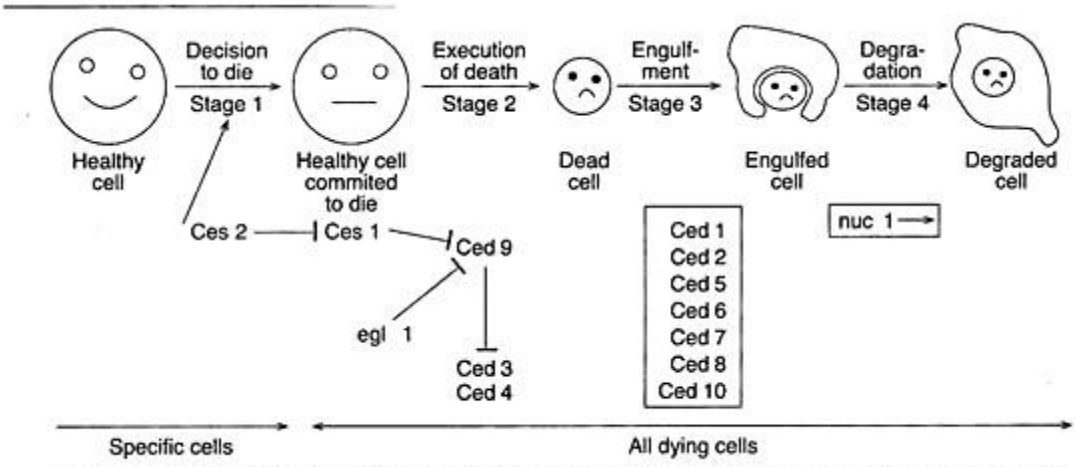


Fig. 5.35: Model for molecular basis of apoptosis in the nematode, Caenorhabditis elegans (after Steller, 1995)

Ced-3 and Ced-4 promote apoptosis, while Ced-9 Is anti-apoptotic and protects cells from apoptosis by antagonizing Ced-3 and Ced-4. The Ced genes are responsible for all programmed cell death.

Caspases are cysteine proteases which cleave the substrates at the C-terminal of an aspartic acid residue. Different caspases have different substrate recognition preferences and cleavage of substrates by caspases results in disassembly and consequent death of cell in a highly organized manner.

Death receptors are important in **'instructive'** apoptosis where ceil death is brought about by the secretion of death legends which bind to death receptors on the target cell (Fig. 5.36).

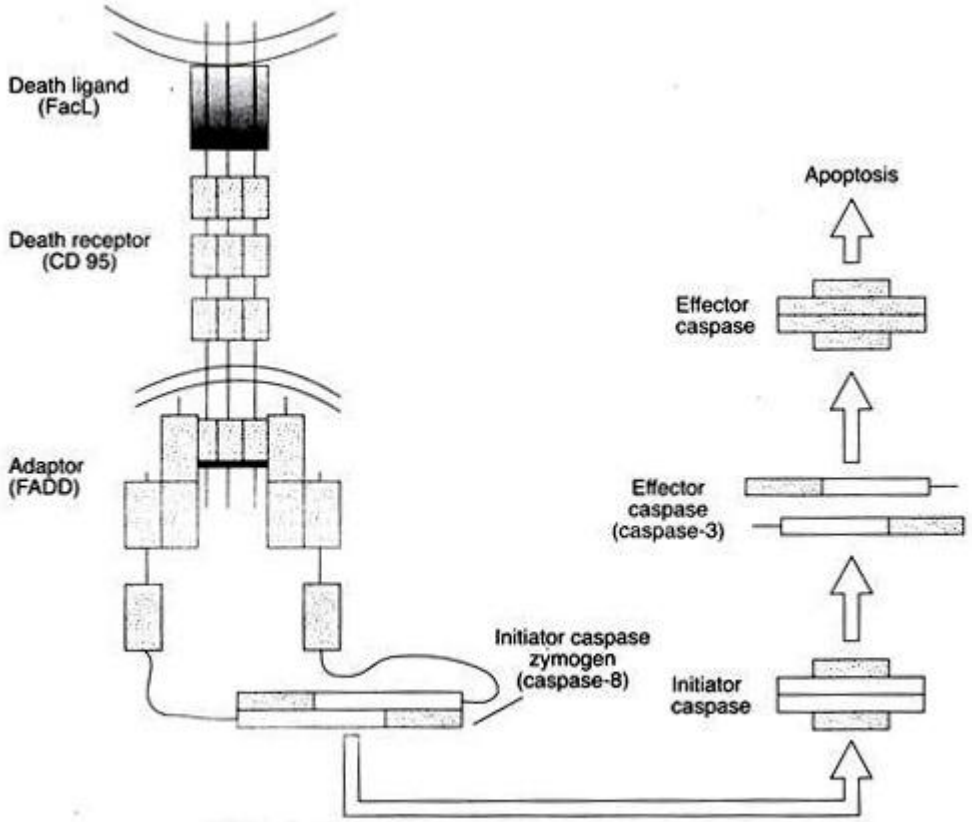


Fig. 5.36: A model showing instructive apoptosis by secretion of death ligands (from Rastogi)

Importance of Apoptosis:

It is a necessary mechanism complementary to proliferation to ensure homeostasis in all tissues. Removal of a number of vestigeal structures (developmental structure, e.g., tail) is caused by programmed cell death. Apoptosis is considered a necessary anticancer mechanism, as defect in this process leads to neoplastic and tumorigenic cell development.

Irregular apoptosis and Diseases

Apoptosis has a duality. While it is a pathway used in the normal maintenance and development of tissues in healthy organisms it also had a dark side. Apoptosis occurs during the normal development of multicellular organisms and continues throughout adult life. The combination of apoptosis and cell proliferation is responsible for shaping tissues and organs in developing embryos. For example the apoptosis of cells located in-between the toes allows for their separation.

As you can imagine, apoptosis is a tightly regulated process – controlled by the integration of multiple pro- and anti-apoptotic signals. Ultimately the induction of apoptosis occurs through the activation of the caspase proteases that are responsible for coordinating the hallmarks of an apoptotic death: cell shrinkage, chromatin condensation, membrane blebbing and DNA fragmentation. Apoptosis is also an important part of the regulation of the immune system. T lymphocytes are cells of the immune system that are responsible for destroying infected or damaged cells in the body. They mature in the thymus, but before they can enter the bloodstream they are tested to ensure that they are effective against foreign antigens and are also not reactive against normal, healthy cells. Any ineffective or self-reactive T-cells are removed through the induction of apoptosis.

Problems with the regulation of apoptosis have been implicated in a number of diseases. Cancer is a disease that is often characterized by too little apoptosis.

Understandably, bad things happen when apoptotic pathways are disrupted. Below are some examples of what happens when apoptosis is not well-regulated.

Apoptosis in cancer

Almost all cancer cells are resistant to apoptosis. While unchecked proliferation of cells normally stimulates the apoptotic pathway, a shutdown of the pathway through mutation allows for growth of the cancer. To avoid apoptosis, mutations in cancer cells will either inhibit expression of pro-apoptotic proteins, such as the p53 tumor suppressor, or increase expression of anti-apoptotic signals. Indeed, malignant cells often show over-expression of inhibitors of apoptosis protein (IAP) family members. To counteract this, pro-apoptotic drugs can be used for chemotherapy (such as IAP antagonists).

Viruses use the cell death pathways for their own survival.

If cancer is a disease where too little apoptosis occurs there are other diseases where too much apoptosis is thought to be part of the problem. For example in neurodegenerative diseases such as Parkinson's or Alzheimer's Diseases apoptosis is thought to account for much of the cell death and the progressive loss of neurons.

During pregnancy trophoblast cells from the placenta invade the uterine environment in order to remodel the maternal blood vessels and help establish and maintain a successful pregnancy. Strict control over cell proliferation and apoptosis is required to achieve this. In some cases this process can be compromised and excessive apoptosis of the trophoblast cells is thought to be implicated in the failure to fully remodel the maternal environment that is observed in complications of pregnancy such as preeclampsia.

Apoptosis is also thought to play a role in the progression of many auto-immune diseases. For example, in the case of rheumatoid arthritis excessive proliferation of synovial cells is thought to be due in part to the resistance of these cells to apoptotic stimuli. In other cases poor regulation

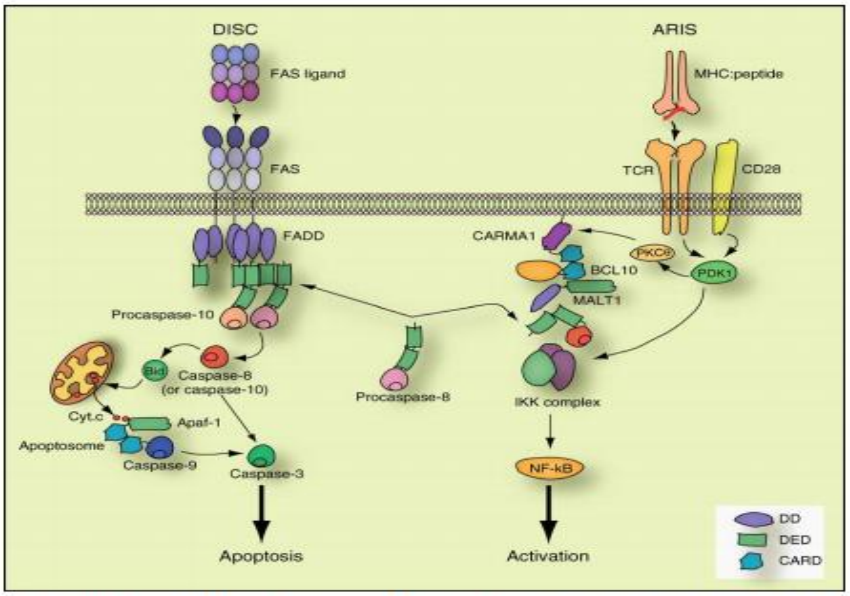
of apoptosis in T-lymphocytes can result in auto-reactive T-cells entering the circulation and contributing to the onset of autoimmune diseases.
Infection with intracellular pathogens, such as viruses, can trigger apoptotic pathways – it is a great way to prevent the spread of infection. Therefore, many viruses take control over the apoptotic system of the host cell upon infection.

Shutting down apoptosis
Viruses from many different families inhibit apoptosis of cells. Herpesviruses are excellent examples of viruses that shut down apoptosis. As part of their lifecycle, herpesviruses enter a state of latency, which means they hide out in the host cell for extended periods with little to no virus production. This is complemented by the lytic cycle, during which virus particles are rapidly produced and assembled before the virus is released. While in the latent phase, the virus keeps the host cell alive by suppressing apoptosis. As an argument for how important this is for the virus, herpesviruses use multiple proteins and multiple mechanisms to inhibit apoptosis. While this is advantageous to the virus, it means that the host can never completely clear the virus and will always be infected.
Inducing apoptosis
Alternatively, some viruses can increase apoptosis in certain cell types. Infection with human immunodeficiency virus (HIV) brings about a 95% depletion of non-infected CD4 T-cells (T-helper cells). Until recently, this was thought to be executed through up-regulation of a pro-apoptotic signal in T-cells which then initiates apoptosis. However this year, it has become clear that these cells undergo a different form of regulated cell death, called pyroptosis. Pyroptosis is accompanied by a strong inflammatory reaction and induced by activated caspase 1, marked by the release of the cytokine Interleukin 1-?. This finding makes caspase 1 a promising new drug target for HIV therapy.
Neurological disorders
Cell death accompanies neurodegenerative disorders, which are characterized by loss of neurons. There is much left to learn, but the involvement of caspases has been characterized to some extend:
– In Alzheimer's disease, the cleavage of amyloid precursor protein (APP) into A? peptides leads to their accumulation in the form of plaques, and tau proteins form neurofibrillary tangles. APP has been shown to be cleaved by the apoptotic kinases caspase 3 and caspase 6, and tau can serve as a substrate for caspase 3. Interestingly, inhibiting apoptosis by overexpression of the anti-apoptotic regulatory protein bcl-2 can lead to a reduction of Alzheimer's symptoms.
– A common mutation found in Parkinson's leads to the inactivation of a gene encoding the mitochondrial kinase PINK1. This kinase has a function in inhibition of apoptosis and thus its loss leads to an increase in caspase activity.
– Caspases are also involved in Huntington's disease, which is caused by a mutation in the huntingtin protein (htt). The mutant protein can be cleaved by caspases; in particular, caspase 6 has been implicated.
Like so many other biological pathways, the maintenance of a healthy organism requires a delicate balance of apoptotic signals. Stay tuned for my final article in which I will show you different techniques to determine if this balance is being maintained.

Death causing genes

Caspase-8 deficiency state (CEDS)

- Caspase-8 is a member of a family of intracellular cysteine proteases called caspases that cleave target proteins after specific aspartic acid residues.
- The caspase-8 zymogen possesses a prodomain containing death effector domains (DED), a large enzyme subunit, and a small enzyme subunit.
- Caspase-8 deficiency state (CEDS) is characterized by a prominent combined lymphocyte immunodeficiency superimposed upon a mild lymphoaccumulation with autoimmunity.
- Patients have impaired lymphocyte activation, resulting in hypogammaglobulinemia, recurrent sinopulmonary infections with bronchiectasis, and mucocutaneous herpesvirus infections. There is persistent but mild lymphadenopathy and splenomegaly, marginally elevated DNTs (double negative T cells, CD4−CD8−TCRα/β+),and autoantibodies.
- Although the lymphoaccumulation and autoimmunity are reminiscent of autoimmune lymphoproliferative syndrome (ALPS), the prominent feature of immunodeficiency distinguishes this as a separate clinical entity.



Caspase-8 Deficiency State. Figure 1 Caspase-8 participates in two signalling complexes: the activation receptor induced signalosome (ARIS) and the death inducing signaling complex (DISC). In the former, immunoreceptor stimulation leads to caspase-8-depending recruitment of the CBM-IKK complex and NF-κB activation. In the latter, death receptor stimulation results in the caspase-8 activation and cleavage of downstream caspases, leading to the cell's death. Adapted from [2].

Proteins - Caspases

Cysteine aspartic proteases (caspases) play critical roles in regulating apoptosis and inflammation. Caspases involved in apoptosis are classified into distinct subsets based on function, effector caspases (caspases-3, -6 and -7) and initiator caspases (caspases-2, -8, -9 and -10). Caspases-1, -4, -5, -11 and -12 are involved in inflammation.

All caspases are synthesized in inactive forms, referred to as pro-caspases, that require dimerization/oligomerization and subsequent cleavage for activation. Extrinsic or intrinsic death signals mediate caspase activation and apoptosis via two distinct pathways (see image

1). The extrinsic pathway is triggered by ligand binding to cell surface death receptors, and the intrinsic pathway is activated by factors such as DNA damage, growth factor withdrawal or loss of extracellular matrix contact and is mediated through the mitochondria.

Apoptotic caspases are activated upon the receipt of either an extrinsic or an intrinsic death signal. The extrinsic pathway (green arrows) is initiated by ligand binding to cell surface death receptors (TNF RI, Fas/CD95, DR3, TRAIL R1/DR4, TRAIL R2/DR5) followed by receptor oligomerization and cleavage of Pro-caspase-8 and -10. Activation of Caspase-8 and Caspase-10 results in the cleavage of BID and downstream effector caspases. The intrinsic pathway of caspase activation (purple arrows) is initiated by events such as DNA damage, growth factor withdrawal, or loss of contact with the extracellular matrix. These events lead to changes in the integrity of the mitochondrial membrane that result in the release of pro-apoptotic proteins including Cytochrome c, Smac/Diablo, HTRA2/Omi, Apoptosis-Inducing Factor (AIF), and Endonuclease G.

Two types:

- those related to caspase 1 (Caspases 1, 4, 5, 13, and 14); role in cytokine processing during inflammation
- those involved in apoptosis (Caspases 2, 3, 6, 7, 8, 9 and 10)

Initiators – activate downstream effector caspases to initate activation cascades

Effectors - cleave target proteins resulting in morphological and biochemical markers of apoptosis

Effector Caspases

Activated effector caspases cleave target proteins:

Nuclear Lamins – scaffold proteins of nuclear envelope; leads to nuclear shrinkage and fragmentation

Cytoskeleton proteins – e.g.:Fodrin; leads to loss of cell shape and membrane blebbing;

Gelsolin (an actin depolymerizing enzyme); cleaved by caspase 3; role in cell morphology during apoptosis (blebbing etc.)

ICAD (inhibitor of Caspase Activated Dnase) – DNA now cut up by CAD

Components of focal adhesion complex; leads to detachment of apoptotic cells from other cells

Mitochondrial membrane permeability is regulated by the Bcl-2 family of proteins. The balance between pro- and anti-apoptotic family members determines whether or not a cell will undergo apoptosis. In healthy cells, Bcl-2 and Bcl-xL inhibit apoptosis by binding to the pro-apoptotic Bax and BAK proteins. Bad is also phosphorylated and sequestered in the cytoplasm by the 14-3-3 protein in these cells. If cytoplasmic levels of free BAD increase, Bcl-2 and Bcl-xL bind to Bad and release Bax and BAK. Bax and BAK, or processed forms of these proteins, can then insert into the mitochondrial membrane, compromising its integrity.

Initiator caspases are activated in three distinct protein complexes, the death-inducing signaling complex (DISC; Caspase-8 and -10), the apoptosome (Caspase-9), and the PIDDosome (Caspase-2). The DISC is formed following ligand binding and death receptor oligomerization. Pro-caspase-8 and Pro-caspase-10 are recruited to the death receptors through their interactions with the adaptor protein, FADD. This interaction is mediated by their shared death effector domains (DED). Clustering of pro-caspases near the death receptors results in their cleavage and activation. In contrast, Pro-caspase-9 is activated following an intrinsic change associated with the release of Cytochrome c from the mitochondria. In the cytoplasm, Cytochrome c interacts

with APAF-1, which recruits Pro-caspase-9 by way of its caspase recruitment domain (CARD) to form the apoptosome. Formation of the apoptosome leads to the cleavage and activation of Caspase-9. Intrinsic cellular changes can also lead to the activation of Caspase-2. Following DNA damage, p53 induces the expression of p53-induced protein with a death domain (PIDD), which associates with the CRADD/RAIDD adaptor protein and Pro-caspase-2 to form the PIDDosome. The association between CRADD/RAIDD and PIDD is mediated by their shared death domains (DD), while CARD domains mediate the interaction between CRADD/RAIDD and Pro-caspase-2. Formation of the PIDDosome leads to the cleavage of Pro-caspase-2. Autocatalytic cleavage of the initiator pro-caspases occurs at aspartic acid residues located after the pro-domain, and in between the large and the small subunits. Upon cleavage, mature caspases form a proteolytically active heterotetramer consisting of two small and two large subunits. Once activated, initiator caspases cleave downstream effector caspases that promote the ordered disassembly of the cell by targeting a number of critical cellular proteins including structural proteins, DNA repair proteins, and proteins involved in signal transduction pathways.

Procaspases - Contain N terminal pro-domain followed by region that forms a 2 subunit catalytic effector domain

Prodomain: for prot-prot interactions; allows it to bind upstream regulators and effector proteins; examples include:

DED – death effector domain (e.g. caspase 8)

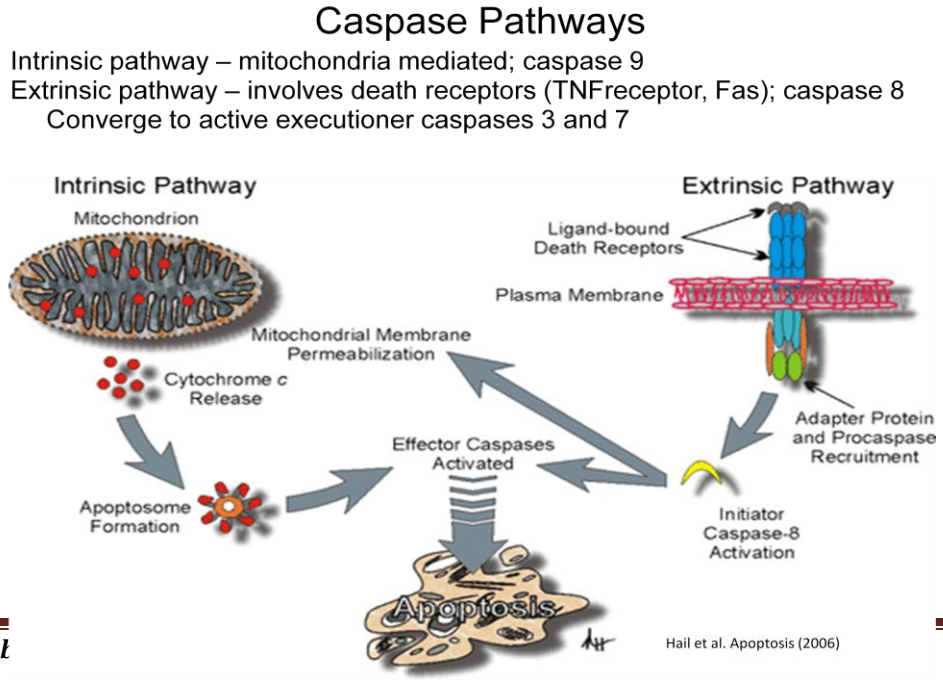
CARD – caspase activation and recruitment domain (e.g. caspase 9)

Active caspases – heterotetramers composed of two large and two small subunits with two active sites per molecule

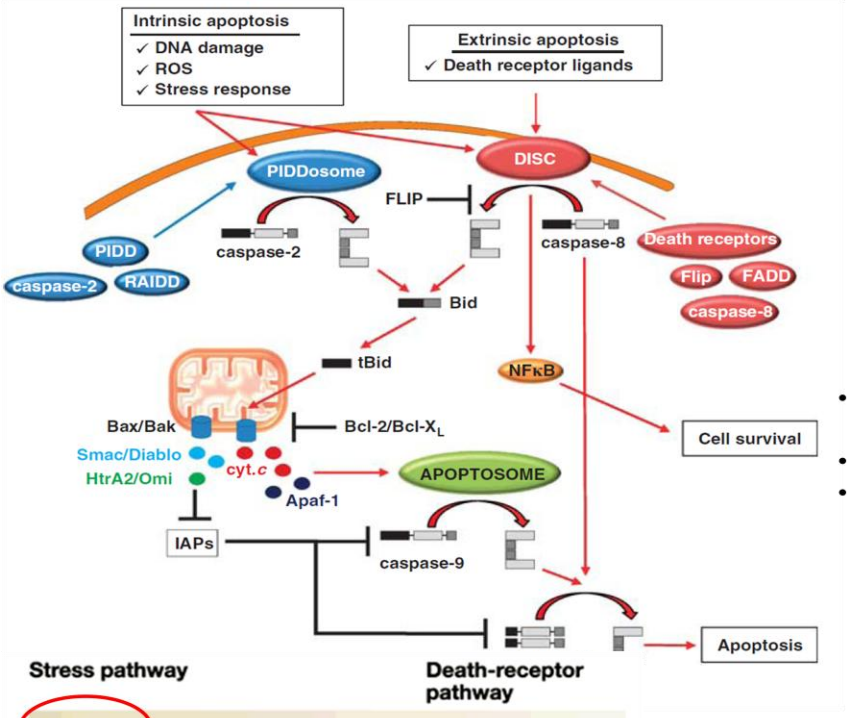
Other caspase dependent features:

Cleavage of PAK2 (member of p21-activated kinase family); results in formation of apoptotic bodies and other signaling cascades

Exposure of phosphatidylserine on outer membrane; probably due to down-regulation of phospholipid translocase activity and/or activation of lipid scramblase



Caspase cascades and their inhibitors



Formation of multicomponent complexes triggers initiator caspase dimerization sufficient for their activation:

- DISC: Death-inducing signaling complex
- Apoptosome
- (PIDD)osome: p53-induced protein with a death domain

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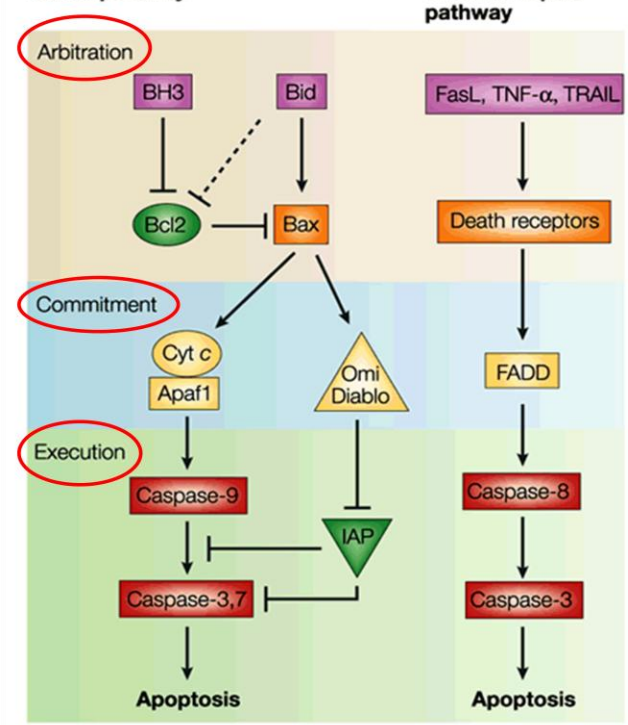
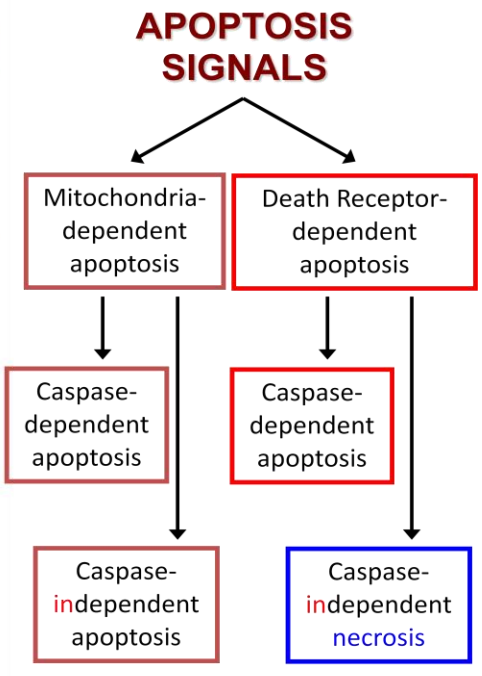




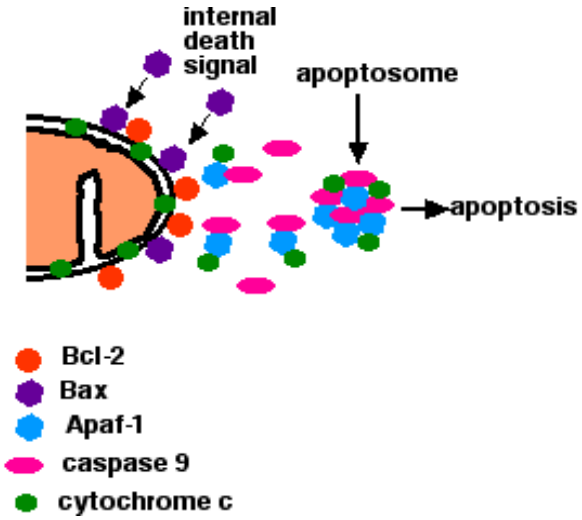
Nature Reviews Cancer **2**; 647-656 (2002)

Mechanism of programmed cell death (PCD)

There are 3 different mechanisms by which a cell commits suicide by apoptosis.

1. One generated by signals arising within the cell;
2. another triggered by **death activators** binding to receptors at the cell surface:
   - TNF-α
   - Lymphotoxin
   - Fas ligand (**FasL**)
3. A third that may be triggered by dangerous reactive oxygen species.

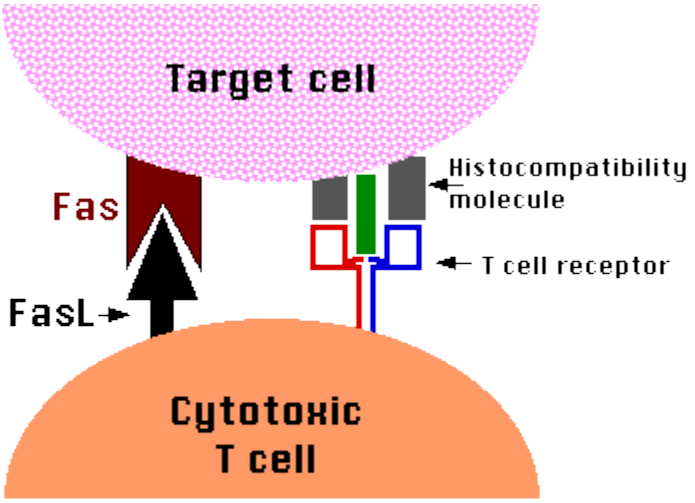
1. Apoptosis triggered by internal signals: the intrinsic or mitochondrial pathway



- In a healthy cell, the outer membranes of its mitochondria display the protein **Bcl-2** on their surface. Bcl-2 **inhibits** apoptosis.
- Internal damage to the cell
  - causes a related protein, **Bax**, to migrate to the surface of the mitochondrion where it inhibits the protective effect of Bcl-2 and inserts itself into the outer mitochondrial membrane punching holes in it and causing
  - **cytochrome c** to leak out.
- The released cytochrome c binds to the protein **Apaf-1** ("apoptotic protease activating factor-1").
- Using the energy provided by ATP,
- these complexes aggregate to form **apoptosomes**.
- The apoptosomes bind to and activate **caspase-9**.
- Caspase-9 is one of a family of over a dozen caspases. They are all proteases. They get their name because they cleave proteins — mostly each other — at aspartic acid (Asp) residues).
- Caspase-9 cleaves and, in so doing, activates other caspases (caspase-3 and -7).
- The activation of these "executioner" caspases creates an expanding cascade of proteolytic activity (rather like that in blood clotting and complement activation) which leads to
  - digestion of structural proteins in the cytoplasm,
  - degradation of chromosomal DNA, and
- phagocytosis of the cell.

2. Apoptosis triggered by external signals: the extrinsic or death receptor pathway

- **Fas** and the **TNF receptor** are integral membrane proteins with their receptor domains exposed at the surface of the cell
- binding of the complementary **death activator** (**FasL** and **TNF** respectively) transmits a signal to the cytoplasm that leads to
- activation of **caspase 8**
- caspase 8 (like caspase 9) initiates a cascade of caspase activation leading to
- phagocytosis of the cell.



One method by which cytotoxic T cells induce their targets (e.g., virus-infected cells) to commit suicide (apoptosis)

Example (right): When cytotoxic T cells recognize (bind to) their target,

- they produce more **FasL** at their surface.
- This binds with the **Fas** on the surface of the target cell leading to its death by apoptosis.

The early steps in apoptosis are reversible — at least in C. elegans. In some cases, final destruction of the cell is guaranteed only with its engulfment by a phagocyte.

3. Apoptosis-Inducing Factor (AIF)

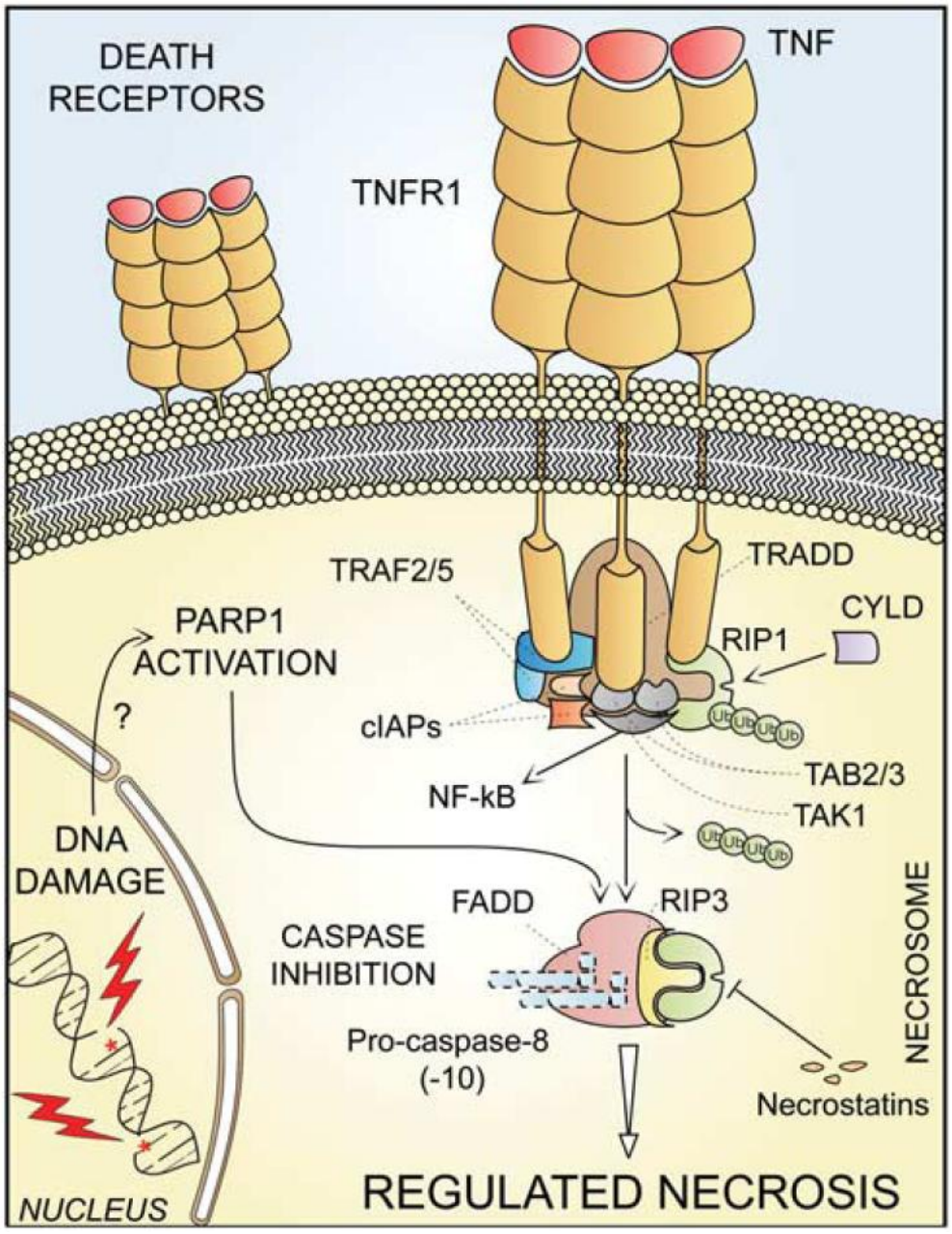
Neurons, and perhaps other cells, have another way to self-destruct that — unlike the two paths described above — does not use caspases.

Apoptosis-inducing factor (**AIF**) is a protein that is normally located in the intermembrane space of mitochondria. When the cell receives a signal telling it that it is time to die, AIF

- is released from the mitochondria (like the release of cytochrome c in the first pathway);
- migrates into the nucleus;
- binds to DNA, which
- triggers the destruction of the DNA and cell death.

Regulated Necrosis:

necrosis can occur in a regulated manner in addition to spontaneous cell death

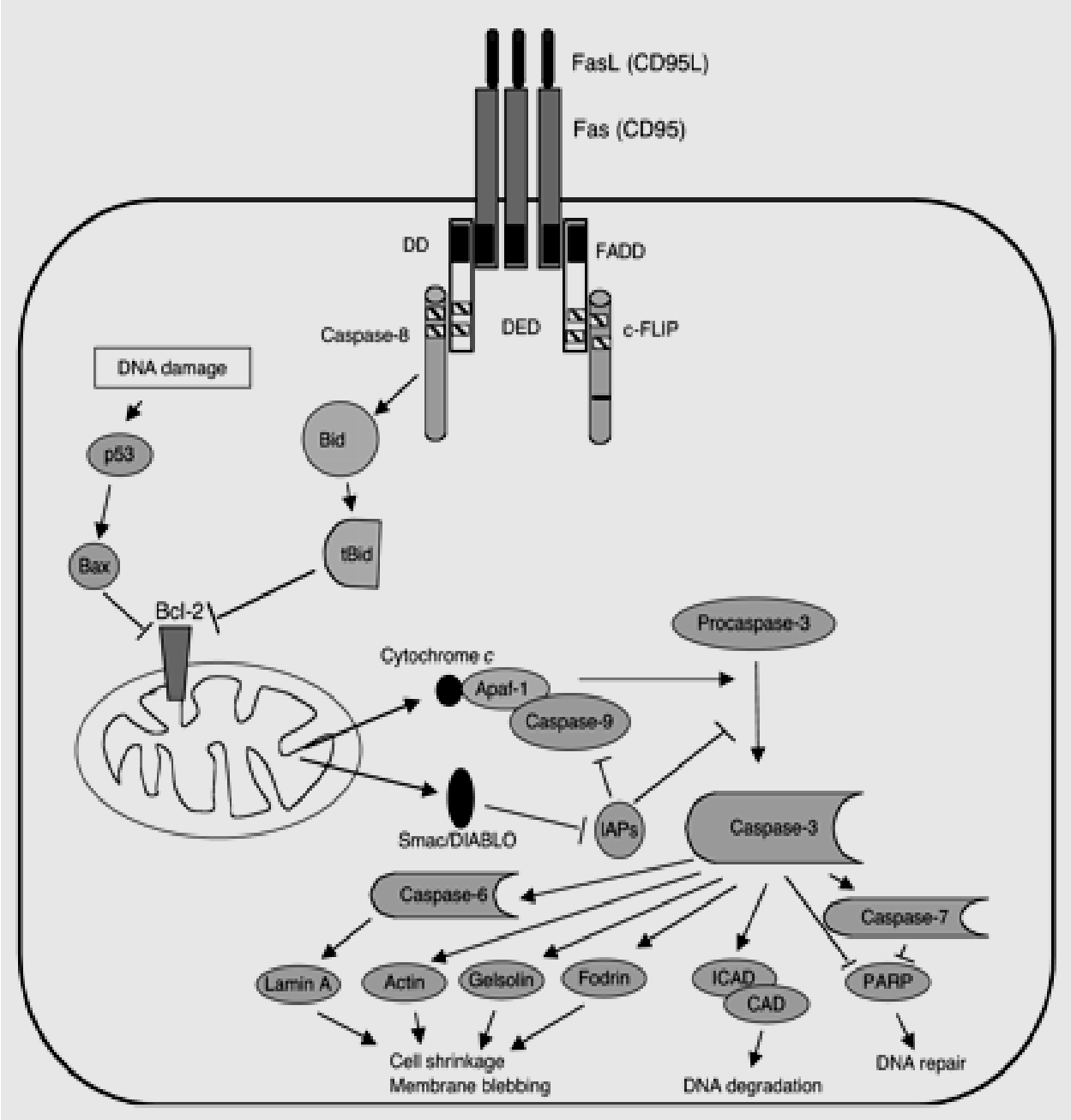


Upon tumor necrosis factor a (TNFa) binding, the cytoplasmic tails of TNF receptor 1 (TNFR1, a prototypic death receptor) trimers recruit TNFR-associated death domain (TRADD), receptor-interacting protein kinase 1 (RIP1), cellular inhibitor of apoptosis 1 (cIAP1), cIAP2, TNFR-associated factor 2 (TRAF2) and TRAF5. Within the so-called complex I, RIP1 is polyubiquitinated by cIAPs, thereby providing a docking site for the recruitment of transforming growth factor b (TGFb)-activated kinase 1 (TAK1), TAK1-binding protein 2 (TAB2) and TAB3 (which together deliver a pro-survival signal by activating the transcription factor NF-kB). In some patho-physiological and experimental settings, and in particular when caspase-8 is absent or when caspases are inhibited by pharmacological agents, cylindromatosis (CYLD)-deubiquitinated RIP1 engage in physical and functional interactions with its homolog RIP3,

ultimately activating the execution of necrotic cell death. Regulated necrosis can also be induced by alkylating DNA damage (possibly by the overactivation of poly(ADP-ribose) polymerase 1, PARP1). In some (but not all) instances, regulated necrosis requires the kinase activity of RIP1, that is, it can be blocked by the RIP1-targeting compounds necrostatins. FADD, FAS-associated protein with a death domain

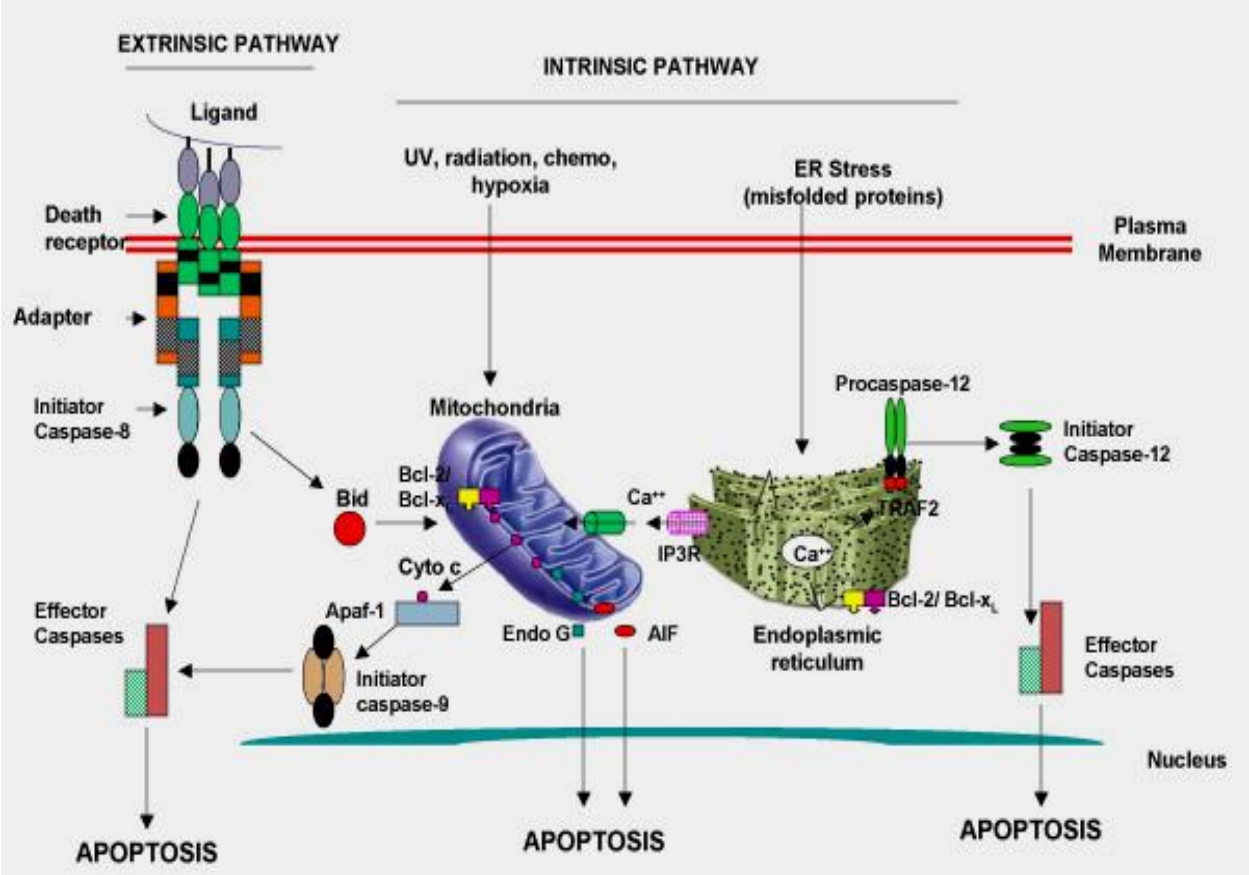
Pathways of apoptosis-intrinsic and extrinsic

Extrinsic or Death Receptor Pathway



- Binding of Fas by FasL induces recruitment of FADD to the cytoplasmic tail of Fas

- The opposite end of FADD contains a death effector domain (hatched boxes); recruitment of either procaspase-8 or c-FLIP
- Caspase-8 can cleave Bid
- truncated Bid (tBid) can inactivate Bcl-2 in the mitochondrial membrane.
- This allows the escape of cytochrome *c*, which clusters with Apaf-1 and caspase-9 in the presence of dATP to activate caspase-9.
- Smac/DIABLO is also released from the mitochondria and inactivates inhibitors of apoptosis (IAPs).
- breakdown of several cytoskeletal proteins and degradation of the inhibitor of caspase-activated DNase (ICAD).



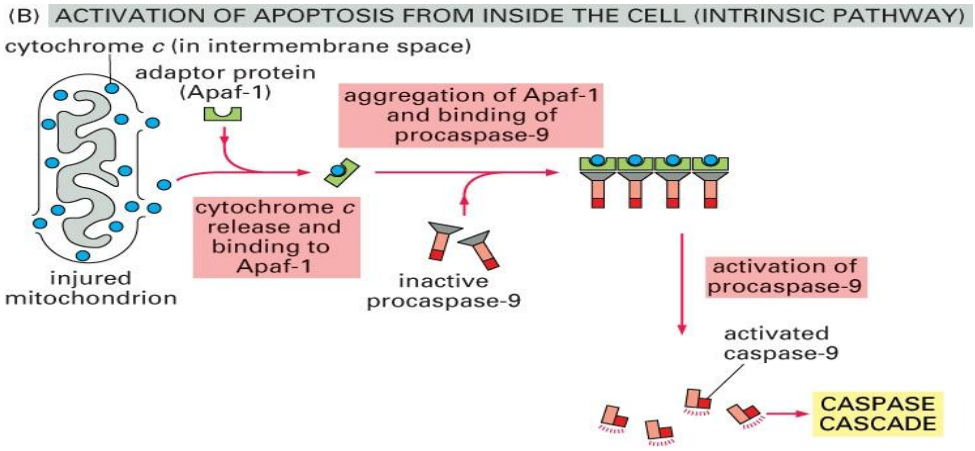
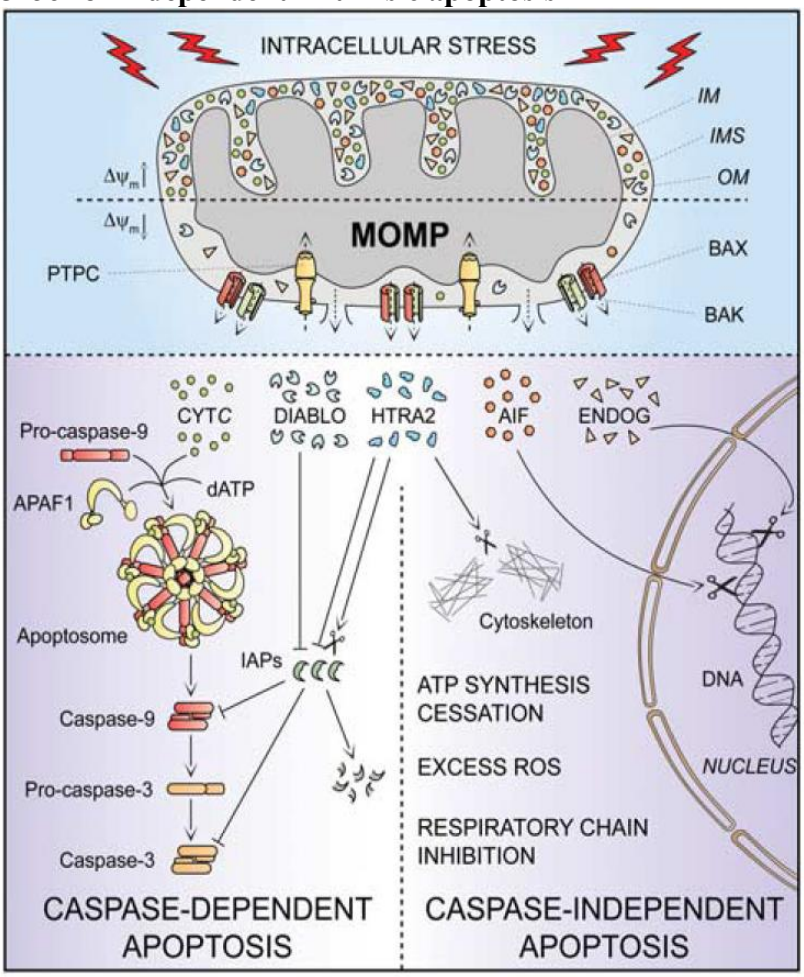


Figure 17–39 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Caspase-dependent and -independent 'intrinsic apoptosis'



In response to multiple intracellular stress conditions (e.g., DNA damage, cytosolic Ca2þ overload), pro-survival and pro-death signals are generated and converge to a mitochondrion-centered control mechanism. When lethal signals prevail, mitochondrial outer membrane permeabilization (MOMP) occurs and leads to mitochondrial trans-membrane potential (Dcm) dissipation, arrest of mitochondrial ATP synthesis and Dcm-dependent transport activities. Moreover, the respiratory chains gets uncoupled, leading to generation of reactive oxygen species (ROS), and proteins that are normally confined within the mitochondrial inter-membrane space (IMS) are released into the cytosol. Among these, cytochrome c (CYTC) drives – together with the cytoplasmic adaptor protein APAF1 and dATP – the assembly of the so-called apoptosome, a multi-protein complex that triggers the caspase-9-caspase-3 proteolytic cascade. Direct IAP-binding protein with low pI (DIABLO, also known as second mitochondria-derived activator of caspases, SMAC) and high temperature requirement protein A2 (HTRA2) facilitate caspase activation by sequestering and/or degrading several members of the inhibitor of apoptosis protein (IAP) family. On the contrary, apoptosis-inducing factor (AIF) and endonuclease G (ENDOG) function in a caspase-independent manner by relocating to the nucleus and mediating large-scale DNA fragmentation. Of note, the serine protease HTRA2 also contributes to caspase-independent apoptosis by cleaving a wide array of cellular substrates (including cytoskeletal proteins). IM, mitochondrial inner membrane; OM, mitochondrial outer membrane; PTPC, permeability transition pore complex.

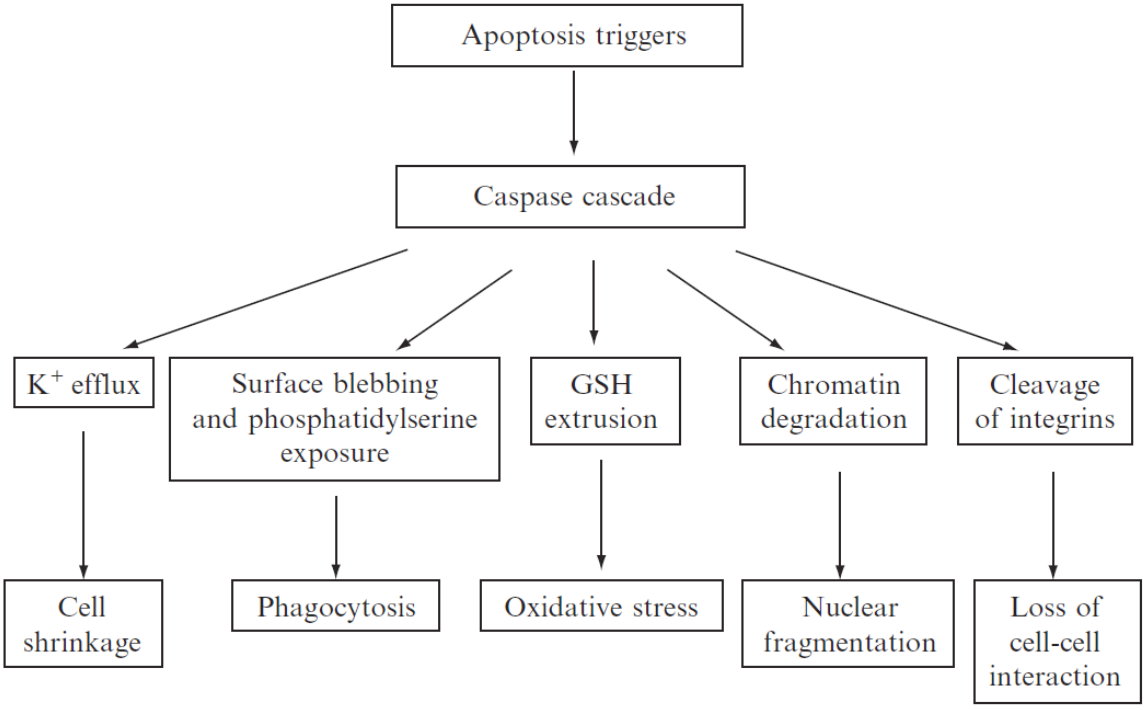


Figure 8.1 Selected consequences of caspase actions during apoptosis. Apoptosis-inducing compounds trigger activation of the caspase cascade, which leads to the cleavage of target proteins and results in various biochemical and morphological manifestations of cell death.

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DEPARTMENT OF BIOCHEMISTRY
III-B.Sc., BIOCHEMISTRY
CANCER BIOLOGY (15BCU505C)
MULTIPLE CHOICE QUESTIONS
UNIT IV

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	In which of the following situations would cells die by necrosis, not apoptosis?	Removal of cells with damaged DNA that cannot be repaired	Removal of developing neurones that fail to make profitable connections with other cells.	Removal of heart muscle cells damaged by oxygen depletion following cardiac infarction.	Removal of virus infected cells.	**Removal of heart muscle cells damaged by oxygen depletion following cardiac infarction.**
2	Which cellular organelles are involved in the initiation of the intrinsic pathway of apoptosis?	endoplasmic reticulum	lysosomes	mitochondria	peroxisomes	**mitochondria**
3	What roles in regulating the intrinsic pathway of apoptosis are played by the Bcl-2 protein family members Bax and Bcl-2?	Bax inhibits apoptosis while Bcl-2 stimulates apoptosis.	Bax stimulates apoptosis while Bcl-2 inhibits apoptosis.	Both Bax and Bcl-2 inhibit apoptosis.	Both Bax and Bcl-2 stimulate apoptosis.	**Bax stimulates apoptosis while Bcl-2 inhibits apoptosis.**
4	Which of the following are killed by the extrinsic apoptosis pathway?	Cells with damaged DNA.	Developing nerve cells that fail to make profitable connections.	Irradiated cells.	Virus infected cells.	**Virus infected cells.**
5	Which of the following proteins is a death receptor which triggers the extrinsic pathway of apoptosis?	caspase-8	FADD	Fas	Fas ligand	**Fas**
6	The triggering of the intrinsic pathway of apoptosis involves a balance between pro-apoptotic and anti-apoptotic proteins. Which of the following is anti-apoptotic?	Bax.	Bad.	Bcl-2	Cytochrome C	**Bcl-2**
7	What is the function of apoptosis?	to weed out cells that grow uncontrollably	to allow the immune system to distinguish self from non-self .	to shape organs during development	All of these are correct	**All of these are correct**
8	Apoptosis begins when _____.	the cell is injured	a “death receptor” on the cell membrane receives the signal to die	caspases become activated	killer enzymes tear up the cytoskeleton	**a “death receptor” on the cell membrane receives the signal to die**
9	Molecules that act locally to stimulate mitosis are called _____.	growth factors	harmones	stimulants	kinases	**growth factors**
10	The type of programmed cell death that is a part is normal development is called _____.	mitosis	apoptosis	cytokinesis	angiogenesis	**apoptosis**
11	Special enzymes are released during necrosis from	lysosomes	vacuoles	cytoplasm	Golgi bodies	**lysosomes**
12	Number of cells that are destroyed in adults by apoptosis are	20 to 35 billion cells	50 to 70 billion cells	10 to 20 billion cells	15 to 25 billion cells	**50 to 70 billion cells**
13	Causes of necrosis includes	injury	cancer	infection	all of above	**all of above**
14	Biebs that are broke off from cell are called	apoptotic bodies	necrosis bodies	tuberculosis bodies	cytokinetic bodies	**apoptotic bodies**
15	Apoptosis is classified as	programmed cell death	non-programmed cell death	accidental cell death	mitotic cell death	**programmed cell death**

16	Which of the following statements is not suitable to describe apoptosis?	Tightly regulated involving activation and enzymatic steps	Shrinking of cytoplasm and condensation of nucleus	Significant inflammatory response	Energy (ATP) dependant	**Significant inflammatory response**
17	Select the one correct statement. Apoptosis is a process which occurs:	as a result of damage to p53	to help adapt amphibians to wet environments	due to Helper T (CD4) cell action	None of the above	**None of the above**
18	Growth factors are an example of a positive signal which helps avert apoptosis. Which of the following are two examples of growth factors that allow their target cells to avoid apoptosis?	IL-2 and Bid	IL-2 and NGF	NGF and TNF	TNF and FasL	**IL-2 and NGF**
19	UV radiation is a 'negative signal' which can induce apoptosis in cells as a result of various forms of cell damage. If DNA damage is irreparable which of the following is activated as part of the apoptotic cascade?	Bid (to form truncated Bid)	p53	c-FLIP	All of the above	**p53**
20	The integral components strongly associated with the intrinsic and extrinsic pathways are...	the mitochondria and 'death receptors' respectively	death receptors' and the mitochondria respectively	effector caspases and initiator caspases respectively	the mitochondria and Bcl-2 family proteins respectively	**the mitochondria and 'death receptors' respectively**
21	Which of these statements is true of the intrinsic pathway of apoptosis?	The intrinsic pathway makes use of procaspase-8	TNF directly stimulates mitochondria to induce apoptosis	FADD and TRADD serve as protein kinases in the intrinsic pathway	the intrinsic pathway involves the movement of cytochrome c into the cytoplasm from the mitochondria	**the intrinsic pathway involves the movement of cytochrome c into the cytoplasm from the mitochondria**
22	Bcl-2 family proteins undergo a direct battle with each other during apoptosis. Some members of the family are pro-apoptotic and promote release of apoptotic factors from the mitochondria, others are anti-apoptotic. From the options below which are the pro-apoptotic family members?	Bax and Bcl-2	Bid and Bax	Bid and Bcl-2	Bcl-2 and Bcl-xL	**Bid and Bax**
23	What is the apoptosome complex constructed from?	cytochrome c, Apaf-1 and procaspase-8	cytochrome c, Bcl-2 and procaspase-9	FasL, CD95 and FADD	Apaf-1, cytochrome c and procaspase-9	**Apaf-1, cytochrome c and procaspase-9**
24	Which of the following is the correct order of events involved in the extrinsic pathway of apoptosis?	cell-surface ligand binding, recruitment of procaspase-8, recruitment of death domains, activation of caspase-8, activation of down stream effectors, apoptosis	DNA damage, release of apoptotic factors from mitochondria, formation of apoptosome, activation of down stream effectors, apoptosis	cell-surface ligand binding, recruitment of death domains, recruitment of procaspase-8, activation of caspase-8, activation of down stream effectors, apoptosis	None of the above	**cell-surface ligand binding, recruitment of death domains, recruitment of procaspase-8, activation of caspase-8, activation of down stream effectors, apoptosis**
25	As a result of apoptotic stimuli what action is taken by apoptosis inducing factor (AIF)?	translocates to the mitochondria to aid pro-apoptotic Bcl-2 family proteins	cleaves initiator pro-caspases to their active form	translocates to the nucleus causing DNA fragmentation	inhibits IAPs to promote apoptosis	**translocates to the nucleus causing DNA fragmentation**
26	Which of the following statements about caspases is true?	pro-caspases are zymogens	caspases are cysteine proteases	both a) and b) are true	both a) and b) are false	**both a) and b) are true**
27	The effector caspases actually initiate the death of the cell and they are smaller than the upstream initiator caspases. Select the list which correctly represents the effector	caspase-3,-6,-7	caspase-3,-6,-8	caspase-8,-9,-2	caspase-8,-9	**caspase-3,-6,-7**

	caspases.					
28	Identify the components of the death-inducing-signalling-complex (DISC)	NGF, CD95, FADD/TRADD, procaspase-8	Ligand, CD95, FADD/TRADD, procaspase-8	Ligand, CD95, FADD/TRADD, procaspase-9	Ligand, CD95, Apaf-1, procaspase-9	**Ligand, CD95, FADD/TRADD, procaspase-8**
29	Association with a regulatory subunit is one means of initiator caspase activation, which of these statements is false?	procaspase-9 associates with a protein cofactor called Apaf-1	apoptosome formation is ATP independent	CARD-CARD interactions are important in apoptosome formation	seven Apaf-1 molecules contribute to a wheel structure	**apoptosome formation is ATP independent**
30	Excessive rate of apoptosis causes	AIDS	fever	sneezing	atrophy	**atrophy**
31	Necrosis it is death:	of cells due to metabolic disorders	of parenchymatous cells only	of cells and tissues in a living organism	programmed, genetically determined death of cells	**of cells and tissues in a living organism**
32	Causes of necrosis are following:	infectious agents	allergic factors	chemical substances	all the enumerated	**all the enumerated**
33	Cell morphological type of necrosis:	vascular	allergic	coagulative	traumatic	**coagulative**
34	Which of the following indicates the progressive stages of cancer development in the correct order?	in situcancer→dysplasia→malignant tumor→metastasis	hyperplasia→dysplasia→in situcancer→malignant tumor	dysplasia→hyperplasia→metastasis→malignant tumor	in situcancer→hyperplasia→metastasis→malignant tumor	**hyperplasia→dysplasia→in situcancer→malignant tumor**
35	Morphological changes of apoptosis include	Inflammation	Nuclear fragmentation	Spindle formation	Cell swelling	**Nuclear fragmentation**
36	Starting point of apoptosis for programme cell death is -	Activation of endonuclease	Release of enzyme	Accumulation of calcium	Destruction by macrophages	**Activation of endonuclease**
37	One of the following is an apoptosis inhibitor gene	p53′	BcL-2	Rb	c-Myc	**BcL-2**
38	The process of programmed gene directed celldeath characterized by cell-shrinkage, nuclearcondensation and fragmentation is known as -	Necrosis	Chromatolysis	Pyknosis	Apoptosis	**Apoptosis**
39	Internucleosomal Cleavage of DNA is characteristic of	Reversible cell injury	Irreversible cell injury	Necrosis	Apoptosis	**Apoptosis**
40	Hypertrophy of a muscle is due to	Increase in number of cells	Increase in size of cells	Decrease in number of cells	Abnormal shape od cells	**Increase in size of cells**
41	Metaplasia is a	reversible change.	Irreversible change	Inflammatory reaction	Post-infectious state.	**reversible change.**
42	Dysplasia refers of	Change in morphology	Change in number	Change in cell type	Change in cell size	**Change in morphology**
43	Irreversible cell injury is seen	Apoptosis	Hypertrophy	Hyaline change	Fatty change	**Apoptosis**
44	All are features of apoptosis except	Increase in cell size	Cell bodies	Cytoplasmic blebs	Chromatin Condensation	**Increase in cell size**
45	Apoptosis can be defined as	Programmed cell growth	Programmed cell death	Programmed cell maturity	Programmed cell modulation	**Programmed cell death**
46	Anti-apoptotic proteins are	Bcl 2 & Bc1 x	Apaf – 1	Bax & Bim	Bim & Bid	**Bcl 2 & Bc1 x**

47	Cellular response that results from reprogramming of stem cells is	Anaplastic	Metaplasia	Hyperplasia	Hypertrophy	**Metaplasia**
48	Phagocytosis was discovered by	Ehrlich	Metchnikoff	Celsus	Virchow	**Metchnikoff**
49	Locomotion across chemical gradient is called	Diapedesis	Chemotaxis	Pavement	Margination	**Chemotaxis**
50	Wound contraction is due to	Myocyte	Fibroblast	Myofibroblast	Skeletal muscle fiber	**Myofibroblast**
51	Myofibroblasts are seen in	Healing wounds	Cancerous site	Adipose tissue	Muscle septae	**Healing wounds**
52	Natural response to injury are all except	Immobility	Anorexia	Anabolism	Catabolism	**Anabolism**
53	Which of the following type of cell death involved in tissue damage	Apoptosis	Necrosis	Pyroptosis	Phagocytosis	**Necrosis**
54	Major steps of apoptosis except	Cell shrinks	Cytoskeleton collapses	Cell fragment	Inflammation	Inflammation
55	The combination of apoptosis and _____ is responsible for shaping tissues and organs in developing embryos.	cell proliferation	cell inflammation	Growth hormones	Autophagy	**cell proliferation**
56	Which virus causes liver cancer	HPV	HSV	EBV	HBV	**HBV**
57	Which virus causes oral cancer	HPV	HSV	EBV	HBV	**HPV**
58	Activation of which gene continues to programmed cell death?	Bid	Bad	Bcl-Xl	Caspases	**Bid**
59	Which of the following protein involved in the apoptotic pathway	Caspases	Nfkb	MMP-2	Akt	**Caspases**
60	Apoptosis-inducing factor (**AIF**) is a protein that is normally located in _____.	intermembrane space of mitochondria	intermembrane space of golgi apparatus	Matrix of mitochondria	cytosol	**intermembrane space of mitochondria**

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DEPARTMENT OF BIOCHEMISTRY

SYLLABUS

SUBJECT NAME: CORE ELECTIVE I - CANCER BIOLOGY

SUB.CODE: 15BCU505C

SEMESTER: V **CLASS: III B.Sc., BIOCHEMISTRY**

UNIT V

Treatment of cancer: Early detection of cancer, molecular diagnosis, treatment -radio therapy, chemotherapy, immunotherapy and use of RNAi techniques and stem cells.

TEXT BOOKS

1. Papachristodoulou, D., Snape, A., Elliott, W. H., Elliott, D.C. (2014). *Biochemistry and Molecular Biology* (5th ed.). New York, Oxford University Press.
2. Hayat, M.A. (2010). *Methods of Cancer Diagnosis, Therapy and Prognosis*. Springer Science. ISBN: 978--420-8441-6.

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2. Lodish, H., Ber, A., Zipuoskry, L.S., Matsudaira, P., Bahimore, D., Damell, J. (2017) *Molecular Biology*. (8th ed.). W.H Freeman G Co.

Early detection of cancer

Early detection of cancer greatly increases the chances for successful treatment. There are two major components of early detection of cancer: education to promote early diagnosis and screening.

Recognizing possible warning signs of cancer and taking prompt action leads to early diagnosis. Increased awareness of possible warning signs of cancer, among physicians, nurses and other health care providers as well as among the general public, can have a great impact on the disease. Some early signs of cancer include lumps, sores that fail to heal, abnormal bleeding, persistent indigestion, and chronic hoarseness. Early diagnosis is particularly relevant for cancers of the breast, cervix, mouth, larynx, colon and rectum, and skin.

Screening

Screening refers to the use of simple tests across a healthy population in order to identify individuals who have disease, but do not yet have symptoms. Examples include breast cancer screening using mammography and cervical cancer screening using cytology screening methods, including Pap smears.

Screening programmes should be undertaken only when their effectiveness has been demonstrated, when resources (personnel, equipment, etc.) are sufficient to cover nearly all of the target group, when facilities exist for confirming diagnoses and for treatment and follow-up of those with abnormal results, and when prevalence of the disease is high enough to justify the effort and costs of screening.

Based on the existing evidence, mass population screening can be advocated only for breast and cervical cancer, using mammography screening and cytology screening, in countries where resources are available for wide coverage of the population. Several ongoing studies are currently evaluating low cost approaches to screening that can be implemented and sustained in low-resource settings. For example visual inspection with acetic acid may prove to be an effective screening method for cervical cancer in the near future. More studies that evaluate low cost alternative methods to mammography screening, such as clinical breast examination, are needed

Screening for various cancers

Screening is the presumptive identification of unrecognized disease or defects by means of tests, examinations, or other procedures that can be applied rapidly.

In advocating screening programmes as part of early detection of cancer, it is important for national cancer control programmes to avoid imposing the "high technology" of the developed world on countries that lack the infrastructure and resources to use the technology appropriately or to achieve adequate coverage of the population. The success of screening depends on having sufficient numbers of personnel to perform the screening tests and on the availability of facilities that can undertake subsequent diagnosis, treatment, and follow-up.

A number of factors should be taken into account when the adoption of any screening technique is being considered:

- Sensitivity: the effectiveness of a test in detecting a cancer in those who have the disease;
- Specificity: the extent to which a test gives negative results in those that are free of the disease;
- Positive predictive value: the extent to which subjects have the disease in those that give a positive test result;
- Negative predictive value: the extent to which subjects are free of the disease in those that give a negative test result;
- Acceptability: the extent to which those for whom the test is designed agree to be tested.

A screening test aims to be sure that as few as possible with the disease get through undetected (high sensitivity) and as few as possible without the disease are subject to further diagnostic tests (high specificity). Given high sensitivity and specificity, the likelihood that a positive screening test will give a correct result (positive predictive value) strongly depends on the prevalence of the disease within the population. If the prevalence of the disease is very low, even the best screening test will not be an effective public health programme.

Policies on early cancer detection will differ markedly between countries. An industrialized country may conduct screening programmes for cervical and breast cancer. Such programmes are not, however, recommended in the least developed countries in which there is a low prevalence of cancer and a weak health care infrastructure. Further, only organized

screening programmes are likely to be fully successful as a means of reaching a high proportion of the at-risk population.

Countries that favour cancer detection remaining part of routine medical practice, or that simply encourage people to seek specific tests at regular intervals, are unlikely to realize the full potential of screening.

The success of screening programmes depends on a number of fundamental principles:

- The target disease should be a common form of cancer, with high associated morbidity or mortality;
- Effective treatment, capable of reducing morbidity and mortality, should be available;
- Test procedures should be acceptable, safe, and relatively inexpensive.

In a national cancer control programme, screening programmes should be organized to ensure that a large proportion of the target group is screened and that those individuals in whom abnormalities are observed receive appropriate diagnosis and therapy. Agreement needs to be reached on guidelines to be applied in the national cancer control programme concerning:

- The frequency of screening and ages at which screening should be performed;
- Quality control systems for the screening tests;
- Defined mechanisms for referral and treatment of abnormalities;
- An information system that can: -send out invitations for initial screening; - recall individuals for repeat screening; - follow those with identified abnormalities; - monitor and evaluate the programme.

For a number of reasons, patients often fail to adhere to recommended cancer screening activities. While in many cases both the patients and the health care providers understand the concept of early detection, they fail to comply with recommendations. Non-compliance is a general health problem and one that should be addressed in a comprehensive manner to improve outcome and reduce the waste of resources.

Screening that concentrates solely on a high-risk group is rarely justified, as identified risk groups usually represent only a small proportion of the cancer burden in a country. In planning the coverage of screening programmes, however, steps must be taken to ensure that all those at high risk are included. This requirement may be difficult to fulfill. In screening for cancer of the cervix, for example, those at high risk are often difficult to recruit into screening.

Breast cancer

There are two early detection methods:

- early diagnosis or awareness of early signs and symptoms in symptomatic populations in order to facilitate diagnosis and early treatment, and
- screening that is the systematic application of a screening test in a presumably asymptomatic population. It aims to identify individuals with an abnormality suggestive of cancer.

A screening programme is a far more complex undertaking that an early diagnosis programme. Irrespective of the early detection method used, central to the success of population based early detection are careful planning and a well organized and sustainable programme that targets the right population group and ensures coordination, continuity and quality of actions across the whole continuum of care. Targeting the wrong age group, such as, younger women with low risk of breast cancer, could cause a lower number of breast cancers found per woman screened and therefore reduce its cost-effectiveness. In addition,

targeting younger women would lead to more evaluation of benign tumours, which causes unnecessary overload of health care facilities due to the use of addition diagnostic resources

Early diagnosis

Early diagnosis remains an important early detection strategy, particularly in low- and middle-income countries where the diseases is diagnosed in late stages and resources are very limited. There is some evidence that this strategy can produce "down staging" (increasing in proportion of breast cancers detected at an early stage) of the disease to stages that are more amenable to curative treatment (Yip et al., 2008).

Mammography screening

Mammography screening is the only screening method that has proven to be effective. Although there is evidence that organized population-based mammography screening programmes can reduce breast cancer mortality by around 20% in the screened group versus the unscreened group across all age groups, in general there appears to be a narrow balance of benefits compared with harms, particularly in younger and older women. There is uncertainty about the magnitude of the harms – particularly over diagnosis and overtreatment. Mammography screening is very complex and resource intensive and no research of its effectiveness has been conducted in low resource settings.

Breast self examination (BSE)

There is no evidence on the effect of screening through breast self-examination (BSE). However, the practice of BSE has been seen to empower women, taking responsibility for their own health. Therefore, BSE is recommend for raising awareness among women at risk rather than as a screening method.

Clinical Breast Examination (CBE)

Research is underway to evaluate CBE as a low-cost approach to breast cancer screening that can work in less affluent countries. Promising preliminary results show that the age-standardized incidence rate for advanced-stage breast cancer is lower in the screened group compared to the unscreened group.

Screening for cervical cancer

Screening is testing of all women at risk of cervical cancer, most of whom will be without symptoms.

Screening aims to detect precancerous changes, which, if not treated, may lead to cancer.

Screening is only effective if there is a well organized system for follow-up and treatment.

Women who are found to have abnormalities on screening need follow-up, diagnosis and possibly treatment, in order to prevent the development of cancer or to treat cancer at an early stage.

Several tests can be used in screening for cervical cancer. The Pap smear (cytology) is the only test that has been used in large populations and that has been shown to reduce cervical cancer incidence and mortality. Other tests (VIA, VILI, HPV) show promise but there is as yet no comparable evidence on their effectiveness. Large studies are still under way.

Regardless of the test used, the key to an effective programme is to reach the largest proportion of women at risk with quality screening and treatment.

Organized screening programmes designed and managed at the central level to reach most women at risk are preferable to opportunistic screening.

Screening for colorectal cancer

Evidence to suggest that sigmoidoscopy may be effective for colorectal cancer screening, with benefits lasting for up to ten years, has come from two case-controlled studies (Selby et al., 1992; Newcomb et al., 1992). As such studies cannot eliminate the effect of selection bias, however, this benefit may have been overestimated. Trials are now under way to evaluate flexible sigmoidoscopy and colonoscopy for screening.

Several trials have evaluated the effect of the fecal occult blood test (FOBT). A trial in Minnesota, United States of America, used the FOBT annually in one group and biennially in another. This initially indicated that annual, but not biennial, FOBTs reduce mortality from colorectal cancer after about a ten year period (Mandel et al., 1993). A more recent report, with follow-up for up to 18 years, showed mortality reduction at a lower level from biennial screening (Mandel et al., 1999). Trials in Europe also showed mortality reduction from biennial screening (Hardcastle et al., 1996; Kronborg et al., 1996).

It is clear that a major difficulty with screening using the FOBT is lack of specificity, especially if the test is rehydrated, which substantially increases the costs of programmes. Further, there seems to be a lack in sensitivity for detecting adenomas.

Taken together, the FOBT trials suggest that, after an interval of about 10 years, there could be a reduction of up to 20% in colorectal cancer mortality from biennial screening, and a greater reduction as a result of annual screening. Unless high compliance with the test can be achieved, however, the benefit that could be obtained in the general population would be much less, and not commensurate with the expense of the screening programme.

Colon and rectal cancer and polyps

Starting at age 50, both men and women should follow one of these testing plans:

Tests that find polyps and cancer

- Colonoscopy every 10 years, or
- CT colonography (virtual colonoscopy) every 5 years*, or
- Flexible sigmoidoscopy every 5 years*, or
- Double-contrast barium enema every 5 years*

Tests that mostly find cancer

- Yearly fecal immunochemical test (FIT)**, or
- Yearly guaiac-based fecal occult blood test (gFOBT)**, or
- Stool DNA test (sDNA) every 3 years*

*If the test is positive, a colonoscopy should be done.

** The multiple stool take-home test should be used. One test done in the office is not enough. A colonoscopy should be done if the test is positive.

The tests that can find both early cancer and polyps should be your first choice if these tests are available and you're willing to have one of them. But the most important thing is to get tested, no matter which test you choose. Talk to a health care provider about which tests might be right for you.

If you are at high risk of colon cancer based on family history or other factors, you may need to be screened using a different schedule. Talk with a health care provider about your history and the testing plan that's best for you.

Cervical cancer

- **Cervical cancer testing should start at age 21.** Women under age 21 should not be tested.

- **Women between the ages of 21 and 29** should have a Pap test done every 3 years. HPV testing should not be used in this age group unless it's needed after an abnormal Pap test result.
- **Women between the ages of 30 and 65** should have a Pap test plus an HPV test (called "co-testing") done every 5 years. This is the preferred approach, but it's OK to have a Pap test alone every 3 years.
- **Women over age 65** who have had regular cervical cancer testing in the past 10 years with normal results should not be tested for cervical cancer. Once testing is stopped, it should not be started again. Women with a history of a serious cervical pre-cancer should continue to be tested for at least 20 years after that diagnosis, even if testing goes past age 65.
- **A woman who has had her uterus and cervix removed (a total hysterectomy)** for reasons not related to cervical cancer and who has no history of cervical cancer or serious pre-cancer should not be tested.
- **All women who have been vaccinated against HPV** should still follow the screening recommendations for their age groups.

Some women – because of their health history (HIV infection, organ transplant, DES exposure, etc.) – may need a different screening schedule for cervical cancer. Talk to a health care provider about your history.

Endometrial (uterine) cancer

The American Cancer Society recommends that at the time of menopause, all women should be told about the risks and symptoms of endometrial cancer. Women should report any unexpected vaginal bleeding or spotting to their doctors.

Some women – because of their history – may need to consider having a yearly endometrial biopsy. Please talk with a health care provider about your history.

Lung cancer

The American Cancer Society does not recommend tests to check for lung cancer in people who are at average risk. But, we do have screening guidelines for those who are at high risk of lung cancer due to cigarette smoking. Screening might be right for you if you are all of the following:

- 55 to 74 years of age
- In good health
- Have at least a 30 pack-year smoking history AND are either still smoking or have quit within the last 15 years (A pack-year is the number of cigarette packs smoked each day multiplied by the number of years a person has smoked. Someone who smoked a pack of cigarettes per day for 30 years has a 30 pack-year smoking history, as does someone who smoked 2 packs a day for 15 years.)

Screening is done with an annual low-dose CT scan (LDCT) of the chest. If you fit the list above, talk to a health care provider if you want to start screening.

Prostate cancer

The American Cancer Society recommends that men make an informed decision with a health care provider about whether to be tested for prostate cancer. Research has not yet proven that the potential benefits of testing outweigh the harms of testing and treatment. We believe that men should not be tested without first learning about what we know and don't know about the risks and possible benefits of testing and treatment.

Starting at age 50, men should talk to a health care provider about the pros and cons of testing so they can decide if testing is the right choice for them.

If you are African American or have a father or brother who had prostate cancer before age 65, you should have this talk with a health care provider starting at age 45.
If you decide to be tested, you should get a PSA blood test with or without a rectal exam. How often you're tested will depend on your PSA level.

Take control of your health, and help reduce your cancer risk.

- Stay away from all forms of tobacco.
- Get to and stay at a healthy weight.
- Get moving with regular physical activity.
- Eat healthy with plenty of fruits and vegetables.
- Limit how much alcohol you drink (if you drink at all).
- Protect your skin.
- Know yourself, your family history, and your risks.
- Get regular check-ups and cancer screening tests.

Molecular diagnosis

Molecular diagnostics could allow a clinician to differentiate a breast cancer patient who will have a recurrence of the disease from one who will not, or to distinguish a cancer that originated in the thyroid from one that began in the lung.
As cancer develops — becomes more aggressive, becomes metastatic — there are further molecular changes that can occur, that then further define the cancer. There are a number of molecular diagnostics for cancer on the market, and more are coming. Physicians are beginning to order tests and there are indications that some molecular diagnostics may be cost-effective. The technologies that many tests are based on — microarrays and PCR — may soon be shaken up as sequencing enters the clinical market.

Development

The molecular diagnostic field began to grow rapidly about 10 years ago with a number of tests emerging based on gene expression signatures.
The most effective way to deal with cancer would be to prevent development of the disease. Many cancers can be cured by localized treatments, such as surgery or radiation, if they are detected before they metastasize into the body. The initial diagnosis of most cancers is through screening by the appearance of signs or symptoms which does not lead to a definitive diagnosis, and thus requires the examination of a tissue sample by a pathologist. Before molecular diagnostics, clinicians categorized cancer cells according to biopsy samples and by observing their appearance under a microscope. Molecular analysis of the oncogenes and tumor suppressor genes involved in particular types of tumors has the potential of providing information that is useful in the diagnosis of cancer and in monitoring the effects of treatment. Nowadays molecular diagnosis is emerging as a new and eye opening approach that merges genomics and proteomics for early detection and diagnosis of cancer. Merging two new disciplines, genomics and proteomics, molecular diagnostics categorizes cancer using technologies such as microarrays and mass spectrometry. Genomics is the branch of science which deals with study of all the genes in a cell or organism, while proteomics is the study of all the proteins. Through molecular diagnostics the interaction of genes and proteins in a cell can be determined. Molecular diagnostics uncovers the sets of changes in a normal cell and captures this information as expression patterns. The expression patterns are also called as "molecular signatures, "which help in improving the clinicians' ability to diagnose cancer (A). Genomics and proteomics tools are used to detect different molecular signatures like

genetic and epigenetic signatures, changes in gene expressions, protein biomarker profiles and other metabolite profile changes, which in turn allows to identify the combinations of biomarkers which may detect best the presence or risk of cancer or monitor cancer therapies.

CANCER DIAGNOSIS AND GENOMICS

Genomics applies recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyze the function and structure of the complete set of DNA within a single cell of an organism. The field is used to determine the entire DNA sequence of organisms.

CANCER DIAGNOSIS THROUGH MICROARRAYS

A major emphasis in molecular diagnosis is on the use of DNA microarrays also called as “gene chips” for determining the expression patterns of genes. The major challenge is to find the genes active in cancer and separate them from all of the others in a cell. DNA microarrays can be used to compare the patterns of gene expression in two different cell populations, such as a population of cancer cells with a population of normal cells. In this case, two different fluorescent dyes are used (A).

Microarrays allow researchers to "see" the expression of hundreds or thousands of genes all at once and their comparison simultaneously. The results obtained from microarray experiments are dramatically changing cancer-treatment decisions. Cancer research make use of certain specific standard arrays which include lymph chip and other arrays that contain strands of DNA called single nucleotide polymorphisms, or SNPs ("snips"), which are common variations in DNA.) DNA Microarray technology measures the relative activity of previously identified target genes. In recent years scientists have focused on machine learning algorithms for classification and diagnosis of cancer based on expression profiles.

Supervised methods that have been applied to molecular classification of cancer tissues include correlation-based classification methods, artificial neural networks (ANNs) and support vector machines (SVMs). However one of the difficulties in using gene expression profiles to predict cancer is how to effectively select a few informative genes to construct accurate prediction models from thousands or ten thousands of genes. Microarrays can also be used to detect differences in patterns of gene activity even within the same tumor type, as a single type of cancer categorized microscopically can be of more subtypes, each with a distinct gene expression pattern.

CANCER DIAGNOSIS THROUGH MICRO RNAs

Micro RNA (abbr. miRNA), a small non-coding RNA molecule encoded by eukaryotic nuclear DNA, found in plants and animals, functions in transcriptional and post-transcriptional regulation of gene expression. miRNAs function via base-pairing with complementary sequences within mRNA molecules, usually resulting in gene silencing via translational repression or target degradation. The human genome may encode over 1000 miRNAs, which may target about 60% of mammalian genes and are abundant in many human cell types.

CANCER DIAGNOSIS THROUGH REAL TIME PCR

A quantitative polymerase chain reaction (qPCR), also called real-time polymerase chain reaction, is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR), which is used to amplify and simultaneously quantify a targeted DNA molecule. For one or more specific sequences in a DNA sample, quantitative PCR enables both detection and quantification. The quantity can be either an absolute number of copies or a relative amount when normalized to DNA input or additional normalizing genes.

Treatment

Radiation Therapy

Radiation therapy uses high-energy radiation to shrink tumors and kill cancer cells (1). X-rays, gamma rays, and charged particles are types of radiation used for cancer treatment.

The radiation may be delivered by a machine outside the body (external-beam radiation therapy), or it may come from radioactive material placed in the body near cancer cells (internal radiation therapy, also called brachytherapy).

Systemic radiation therapy uses radioactive substances, such as radioactive iodine, that travel in the blood to kill cancer cells.

About half of all cancer patients receive some type of radiation therapy sometime during the course of their treatment.

Radiation therapy kills cancer cells by damaging their DNA (the molecules inside cells that carry genetic information and pass it from one generation to the next) (1). Radiation therapy can either damage DNA directly or create charged particles (free radicals) within the cells that can in turn damage the DNA.

Cancer cells whose DNA is damaged beyond repair stop dividing or die. When the damaged cells die, they are broken down and eliminated by the body's natural processes.

Doctors take potential damage to normal cells into account when planning a course of radiation therapy. The amount of radiation that normal tissue can safely receive is known for all parts of the body. Doctors use this information to help them decide where to aim radiation during treatment.

Radiation therapy is sometimes given with curative intent (that is, with the hope that the treatment will cure a cancer, either by eliminating a tumor, preventing cancer recurrence, or both). In such cases, radiation therapy may be used alone or in combination with surgery, chemotherapy, or both.

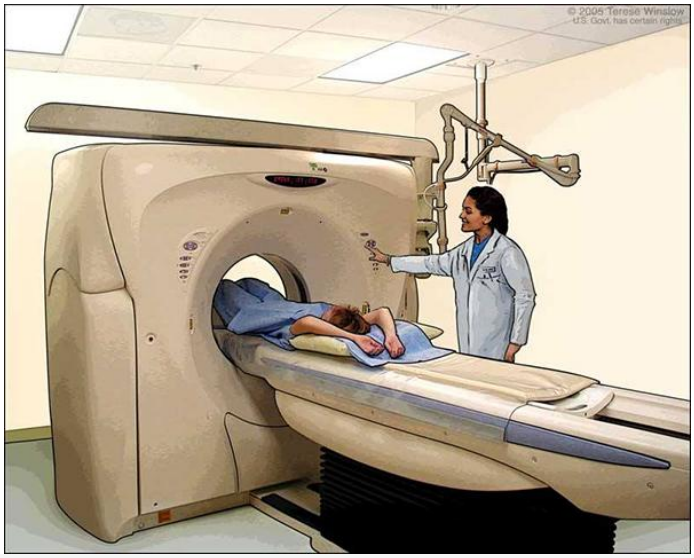
Radiation therapy may also be given with palliative intent. Palliative treatments are not intended to cure. Instead, they relieve symptoms and reduce the suffering caused by cancer.

Some examples of palliative radiation therapy are:

- Radiation given to the brain to shrink tumors formed from cancer cells that have spread to the brain from another part of the body (metastases).
- Radiation given to shrink a tumor that is pressing on the spine or growing within a bone, which can cause pain.
- Radiation given to shrink a tumor near the esophagus, which can interfere with a patient's ability to eat and drink.

A radiation oncologist develops a patient's treatment plan through a process called treatment planning, which begins with simulation.

During simulation, detailed imaging scans show the location of a patient's tumor and the normal areas around it. These scans are usually computed tomography (CT) scans, but they can also include magnetic resonance imaging (MRI), positron emission tomography(PET), and ultrasound scans.



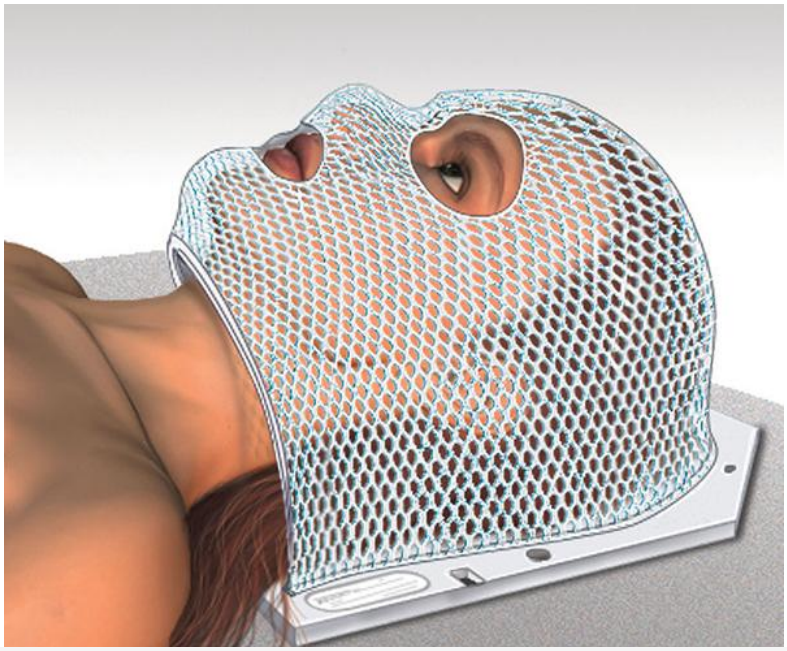
Computed Tomography Scanner. CT scans are often used in treatment planning for radiation therapy. During CT scanning, pictures of the inside of the body are created by a computer linked to an x-ray machine.

CT scans are often used in treatment planning for radiation therapy. During CT scanning, pictures of the inside of the body are created by a computer linked to an x-ray machine.

During simulation and daily treatments, it is necessary to ensure that the patient will be in exactly the same position every day relative to the machine delivering the treatment or doing the imaging. Body molds, head masks, or other devices may be constructed for an individual patient to make it easier for a patient to stay still. Temporary skin marks and even tattoos are used to help with precise patient positioning.

Patients getting radiation to the head may need a mask. The mask helps keep the head from moving so that the patient is in the exact same position for each treatment.

After simulation, the radiation oncologist then determines the exact area that will be treated, the total radiation dose that will be delivered to the tumor, how much dose will be allowed for the normal tissues around the tumor, and the safest angles (paths) for radiation delivery.



Radiation Therapy Head Mask. Patients getting radiation to the head may need a mask. The mask helps keep the head from moving so that the patient is in the exact same position for each treatment.

The staff working with the radiation oncologist (including physicists and dosimetrists) use sophisticated computers to design the details of the exact radiation plan that will be used. After approving the plan, the radiation oncologist authorizes the start of treatment. On the first day of treatment, and usually at least weekly after that, many checks are made to ensure that the treatments are being delivered exactly the way they were planned.

Radiation doses for cancer treatment are measured in a unit called a gray (Gy), which is a measure of the amount of radiation energy absorbed by 1 kilogram of human tissue. Different doses of radiation are needed to kill different types of cancer cells.

Radiation can damage some types of normal tissue more easily than others. For example, the reproductive organs (testicles and ovaries) are more sensitive to radiation than bones. The radiation oncologist takes all of this information into account during treatment planning.

If an area of the body has previously been treated with radiation therapy, a patient may not be able to have radiation therapy to that area a second time, depending on how much radiation was given during the initial treatment. If one area of the body has already received the maximum safe lifetime dose of radiation, another area might still be treated with radiation therapy if the distance between the two areas is large enough.

The area selected for treatment usually includes the whole tumor plus a small amount of normal tissue surrounding the tumor. The normal tissue is treated for two main reasons:

- To take into account body movement from breathing and normal movement of the organs within the body, which can change the location of a tumor between treatments.
- To reduce the likelihood of tumor recurrence from cancer cells that have spread to the normal tissue next to the tumor (called microscopic local spread).

How is radiation therapy given to patients?

Radiation can come from a machine outside the body (external-beam radiation therapy) or from radioactive material placed in the body near cancer cells (internal radiation therapy, more

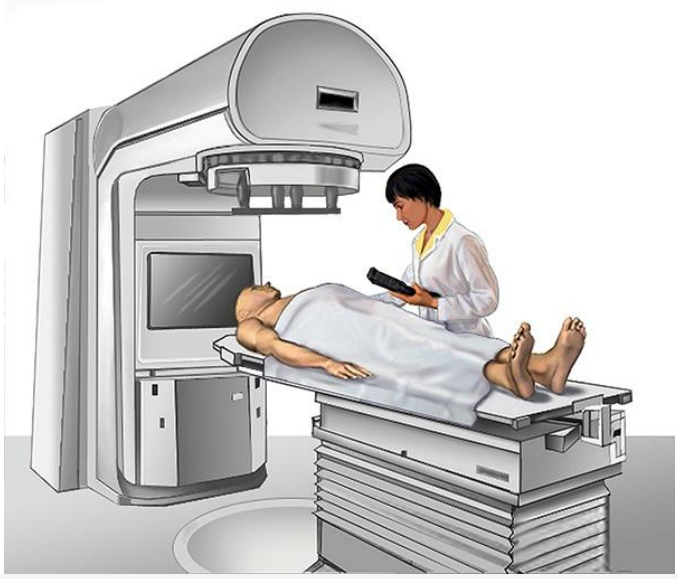
commonly called brachytherapy). Systemic radiation therapy uses a radioactive substance, given by mouth or into a vein, that travels in the blood to tissues throughout the body.

The type of radiation therapy prescribed by a radiation oncologist depends on many factors, including:

- The type of cancer.
- The size of the cancer.
- The cancer's location in the body.
- How close the cancer is to normal tissues that are sensitive to radiation.
- How far into the body the radiation needs to travel.
- The patient's general health and medical history.
- Whether the patient will have other types of cancer treatment.
- Other factors, such as the patient's age and other medical conditions.

External-beam radiation therapy

External-beam radiation therapy is most often delivered in the form of photon beams (either x-rays or gamma rays) (1). A photon is the basic unit of light and other forms of electromagnetic radiation. It can be thought of as a bundle of energy. The amount of energy in a photon can vary. For example, the photons in gamma rays have the highest energy, followed by the photons in x-rays.



Linear Accelerator Used for External-beam Radiation Therapy. Many types of external-beam radiation therapy are delivered using a machine called a linear accelerator (also called a LINAC). A LINAC uses electricity to form a stream of fast-moving subatomic particles. This creates high-energy radiation that may be used to treat cancer.

Many types of external-beam radiation therapy are delivered using a machine called a linear accelerator (also called a LINAC). A LINAC uses electricity to form a stream of fast-moving subatomic particles. This creates high-energy radiation that may be used to treat cancer.

Patients usually receive external-beam radiation therapy in daily treatment sessions over the course of several weeks. The number of treatment sessions depends on many factors, including the total radiation dose that will be given.

One of the most common types of external-beam radiation therapy is called **3-dimensional conformal radiation therapy (3D-CRT)**. 3D-CRT uses very sophisticated computer software and advanced treatment machines to deliver radiation to very precisely shaped target areas.
Many other methods of external-beam radiation therapy are currently being tested and used in cancer treatment. These methods include:

- **Intensity-modulated radiation therapy (IMRT)**: IMRT uses hundreds of tiny radiation beam-shaping devices, called collimators, to deliver a single dose of radiation. The collimators can be stationary or can move during treatment, allowing the intensity of the radiation beams to change during treatment sessions. This kind of dose modulation allows different areas of a tumor or nearby tissues to receive different doses of radiation.
  Unlike other types of radiation therapy, IMRT is planned in reverse (called inverse treatment planning). In inverse treatment planning, the radiation oncologist chooses the radiation doses to different areas of the tumor and surrounding tissue, and then a high-powered computer program calculates the required number of beams and angles of the radiation treatment. In contrast, during traditional (forward) treatment planning, the radiation oncologist chooses the number and angles of the radiation beams in advance and computers calculate how much dose will be delivered from each of the planned beams.
  The goal of IMRT is to increase the radiation dose to the areas that need it and reduce radiation exposure to specific sensitive areas of surrounding normal tissue. Compared with 3D-CRT, IMRT can reduce the risk of some side effects, such as damage to the salivary glands (which can cause dry mouth, or xerostomia), when the head and neck are treated with radiation therapy. However, with IMRT, a larger volume of normal tissue overall is exposed to radiation. Whether IMRT leads to improved control of tumor growth and better survival compared with 3D-CRT is not yet known.
- **Image-guided radiation therapy (IGRT)**: In IGRT, repeated imaging scans (CT, MRI, or PET) are performed during treatment. These imaging scans are processed by computers to identify changes in a tumor's size and location due to treatment and to allow the position of the patient or the planned radiation dose to be adjusted during treatment as needed. Repeated imaging can increase the accuracy of radiation treatment and may allow reductions in the planned volume of tissue to be treated, thereby decreasing the total radiation dose to normal tissue.
- **Tomotherapy:** Tomotherapy is a type of image-guided IMRT. A tomotherapy machine is a hybrid between a CT imaging scanner and an external-beam radiation therapy machine. The part of the tomotherapy machine that delivers radiation for both imaging and treatment can rotate completely around the patient in the same manner as a normal CT scanner.
  Tomotherapy machines can capture CT images of the patient's tumor immediately before treatment sessions, to allow for very precise tumor targeting and sparing of normal tissue.
  Like standard IMRT, tomotherapy may be better than 3D-CRT at sparing normal tissue from high radiation doses. However, clinical trials comparing 3D-CRT with tomotherapy have not been conducted.
- **Stereotactic radiosurgery:** Stereotactic radio surgery (SRS) can deliver one or more high doses of radiation to a small tumor. SRS uses extremely accurate image-guided tumor targeting and patient positioning. Therefore, a high dose of radiation can be given without excess damage to normal tissue.
  SRS can be used to treat only small tumors with well-defined edges. It is most commonly used in the treatment of brain or spinal tumors and brain metastases from other cancer

types. For the treatment of some brain metastases, patients may receive radiation therapy to the entire brain (called whole-brain radiation therapy) in addition to SRS.
SRS requires the use of a head frame or other device to immobilize the patient during treatment to ensure that the high dose of radiation is delivered accurately.

- **Stereotactic body radiation therapy:** Stereotactic body radiation therapy (SBRT) delivers radiation therapy in fewer sessions, using smaller radiation fields and higher doses than 3D-CRT in most cases. By definition, SBRT treats tumors that lie outside the brain and spinal cord. Because these tumors are more likely to move with the normal motion of the body, and therefore cannot be targeted as accurately as tumors within the brain or spine, SBRT is usually given in more than one dose. SBRT can be used to treat only small, isolated tumors, including cancers in the lung and liver.
- **Proton therapy:** External-beam radiation therapy can be delivered by proton beams as well as the photon beams described above. Protons are a type of charged particle.
  Proton beams differ from photon beams mainly in the way they deposit energy in living tissue. Whereas photons deposit energy in small packets all along their path through tissue, protons deposit much of their energy at the end of their path (called the Bragg peak) and deposit less energy along the way.
  In theory, use of protons should reduce the exposure of normal tissue to radiation, possibly allowing the delivery of higher doses of radiation to a tumor. Proton therapy has not yet been compared with standard external-beam radiation therapy in clinical trials.
- **Other charged particle beams:** Electron beams are used to irradiate superficial tumors, such as skin cancer or tumors near the surface of the body, but they cannot travel very far through tissue. Therefore, they cannot treat tumors deep within the body.

Patients can discuss these different methods of radiation therapy with their doctors to see if any is appropriate for their type of cancer and if it is available in their community or through a clinical trial.

Internal radiation therapy

Internal radiation therapy (brachytherapy) is radiation delivered from radiation sources (radioactive materials) placed inside or on the body. Several brachytherapy techniques are used in cancer treatment. Interstitial brachytherapy uses a radiation source placed within tumor tissue, such as within a prostate tumor. Intracavitary brachytherapy uses a source placed within a surgical cavity or a body cavity, such as the chest cavity, near a tumor. Episcleral brachytherapy, which is used to treat melanoma inside the eye, uses a source that is attached to the eye.

In brachytherapy, radioactive isotopes are sealed in tiny pellets or "seeds." These seeds are placed in patients using delivery devices, such as needles, catheters, or some other type of carrier. As the isotopes decay naturally, they give off radiation that damages nearby cancer cells.

If left in place, after a few weeks or months, the isotopes decay completely and no longer give off radiation. The seeds will not cause harm if they are left in the body. Brachytherapy may be able to deliver higher doses of radiation to some cancers than external-beam radiation therapy while causing less damage to normal tissue.

Brachytherapy can be given as a low-dose-rate or a high-dose-rate treatment:

- In low-dose-rate treatment, cancer cells receive continuous low-dose radiation from the source over a period of several days.
- In high-dose-rate treatment, a robotic machine attached to delivery tubes placed inside the body guides one or more radioactive sources into or near a tumor, and then removes the

sources at the end of each treatment session. High-dose-rate treatment can be given in one or more treatment sessions.

The placement of brachytherapy sources can be temporary or permanent:

- For permanent brachytherapy, the sources are surgically sealed within the body and left there, even after all of the radiation has been given off. The remaining material (in which the radioactive isotopes were sealed) does not cause any discomfort or harm to the patient. Permanent brachytherapy is a type of low-dose-rate brachytherapy.
- For temporary brachytherapy, tubes (catheters) or other carriers are used to deliver the radiation sources, and both the carriers and the radiation sources are removed after treatment. Temporary brachytherapy can be either low-dose-rate or high-dose-rate treatment.

Doctors can use brachytherapy alone or in addition to external-beam radiation therapy to provide a "boost" of radiation to a tumor while sparing surrounding normal tissue.

Systemic radiation therapy

In systemic radiation therapy, a patient swallows or receives an injection of a radioactive substance, such as radioactive iodine or a radioactive substance bound to a monoclonal antibody. Radioactive iodine (^{131}I) is a type of systemic radiation therapy commonly used to help treat some types of thyroid cancer. Thyroid cells naturally take up radioactive iodine.

Why are some types of radiation therapy given in many small doses?

Patients who receive most types of external-beam radiation therapy usually have to travel to the hospital or an outpatient facility up to 5 days a week for several weeks. One dose (a single fraction) of the total planned dose of radiation is given each day. Occasionally, two treatments a day are given.

Most types of external-beam radiation therapy are given in once-daily fractions. There are two main reasons for once-daily treatment:

- To minimize the damage to normal tissue.
- To increase the likelihood that cancer cells are exposed to radiation at the points in the cell cycle when they are most vulnerable to DNA damage (1, 14).

In recent decades, doctors have tested whether other fractionation schedules are helpful (1), including:

- Accelerated fractionation—treatment given in larger daily or weekly doses to reduce the number of weeks of treatment.
- Hyperfractionation—smaller doses of radiation given more than once a day.
- Hypofractionation—larger doses given once a day or less often to reduce the number of treatments.

Researchers hope that different types of treatment fractionation may either be more effective than traditional fractionation or be as effective but more convenient.

When will a patient get radiation therapy?

A patient may receive radiation therapy before, during, or after surgery. Some patients may receive radiation therapy alone, without surgery or other treatments. Some patients may receive radiation therapy and chemotherapy at the same time. The timing of radiation therapy depends on the type of cancer being treated and the goal of treatment (cure or palliation).

Radiation therapy given before surgery is called pre-operative or neoadjuvant radiation. Neoadjuvant radiation may be given to shrink a tumor so it can be removed by surgery and be less likely to return after surgery.

Radiation therapy given during surgery is called intraoperative radiation therapy (IORT). IORT can be external-beam radiation therapy (with photons or electrons) or brachytherapy. When radiation is given during surgery, nearby normal tissues can be physically shielded from radiation exposure. IORT is sometimes used when normal structures are too close to a tumor to allow the use of external-beam radiation therapy.

Radiation therapy given after surgery is called post-operative or adjuvant radiation therapy.

Radiation therapy given after some types of complicated surgery (especially in the abdomen or pelvis) may produce too many side effects; therefore, it may be safer if given before surgery in these cases.

The combination of chemotherapy and radiation therapy given at the same time is sometimes called chemoradiation or radiochemotherapy. For some types of cancer, the combination of chemotherapy and radiation therapy may kill more cancer cells (increasing the likelihood of a cure), but it can also cause more side effects.

After cancer treatment, patients receive regular follow-up care from their oncologists to monitor their health and to check for possible cancer recurrence. Detailed information about follow-up care can be found at NCI's Follow-up Medical Care page.

Does radiation therapy make a patient radioactive?

External-beam radiation does not make a patient radioactive.

During temporary brachytherapy treatments, while the radioactive material is inside the body, the patient is radioactive; however, as soon as the material is removed, the patient is no longer radioactive. For temporary brachytherapy, the patient will usually stay in the hospital in a special room that shields other people from the radiation.

During permanent brachytherapy, the implanted material will be radioactive for several days, weeks, or months after the radiation source is put in place. During this time, the patient is radioactive. However, the amount of radiation reaching the surface of the skin is usually very low. Nonetheless, this radiation can be detected by radiation monitors and contact with pregnant woman and young children may be restricted for a few days or weeks.

Some types of systemic radiation therapy may temporarily make a patient's bodily fluids (such as saliva, urine, sweat, or stool) emit a low level of radiation. Patients receiving systemic radiation therapy may need to limit their contact with other people during this time, and especially avoid contact with children younger than 18 and pregnant women.

A patient's doctor or nurse will provide more information to family members and caretakers if any of these special precautions are needed. Over time (usually days or weeks), the radioactive material retained within the body will break down so that no radiation can be measured outside the patient's body.

What are the potential side effects of radiation therapy?

Radiation therapy can cause both early (acute) and late (chronic) side effects. Acute side effects occur during treatment, and chronic side effects occur months or even years after treatment ends. The side effects that develop depend on the area of the body being treated, the dose given per day, the total dose given, the patient's general medical condition, and other treatments given at the same time.

Acute radiation side effects are caused by damage to rapidly dividing normal cells in the area being treated. These effects include skin irritation or damage at regions exposed to the radiation beams. Examples include damage to the salivary glands or hair loss when the head or neck area is treated, or urinary problems when the lower abdomen is treated.

Most acute effects disappear after treatment ends, though some (like salivary glanddamage) can be permanent. The drug amifostine can help protect the salivary glands from radiation damage if it is given during treatment. Amifostine is the only drug approved by the FDA to protect normal tissues from radiation during treatment. This type of drug is called a radioprotector. Other potential radioprotectors are being tested in clinical trials.

Fatigue is a common side effect of radiation therapy regardless of which part of the body is treated. Nausea with or without vomiting is common when the abdomen is treated and occurs sometimes when the brain is treated. Medications are available to help prevent or treat nausea and vomiting during treatment.

Late side effects of radiation therapy may or may not occur. Depending on the area of the body treated, late side effects can include (1):

- Fibrosis (the replacement of normal tissue with scar tissue, leading to restricted movement of the affected area).
- Damage to the bowels, causing diarrhea and bleeding.
- Memory loss.
- Infertility (inability to have a child).
- Rarely, a second cancer caused by radiation exposure.

Second cancers that develop after radiation therapy depend on the part of the body that was treated. A more comprehensive discussion of acute and late side effects from radiation therapy, as well as ways to cope with these side effects, can be found in the NCI publications Radiation Therapy and You: Support for People With Cancer and the Radiation Therapy Side Effects Series.

Chemotherapy

(also called chemo) is a type of cancer treatment that uses drugs to kill cancer cells.

Chemotherapy works by stopping or slowing the growth of cancer cells, which grow and divide quickly. Chemotherapy is used to:

- Treat cancer
  Chemotherapy can be used to cure cancer, lessen the chance it will return, or stop or slow its growth.
- Ease cancer symptoms
  Chemotherapy can be used to shrink tumors that are causing pain and other problems.

When used with other treatments, chemotherapy can:

- Make a tumor smaller before surgery or radiation therapy. This is called neoadjuvant chemotherapy.
- Destroy cancer cells that may remain after treatment with surgery or radiation therapy. This is called adjuvant chemotherapy.
- Help other treatments work better.
- Kill cancer cells that have returned or spread to other parts of your body.

Chemotherapy not only kills fast-growing cancer cells, but also kills or slows the growth of healthy cells that grow and divide quickly. Examples are cells that line your mouth and intestine and those that cause your hair to grow. Damage to healthy cells may cause side effects, such as mouth sores, nausea, and hair loss. Side effects often get better or go away after you have finished chemotherapy.

Chemotherapy may be given in many ways. Some common ways include:

- Oral
  The chemotherapy comes in pills, capsules, or liquids that you swallow
- Intravenous (IV)
  The chemotherapy goes directly into a vein
- Injection
  The chemotherapy is given by a shot in a muscle in your arm, thigh, or hip, or right under the skin in the fatty part of your arm, leg, or belly
- Intrathecal
  The chemotherapy is injected into the space between the layers of tissue that cover the brain and spinal cord
- Intraperitoneal (IP)
  The chemotherapy goes directly into the peritoneal cavity, which is the area in your body that contains organs such as your intestines, stomach, and liver
- Intra-arterial (IA)
  The chemotherapy is injected directly into the artery that leads to the cancer
- Topical
  The chemotherapy comes in a cream that you rub onto your skin

Chemotherapy is often given through a thin needle that is placed in a vein on your hand or lower arm. Your nurse will put the needle in at the start of each treatment and remove it when treatment is over. IV chemotherapy may also be given through catheters or ports, sometimes with the help of a pump.

- Catheter
  A catheter is a thin, soft tube. A doctor or nurse places one end of the catheter in a large vein, often in your chest area. The other end of the catheter stays outside your body. Most catheters stay in place until you have finished your chemotherapy treatments. Catheters can also be used to give you other drugs and to draw blood. Be sure to watch for signs of infection around your catheter. See the section about infection for more information.
- Port
  A port is a small, round disc that is placed under your skin during minor surgery.
  A surgeon puts it in place before you begin your course of treatment, and it remains there until you have finished. A catheter connects the port to a large vein, most often in your chest. Your nurse can insert a needle into your port to give you chemotherapy or draw blood. This needle can be left in place for chemotherapy treatments that are given for longer than one day. Be sure to watch for signs of infection around your port. See the section about infection for more information.
- Pump
  Pumps are often attached to catheters or ports. They control how much and how fast chemotherapy goes into a catheter or port, allowing you to receive your chemotherapy outside of the hospital. Pumps can be internal or external. External pumps remain outside your body. Internal pumps are placed under your skin during surgery.

Immunotherapy

is treatment that uses certain parts of a person's immune system to fight diseases such as cancer. Immunotherapy includes treatments that work in different ways. Some boost the body's immune system in a very general way. Others help train the immune system to attack cancer cells specifically. Immunotherapy works better for some types of cancer than for others. It's used by

itself for some of these cancers, but for others it seems to work better when used with other types of treatment.

Types of cancer immunotherapy

The main types of immunotherapy now being used to treat cancer include:

- **Monoclonal antibodies:** These are man-made versions of immune system proteins. Antibodies can be very useful in treating cancer because they can be designed to attack a very specific part of a cancer cell.
- **Immune checkpoint inhibitors:** These drugs basically take the 'brakes' off the immune system, which helps it recognize and attack cancer cells.
- **Cancer vaccines:** Vaccines are substances put into the body to start an immune response against certain diseases. We usually think of them as being given to healthy people to help prevent infections. But some vaccines can help prevent or treat cancer.
- **Other, non-specific immunotherapies:** These treatments boost the immune system in a general way, but this can still help the immune system attack cancer cells.

Immunotherapy drugs are now used to treat many different types of cancer. For more information about immunotherapy as a treatment for a specific cancer, please see our information on that type of cancer.

Use of RNAi technique

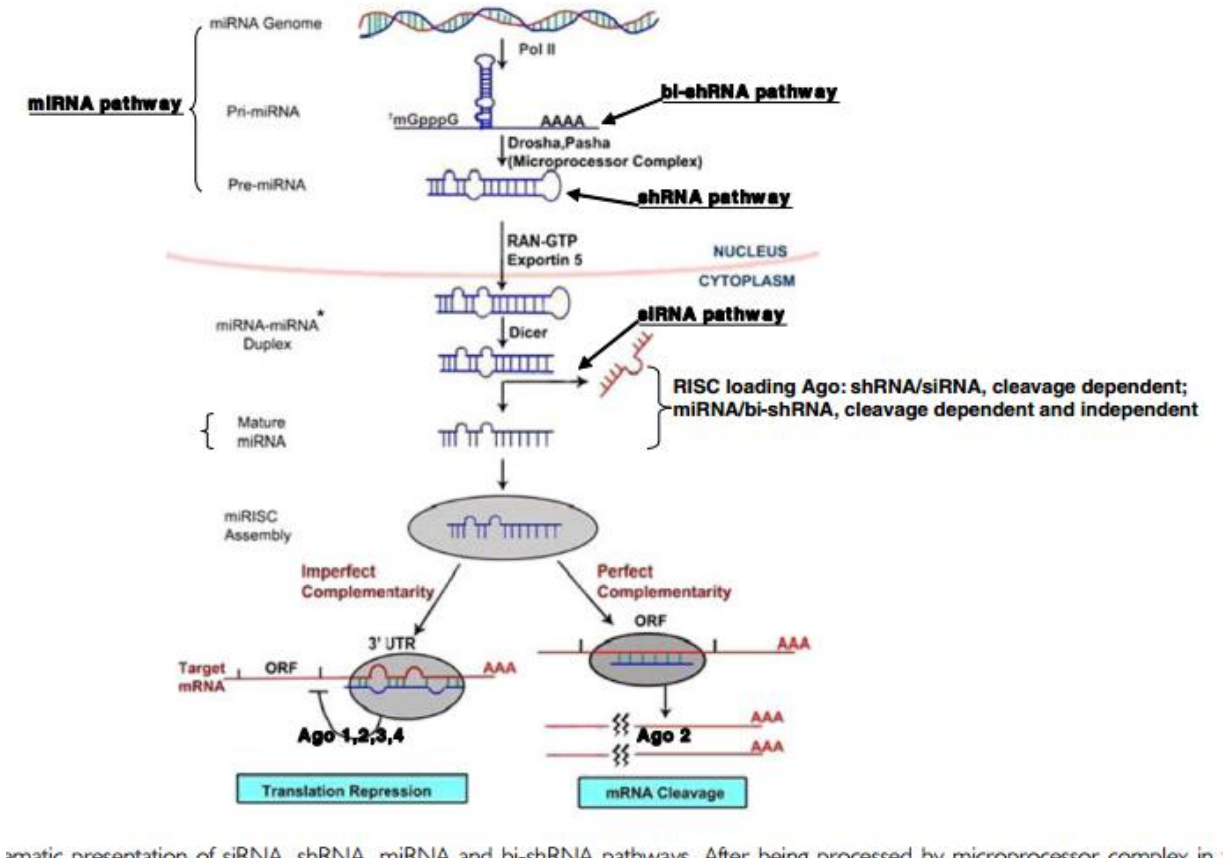
RNAi, discovered by Fire and Mello in 1998, is defined as a mechanism of gene-silencing produced by small RNAs, which include endogenous microRNA (miRNA) and exogenous siRNA or shRNA. This gene silencing is an evolutionarily conserved process and is highly dependent on gene sequence. Due to the inherent difficulties of inhibiting potential targets with small molecular drugs, recombinant proteins and monoclonal antibodies, researchers and clinicians have looked towards RNAi as a revolutionary approach to target "undruggable" targets with robustness and specificity. The mechanism of RNAi has been thoroughly investigated. Briefly, a double-stranded small RNA is incorporated into the pre-RISC (RNA Induced Silencing Complex) followed by the cleavage-dependent (in the case of matched guide and passenger strands) or—independent (unmatched guide and passenger strands) release of the passenger strand forming the guide strand containing RISC. The guide-strand (anti-sense strand) guides RISC to the complementary or near-complementary region of target mRNA. In general, siRNA (small interfering RNA from the cleavage-dependent RISC) with a perfect match to its target cleaves the target mRNA via the endonuclease Ago2 whereas miRNA (microRNA), with an imperfect match to its target, induces mRNA degradation (or sequestration in the p-body (processing body) and translational inhibition. Duplex siRNA and vector encoded shRNA were first introduced as a way to silence gene expression in animals in 2002.

Synthetic siRNA was the first RNAi technology to be introduced into mammalian cells in order to accomplish sequence specific gene silencing. This type of small RNA directly incorporates into RISC, where its guide-strand binds to and cleaves the complementary mRNA. When the cleaved mRNA is released and further degraded, the guide strand-bound RISC binds to another mRNA and starts a new cycle of cleavage (Fig). siRNA is able to cleave target RNA in both the cytoplasm and the nucleus. As a result of this efficient suppression machinery, a well-designed siRNA is capable of inhibiting gene expression at picomolar concentrations in vitro. However, there are limitations to in vivo use. First, siRNA is sensitive to nucleases present in plasma and therefore must be protected for delivery to target tissues. Second, frequent dosing is required since there is no endogenous production of the delivered "therapeutic" and siRNA

pharmacokinetics are characterized by a short half-life as well as rapid clearance. Therefore, chemical modification is required to improve stability and circulation half-life.

In cancer, siRNA and shRNA have been extensively used to silence cancer-related targets. A large number of preclinical studies have presented favorable outcomes by silencing genes critical for tumor cell growth, metastasis, angiogenesis and chemoresistance. RNAi technology has been used to inhibit tumor metastasis.

RNAi has been employed to block immunosuppressive pathways in dendritic cells, T cells and tumor cells. For instance, siRNA has been used to knockdown expression of 3 inducible proteosome subunits in mature dendritic cells (DCs). The transected DCs had increased expression of constitutive proteosomes, leading to an altered repertoire of tumor antigenic peptides. The DCs generated from melanoma patients were transfected with the immunoprotesome siRNA and subsequently induced antigen-specific CTL activity against autologous melanoma cells. Suppressor of cytokine signaling 1(SOCS1) negatively regulates JAK/STAT pathway in T cells, DCs and other immune cells. It specifically attenuates the extent of antigen presentation in DCs. Silencing SOCS1 in DCs using lentivirus-encoded shRNA stimulated antigen presentation and elicited antigen-specific CTL activity. Moreover, in a mouse melanoma xenograft model, immunization with DCs previously transduced with SOCS1 shRNA induced potent antigen-specific anti-tumor immunity and inhibited tumor growth. Likewise, zinc-finger protein A20 is an ubiquitin-modifying enzyme and downregulates TNFR and TLR signaling pathways. Silencing A20 with shRNA delivered by lentivirus increased the expression of costimulator molecules and proinflammatory cytokines, and abolished regulatory T cells (Treg cells)-mediated suppression in an antigen-specific manner, Immunization with A20-silenced DCs elicited antitumor immunity, a result of tumor infiltration of suppressed Treg cells and hyperactivated CTLs and T helper cells. RNAi has the potential to lessen the immunotolerance, which allows tumors to escape immune surveillance. IDO is an enzyme that catalyzes the degradation of tryptophan, an essential amino acid for T cell viability and proliferation. It suppresses immune response by inducing T cell apoptosis. IDO is constitutively expressed in most human tumors. Silencing of IDO reduced T cells apoptosis and enhanced T cell proliferation. Intratumoral delivery of IDO siRNA significantly reduced tumor growth in a melanoma xenograft model. Besides the adaptive immune system, the innate immune response can be harnessed to increase tumor cell immunogenicity. For example, a Bcl-2-specific siRNA with 5′-triphosphate ends combined the activation of the innate immune response with target-specific gene silencing. Recognition of 5′-triphosphate by antiviral helicase retinoic acid-induced protein I (Rig-I) induced type I IFN and activated NK cells in tumors, synergized with siRNA mediated Bcl-2 gene silencing. This combinatorial approach was validated in various animal models and human melanoma cells. In another study, a siRNA targeting Stat3 was synthetically linked to an oligonucleotide agonist of toll-like receptor 9 (TLR9) to simultaneously induce antitumor immunity and gene silencing in tumor–associated TLR9+ myeloid cells and B cells. Intratumor and systemic delivery of CpG-Stat3 siRNA reduced tumor growth in xenograft melanoma models.



ematic presentation of siRNA, shRNA, miRNA and bi-shRNA pathways. After being processed by microprocessor complex in

Use of Stem cells

Stem cell transplants are procedures that restore blood-forming stem cells in people who have destroyed by the very high doses of chemotherapy or radiation therapy that are used to treat certain cancers.

Blood-forming stem cells are important because they grow into different types of blood cells. The main types of blood cells are:

- White blood cells, which are part of your immune system and help your body fight infection
- Red blood cells, which carry oxygen throughout your body
- Platelets, which help the blood clot

In a stem cell transplant, you receive healthy blood-forming stem cells through a needle in your vein. Once they enter your bloodstream, the stem cells travel to the bone marrow, where they take the place of the cells that were destroyed by treatment. The blood-forming stem cells that are used in transplants can come from the bone marrow, bloodstream, or umbilical cord.

Transplants can be:

- Autologous, which means the stem cells come from you, the patient
- Allogeneic, which means the stem cells come from someone else. The donor may be a blood relative but can also be someone who is not related.
- Syngeneic, which means the stem cells come from your identical twin, if you have one

To reduce possible side effects and improve the chances that an allogeneic transplant will work, the donor's blood-forming stem cells must match yours in certain ways.

Stem cell transplants do not usually work against cancer directly. Instead, they help you recover your ability to produce stem cells after treatment with very high doses of radiation therapy, chemotherapy, or both. However, in multiple myeloma and some types of leukemia, the stem cell transplant may work against cancer directly. This happens because of an effect called graft-versus-tumor that can occur after allogeneic transplants. Graft-versus-tumor occurs when white blood cells from your donor (the graft) attack any cancer cells that remain in your body (the tumor) after high-dose treatments. This effect improves the success of the treatments.

Depending on the type of transplant that's done, there are 3 possible sources of stem cells to use for transplants:

- Bone marrow (from you or someone else)
- The bloodstream (peripheral blood – from you or someone else)
- Umbilical cord blood from newborns

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
III-B.Sc., BIOCHEMISTRY
CANCER BIOLOGY (15BCU505C) - UNIT V
MULTIPLE CHOICE QUESTIONS

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	Blastoma is a cancer involving which tissue	bones	connective tissue	epithelial cells	embryonic tissue	**embryonic tissue**
2	Migration of cancerous cells from the site of origin to other part of the body forming secondary tumours is called	diapedesis	metastasis	proliferation	none of these	**metastasis**
3	Which on of the following is used in treatment of thyroid cancer?	U-238	I-131	C-14	rA-240	**I-131**
4	A patient is suspicious of having breast cancer. What type of test will a physician conduct to diagnose the cancer	blood test	pap test	CT scan	mammography	**mammography**
5	Which one of the following therapies will involve only the cancerous cells not the normal cells in treatment	immunotherapy	surgery	aromatherapy	chemotherapy	**immunotherapy**
6	Which one of the following cancers does not form a solid neoplasm	leukemia	lymphoma	lipoma	sarcoma	**leukemia**
7	Why is genetic counseling for familial breast cancer difficult?	BRCA1 and BRCA2 are incompletely penetrant	Breast cancer can occur in other ways.	Not all mutations are associated with disease	all of the above	**all of the above**
8	Which of the following is not a traditional cancer treatment?	blocking telomerase	inhibiting angiogenesis	stimulating specialization	none of the above	**blocking telomerase**
9	Which of the following is NOT among the uses of PET imaging in the management of cervical cancer?	Initial diagnosis	Staging	Treatment planning	Assessment of prognosis	**Initial diagnosis**
10	The primary treatment for locoregionally advanced head and neck cancer consists of:	Surgery	Radiation therapy	Chemoradiation therapy	All of the above	**Chemoradiation therapy**
11	What sort of therapy involves administration of an antibody linked to an enzyme, such that the enzyme activates a prodrug?	ADAPT	GDAPT	ADEPT	GDEPT	**ADEPT**
12	Which of the following inhibits angiogenesis?	VEGF	FGF-2	Angiostatin	Interleukin-6	**Angiostatin**
13	Mechanism of cellular resistance to polyfunctional alkylating drugs:	decreased DNA repair capability	reduced production of glutathione	increased permeability to the drug	none of the above	**none of the above**
14	Polyfunctional alkylating agent used specifically for chronic myeloid leukemia:	cyclophosphamide (Cytoxan)	busulfan (Myleran)	thiopeta (Thioplex)	dacarbazine (DTIC)	**busulfan (Myleran)**
15	Major toxicity of alkylating drugs:	alopecia	myelosuppression	renal damage	hepatic failure	**myelosuppression**
16	Anticancer drug that is also used to treat psoriasis and rheumatoid arthritis:	mercaptomurine (6-MP)	methotrexate	procarbazine (Matulane)	allopurinol (Zyloprim, Purinol)	**methotrexate**

17	The treatment of choice for anaplastic carcinoma of thyroid infiltrating trachea and sternum will be?	radial excision	radiotherapy	palliative therapy	chemotherapy	**chemotherapy**
18	The chemotherapeutic agent, most commonly administered by continious infusion is?	Ara-C	5-FU	Cisplatin	Etoposide	**Ara-C**
19	The treatment of choice for stage I cancer larynx is?	Radical surgery	chemotherapy	radiotherapy	surgery followed by radiotherapy	**radiotherapy**
20	sodium-2-mercapto ethane sulfonate is used as a protective agent in?	radiotherapy	cancer chemotherapy	Lithotripsy	Hepatic encephalopathy	**cancer chemotherapy**
21	chemotherapeutic drugs can cause	necrosis	apoptosis	both (a) & (b)	anoikis	**both (a) & (b)**
22	The most common side effects of chemotherapy administration is	Nausea	alopecia	myelosuppression	renal distinction	**nausea**
23	Which of these are thought to have anti-cancer benefits?	Heterocyclic aromatic amines (HAs)	Cruciferous vegetables such as broccoli	Red meats	Baked potatoes	**Cruciferous vegetables such as broccoli**
24	The use of drugs to treat cancer is called	radiotherapy	chemotherapy	physiotherapy	surgery	**chemotherapy**
25	The following reduces the side â€“effects of cancer treatment	geeting up in the morning	a well balanced diet	meditation	going for walk	**a well balanced diet**
26	Each of the following is an example of a way to detect cancer EXCEPT _____.	mammograms	MRI (magnetic resonance imaging)	PSA blood test	magnetism	**magnetism**
27	Cancer in its early stages is usually _____.	easy to diagnose using physical symptoms	easy to diagnose using blood tests	metastasized to distant organs	asymptomatic and undiagnosed	**asymptomatic and undiagnosed**
28	The HPV vaccine (Gardasil) is recommended for the prevention of _____.	lung cancer	skin cancer	prostrate cancer	cervical cancer	**cervical cancer**
29	One cancer treatment involves "starving" the cancer cells by inhibiting _____.	immune function	angiogenesis	Digestion	metastasis	**angiogenesis**
30	The immune system can help to defend against cancer cells because _____.	cancer is able to hide inside body organs	cancer cells metastasize	cancer cells may stop displaying normal "self" proteins on the cell surface	cancer antigens attack T cells	**cancer cells may stop displaying normal "self" proteins on the cell surface**
31	Which form of skin cancer is the least common, but most dangerous?	actinic keratosis	melanoma	squamous cell carcinoma	basal cell carcinoma	**melanoma**
32	Which source of carcinogens is associated with approximately 30% of all cancer deaths?	UV radiation	tobacco	X-ray and gamma radiation	viruses	**tobacco**
33	Which of the following is NOT a classic treatment for cancer?	chemotherapy	physical therapy	Radiation	surgery	**physical therapy**
34	Which of the following is NOT a risk factor for breast cancer?	taking estrogen supplements after menopause	smoking	early menstruation	age	**smoking**

35	You can inherit a susceptibility to cancer because _____.	cancer abnormalities may be passed in the cytoplasm of an oocyte	cancer involves abnormal genes, some of which may be inherited	cancer begins as a weakness in the tissues of an organ	cancer genes develop primarily during meiotic cell divisions	**cancer involves abnormal genes, some of which may be inherited**
36	Tumor marker for primary hepatocellular carcinoma are all except	Alpha feto protein	Alpha 2 macroglobulin	PIVKA-2	Neurotensin	**Alpha 2 macroglobulin**
37	Which cancer classification is NOT matched with its description?	leukemias - cancers of the blood	carcinomas - cancer of epithelial tissue	lymphomas - cancers of lymphatic tissue	sarcomas - cancers of the liver	**sarcomas - cancers of the liver**
38	The most common cancer in males is:	lung cancer	colorectal cancer	prostate cancer	leukemia	**prostate cancer**
39	The most common cancer in females is:	non-Hodgkin lymphoma	lung cancer	ovarian cancer	breast cancer	**breast cancer**
40	Which type of cancer causes the most deaths in both males and females?	skin cancer	Lung cancer	colorectal cancer	reproductive organ cancer	**Lung cancer**
41	____ percent of all cancers are related to tobacco smoke.	80	90	70	60	**80**
42	What DNA virus has been linked to a type of human cancer?	hepatitis B virus	human papillomavirus	Epstein-Barr virus .	All of these are correct	**All of these are correct**
43	Which of the following is NOT a warning signal of possible cancer?	change in bowel habits	thickening or lump	cold-like symptoms	a sore that does not heal	**cold-like symptoms**
44	Which of the following is a correct sign of possible malignant melanoma?	uniform color of a mole	irregular border of a mole	symmetrical appearance of a mole	a mole with a diameter of 2mm	**irregular border of a mole**
45	The PAP smear is a test for _____ cancer.	Colon	cervical	Breast	ovarian	**cervical**
46	_____ is an X-ray study of the breast used to detect tumors too small to be felt.	Sigmoidoscopy	Mammography	Tomography	Chemotherapy	**Mammography**
47	Sigmoidoscopy is a test for _____ cancer.	Breast	colon	Cervical	brain	**colon**
48	The standard methods of treatment for cancer are _____.	Surgery	radiation	chemotherapy	All of these are correct	**All of these are correct**
49	Most chemotherapeutic drugs kill cells by damaging _____.	DNA	protein	nearby blood vessels	All of these are correct	**DNA**
50	_____ involves genetically engineered antigen-presenting cells that give tumor antigens to cytotoxic T-cells that kill the tumor cells.	Immunotherapy	Chemotherapy	Angiogenic therapy	Biopsy	**Immunotherapy**
51	Which of the following clinical features are characteristic of familial cancer syndromes?	Observed tumor types are rarely seen as sporadic cancers	Two or more independent primary tumors in a single individual	a & b	none of the above	**a & b**
52	Which process is used to insert normal genes into human cells to correct disorders?	Gene therapy	Live vector vaccines	Molecular cloning	Stem cell therapy	**Gene therapy**
53	Most current gene therapy trials target…	SCID deficiency	Cancer	Cystic fibrosis	HIV	**Cancer**
54	Which vehicles are often used for gene therapy to carry a healthy gene?	Bacteria	Plastic capsules	Powder balls	Viruses	**Viruses**

55	Gene therapy targeting the germ-line is…	Heritable	Not heritable	Sometimes heritable	Unrelated to heritability	**Heritable**
56	Which part of the human body are bone marrow cells removed from to perform ex vivo SCID gene therapy?	Lung	Skull	Hip bone	Spinal cord	**Hip bone**
57	In gene therapy, in order to be successful, the healthy gene inserted into a target cell must…	Take over and kill the defective gene	Be inserted manually into the cell's mitochondria	Become attached to the cell's mRNA molecules	Be able to make the correct amount of the protein needed	**Be able to make the correct amount of the protein needed**
58	When was the first gene therapy patient treated?	1988	1990	1993	1999	**1990**
59	In which country was the first commercial gene therapy product, Gendicine, registered for the treatment of head and neck carcinoma?	China	USA	United Kingdom	Germany	**China**
60.	Which diagnostic therapy preliminarily was used in breast cancer?	Mammogram	Range test PSA	CT Scan	Biopsy	**Mammogram**

Reg. No.....

[16RBC302]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2016 onwards)

M.Phil., / Ph.D., DEGREE EXAMINATION, JUNE 2016

BIOCHEMISTRY

CANCER BIOLOGY AND IMMUNOLOGY

Time: 3 hours Maximum : 100 marks

Answer ALL the Questions
(5 x 20 = 100 Marks)

1. a. Explain cell cycle and its control with a schematic representation

   Or

   b. Explain the role of protein kinase and cellular response in biology of cancer

2. a. Explain apoptosis and the process involved in normal and cancer cell.

   Or

   b. Explain Bcl-2 and IAP family proteins.

3. a. Explain tumor suppressor genes

   Or

   b. Explain DNA repair mechanism

4. a. Explain cell mediated immunity.

   Or

   b. Discuss the clinical laboratory methods for the detection of antigens and antibodies.

5. a. Explain Real-time PCR

   Or

   b. Explain ELISA assay

Reg. No.....

[12RBC302]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

(For the candidates admitted from 2012 onwards)

M.Phil., / Ph.D., DEGREE EXAMINATION, DECEMBER 2012

BIOCHEMISTRY

CANCER BIOLOGY AND IMMUNOLOGY

Time: 3 hours

Maximum: 100 marks

Answer ALL the Questions

(5 x 20 = 100 Marks)

1. a. Explain Tumor viruses.

Or

b. Explain cell cycle and its role in cancer.

2. a. Explain briefly a. intracellular proteolytic cascade. B. Bcl-2 family protein.

Or

b. Explain tumor necrosis factor.

3. a. Explain oncogenic mutations.

Or

b. Explain DNA repair mechanism.

4. a. Explain cell mediated immunity.

Or

b. Discuss the clinical laboratory methods for the detection of antigens and antibodies.

5. a. Explain : a. Western blotting b. ELISA assay.

Or

b. Explain immunocytochemistry and immunohistochemistry
