CANCER

- **Neoplasm or tumour** is *'a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells even after cessation of stimulus for growth which caused it'.*
- *The* branch of science dealing with the study of neoplasms or tumours is called **oncology** (*oncos=tumour, logos=study).*
- Neoplasms may be **'benign'** when they are slow-growing and localised without causing much difficulty to the host, or **'malignant'** when they proliferate rapidly, spread throughout the body and may eventually cause death of the host.
- The common term used for all malignant tumours is **cancer.**

- The tumours derive their nomenclature on the basis of the parenchymal component comprising them.
- The suffix *'**-oma**' is added to denote benign tumours.*
- *Malignant tumours* of epithelial origin are called ***carcinomas**, while malignant* mesenchymal tumours are named ***sarcomas***.

TABLE 8.1: Classification of Tumours.

Tissue of Origin	Benign	Malignant
I. TUMOURS OF ONE PARENCHYMAL CELL TYPE		
A. Epithelial Tumours		
1. Squamous epithelium	Squamous cell papilloma	Squamous cell (Epidermoid) carcinoma
2. Transitional epithelium	Transitional cell papilloma	Transitional cell carcinoma
3. Glandular epithelium	Adenoma	Adenocarcinoma
4. Basal cell layer skin	—	Basal cell carcinoma
5. Neuroectoderm	Naevus	Melanoma (Melanocarcinoma)
6. Hepatocytes	Liver cell adenoma	Hepatoma (Hepatocellular carcinoma)
7. Placenta (Chorionic epithelium)	Hydatidiform mole	Choriocarcinoma
B. Non-epithelial (Mesenchymal) Tumours		
1. Adipose tissue	Lipoma	Liposarcoma
2. Adult fibrous tissue	Fibroma	Fibrosarcoma
3. Embryonic fibrous tissue	Myxoma	Myxosarcoma
4. Cartilage	Chondroma	Chondrosarcoma
5. Bone	Osteoma	Osteosarcoma
6. Synovium	Benign synovioma	Synovial sarcoma
7. Smooth muscle	Leiomyoma	Leiomyosarcoma
8. Skeletal muscle	Rhabdomyoma	Rhabdomyosarcoma
9. Mesothelium	—	Mesothelioma

TABLE 8.1: Classification of Tumours.

Tissue of Origin	Benign	Malignant
10. Blood vessels	Haemangioma	Angiosarcoma
11. Lymph vessels	Lymphangioma	Lymphangiosarcoma
12. Glomus	Glomus tumour	–
13. Meninges	Meningioma	Invasive meningioma
14. Haematopoietic cells	–	Leukaemias
15. Lymphoid tissue	Pseudolymphoma	Malignant lymphomas
16. Nerve sheath	Neurilemmoma, Neurofibroma	Neurogenic sarcoma
17. Nerve cells	Ganglioneuroma	Neuroblastoma
II. MIXED TUMOURS		
Salivary glands	Pleomorphic adenoma (mixed salivary tumour)	Malignant mixed salivary tumour
III. TUMOURS OF MORE THAN ONE GERM CELL LAYER		
Totipotent cells in gonads or in embryonal rests	Mature teratoma	Immature teratoma

SPECIAL CATEGORIES OF TUMOURS.

- Following categories of tumours are examples which defy the generalisation in nomenclature given above:

1. **Mixed tumours.** When two types of tumours are combined in the same tumour, it is called a mixed tumour.

- For example: *Adenosquamous carcinoma is the combination of* adenocarcinoma and squamous cell carcinoma in the endometrium.

2. **Teratomas.** These tumours are made up of a mixture of various tissue types arising from totipotent cells derived from the three germ cell layers—ectoderm, mesoderm and endoderm.

- Most common sites for teratomas are ovaries and testis (*gonadal teratomas).*

3. **Blastomas (Embryomas).** Blastomas or embryomas are a group of malignant tumours which arise from embryonal or partially differentiated cells which would normally form blastema of the organs and tissue during embryogenesis.

- Eg: Hepatoblastoma, Retinoblastoma

4. **Hamartoma.** Hamartoma is benign tumour which is made of mature but disorganised cells of tissues indigenous to the particular organ.

- e.g. hamartoma of the lung consists of mature cartilage, mature smooth muscle and epithelium.

5. **Choristoma.** Choristoma is the name given to the ectopic islands of normal tissue.

- Thus, choristoma is heterotopia but is not a true tumour, though it sounds like one.

CHARACTERISTICS OF TUMOURS

- The characteristics of tumors are described under the following headings:

I. Rate of growth

II. Cancer phenotype and stem cells

III. Clinical and gross features

IV. Microscopic features

V. Local invasion (Direct spread)

VI. Metastasis (Distant spread).

I. RATE OF GROWTH

- The tumour cells generally proliferate more rapidly than the normal cells. In general, benign tumours grow slowly and malignant tumours rapidly.
- The rate at which the tumour enlarges depends upon 2 main factors:

1. Rate of cell production, growth fraction and rate of cell loss

2. Degree of differentiation of the tumour.

1. Rate of cell production, growth fraction and rate of cell loss.

Rate of growth of a tumour depends upon 3 important parameters:

i) doubling time of tumour cells,

ii) number of cells remaining in proliferative pool (growth fraction), and

iii) rate of loss of tumour cells by cell shedding.

2. Degree of differentiation.

- Secondly, the rate of growth of malignant tumour is directly proportionate to the degree of differentiation.
- The regulation of tumour growth is under the control of growth factors secreted by the tumour cells.
- Out of various growth factors, important ones modulating tumour biology are listed below and discussed later:

i) Epidermal growth factor (EGF)

ii) Fibroblast growth factor (FGF)

iii) Platelet-derived growth factor (PDGF)

iv) Colony stimulating factor (CSF)

v) Transforming growth factors-β (TGF-β)

vi) Interleukins (IL)

vii) Vascular endothelial growth factor (VEGF)

II. CANCER PHENOTYPE AND STEM CELLS

- Normally growing cells in an organ are related to the neighbouring cells—they grow under normal growth controls, perform their assigned function and there is a balance between the rate of cell proliferation and the rate of cell death including cell suicide (i.e. apoptosis).
- However, cancer cells exhibit antisocial behaviour as under:

i) Cancer cells disobey the growth controlling signals in the body and thus *proliferate rapidly.*

ii) Cancer cells escape death signals and achieve *immortality.*

iii) Imbalance between cell proliferation and cell death in cancer causes *excessive growth.*

iv) Cancer cells lose properties of differentiation and thus perform *little or no function.*

v) Due to loss of growth controls, cancer cells are genetically unstable and develop *newer mutations.*

vi) Cancer cells overrun their neighbouring tissue and *invade locally.*

vii) Cancer cells have the ability to travel from the site of origin to other sites in the body where they colonise and establish *distant metastasis.*

- Cancer cells arise from stem cells normally present in the tissues in small number and are not readily identifiable.
- These stem cells have the properties of prolonged self-renewal, asymmetric replication and transdifferentiation (i.e. plasticity).
- These cancer stem cells are called tumour-initiating cells.

III. CLINICAL AND GROSS FEATURES

- Clinically, benign tumours are generally slow growing, and depending upon the location, may remain asymptomatic (e.g. subcutaneous lipoma), or may produce serious symptoms (e.g. meningioma in the nervous system).
- On the other hand, malignant tumours grow rapidly, may ulcerate on the surface, invade locally into deeper tissues, may spread to distant sites (metastasis), and also produce systemic features such as weight loss, anorexia and anemia.
- In fact, two of the cardinal clinical features of malignant tumours are: i*nvasiveness and metastasis*

- **Gross appearance of benign and malignant tumours may** be quite variable and the features may not be diagnostic on the basis of gross appearance alone.
- *Benign tumours are generally spherical or ovoid in shape.*
- They are encapsulated or well-circumscribed, more often firm and uniform, unless secondary changes like haemorrhage or infarction supervene
- *Malignant tumours, on the other hand, are usually* irregular in shape, poorly-circumscribed and extend into the adjacent tissues.
- Secondary changes like haemorrhage, infarction and ulceration are seen more often.
- Sarcomas typically have fish-flesh like consistency while carcinomas are generally firm.

IV. MICROSCOPIC FEATURES

- For recognising and classifying the tumours, the microscopic characteristics of tumour cells are of greatest importance.
- These features which are appreciated in histologic sections are as under:

1. microscopic pattern
2. cytomorphology of neoplastic cells (differentiation and anaplasia);
3. tumour angiogenesis and stroma; and
4. inflammatory reaction.

1. Microscopic Pattern

- The tumour cells may be arranged in a variety of patterns in different tumours as under:
- The *epithelial tumours generally consist of acini, sheets,* columns or cords of epithelial tumour cells that may be arranged in solid or papillary pattern
- The *mesenchymal tumours have mesenchymal tumour cells* arranged as interlacing bundles, fasicles or whorls, lying separated from each other usually by the intercellular matrix substance such as hyaline material in leiomyoma
- Generally, most benign tumours and low grade malignant tumours *reduplicate the normal structure of origin more* closely so that there is little difficulty in identifying and classifying such tumours
- **However, anaplastic** tumours differ greatly from the arrangement in normal tissue of origin of the tumour and may occasionally pose problems in classifying the tumour.

2. Cytomorphology of Neoplastic Cells (Differentiation and Anaplasia)

- The neoplastic cell is characterised by morphologic and functional alterations, the most significant of which are *'differentiation' and 'anaplasia'.*
- **Differentiation is defined as the extent of morphological** and functional resemblance of parenchymal tumour cells to corresponding normal cells.
- If the deviation of neoplastic cell in structure and function is minimal as compared to normal cell, the tumour is described as *'well-differentiated' such as* most benign and low-grade malignant tumours.
- *'Poorly differentiated', 'undifferentiated' or 'dedifferentiated' are* synonymous terms for poor structural and functional resemblance to corresponding normal cell.

- As a result of anaplasia, noticeable morphological and functional alterations in the neoplastic cells are observed.

i) *Loss of polarity :* tumour cells lose their basal polarity so that the nuclei tend to lie away from the basement membrane.

ii) *Pleomorphism. The term pleomorphism means variation* in size and shape of the tumour cells.

iii) *N:C ratio : N*ucleocytoplasmic ratio is increased from normal 1:5 to 1:1

iv) *Anisonucleosis. Just like cellular pleomorphism, the nuclei* too, show variation in size and shape in malignant tumour cells

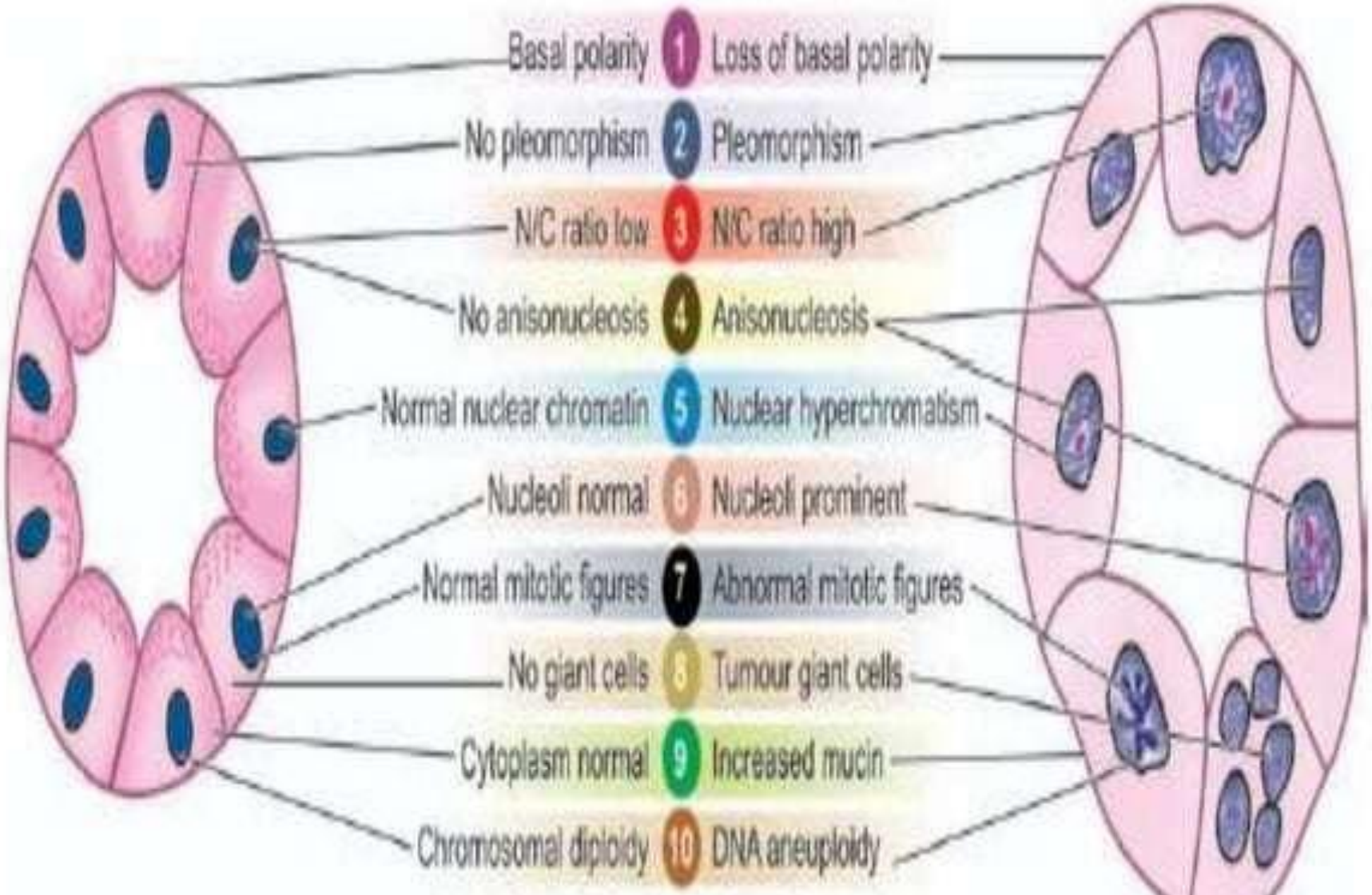
v) *Hyperchromatism. Characteristically, the nuclear* chromatin of malignant cell is increased and coarsely clumped. This is due to increase in the amount of nucleoprotein resulting in dark-staining nuclei.

vi) *Nucleolar changes. Malignant cells frequently have a* prominent nucleolus or nucleoli in the nucleus reflecting increased nucleoprotein synthesis.

vii) *Mitotic figures. The parenchymal cells of poorly differentiated* tumours often show large number of mitoses as compared with benign tumours and well-differentiated malignant tumours.

viii) *Tumour giant cells. Multinucleate tumour giant cells or* giant cells containing a single large and bizarre nucleus.

x) *Chromosomal abnormalities.* Most malignant tumours show *DNA aneuploidy,* often in the form of an increase in the number of chromosomes, reflected morphologically by the increase in the size of nuclei.



A. NORMAL MORPHOLOGY

B. CYTOMORPHOLOGY IN CANCER

3. Tumour Angiogenesis and Stroma

- **TUMOUR ANGIOGENESIS :** In order to provide nourishment to growing tumour, new blood vessels are formed from pre-existing ones (angiogenesis).
- How this takes place under the influence of angiogenic factors elaborated by tumour cells such as vascular endothelium growth factor (VEGF).
- However, related morphologic features are as under:

i) Microvascular density

ii) Central necrosis.

TUMOUR STROMA. The collagenous tissue in the stroma may be scanty or excessive.

- In the former case, the tumour is soft and fleshy (e.g. in sarcomas, lymphomas), while in the latter case the tumour is hard and gritty (e.g. infiltrating duct carcinoma breast).
- Growth of fibrous tissue in tumour is stimulated by basic fibroblast growth factor (bFGF) elaborated by tumour cells.

4. Inflammatory Reaction

- At times, prominent inflammatory reaction is present in and around the tumours.
- It could be the result of ulceration in the cancer when there is secondary infection.

- The inflammatory reaction in such instances may be acute or chronic.
- However, some tumours show chronic inflammatory reaction, chiefly of lymphocytes, plasma cells and macrophages, and in some instances granulomatous reaction, in the absence of ulceration.
- This is due to cell-mediated immunologic response by the host in an attempt to destroy the tumour.

Eg: malignant melanoma of the skin

TABLE 8.2: Contrasting Features of Benign and Malignant Tumours.

Feature	Benign	Malignant
I. CLINICAL AND GROSS FEATURES		
1. *Boundaries*	Encapsulated or well-circumscribed	Poorly-circumscribed and irregular
2. *Surrounding tissue*	Often compressed	Usually invaded
3. *Size*	Usually small	Often larger
4. *Secondary changes*	Occur less often	Occur more often
II. MICROSCOPIC FEATURES		
1. *Pattern*	Usually resembles the tissue of origin closely	Often poor resemblance to tissue of origin
2. *Basal polarity*	Retained	Often lost
3. *Pleomorphism*	Usually not present	Often present
4. *Nucleo-cytoplasmic ratio*	Normal	Increased
5. *Anisonucleosis*	Absent	Generally present
6. *Hyperchromatism*	Absent	Often present
7. *Mitoses*	May be present but are always typical mitoses	Mitotic figures increased and are generally atypical and abnormal

TABLE 8.2: Contrasting Features of Benign and Malignant Tumours.

Feature	Benign	Malignant
8. *Tumour giant cells*	May be present but without nuclear atypia	Present with nuclear atypia
9. *Chromosomal abnormalities*	Infrequent	Invariably present
10. *Function*	Usually well maintained	May be retained, lost or become abnormal
III. GROWTH RATE	Usually slow	Usually rapid
IV. LOCAL INVASION	Often compresses the surrounding tissues without invading or infiltrating them	Usually infiltrates and invades the adjacent tissues
V. METASTASIS	Absent	Frequently present
VI. PROGNOSIS	Local complications	Death by local and metastatic complications

V. LOCAL INVASION (DIRECT SPREAD)

- **BENIGN TUMOURS.** Most benign tumours form encapsulated or circumscribed masses that *expand and push aside the surrounding normal tissues without actually* invading, infiltrating or metastasising.
- **MALIGNANT TUMOURS.** Malignant tumours also enlarge by expansion and some well-differentiated tumours may be partially encapsulated as well e.g. follicular carcinoma thyroid.
- But characteristically, they are distinguished from benign tumours by *invasion, infiltration and destruction of the* surrounding tissue, besides distant metastasis

VI. METASTASIS (DISTANT SPREAD)

- Metastasis (*meta = transformation, stasis = residence) is* defined as spread of tumour by invasion in such a way that discontinuous secondary tumour mass/masses are formed at the site of lodgement.
- *Metastasis and invasiveness are the two most important features to distinguish malignant from benign tumours.*

Routes of Metastasis

- Cancers may spread to distant sites by following pathways:

1. Lymphatic spread
2. Haematogenous spread
3. Spread along body cavities and natural passages

1. LYMPHATIC SPREAD.

- ***In general, carcinomas metastasise*** *by lymphatic route while sarcomas favour haematogenous route.*
- However, sarcomas may also spread by lymphatic pathway.
- The involvement of lymph nodes by malignant cells may be of two forms:

i) Lymphatic permeation. The walls of lymphatics are readily invaded by cancer cells and may form a continuous growth in the lymphatic channels called lymphatic permeation.

ii) Lymphatic emboli. Alternatively, the malignant cells may detach to form tumour emboli so as to be carried along the lymph to the next draining lymph node.

- Later, of course, the whole lymph node may be replaced and enlarged by the metastatic tumour.
- Generally, regional lymph nodes draining the tumour are invariably involved producing *regional nodal metastasis e.g.*from carcinoma breast to axillary lymph nodes.
- All regional nodal enlargements are not due tonodal metastasis because necrotic products of tumour andantigens may also incite regional lymphadenitis of *sinushistiocytosis*.

2. HAEMATOGENOUS SPREAD. *Blood-borne metastasis is the common route for sarcomas but certain carcinomas also frequently metastasise by this mode, especially those of the lung,* breast, thyroid, kidney, liver, prostate and ovary.

- *Systemic veins drain blood into vena cavae from limbs,* head and neck and pelvis. Therefore, cancers of these sites more often metastasise to the lungs.
- *Portal veins drain blood from the bowel, spleen and* pancreas into the liver. Thus, tumours of these organs frequently have secondaries in the liver.
- ***Grossly, blood-borne metastases in an organ appear as*** multiple, rounded nodules of varying size, scattered throughout the organ
- ***Microscopically, the secondary deposits generally*** reproduce the structure of primary tumour

3. SPREAD ALONG BODY CAVITIES AND NATURAL PASSAGES.

i) Transcoelomic spread.

- *Carcinoma of the stomach*
- *Carcinoma of the ovary*

ii) Spread along epithelium-lined surfaces.

Malignant tumour may spread through:

- a) the fallopian tube from the endometrium to the ovaries or *vice-versa;*
- b) through the bronchus into alveoli; and
- c) through the ureters from the kidneys into lower urinary tract.

iii) Spread via cerebrospinal fluid.

- Malignant tumour of leptomeninges

iv) Implantation. Rarely, a tumour may spread by implantation by surgeon's scalpel, needles, sutures, or may be implanted by direct contact such as transfer of cancer of the lower lip to the apposing upper lip.

MECHANISM AND BIOLOGY OF INVASION AND METASTASIS

The following steps are involved at the molecular level:

1. Aggressive clonal proliferation and angiogenesis.

- The first step in the spread of cancer cells is the development of rapidly proliferating clone of cancer cells.
- Tumour angiogenesis plays a very significant role in metastasis since the new vessels formed as part of growing tumour are more vulnerable to invasion as these evolving vessels are directly in contact with cancer cells.

2. Tumour cell loosening.

- Normal cells remain glued to each other due to presence of cell adhesion molecules (CAMs) i.e.E (epithelial)-cadherin.
- In epithelial cancers, there is either loss or inactivation of E-cadherin and also other CAMs of immunoglobulin superfamily, all of which results in loosening of cancer cells.

3. Tumour cell-ECM interaction.

- Loosened cancer cells are now attached to ECM proteins, mainly *laminin and fibronectin*.
- There is also loss of *integrins, the transmembrane receptors, further* favouring invasion.

4. Degradation of ECM.

- Tumour cells overexpress *proteases* and matrix-degrading enzymes, *metalloproteinases, that* includes collagenases and gelatinase, while the inhibitors of metalloproteinases are decreased.
- Another protease, cathepsin D, is also increased in certain cancers.
- These enzymes bring about dissolution of ECM—firstly basement membrane of tumour itself, then make way for tumour cells through the interstitial matrix, and finally dissolve the basement membrane of the vessel wall.

5. Entry of tumour cells into capillary lumen.

The tumour cells after degrading the basement membrane are ready to migrate into lumen of capillaries or venules for which the following mechanisms play a role:

i) Autocrine motility factor (AMF) is a cytokine derived from tumour cells and stimulates receptor-mediated motility of tumour cells.

ii) Cleavage products of matrix components which are formed following degradation of ECM have properties of tumour cell chemotaxis, growth promotion and angiogenesis in the cancer.

- After the malignant cells have migrated through the breached basement membrane, these cells enter the lumen of lymphatic and capillary channels.

6. Thrombus formation.

- The tumour cells protruding in the lumen of the capillary are now covered with constituents of the circulating blood and form the thrombus.
- Thrombus provides nourishment to the tumour cells and also protects them from the immune attack by the circulating host cells.
- In fact, normally a large number of tumour cells are released into circulation but they are attacked by the host immune cells.
- Actually a very small proportion of malignant cells (less than 0.1%) in the blood stream survive to develop into metastasis.

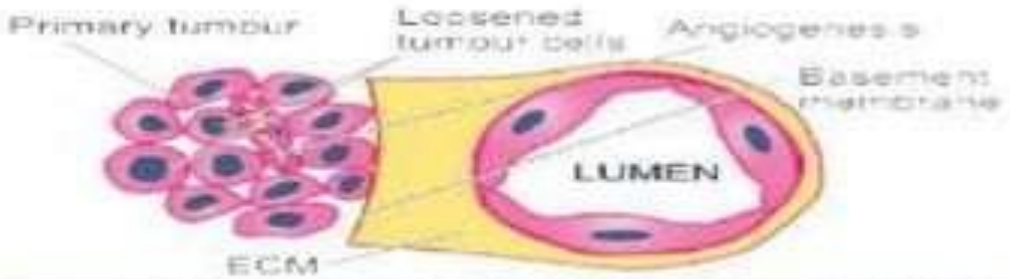
7. Extravasation of tumour cells.

- Tumour cells in the circulation (capillaries, venules, lymphatics) may mechanically block these vascular channels and attach to vascular endothelium.
- In this way, the sequence similar to local invasion is repeated and the basement membrane is exposed.

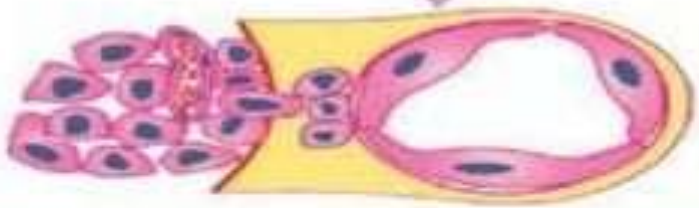
8. Survival and growth of metastatic deposit.

- The extravasated malignant cells on lodgement in the right environment grow further under the influence of growth factors produced by host tissues, tumour cells and by cleavage products of matrix components.

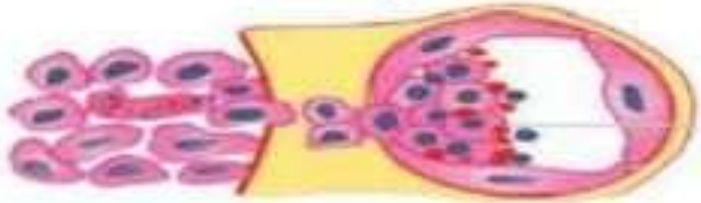
- These growth factors in particular include: PDGF, FGF, TGF-β and VEGF.
- The metastatic deposits grow further if the host immune defense mechanism fails to eliminate it.
- Metastatic deposits may further metastasise to the same organ or to other sites by forming emboli.



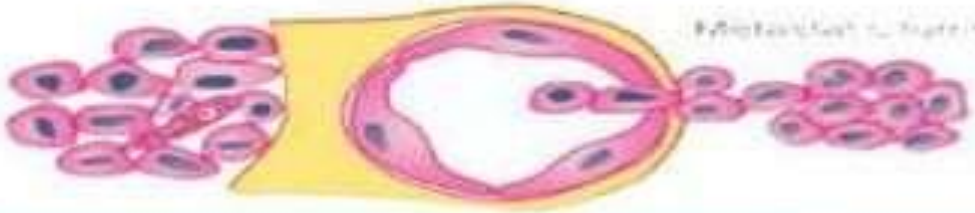
1,2 AGGRESSIVE CLONE WITH ANGIOGENESIS, LOOSENING OF TUMOUR CELLS



3,4 TUMOUR CELL-ECM INTERACTION, DEGRADATION OF ECM



5,6 ENTRY OF TUMOUR CELLS IN LUMEN, THROMBUS FORMATION



7,8 EXTRAVASATION OF TUMOUR CELLS, FORMATION OF METASTASIS

GRADING AND STAGING OF CANCER

- *Grading is defined as the gross and microscopic degree of differentiation of the tumour, while staging means extent of spread of the tumour within the patient.*

Grading

- Grading is largely based on 2 important histologic features: *the degree of anaplasia, and the rate of growth.* ***Broders' grading*** *is as under:*
- *Grade I: Well-differentiated (less than 25% anaplastic cells).*
- *Grade II: Moderately-differentiated (25-50% anaplastic cells).*
- *Grade III: Moderately-differentiated (50-75% anaplastic cells).*
- *Grade IV: Poorly-differentiated or anaplastic (more than 75%* anaplastic cells).

- **Staging**
- **TNM staging.** (T for primary *tumour, N for regional nodal* involvement, and M for distant *metastases)*
- *T0 to T4: In situ lesion to largest and most extensive* primary tumour.
- *N0 to N3: No nodal involvement to widespread lymph* node involvement.
- *M0 to M2: No metastasis to disseminated haematogenous* metastases.
- **AJC staging. American Joint Committee staging** divides all cancers into stage 0 to IV, and takes into account all the 3 components of the preceding system (primary tumour, nodal involvement and distant metastases) in each stage.

CARCINOGENESIS: ETIOLOGY AND PATHOGENESIS OF CANCER

- *Carcinogenesis or oncogenesis or tumorigenesis means* mechanism of induction of tumours (*pathogenesis of cancer);* agents which can induce tumours are called *carcinogens* (*etiology of cancer)*
- The etiology and pathogenesis of cancer is discussed under the following 4 broad headings:

A. Molecular pathogenesis of cancer (genes and cancer)

B. Chemical carcinogens and chemical carcinogenesis

C. Physical carcinogens and radiation carcinogenesis

D. Biologic carcinogens and viral oncogenesis.

A. MOLECULAR PATHOGENESIS OF CANCER (GENETIC MECHANISMS OF CANCER)

- The general concept of molecular mechanisms of cancer is:

1. Monoclonality of tumours. There is strong evidence to support that most human cancers arise from a single clone of cells by genetic transformation or mutation.

For example:

- i) In a case of multiple myeloma (a malignant disorder of plasma cells), there is production of a single type of immunoglobulin or its chain as seen by monoclonal spike in serum electrophoresis.

2. **Field theory of cancer.** In an organ developing cancer, in the backdrop of normal cells, limited number of cells only grow in to cancer after undergoing sequence of changes under the influence of etiologic agents.

- This is termed as 'field effect' and the concept called as field theory of cancer.

3. Multi-step process of cancer growth and progression.

- Carcinogenesis is a gradual multi-step process involving many generations of cells.
- The various causes may act on the cell one after another (*multi-hit process). The same process* is also involved in further progression of the tumour.

- Ultimately, the cells so formed are genetically and phenotypically transformed cells having phenotypic features of malignancy—excessive growth, invasiveness and distant metastasis.

4. Genetic theory of cancer. Cell growth of normal as well as abnormal types is under genetic control.

- In cancer, there are either genetic abnormalities in the cell, or there are normal genes with abnormal expression.
- The abnormalities in genetic composition may be from inherited or induced mutations (induced by etiologic carcinogenic agents namely: chemicals, viruses, radiation).
- The mutated cells transmit their characters to the next progeny of cells and result in cancer.

5. Genetic regulators of normal and abnormal mitosis. In normal cell growth, regulatory genes control mitosis as well as cell aging, terminating in cell death by apoptosis.

In normal cell growth, there are 4 regulatory genes:

i) Proto-oncogenes are growth-promoting genes i.e. they encode for cell proliferation pathway.

ii) Anti-oncogenes are growth-inhibiting or growth suppressor genes.

iii) Apoptosis regulatory genes control the programmed cell death.

iv) DNA repair genes are those normal genes which regulate the repair of DNA damage that has occurred during mitosis and also control the damage to proto-oncogenes and antioncogenes.

- **In cancer, the transformed cells are produced by** abnormal cell growth due to genetic damage to these normal controlling genes.

i) Activation of growth-promoting oncogenes

ii) Inactivation of cancer-suppressor genes

iii) Abnormal apoptosis regulatory genes

iv) Failure of DNA repair genes

Cancer-related Genes and Cell Growth (Hallmarks of Cancer)

1. Excessive and autonomous growth: Growth-promoting oncogenes.
2. Refractoriness to growth inhibition: Growth suppressing anti-oncogenes.
3. Escaping cell death by apoptosis: Genes regulating apoptosis and cancer.
4. Avoiding cellular aging: Telomeres and telomerase in cancer.
5. Continued perfusion of cancer: Cancer angiogenesis.
6. Invasion and distant metastasis: Cancer dissemination.
7. DNA damage and repair system: Mutator genes and cancer.
8. Cancer progression and tumour heterogeneity: Clonal aggressiveness.
9. Cancer a sequential multistep molecular phenomenon: Multistep theory.
10. MicroRNAs in cancer: OncomiRs.

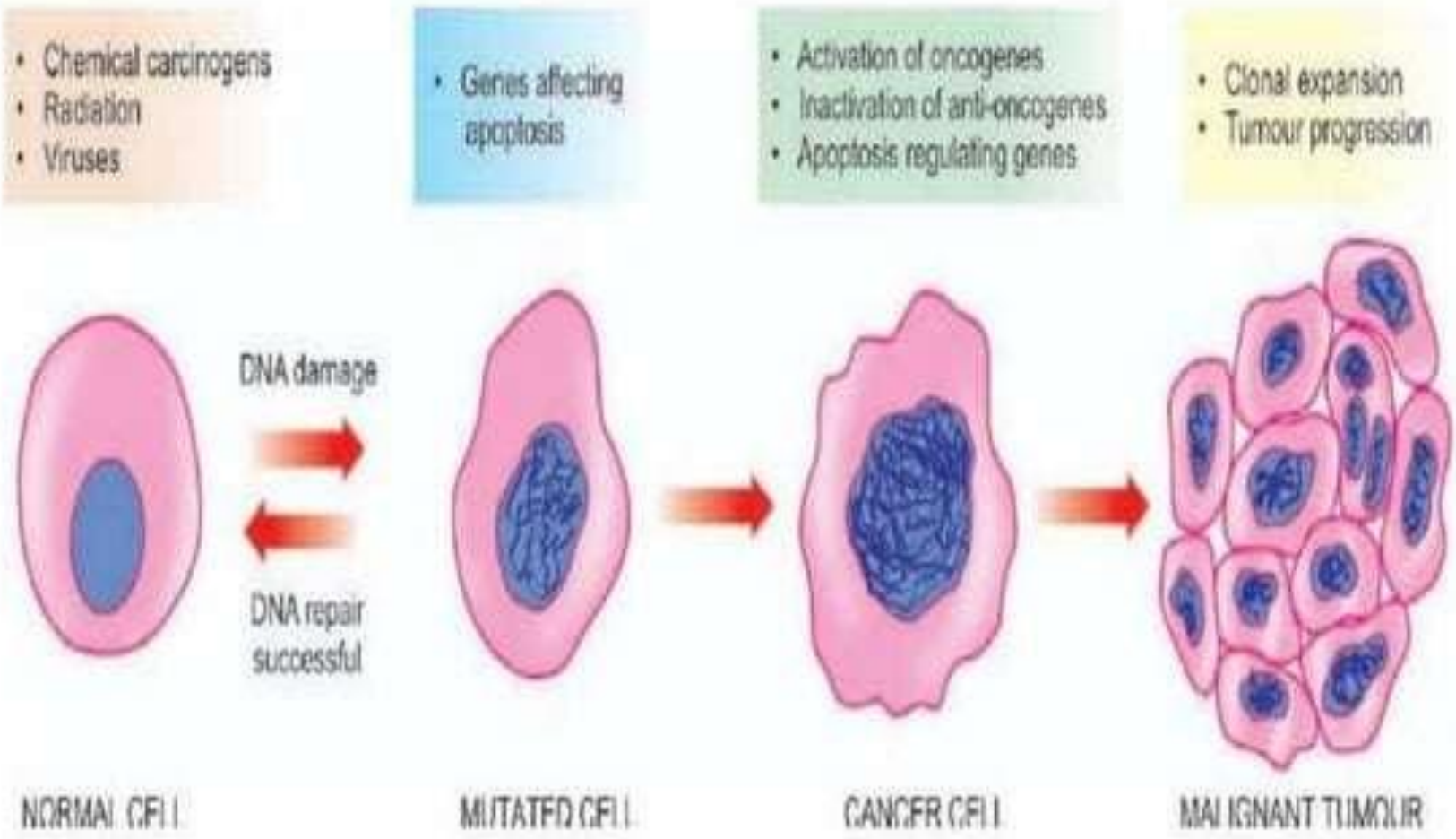


Figure 8.17 Schematic illustration to show molecular basis of cancer.

B. CHEMICAL CARCINOGENESIS

Stages in Chemical Carcinogenesis

- The induction of cancer by chemical carcinogens occurs after a delay—weeks to months in the case of experimental animals, and often several years in man.
- Other factors that influence the induction of cancer are the dose and mode of administration of carcinogenic chemical, individual susceptibility and various predisposing factors.
- The phenomena of cellular transformation by chemical carcinogens (as also other carcinogens) is a progressive process involving 3 sequential stages: **initiation, promotion and progression**

1. INITIATION OF CARCINOGENESIS

- Initiation is the first stage in carcinogenesis induced by initiator chemical carcinogens.
- The change can be produced by a single dose of the initiating agent for a short time, though larger dose for longer duration is more effective.
- The change so induced is sudden, irreversible and permanent.
- Chemical carcinogens acting as initiators of carcinogenesis can be grouped into 2 categories

I. Direct-acting carcinogens. These are a few chemical substances (e.g. alkylating agents, acylating agents) which can induce cellular transformation without undergoing any prior metabolic activation.

II. Indirect-acting carcinogens or procarcinogens. These require metabolic conversion within the body so as to become 'ultimate' carcinogens having carcinogenicity e.g. polycyclic aromatic hydrocarbons, aromatic amines, azo dyes, naturally-occurring products and others.

- In either case, the following steps are involved in transforming 'the target cell' into 'the initiated cell':
- **a) Metabolic activation.** The indirect-acting carcinogens are activated in the liver by the mono-oxygenases of the cytochrome P-450 system in the endoplasmic reticulum.

b) **Reactive electrophiles.** While direct-acting carcinogens are intrinsically electrophilic, indirect-acting substances become electron-deficient after metabolic activation i.e. they become reactive electrophiles.

- Following this step, both types of chemical carcinogens behave alike and their reactive electrophiles bind to electron-rich portions of other molecules of the cell such as DNA, RNA and other proteins.

c) **Target molecules. The primary target of electrophiles is** DNA, producing mutagenesis. The change in DNA may lead to 'the initiated cell' or some form of cellular enzymes may be able to repair the damage in DNA.

d) The initiated cell. The unrepaired damage produced in the DNA of the cell becomes permanent and fixed only if the altered cell undergoes at least one cycle of proliferation.

- This results in transferring the change to the next progeny of cells so that the DNA damage becomes *permanent and irreversible,* which are the characteristics of the initiated cell, vulnerable to the action of promoters of carcinogenesis.

2. PROMOTION OF CARCINOGENESIS

- Promotion is the next sequential stage in the chemical carcinogenesis.
- Promoters of carcinogenesis are substances such as phorbol esters, phenols, hormones, artificial sweeteners and drugs like phenobarbital.
- They differ from initiators in the following respects:

i) They do not produce sudden change.

ii) They require application or administration, as the case may be, *following initiator exposure, for sufficient time and* in sufficient dose.

iii) The change induced may be reversible.

iv) They do not damage the DNA *per se and are thus not* mutagenic but instead enhance the effect of direct-acting carcinogens or procarcinogens.

v) Tumour promoters act by further clonal proliferation and expansion of initiated (mutated) cells, and have reduced requirement of growth factor, especially after *RAS gene* mutation.

3. PROGRESSION OF CARCINOGENESIS

- Progression of cancer is the stage when mutated proliferated cell shows phenotypic features of malignancy.
- These features pertain to morphology, biochemical composition and molecular features of malignancy.
- Such phenotypic features appear only when the initiated cell starts to proliferate rapidly

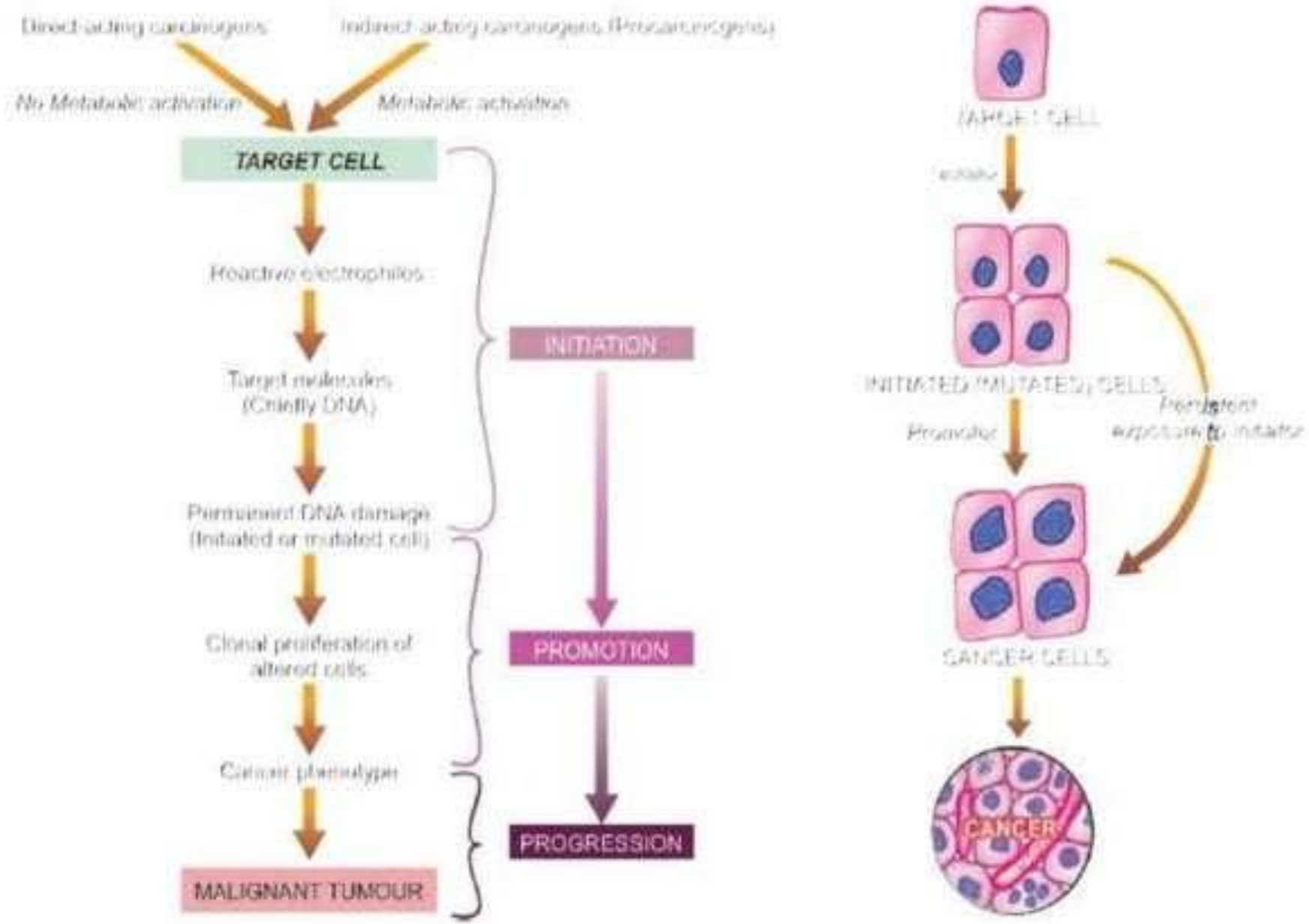


Figure 8.22 Sequential stages in chemical carcinogenesis (*left*) in evolution of cancer (*right*).

TABLE 8.7: Important Chemical Carcinogens.

Carcinogen	Tumour
I. DIRECT-ACTING CARCINOGENS	
a) *Alkylating agents*	
• Anti-cancer drugs (e.g. cyclophosphamide, chlorambucil, busulfan, melphalan, nitrosourea etc)	• Lymphomas
• β-propiolactone	• AML
• Epoxides	
b) *Acylating agents*	• Bladder cancer
• Acetyl imidazole	
• Dimethyl carbamyl chloride	
II. INDIRECT-ACTING CARCINOGENS (PROCARCINOGENS)	
a) *Polycyclic, aromatic hydrocarbons (in tobacco, smoke, fossil fuel, soot, tar, minerals oil, smoked animal foods, industrial and atmospheric pollutants)*	• Lung cancer
• Anthracenes (benza-, dibenza-, dimethyl benza-)	• Skin cancer
• Benzapyrene	• Cancer of upper aerodigestive tract
• Methylcholanthrene	
b) *Aromatic amines and azo-dyes*	
• β-naphthylamine	• Bladder cancer
• Benzidine	
• Azo-dyes (e.g. butter yellow, scarlet red etc)	• Hepatocellular carcinoma

TABLE 8.7: Important Chemical Carcinogens.

Carcinogen	Tumour
c) *Naturally-occurring products*	
• Aflatoxin Bl	• Hepatocellular carcinoma
• Actinomycin D	
• Mitomycin C	
• Safrole	
• Betel nuts	
d) *Miscellaneous*	
• Nitrosamines and nitrosamides	• Gastric carcinoma
• Vinyl chloride monomer	• Angiosarcoma of liver
• Asbestos	• Bronchogenic carcinoma, mesothelioma
• Arsenical compounds	• Cancer, skin, lung
• Metals (e.g. nickel, lead, cobalt, chromium etc)	• Lung cancer
• Insecticides, fungicides (e.g. aldrin, dieldrin, chlordane etc)	• Cancer in experimental animals
• Saccharin and cyclomates	

TABLE 8.8: Contrasting Features of Initiator and Promoter Carcinogens.

Feature	Initiator Carcinogens	Promoter Carcinogens
1. *Mechanism*	Induction of mutation	Not mutagenic
2. *Dose*	Single for a short time	Repeated dose exposure, for a long time
3. *Response*	Sudden response	Slow response
4. *Change*	Permanent, irreversible	Change may be reversible
5. *Sequence*	Applied first, then followed by promoter	Applied after prior exposure to initiator
6. *Effectivity*	Effective alone if exposed in large dose	Not effective alone
7. *Molecular changes*	Most common mutation of *RAS* oncogene, *p53* anti-oncogene	Clonal expansion of mutated cells
8. *Examples*	Most chemical carcinogens, radiation	Hormones, phorbol esters

C. PHYSICAL CARCINOGENESIS

- Physical agents in carcinogenesis are divided into 2 groups:

1. *Radiation, both ultraviolet light and ionising radiation, is* the most important physical agent.
2. *Non-radiation physical agents are the various forms of* injury and are less important.

1. Radiation Carcinogenesis

- Ultraviolet (UV) light and ionising radiation are the two main forms of radiation carcinogens which can induce cancer in experimental animals and in humans.

- A property common between the two forms of radiation carcinogens is the appearance of mutations followed by a long period of latency after initial exposure, often 10-20 years or even later.
- Also, radiation carcinogens may act to enhance the effect of another carcinogen (co-carcinogens) and, like chemical carcinogens, may have sequential stages of initiation, promotion and progression in their evolution.

i) ULTRAVIOLET LIGHT.

- The main source of UV radiation is the sunlight.
- The efficiency of UV light as carcinogen depends upon the extent of light absorbing protective melanin pigmentation of the skin.
- In humans, excessive exposure to UV rays can cause various forms of skin cancers—squamous cell carcinoma, basal cell carcinoma and malignant melanoma.
- Besides, like with other carcinogens, UV radiation also induces mutated forms of oncogenes (in particular *RAS gene)* and anti-oncogenes (*p53 gene).*

Mechanism

- UV radiation may have various effects on the cells.
- The most important is **induction of mutation**; others are **inhibition of cell division, inactivation of enzymes and sometimes causing cell death.**
- The most important biochemical effect of UV radiation is the formation of **pyrimidine dimers in DNA.**
- Such UV-induced DNA damage in normal individuals is repaired, while in the predisposed persons who are excessively exposed to sunlight such damage remain unrepaired.

- The proof in favour of mutagenic effect of UV radiation comes from following recessive hereditary diseases characterised by a defect in DNA repair mechanism and associated with high incidence of cancers:

a) Xeroderma pigmentosum is predisposed to skin cancers at younger age (under 20 years of age).

b) Ataxia telangiectasia is predisposed to leukaemia.

c) Bloom's syndrome is predisposed to all types of cancers.

d) Fanconi's anaemia with increased risk to develop cancer.

ii) IONISING RADIATION.

- Ionising radiation of all kinds like X-rays, α-, β- and γ-rays, radioactive isotopes, protons and neutrons can cause cancer in animals and in man.
- Most frequently, radiation-induced cancers are all forms of leukaemias; others are cancers of the thyroid, skin, breast, ovary, uterus, lung, myeloma, and salivary glands.
- The risk is increased by higher dose and with high LET (linear energy transfer) such as in neutrons and α-rays than with low LET as in X-rays and γ- rays.

- The evidence in support of carcinogenic role of ionising radiation is cited in the following examples:

a) Higher incidence of radiation dermatitis and subsequent malignant tumours of the skin was noted in X-ray workers and radiotherapists

b) Miners in radioactive elements have higher incidence of cancers.

Mechanism. Radiation damages the DNA of the cell by one of the 2 possible mechanisms:

a) It may directly alter the cellular DNA.

b) It may dislodge ions from water and other molecules of the cell and result in formation of highly reactive free radicals that may bring about the damage.

- Damage to the DNA resulting in mutagenesis is the most important action of ionising radiation.
- It may cause chromosomal breakage, translocation, or point mutation.
- The effect depends upon a number of factors such as type of radiation, dose, dose-rate, frequency and various host factors such as age, individual susceptibility, immune competence, hormonal influences and type of cells irradiated.

2. Non-radiation Physical Carcinogenesis

- Mechanical injury to the tissues such as from stones in the gallbladder, stones in the urinary tract, and healed scars following burns or trauma, has been suggested as the cause of increased risk of carcinoma in these tissues.
- Other examples of physical agents in carcinogenesis are the implants of inert materials such as plastic, glass etc in prostheses or otherwise, and foreign bodies observed to cause tumour development in experimental animals.
- However, tumorigenesis by these materials in humans is rare.

D. BIOLOGIC CARCINOGENESIS

- The epidemiological studies on different types of cancers indicate the involvement of transmissible biologic agents in their development, chiefly *viruses, hence,* biologic carcinogenesis is largely ***viral carcinogenesis***.
- Other biologic agents implicated in carcinogenesis are
- **Parasites :** *Schistosoma haematobium* (squamous cell carcinoma of the urinary bladder)
- **Fungus :** *Aspergillus flavus* (hepatocellular carcinoma)
- **Bacteria :** *Helicobacter pylori* (gastric lymphoma and gastric carcinoma)

VIRAL CARCINOGENESIS

i) *Horizontal transmission*. Commonly, viral infection passes from one to another by direct contact, by ingestion of contaminated water or food, or by inhalation as occurs in most contagious diseases.

ii) *By parenteral route such by inoculation as happens in some* viruses by inter-human spread and from animals and insects to humans.

iii) *Vertical transmission, when the infection is genetically* transmitted from infected parents to offsprings.

- Based on their nucleic acid content, oncogenic viruses fall into 2 broad groups:

1. Those containing deoxyribonucleic acid are called *DNA oncogenic viruses.*
2. Those containing ribonucleic acid are termed *RNA oncogenic viruse or retroviruses.*

- Both types of oncogenic viruses usually have 3 genes and are abbreviated according to the coding pattern by each gene:

i) *gag gene: codes for group antigen.*

ii) *pol gene: codes for polymerase enzyme.*

iii) *env gene: codes fro envelope protein.*

- General mode of oncogenesis by each group of DNA and RNA oncogenic viruses is briefly considered below:

1. **Mode of DNA viral oncogenesis.** Host cells infected by DNA oncogenic viruses may have one of the following 2 results

i) Replication. The virus may replicate in the host cell with consequent lysis of the infected cell and release of virions.

ii) Integration. The viral DNA may integrate into the host cell DNA.

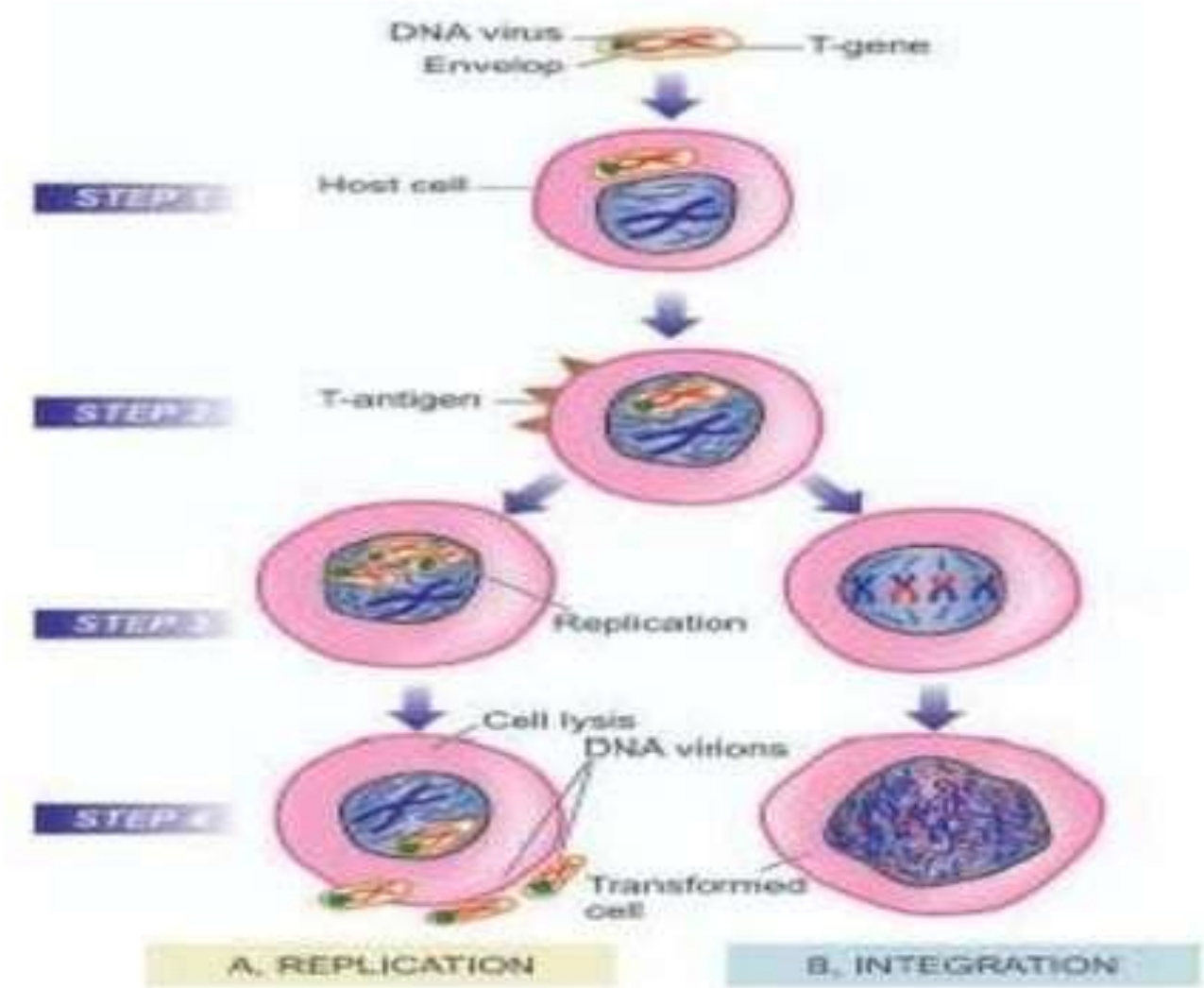


Figure 8.25 Replication and integration of DNA virus in the host cell.
A. Replication: *Step 1*. The DNA virus invades the host cell. *Step 2*. Viral DNA is incorporated into the host nucleus and T-antigen is expressed immediately after infection. *Step 3*. Replication of viral DNA occurs and other components of virion are formed. The new virions are assembled in the cell nucleus. *Step 4*. The new virions are released, accompanied by host cell lysis. B. Integration : *Steps 1 and 2* are similar as in replication. *Step 3*. Integration of viral genome into the host cell genome occurs which requires essential presence of functional T-antigen. *Step 4*. A 'transformed (neoplastic) cell' is formed.

TABLE 8.5: DNA Oncogenic Viruses.

Virus	Host	Associated Tumour
1. PAPOVAVIRUSES		
Human papilloma virus	**Humans**	Cervical cancer and its precursor lesions, squamous cell carcinoma at other sites Skin cancer in epidermodysplasia verruciformis Papillomas (warts) on skin, larynx, genitals (genital warts)
Papilloma viruses	Cotton-tail rabbits	Papillomas (warts)
	Bovine	Alimentary tract cancer
Polyoma virus	Mice	Various carcinomas, sarcomas
SV-40 virus	Monkeys	Harmless
	Hamsters	Sarcoma
	Humans	? Mesothelioma
2. HERPESVIRUSES		
Epstein-Barr virus	**Humans**	Burkitt's lymphoma Nasopharyngeal carcinoma
Human herpesvirus 8 (Kaposi's sarcoma herpesvirus)	**Humans**	Kaposi's sarcoma Pleural effusion lymphoma
Lucke' frog virus	Frog	Renal cell carcinoma
Marek's disease virus	Chickens	T-cell leukaemia-lymphoma
3. ADENOVIRUSES	Hamsters	Sarcomas
4. POXVIRUSES	Rabbits	Myxomatosis
	Humans	Molluscum contagiosum, papilloma
5. HEPADNAVIRUSES		
Hepatitis B virus	**Humans**	Hepatocellular carcinoma

RNA VIRAL ONCOGENESIS

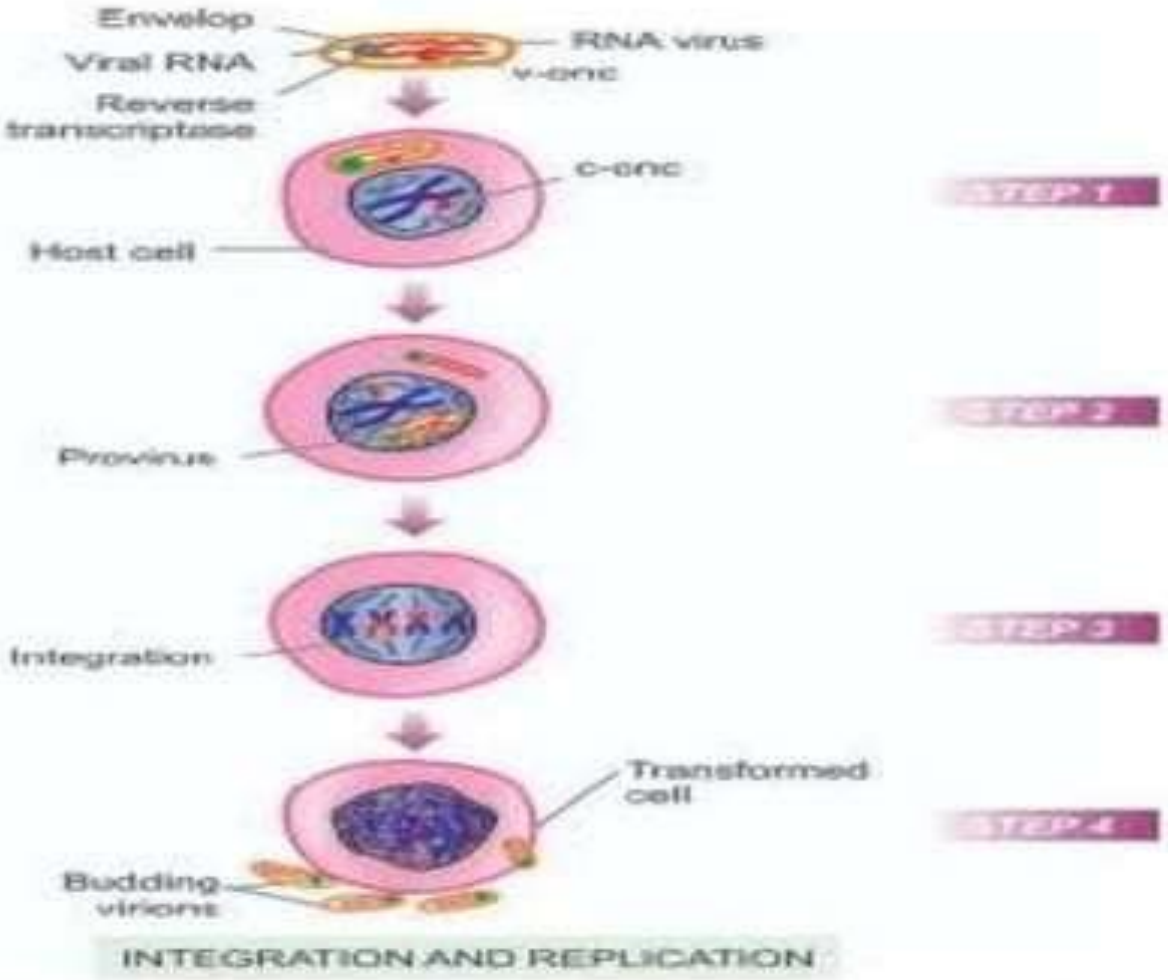


Figure 8.26 ◆ Integration and replication of RNA virus (retrovirus) in the host cell.
Step 1. The RNA virus invades the host cell. The viral envelope fuses with the plasma membrane of the host cell; viral RNA genome as well as reverse transcriptase are released into the cytosol. *Step 2.* Reverse transcriptase acts as template to synthesise single strand of matching viral DNA which is then copied to form complementary DNA resulting in double-stranded viral DNA (provirus). *Step 3.* The provirus is integrated into the host cell genome producing "transformed host cell." *Step 4.* Integration of the provirus brings about replication of viral components which are then assembled and released by budding.

TABLE 8.10: RNA Oncogenic Viruses.

Virus	Host	Associated Tumour
1. ACUTE TRANSFORMING VIRUSES		
Rous sarcoma virus	Chickens	Sarcoma
Leukaemia-sarcoma virus	Avian, feline, bovine, primate	Leukaemias, sarcomas
2. SLOW TRANSFORMING VIRUSES		
	Mice, cats, bovine	Leukaemias, lymphomas
Mouse mammary tumour virus (Bittner milk factor)	Daughter mice	Breast cancer
3. HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV)		
HTLV-I	Human	Adult T-cell leukaemia lymphoma (ATLL)
HTLV-II	Human	T-cell variant of hairy cell leukaemia
4. HEPATITIS C VIRUS		
HCV	Human	Hepatocellular carcinoma

DIAGNOSIS OF CANCER

1. Histological Methods

- These methods are based on microscopic examination of properly fixed tissue (excised tumour mass or open/needle biopsy from the mass)
- The tissue must be fixed in 10% formalin for light microscopic examination and in glutaraldehyde for electron microscopic studies, while quick frozen section and hormonal analysis are carried out on fresh unfixed tissues.
- The histological diagnosis by either of these methods is made on the basis that morphological features of *benign tumours resemble those of normal tissue and that they are* unable to invade and metastasise, while *malignant tumours* are identified by lack of differentiation in cancer cells termed 'anaplasia' or 'cellular atypia' and may invade as well as metastasise.

2. Histochemistry and Cytochemistry

- Histochemistry and cytochemistry are additional diagnostic tools which help the pathologist in identifying the chemical composition of cells, their constituents and their products by special staining methods.

3. Immunohistochemistry

- This is an immunological method of recognising a cell by one or more of its specific components in the cell membrane, cytoplasm or nucleus.
- These cell components (called antigens) combine with specific antibodies on the formalin-fixed paraffin sections or cytological smears.
- The complex of antigen-antibody on slide is made visible for light microscopic identification by either fluorescent dyes ('fluorochromes') or by enzyme system ('chromogens').

TABLE 8.12: Common Histochemical/Cytochemical Stains in Tumour Diagnosis.

	Substance	Stain
1.	*Basement membrane/ collagen*	• Periodic acid-Schiff (PAS) • Reticulin • Van Gieson • Masson's trichrome
2.	*Glycogen*	• PAS with diastase loss
3.	*Glycoproteins, glycolipids,* glycomucins *(epithelial origin)*	• PAS with diastase persistence
4.	*Acid mucin (mesenchymal origin)*	• Alcian blue
5.	*Mucin (in general)*	• Combined Alcian blue-PAS
6.	*Argyrophilic/ argentaffin granules*	• Silver stains
7.	*Cross striations*	• PTAH stain
8.	*Enzymes*	• Myeloperoxidase • Acid phosphatase • Alkaline phosphatase
9.	*Nucleolar organiser regions (NORs)*	• Colloidal silver stain