General Pathology
Chapter 4
Neoplasia

Dr. Al-Saghbini M. S.

MD. PhD. Consultant Pathologist, Assistant Prof.

Al-Balqa'a University

College of Medicine; Department of Pathology

Reference: **ROBBINS BASIC PATHOLOGY,** By Kumar et al. 8th and 10th Ed © 2010/2018: Hand out.

Cancer:

cancer is not one disease but rather many disorders that share a profound growth dysregulation.

Some cancers, are highly curable, whereas others, such as cancer of the pancreas, are virtually always fatal.

The only hope for controlling cancer lies in learning more about its pathogenesis, and great strides have been made in understanding the molecular basis of cancer.

Summary of the fundamental and shared characteristics of cancers:

- **Cancer is a genetic disorder caused by DNA mutations.**
- **Genetic alterations in cancer cells are heritable, being passed to daughter cells upon cell division. As a result, cells harboring these alterations are subject to Darwinian selection (survival of the fittest, arguably the most important scientific concept in biology).**
- **Mutations and epigenetic alterations impart to cancer cells a set of properties that are referred to collectively as *cancer hallmarks.***

NOMENCLATURE

- *Neoplasia* literally means “new growth.”
- All neoplasms depend on the host for their nutrition and blood supply. Neoplasms derived from hormone responsive tissues often also require endocrine support, and such dependencies sometimes can be exploited therapeutically.
- In common medical usage, a neoplasm often is referred to as a *tumor,* and the study of tumors is called *oncology* (from *oncos,* “tumor,” and *logos,* “study of”).

- **A tumor is said to be *benign* when its microscopic and gross characteristics are considered to be relatively innocent, implying that it will remain localized and is amenable to local surgical removal.** Affected patients generally survive.
- ***Malignant*, as applied to a neoplasm, implies that the lesion can invade and destroy adjacent structures and spread to distant sites (metastasize) to cause death.** Malignant tumors are collectively referred to as *cancers.*

- **All tumors, benign and malignant, have two basic components:**

(1) the parenchyma, made up of transformed or neoplastic cells, and

(2) the supporting, host-derived, non-neoplastic *stroma,* made up of connective tissue, blood vessels, and host-derived inflammatory cells.

- **The parenchyma** of the neoplasm largely determines its biologic

behaviour, and it is this component from which the tumor derives its name. The stroma is crucial to the growth of the neoplasm.

Benign Tumors

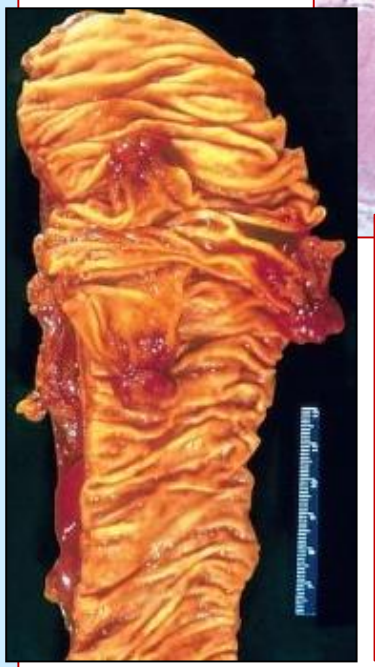
- In general, benign tumors are designated by attaching the suffix *-oma* to the cell type from which the tumor arises. For example, a benign tumor arising in:

Fibrous tissue is a *fibroma;* cartilaginous tumor is a *chondroma.*

Benign epithelial tumors: The term *adenoma* is generally applied

to benign epithelial neoplasms that produce gland-like structures, and also to benign epithelial neoplasms that are derived from glands but lack a glandular growth pattern.

- ***Papillomas*** are benign epithelial neoplasms, growing on any surface, that produce microscopic or macroscopic finger-like fronds.
- **Although this** term commonly is used for benign tumors, some malignant tumors also may grow as polyps, whereas other polyps (such as nasal polyps) are not neoplastic but inflammatory in origin.
- ***Cystadenomas*** are cystic masses that typically arise in the ovary.
- **A *polyp*** is a mass that projects above a mucosal surface, as in the gut, to form a macroscopically visible structure (Fig. 6.1).



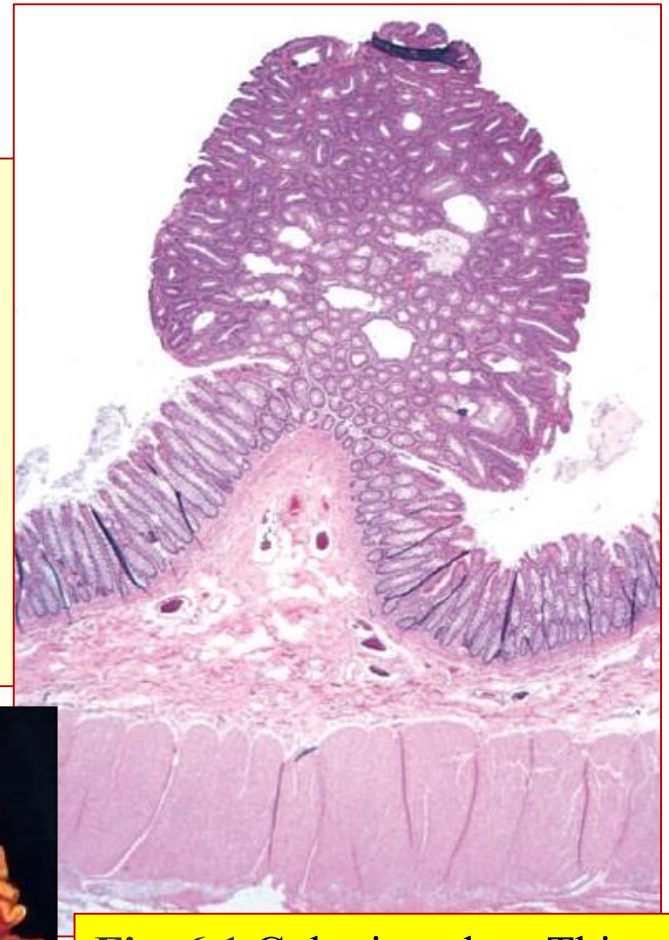


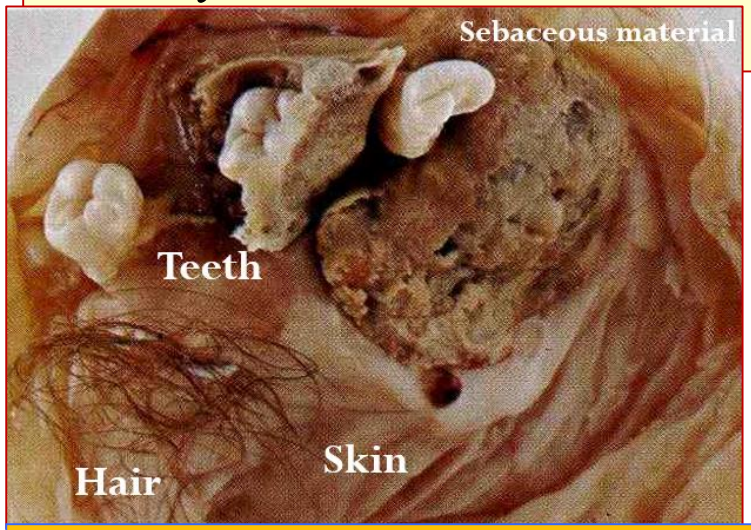
Fig. 6.1 Colonic polyp. This glandular tumor is seen projecting into the colonic lumen. The polyp is attached to the mucosa by a distinct stalk.

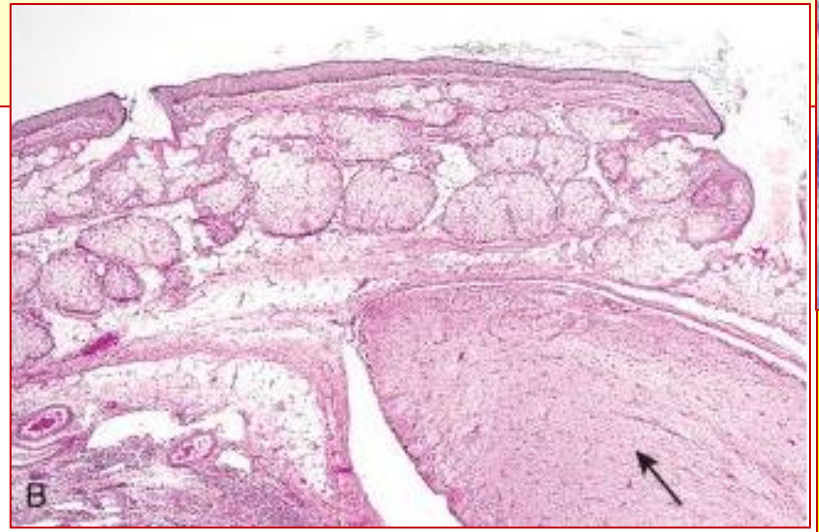
Malignant Tumors

- Malignant neoplasms arising in **"solid" mesenchymal tissues** or its derivatives are called ***sarcomas*** (Liposarcoma, Chondrosarcoma), whereas those arising from the **mesenchymal cells of the blood** are called ***leukemias or lymphomas***.
- Malignant neoplasms of ***epithelial cells*** are called ***carcinomas*** regardless of the tissue of origin. Thus, malignant neoplasms arising in the renal tubular epithelium (mesoderm), the skin (ectoderm), and lining epithelium of the gut (endoderm) are all considered ***carcinomas***. Furthermore, mesoderm may give rise to carcinomas (epithelial), sarcomas (mesenchymal), and hematolymphoid tumors (leukemias and lymphomas).
- ***Carcinomas*** are subdivided further. (**glandular** pattern -*adenocarcinomas,* and those that produce squamous cells are called *squamous cell carcinomas.)*
- Tumors with **little** or **no differentiation** are referred to as ***poorly differen****tiated* or ***undifferentiated*** *carcinoma.*

- In some unusual instances, however, the tumor cells undergo ***divergent differentiation***, creating so-called **"mixed tumors".** Mixed tumors are still of monoclonal origin, but the progenitor cell in such tumors has the capacity to differentiate down more than one lineage (mixed tumor of salivary gland, Fig. 6.2-the preferred designation for these neoplasms is *pleomorphic adenoma*).

- ***Teratoma*** is a special type of mixed tumor that contains recognizable mature or immature cells or tissues derived from more than one germ cell layer, and sometimes all three. Teratomas originate from totipotential germ cells such as those that normally reside in the ovary and testis and that are sometimes abnormally present in midline embryonic rests.





Dermoid cyst : ovary. A gross and microscopic view of a similar tumor shows skin, sebaceous glands, fat cells, and a tract of neural tissue (arrow).

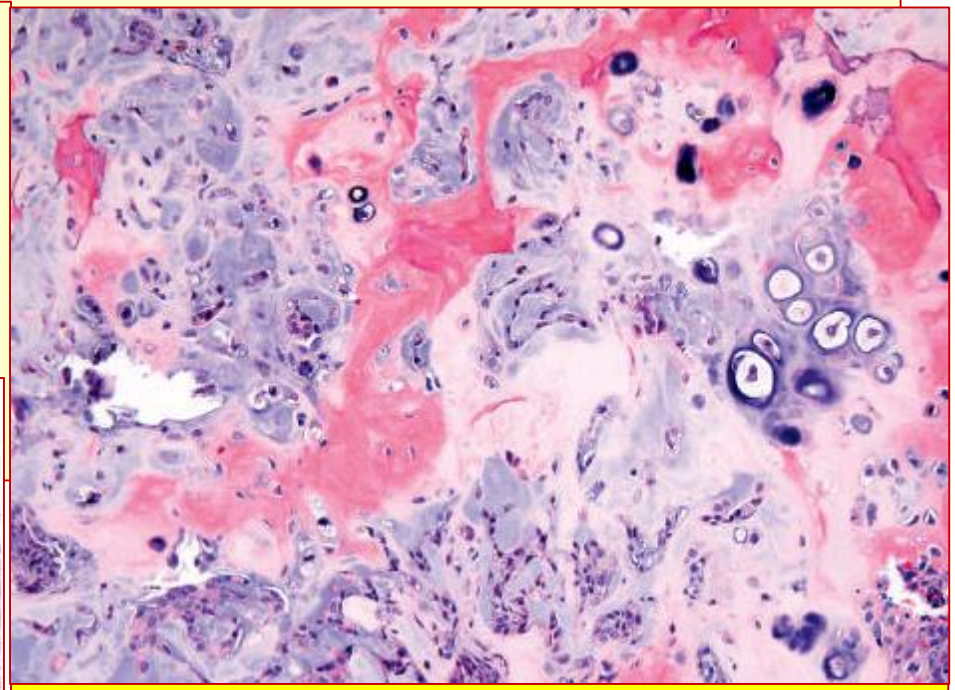


Fig. 6.2 Mixed tumor of the parotid gland. Small nests of epithelial cells and myxoid stroma forming cartilage and bone (an unusual feature) are present in this field.

(Courtesy of Dr. Vicky Jo, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)

Nomenclature of Tumors

- The terms ***lymphoma***, ***mesothelioma***, ***melanoma***, and ***seminoma*** are used for ***malignant neoplasms***.
- ***Hamartoma*** is a mass of disorganized tissue indigenous to the particular site, such as the lung or the liver.
- ***Choristoma*** is a congenital anomaly consisting of a heterotopic nest of cells.

Tissue of Origin	Benign	Malignant
One Parenchymal Cell Type		
Connective tissue and derivatives	Fibroma Lipoma Chondroma Osteoma	Fibrosarcoma Liposarcoma Chondrosarcoma Osteogenic sarcoma
Endothelium and related cell types		
Blood vessels	Hemangioma	Angiosarcoma
Lymph vessels	Lymphangioma	Lymphangiosarcoma
Mesothelium		Mesothelioma
Brain coverings	Meningioma	Invasive meningioma
Blood cells and related cell types		
Hematopoietic cells		Leukemias
Lymphoid tissue		Lymphomas
Muscle		
Smooth	Leiomyoma	Leiomyosarcoma
Striated	Rhabdomyoma	Rhabdomyosarcoma
Skin		
Stratified squamous	Squamous cell papilloma	Squamous cell or epidermoid carcinoma
Basal cells of skin or adnexa		Basal cell carcinoma
Tumors of melanocytes	Nevus	Malignant melanoma
Epithelial lining of glands or ducts	Adenoma Papilloma Cystadenoma	Adenocarcinoma Papillary carcinomas Cystadenocarcinoma
Lung	Bronchial adenoma	Bronchogenic carcinoma
Kidney	Renal tubular adenoma	Renal cell carcinoma
Liver	Liver cell adenoma	Hepatocellular carcinoma
Bladder	Urothelial papilloma	Urothelial carcinoma
Placenta	Hydatidiform mole	Choriocarcinoma
Testicle		Seminoma Embryonal carcinoma
More Than One Neoplastic Cell Type—Mixed Tumors, Usually Derived From One Germ Cell Layer		
Salivary glands	Pleomorphic adenoma (mixed tumor of salivary gland)	Malignant mixed tumor of salivary gland
Renal anlage		Wilms tumor
More Than One Neoplastic Cell Type Derived From More Than One Germ Cell Layer—Teratogenous		
Totipotential cells in gonads or in embryonic rests	Mature teratoma, dermoid cyst	Immature teratoma, teratocarcinoma

Characteristics of Benign and Malignant Neoplasms

There are three fundamental features by which most benign and malignant tumors can be distinguished: **differentiation and anaplasia, local invasion, and metastasis.**

Differentiation and Anaplasia: Differentiation refers to the extent to which neoplasms resemble their parenchymal cells of origin, both morphologically and functionally; lack of differentiation is called *anaplasia*.

- In well-differentiated benign tumors, mitoses are usually rare and are of normal configuration.
- By contrast, while malignant neoplasms exhibit a wide range of parenchymal cell differentiation, most exhibit morphologic alterations that betray their malignant nature.
- In well-differentiated cancers, these features may be quite subtle (Fig. 6.3).

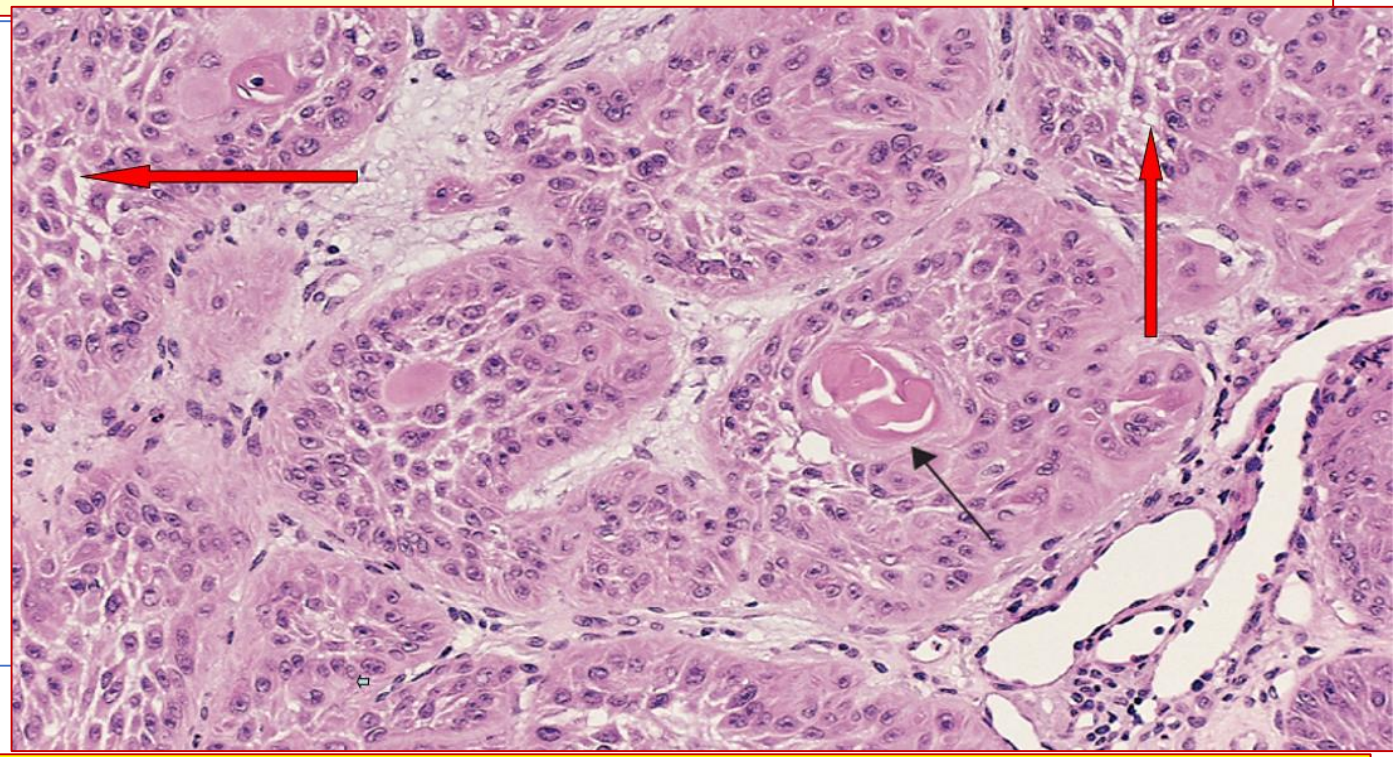


Fig. 6.3 Well-differentiated squamous cell carcinoma of the skin. The tumor cells are strikingly similar to normal squamous epithelial cells, with intercellular bridges (red arrows) and nests of keratin pearls *(black arrow)*. *(Courtesy of Dr. Trace Worrell, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)*

- For example, well-differentiated adenocarcinoma of the thyroid gland may contain normal-appearing follicles, its malignant potential being only revealed by invasion into adjacent tissues or metastasis.
- Certain cancers induce a dense, abundant fibrous stroma (desmoplasia), making them hard, so-called "scirrhous tumors".
- **Tumors composed of undifferentiated cells are said to be *anaplastic*, a feature that is a reliable indicator of malignancy.** The term *anaplasia* literally means "backward formation"—implying dedifferentiation, or loss of the structural and functional differentiation of normal cells.

- Anaplastic cells often display the following morphologic features:

Pleomorphism (i.e., variation in size and shape) (Fig. 6.4)

Nuclear abnormalities, consisting of extreme hyperchromatism (dark-staining), variation in nuclear size and shape, or unusually prominent single or multiple nucleoli. Enlargement of nuclei may result in an increased nuclear-to-cytoplasmic ratio that approaches 1 : 1 instead of the normal 1 : 4 or 1 : 6. Nucleoli may attain astounding sizes, sometimes approaching the diameter of normal lymphocytes.

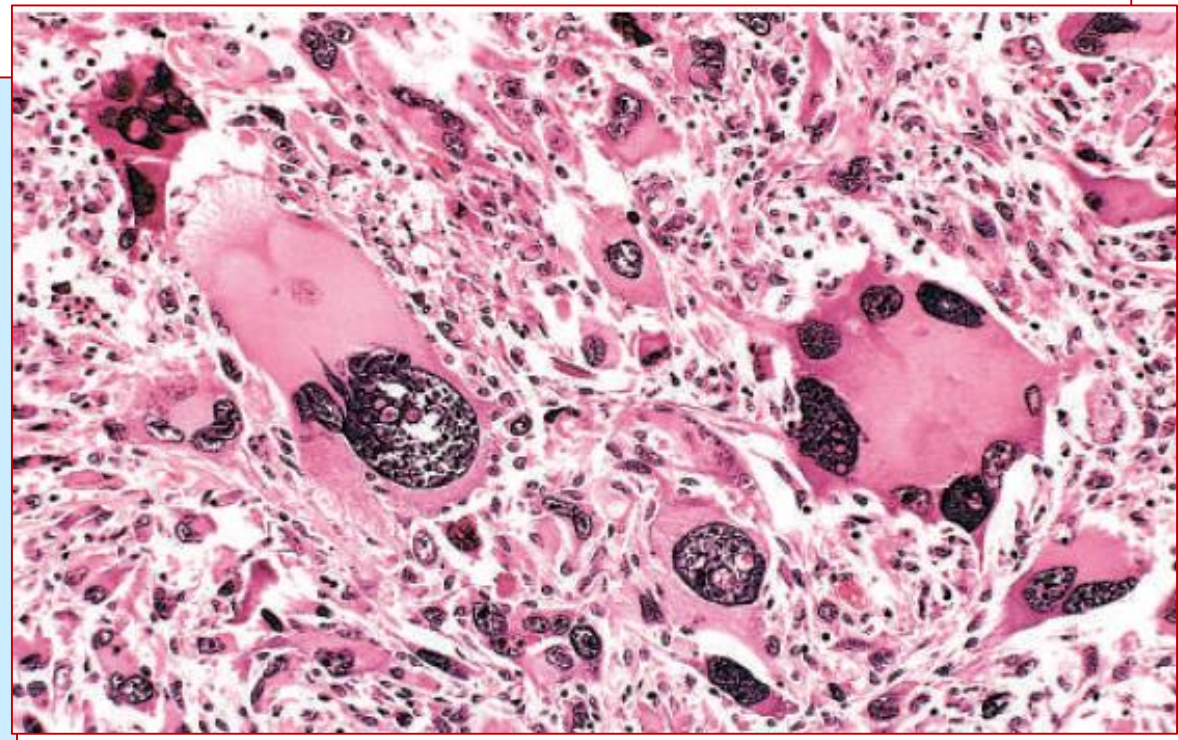


Fig. 6.4 Pleomorphic malignant tumor (rhabdomyosarcoma). Note the marked variation in cell and nuclear sizes, the hyperchromatic nuclei, and the presence of tumor giant cells. *(Courtesy of Dr. Trace Worrell, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)*

- *Tumor giant cells* may be formed.
- ***Atypical mitoses***, which may be numerous. Anarchic multiple spindles may produce tripolar or quadripolar mitotic figures (Fig. 6.5).
- *Loss of polarity,* such that anaplastic cells lack recognizable patterns of orientation to one another.

- Well-differentiated tumor cells are likely to retain the functional capabilities of their normal counterparts, whereas anaplastic tumor cells are much less likely to have specialized functional activities.

For example, benign neoplasms and even well-differentiated cancers of endocrine glands frequently elaborate the hormones characteristic of their cell of origin. Similarly, well-differentiated squamous cell carcinomas produce keratin (see Fig. 6.3), just as well-differentiated hepatocellular carcinomas secrete bile.

Some cancers may express fetal proteins not produced by comparable cells in the adult. Cancers of nonendocrine origin may produce so-called "ectopic hormones." {e.g. certain lung carcinomas may produce adrenocorticotropic hormone (ACTH)}

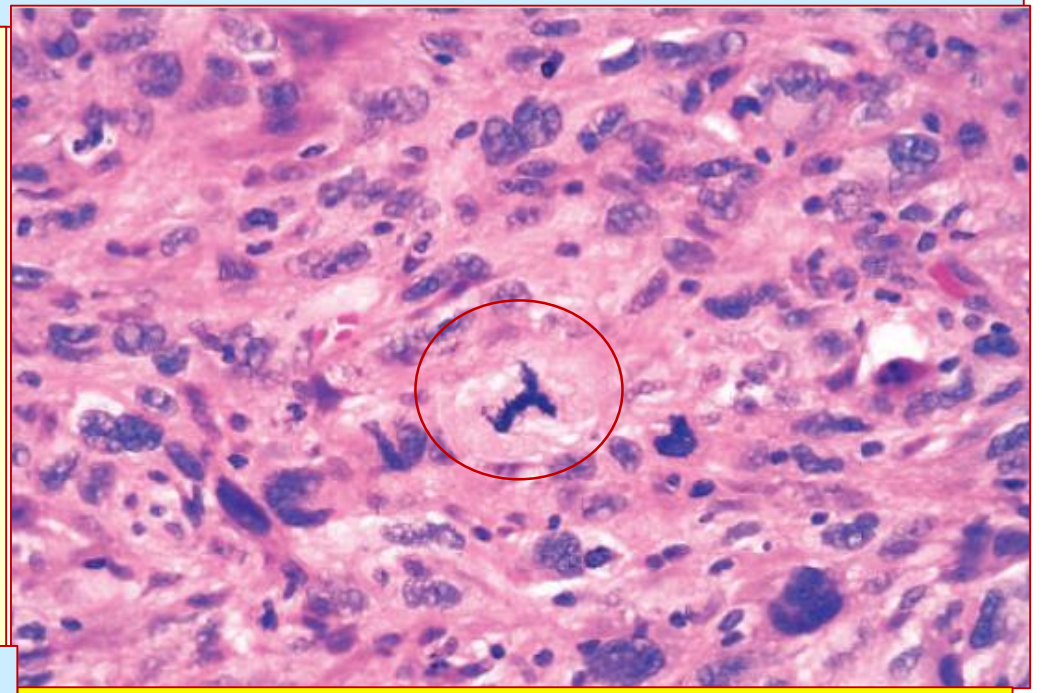


Fig. 6.5 High-power detailed view of anaplastic tumor cells shows cellular and nuclear variation in size and shape. The prominent cell in the center field has an abnormal tripolar spindle.

Also of relevance in the discussion of differentiation and anaplasia is *dysplasia,* referring to disorderly proliferation.

Dysplastic epithelium is recognized by a loss in the uniformity of individual cells and in their architectural orientation.

Dysplastic cells exhibit considerable pleomorphism and often possess abnormally large, hyperchromatic nuclei.

Mitotic figures are more abundant than usual and frequently appear in abnormal locations within the epithelium.

When dysplastic changes are severe and involve the entire thickness of the epithelium, the lesion is referred to as *carcinoma in situ,* a preinvasive stage of cancer (Fig. 6.6).

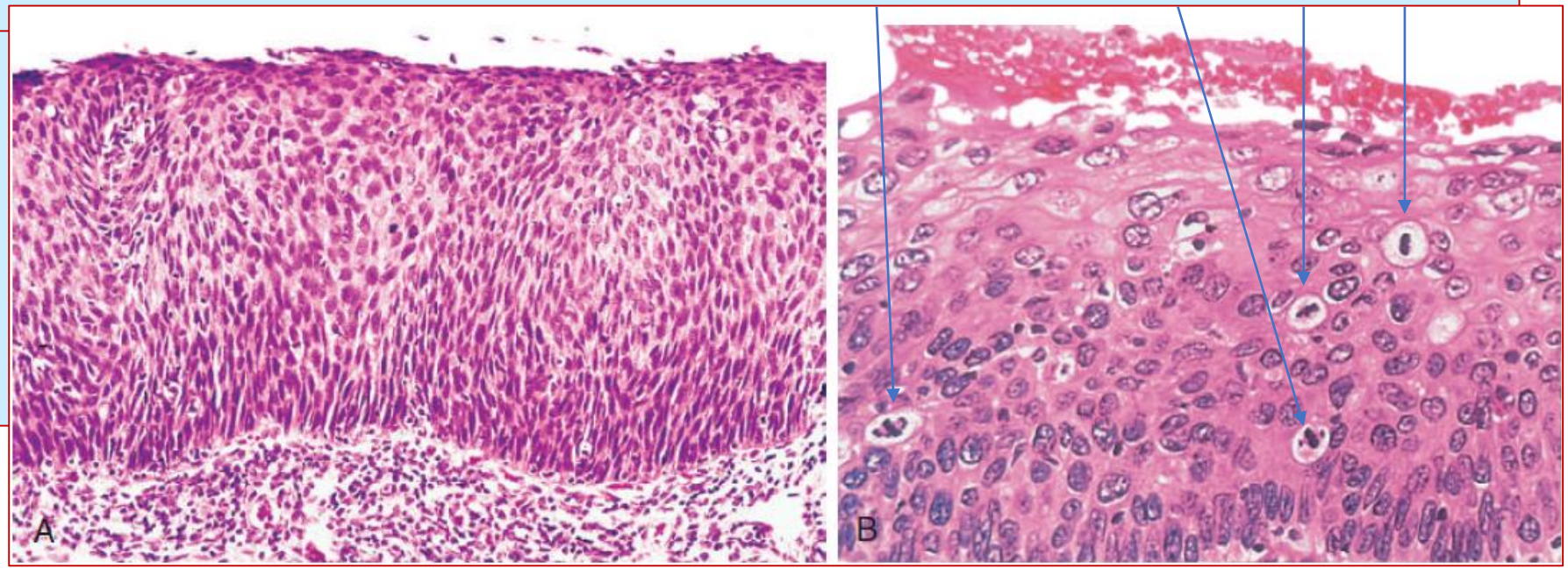


Fig. 6.6 Carcinoma in situ. (A) Low-power view shows that the entire thickness of the epithelium is replaced by atypical dysplastic cells. There is no orderly differentiation of squamous cells. The basement membrane is intact, and there is no tumor in the subepithelial stroma. (B) High-power view of another region shows failure of normal differentiation, marked nuclear and cellular pleomorphism, and numerous mitotic figures extending toward the surface. The intact basement membrane (below) is not seen in this section.

Local Invasion

The growth of cancers is accompanied by progressive infiltration, invasion, and destruction of surrounding tissues, whereas most benign tumors grow as cohesive expansile masses that remain localized to their sites of origin.

Because benign tumors grow and expand slowly, they usually develop a rim of compressed fibrous tissue (Figs. 6.7 and 6.8).

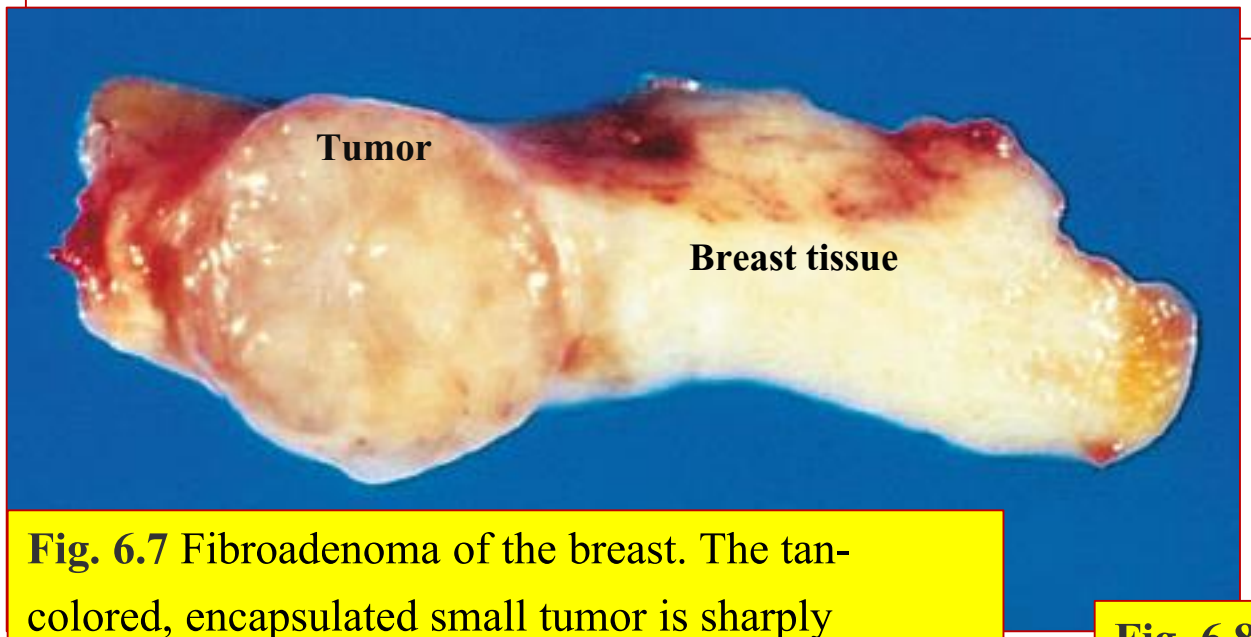


Fig. 6.7 Fibroadenoma of the breast. The tan-colored, encapsulated small tumor is sharply demarcated from the whiter breast tissue.

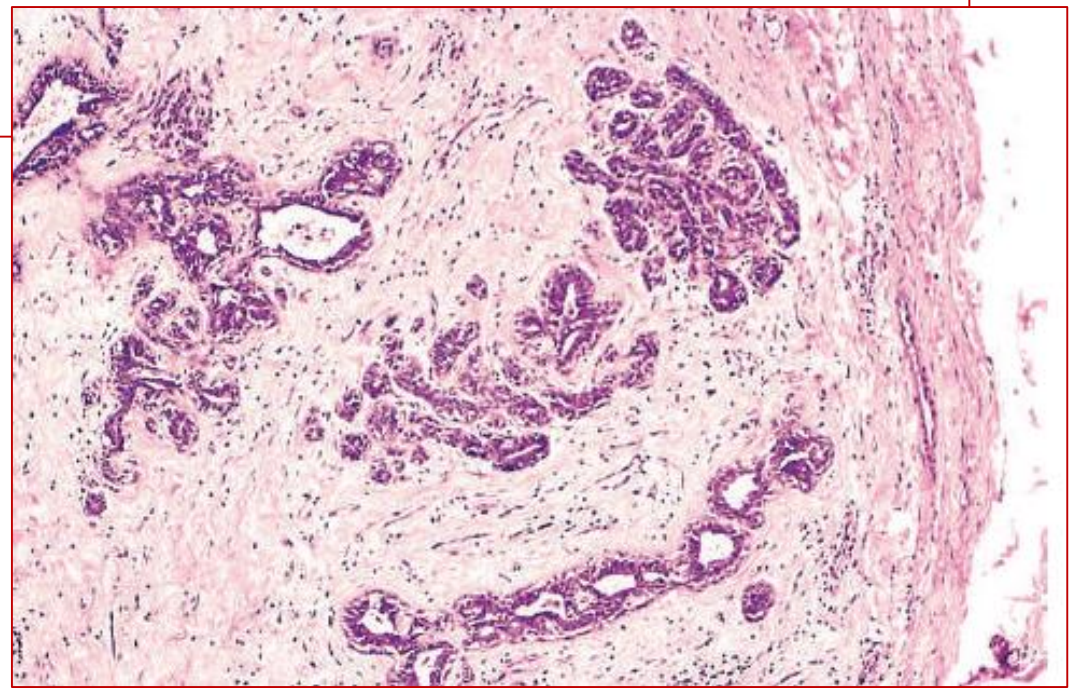


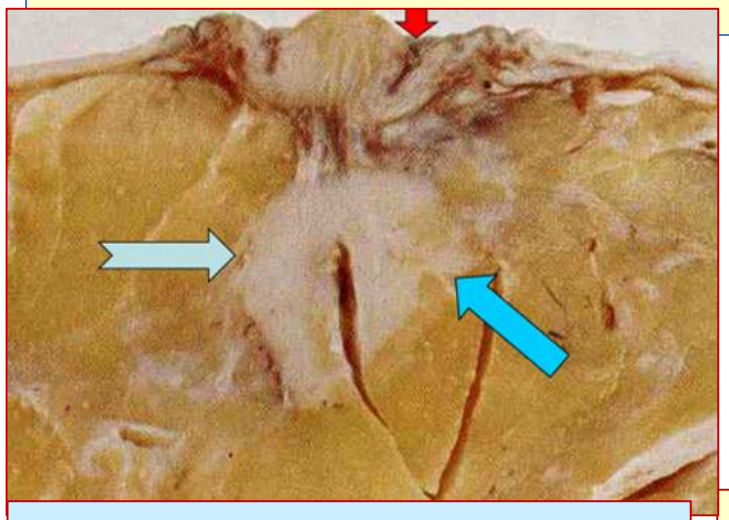
Fig. 6.8 Microscopic view of fibroadenoma of the breast seen in Fig. 6.7. The fibrous capsule *(right)* sharply delimits the tumor from the surrounding tissue. *(Courtesy of Dr. Trace Worrell, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)*

This capsule consists largely of extracellular matrix that is deposited by stromal cells such as fibroblasts. (Why?)

The fibroblasts are activated by hypoxic damage to parenchymal cells resulting from compression by the expanding tumor. Encapsulation creates a tissue plane that makes the tumor discrete, moveable (non-fixed), and readily excisable by surgical enucleation.

Not all benign neoplasms are encapsulated. For example, the leiomyoma of the uterus is discretely demarcated from the surrounding smooth muscle by a zone of compressed and attenuated normal myometrium, but lacks a capsule. A few benign tumors are neither encapsulated nor discretely defined; lack of demarcation is particularly likely in benign vascular neoplasms such as hemangiomas, which understandably may be difficult to excise.

Next to the development of metastases, invasiveness is the feature that most reliably distinguishes cancers from benign tumors (Figs. 6.9 and 6.10). Cancers lack well defined capsules. There are instances in which a slowly growing malignant tumor deceptively appears to be encased by the stroma of the surrounding host tissue, but microscopic examination reveals tiny crablike feet penetrating the margin and infiltrating adjacent structures. This infiltrative mode of growth makes it necessary to remove a wide margin of surrounding normal tissue when surgical excision of a malignant tumor is attempted.



Extension into the nipple ducts has also occurred, with characteristic indrawing (**retraction**) of the nipple.

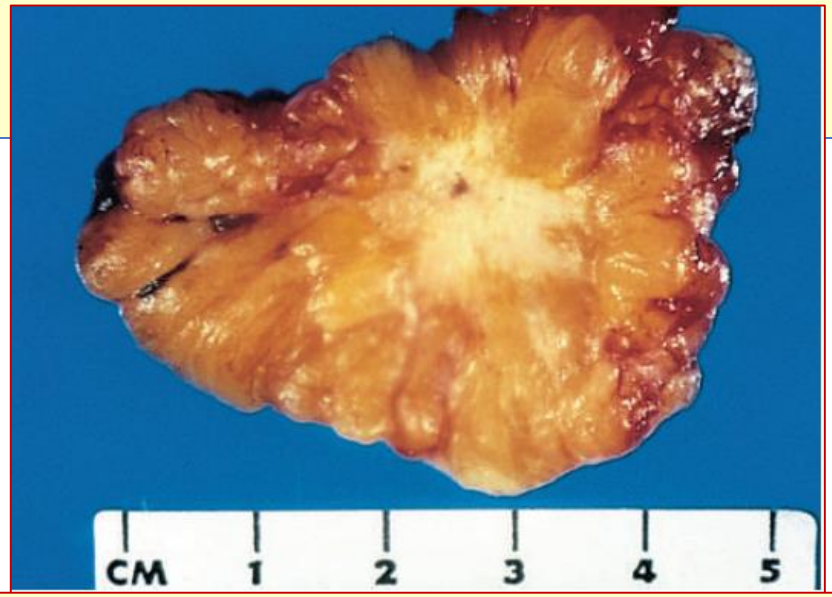


Fig. 6.9 Cut section of invasive ductal carcinoma of the breast. The lesion is retracted, infiltrating the surrounding breast substance, and was stony-hard on palpation.

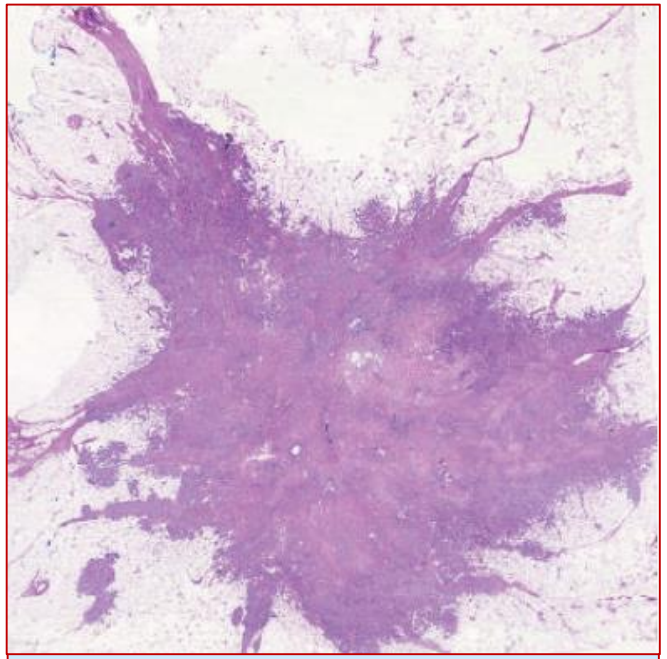


Fig. 6.10 Microscopic view of breast carcinoma seen in Fig. 6.9 illustrates the invasion of breast stroma and fat by nests and cords of tumor cells (compare with Fig. 6.8). Note the absence of a well-defined capsule. *(Courtesy of Dr. Susan Lester, Brigham and Women's Hospital, Boston, Massachusetts.)*

Metastasis

Metastasis is defined by the spread of a tumor to sites that are physically discontinuous with the primary tumor and unequivocally marks a tumor as malignant, as by definition benign neoplasms do not metastasize.

The invasiveness of cancers permits them to penetrate into blood vessels, lymphatics, and body cavities, providing opportunities for spread (Fig. 6.11).

Overall, approximately 30% of patients with newly diagnosed solid tumors (excluding skin cancers other than melanomas) present with clinically evident metastases. An additional 20% have occult (hidden) metastases at the time of diagnosis.

A special circumstance involves so-called “blood cancers”, the leukemias and lymphomas. These tumors are derived from blood-forming cells that normally have the capacity to enter the bloodstream and travel to distant sites; as a result, with only rare exceptions, leukemias and lymphomas are taken to be disseminated diseases at diagnosis and are always considered to be malignant.

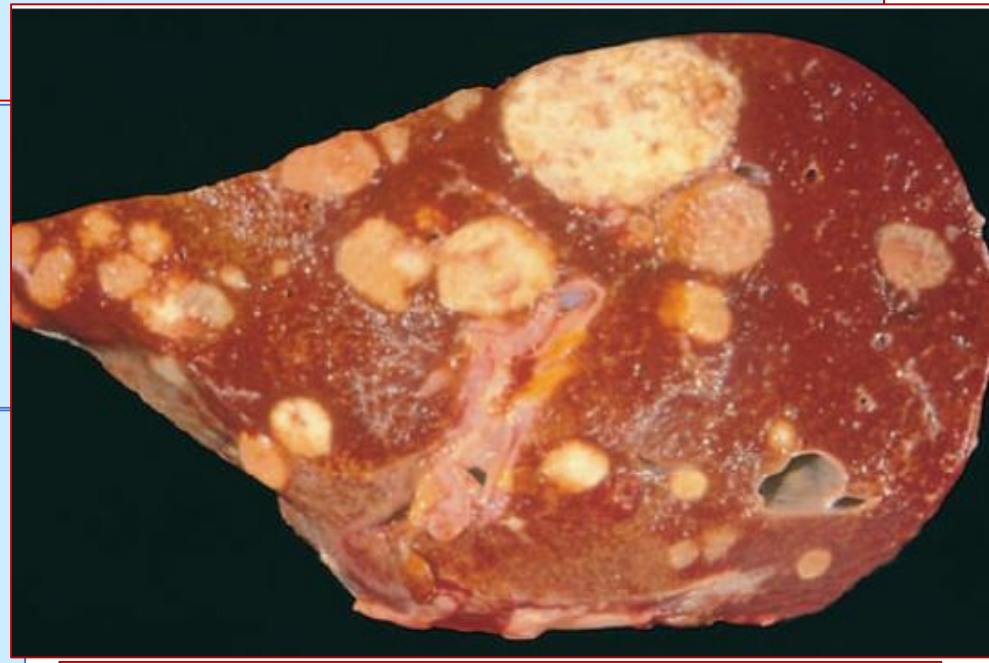


Fig. 6.11 A liver studded with metastatic cancer.

Malignant neoplasms disseminate by one of three pathways:

(1) seeding within body cavities, when neoplasms invade a natural body cavity. (is particularly characteristic of cancers of the ovary, which often cover the peritoneal surfaces widely. Neoplasms of the central nervous system, such as a medulloblastoma or ependymoma, may penetrate the cerebral ventricles and be carried by the cerebrospinal fluid to reimplant on the meningeal surfaces, either within the brain or in the spinal cord.)

(2) lymphatic spread, which is more typical of carcinomas. or

(3) hematogenous spread which is favoured by sarcomas.

There are numerous interconnections, however, between the lymphatic and vascular systems, so all forms of cancer may disseminate through either or both systems.

The pattern of lymph node involvement depends principally on the site of the primary neoplasm and the natural pathways of local lymphatic drainage.

Lung carcinomas arising in the respiratory passages metastasize first to the regional bronchial lymph nodes and then to the tracheobronchial and hilar nodes.

Carcinoma of the breast usually arises in the upper outer quadrant and first spreads to the axillary nodes. However, medial breast lesions may drain through the chest wall to the nodes along the internal mammary artery.

Thereafter, in both instances, the supraclavicular and infraclavicular nodes may be seeded.

In some cases, the cancer cells seem to travel in lymphatic channels within the immediately proximate nodes to be trapped in subsequent lymph nodes, producing so called **"skip metastases."**

A "sentinel lymph node" is the first regional lymph node that receives lymph flow from a primary tumor. It can be identified by injection of **blue dyes** or radiolabelled tracers near the primary tumor.

Biopsy of sentinel lymph nodes allows determination of the extent of spread of tumor and can be used to plan treatment.

Of note, although enlargement of nodes near a primary neoplasm should arouse concern for metastatic spread, it does not always imply cancerous involvement. The necrotic products of the neoplasm and tumor antigens often evoke immunologic responses in the nodes, such as hyperplasia of the follicles (lymphadenitis) and proliferation of macrophages in the subcapsular sinuses (sinus histiocytosis).

Thus, histopathologic verification of tumor within an enlarged lymph node is required.

Since all portal area drainage flows to the liver, and all caval blood flows to the lungs, the liver and lungs are the most frequently involved secondary sites in hematogenous dissemination.

Cancers arising near the vertebral column often embolize through the paravertebral plexus; this pathway probably is involved in the frequent vertebral metastases of carcinomas of the thyroid and prostate glands.

Certain carcinomas have a propensity to grow within veins. **Renal cell carcinoma** often invades the renal vein to grow in a snakelike fashion up the inferior vena cava, sometimes reaching the right side of the heart. **Hepatocellular carcinomas** often penetrate and grow within the radicles of portal and hepatic veins, eventually reaching the main venous channels.

Remarkably, such intravenous growth may not be accompanied by widespread dissemination.

Many observations suggest that the anatomic localization of a neoplasm and its venous drainage cannot wholly explain the systemic distributions of metastases. For example, prostatic carcinoma preferentially spreads to bone, bronchogenic carcinoma tends to involve the adrenal glands and the brain, and neuroblastoma spreads to the liver and bones.

Conversely, skeletal muscles, although rich in capillaries, are rarely sites of tumor metastases.

Thus, numerous features of tumors (Fig. 6.12) usually permit the differentiation of benign and malignant neoplasms.

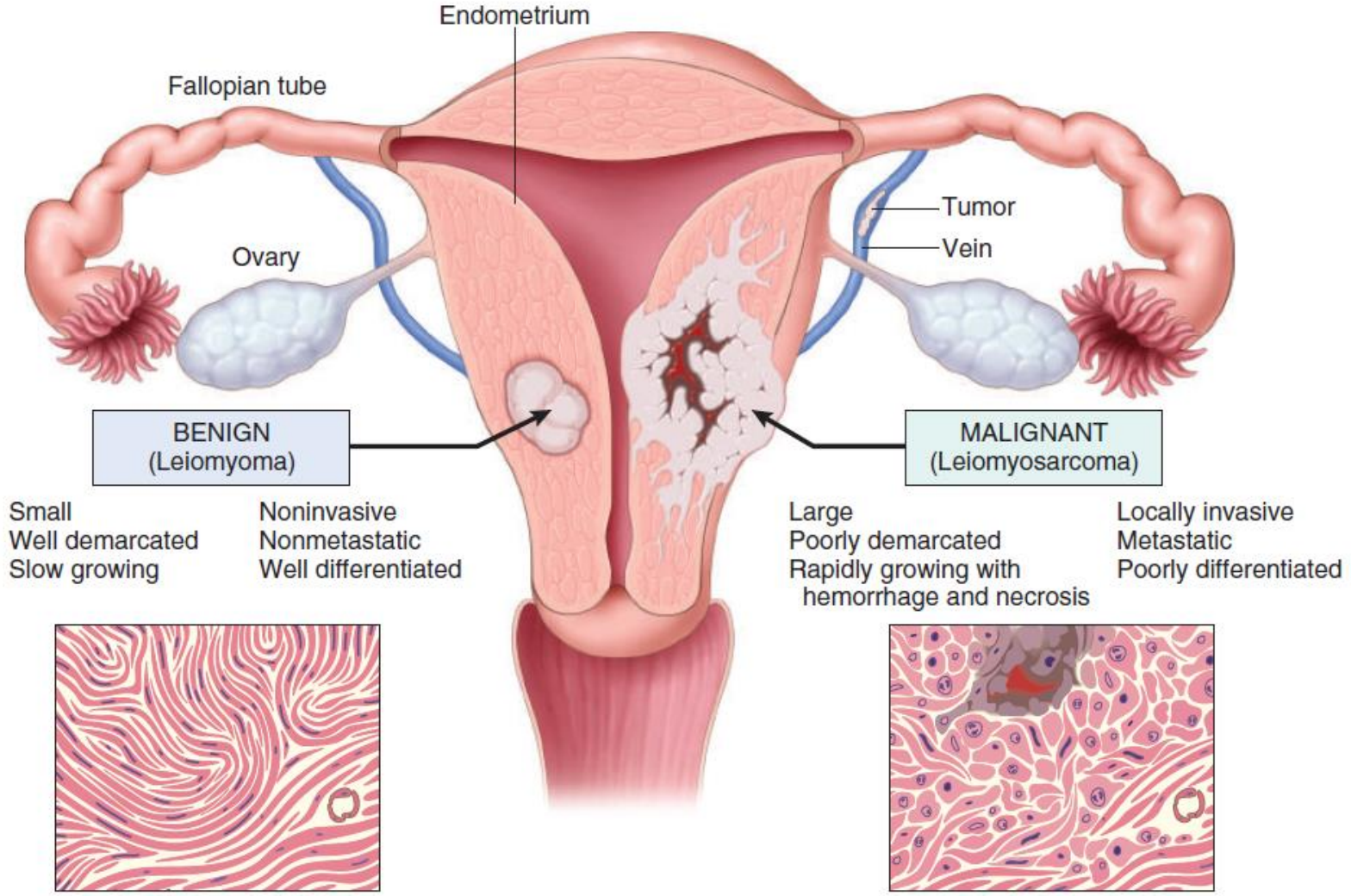


Fig. 6.12 Comparison between a benign tumor of the myometrium (leiomyoma) and a malignant tumor of similar origin (leiomyosarcoma).

EPIDEMIOLOGY

Study of cancer occurrence in populations has contributed substantially to knowledge about its origins.

- cigarette smoking is causally associated with lung cancer.
- dietary fat and fiber content may figure importantly in the causation of colon cancer.

Major insights into the causes of cancer can be obtained by epidemiologic studies that relate particular environmental, racial (possibly hereditary), and cultural influences to the occurrence of specific neoplasms.

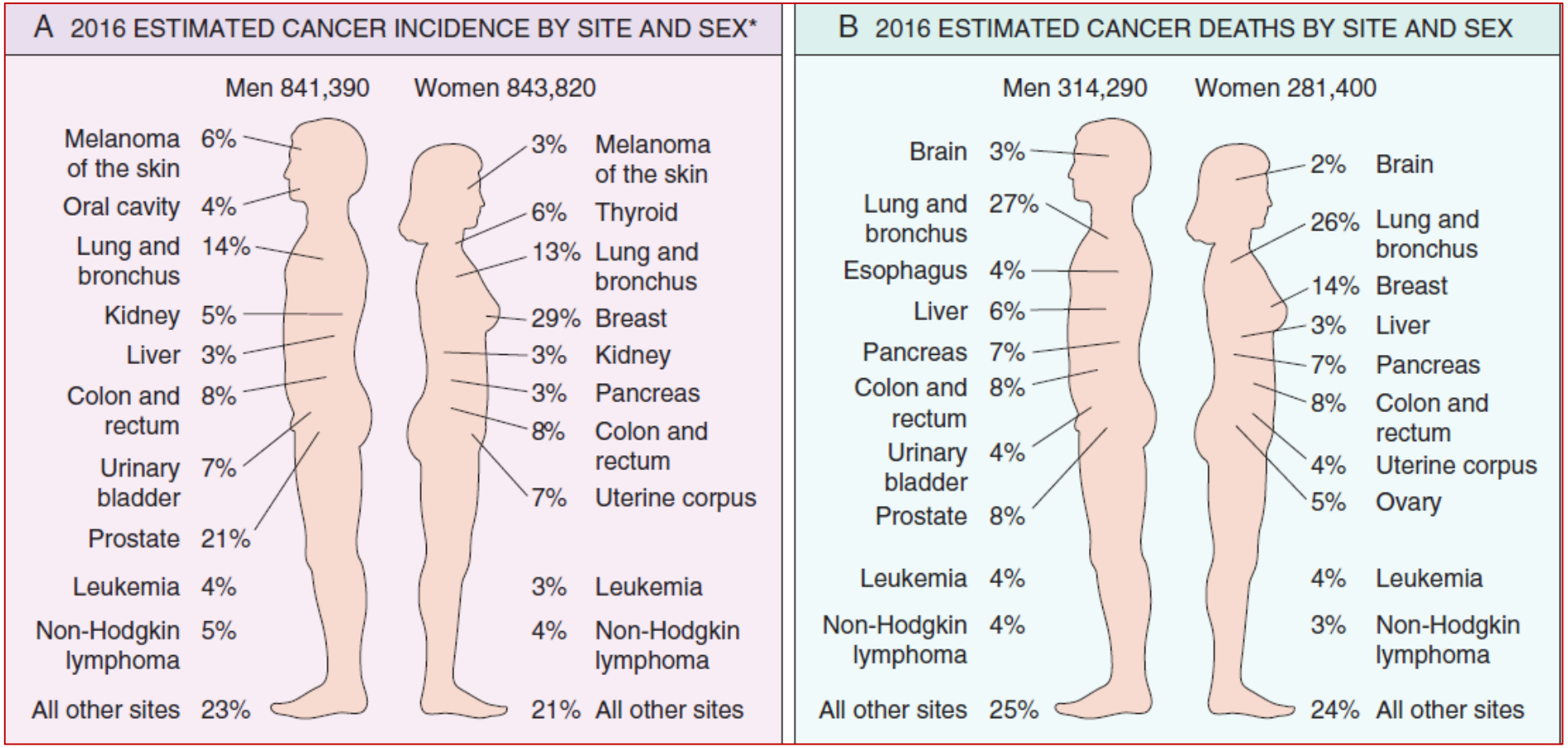
Cancer Incidence

For the year 2012, the World Health Organization (WHO) estimated that there were about 14.1 million new cancer cases worldwide, leading to 8.2 million deaths (approximately 22,500 deaths per day). Moreover, due to increasing population size, by the year 2035 the WHO projects that the numbers of cancer cases and deaths worldwide will

increase to 24 million and 14.6 million, respectively (based on current mortality rates).

Incidence data for the most common forms of cancer, with the major killers identified, are presented in Fig. 6.13.

Fig. 6.13 Estimated cancer incidence and mortality by site and sex in the United States. Excludes basal cell and squamous cell skin cancers and in situ carcinomas, except urinary bladder. *(Adapted from* Cancer facts & figures 2016. *American Cancer Society. www.cancer.org/research/cancer-facts-statistics/all-cancerfacts-figures/cancer-facts-figures-2016.html.)*



Over several decades, the death rates for many forms of cancer have changed.

The cancer death rate has decreased due to the widespread use of early detection methods for various types of cancers, such as breast and colorectal cancer (mammogram and endoscopy techniques respectively), cervical cancer (Pap. Smear, and HPV vaccine), Decreased use of tobacco products is responsible for the reduction in lung cancer deaths

Environmental Factors

Environmental exposures appear to be the dominant risk factors for many common cancers, suggesting that a high fraction of cancers are potentially preventable.

The most important environmental exposures linked to cancer include the following:

- ***Diet***. *(*Certain features of diet have been implicated as predisposing influences.)
- ***Smoking***. Smoking, particularly of cigarettes, has been implicated in cancer of the mouth, pharynx, larynx, esophagus, pancreas, bladder, and, most significantly, the lung, as 90% of lung cancer deaths are related to smoking.
- ***Alcohol consumption***. Alcohol abuse is an independent risk factor for cancers of the oropharynx, larynx, esophagus, and (due to alcoholic cirrhosis) liver.
- ***Reproductive history***. There is strong evidence that lifelong cumulative exposure to estrogen stimulation, particularly if unopposed by progesterone, increases the risk for developing cancers of the endometrium and breast, both of which are estrogen-responsive tissues.
- ***Infectious agents***. It is estimated that infectious agents cause approximately 15% of cancers worldwide.

Table 6.2 Occupational Cancers

Agents or Groups of Agents	Human Cancers for Which Reasonable Evidence Is Available	Typical Use or Occurrence
Arsenic and arsenic compounds	Lung carcinoma, skin carcinoma	By-product of metal smelting; component of alloys, electrical and semiconductor devices, medications and herbicides, fungicides, and animal dips
Asbestos	Lung, esophageal, gastric, and colon carcinoma; mesothelioma	Formerly used for many applications because of fire, heat, and friction resistance; still found in existing construction as well as fire-resistant textiles, friction materials (i.e., brake linings), underlayment and roofing papers, and floor tiles
Benzene	Acute myeloid leukemia	Principal component of light oil; despite known risk, many applications exist in printing and lithography, paint, rubber, dry cleaning, adhesives and coatings, and detergents; formerly widely used as solvent and fumigant
Beryllium and beryllium compounds	Lung carcinoma	Missile fuel and space vehicles; hardener for lightweight metal alloys, particularly in aerospace applications and nuclear reactors
Cadmium and cadmium compounds	Prostate carcinoma	Uses include yellow pigments and phosphors; found in solders; used in batteries and as alloy and in metal platings and coatings
Chromium compounds	Lung carcinoma	Component of metal alloys, paints, pigments, and preservatives
Nickel compounds	Lung and oropharyngeal carcinoma	Nickel plating; component of ferrous alloys, ceramics, and batteries; by-product of stainless-steel arc welding
Radon and its decay products	Lung carcinoma	From decay of minerals containing uranium; potentially serious hazard in quarries and underground mines
Vinyl chloride	Hepatic angiosarcoma	Refrigerant; monomer for vinyl polymers; adhesive for plastics; formerly inert aerosol propellant in pressurized containers

Modified from Stellman JM, Stellman SD: Cancer and the workplace, *CA Cancer J Clin* 46:70–92, 1996, with permission from Lippincott Williams & Wilkins.

Age and Cancer

In general, the frequency of cancer increases with age. Most cancer deaths occur between 55 and 75 years of age; the rate declines, along with the population base, after 75 years of age.

Although cancer preferentially affects older adults, it also is responsible for slightly more than 10% of all deaths among children younger than 15 years of age.

The major lethal cancers in children are leukemias, tumors of the central nervous system, lymphomas, and soft-tissue and bone sarcomas.

Acquired Predisposing Conditions

Acquired conditions that predispose to cancer include disorders associated with chronic inflammation, immunodeficiency states, and precursor lesions. Many chronic inflammatory conditions create a fertile "soil" for the development of malignant tumors (Table 6.3).

Precursor lesions are localized disturbances of epithelial differentiation that are associated with an elevated risk for developing carcinoma. They may arise secondary to chronic inflammation or hormonal disturbances (in endocrine-sensitive tissues), or may occur spontaneously.

However, progression to cancer is not inevitable, and it is important to recognize precursor lesions because their removal or reversal lowers cancer risk.

Table 6.3 Chronic Inflammatory States and Cancer

Pathologic Condition	Associated Neoplasm(s)	Etiologic Agent
Asbestosis, silicosis	Mesothelioma, lung carcinoma	Asbestos fibers, silica particles
Inflammatory bowel disease	Colorectal carcinoma	
Lichen sclerosis	Vulvar squamous cell carcinoma	
Pancreatitis	Pancreatic carcinoma	Alcoholism, germ line mutations (e.g., in the trypsinogen gene)
Chronic cholecystitis	Gallbladder cancer	Bile acids, bacteria, gallbladder stones
Reflux esophagitis, Barrett esophagus	Esophageal carcinoma	Gastric acid
Sjögren syndrome, Hashimoto thyroiditis	MALT lymphoma	
Opisthorchis, cholangitis	Cholangiocarcinoma, colon carcinoma	Liver flukes *(Opisthorchis viverrini)*
Gastritis/ulcers	Gastric adenocarcinoma, MALT lymphoma	*Helicobacter pylori*
Hepatitis	Hepatocellular carcinoma	Hepatitis B and/or C virus
Osteomyelitis	Carcinoma in draining sinuses	Bacterial infection
Chronic cervicitis	Cervical carcinoma	Human papillomavirus
Chronic cystitis	Bladder carcinoma	Schistosomiasis

Adapted from Tlsty TD, Coussens LM: Tumor stroma and regulation of cancer development, *Ann Rev Pathol Mech Dis* 1:119, 2006.

- ***Squamous metaplasia and dysplasia*** *of bronchial mucosa,* seen in in habitual smokers — a risk factor for lung carcinoma.
- ***Endometrial hyperplasia and dysplasia****,* seen in women with unopposed estrogenic stimulation — a risk factor for endometrial carcinoma.
- ***Leukoplakia of the oral cavity, vulva, and penis***, which may progress to squamous cell carcinoma.
- ***Villous adenoma of the colon***, associated with a high risk for progression to colorectal carcinoma

"What is the risk for malignant change in a benign neoplasm?"— or, stated differently, "Are benign tumors precancers?"

Interactions Between Environmental and Genetic Factors

Cancer behaves like an inherited trait in some families, usually due to germ line mutations that affect the function of a gene that suppresses cancer (a so-called "**Tumor Suppressor Gene**").

Lack of family history does not preclude an inherited component. It may in fact be difficult to tease out hereditary and genetic contributions because these factors often interact.

Genetic factors may alter the risk for developing environmentally induced cancers.

Instances where this holds true often involve inherited variation in enzymes such as components of the cytochrome P-450 system that metabolize procarcinogens to active carcinogens. Conversely, environmental factors can influence the risk for developing cancer, even in individuals who inherit well-defined "cancer genes." For instance, breast cancer risk in females who inherit mutated copies of the *BRCA1* or *BRCA2* tumor suppressor genes (discussed later) is almost three-fold higher for women born after 1940 than for women born before that year, perhaps because of changes in reproductive behaviour or increases in obesity in more recent times.

CANCER GENES

Cancer genes can be defined as genes that are recurrently affected by genetic aberrations in cancers, presumably because they contribute directly to the malignant behavior of cancer cells.

Cancer genes fall into one of four major functional classes:

1- Oncogenes are genes that induce a transformed phenotype when expressed in cells by promoting increased cell growth. Oncogenes are mutated or overexpressed versions of normal cellular genes, which are called *proto-oncogenes*.

Most oncogenes encode transcription factors, factors that participate in pro-growth signaling pathways, or factors that enhance cell survival.

They are considered dominant genes because a mutation involving a single allele is sufficient to produce a pro-oncogenic effect.

2- Tumor suppressor genes are genes that normally prevent uncontrolled growth and, when mutated or lost from a cell, allow the transformed phenotype to develop. Tumor suppressor genes can be placed into two general groups, *"governors"* that act as important brakes on cellular proliferation, and *"guardians"* that are responsible for sensing genomic damage. Some guardian genes initiate and choreograph a complex "damage control response" that leads to the cessation of proliferation or, if the damage is too great to be repaired, or induce apoptosis.

3- Genes that regulate apoptosis primarily act by enhancing cell survival, rather than stimulating proliferation *per se*. (are often overexpressed in cancer cells, whereas those that promote apoptosis tend to be under-expressed or functionally inactivated by mutations)

4- ***Genes that regulate interactions between tumor cells and host cells,*** as these genes are also recurrently mutated or functionally altered in certain cancers. Particularly important are genes that enhance or inhibit recognition of tumors cells by the host immune system.

In families in which these germ line mutations are passed from generation to generation, cancer behaves like an inherited trait **(Table 6.4).**

Inherited Predisposition	Gene(s)
Autosomal Dominant Cancer Syndromes	
Retinoblastoma	*RB*
Li-Fraumeni syndrome (various tumors)	*TP53*
Melanoma	*CDKN2A*
Familial adenomatous polyposis/colon cancer	*APC*
Neurofibromatosis 1 and 2	*NF1*, *NF2*
Breast and ovarian tumors	*BRCA1*, *BRCA2*
Multiple endocrine neoplasia 1 and 2	*MEN1*, *RET*
Hereditary nonpolyposis colon cancer	*MSH2*, *MLH1*, *MSH6*
Nevoid basal cell carcinoma syndrome	*PTCH1*
Autosomal Recessive Syndromes of Defective DNA Repair	
Xeroderma pigmentosum	Diverse genes involved in nucleotide excision repair
Ataxia-telangiectasia	*ATM*
Bloom syndrome	*BLM*
Fanconi anemia	Diverse genes involved in repair of DNA cross-links

Genetic Lesions in cancer

The genetic changes found in cancers vary from point mutations involving single nucleotides to abnormalities large enough to produce gross changes in chromosome structure.

In certain neoplasms, genetic abnormalities are non-random and highly characteristic. Specific chromosomal abnormalities have been identified in most leukemias and lymphomas and in an increasing number of nonhematopoietic tumors, while other tumors are characterized by particular point mutations.

Driver and Passenger Mutations

Driver mutations are mutations that alter the function of cancer genes and thereby directly contribute to the development or progression of a given cancer.

They are usually acquired, and occasionally inherited.

By contrast, **passenger mutations are acquired mutations that are neutral in terms of fitness and do not affect cellular behavior; they just come along for the proverbial ride.**

Because they occur at random, passenger mutations are sprinkled throughout the genome, whereas driver mutations tend to be tightly clustered within cancer genes.

Despite their apparently innocuous nature, passenger mutations have nevertheless proven to be important in several ways:

- *In carcinogen-associated cancers, mutational analysis has provided definitive evidence that most genomic damage is directly caused by the carcinogen in question.*

For example, before sequencing of melanoma genomes, the causative role of sun exposure in this cancer was debated. This is no longer so, as most melanomas have thousands of mutations of a type that is specifically linked to damage caused by ultraviolet light.

- *A second, more nefarious effect of passenger mutations is that they create genetic variants that, while initially neutral, may provide tumor cells with a selective advantage in the setting of therapy.*

The evidence for this comes from DNA sequence analyses of tumors at the time of recurrence after drug therapy; in many instances, mutations that lead directly to drug resistance are found in most tumor cells.

Point Mutations:

- Can either **activate** or **inactivate** the protein products of the affected genes depending on their precise position and consequence. Point mutations that convert proto-oncogenes into oncogenes generally produce a gain of-function by altering amino acid residues in a domain that normally holds the protein's activity in check.
- By contrast, point mutations (as well as larger aberrations, such as insertions and deletions) in **tumor suppressor genes** reduce or disable the function of the encoded protein.

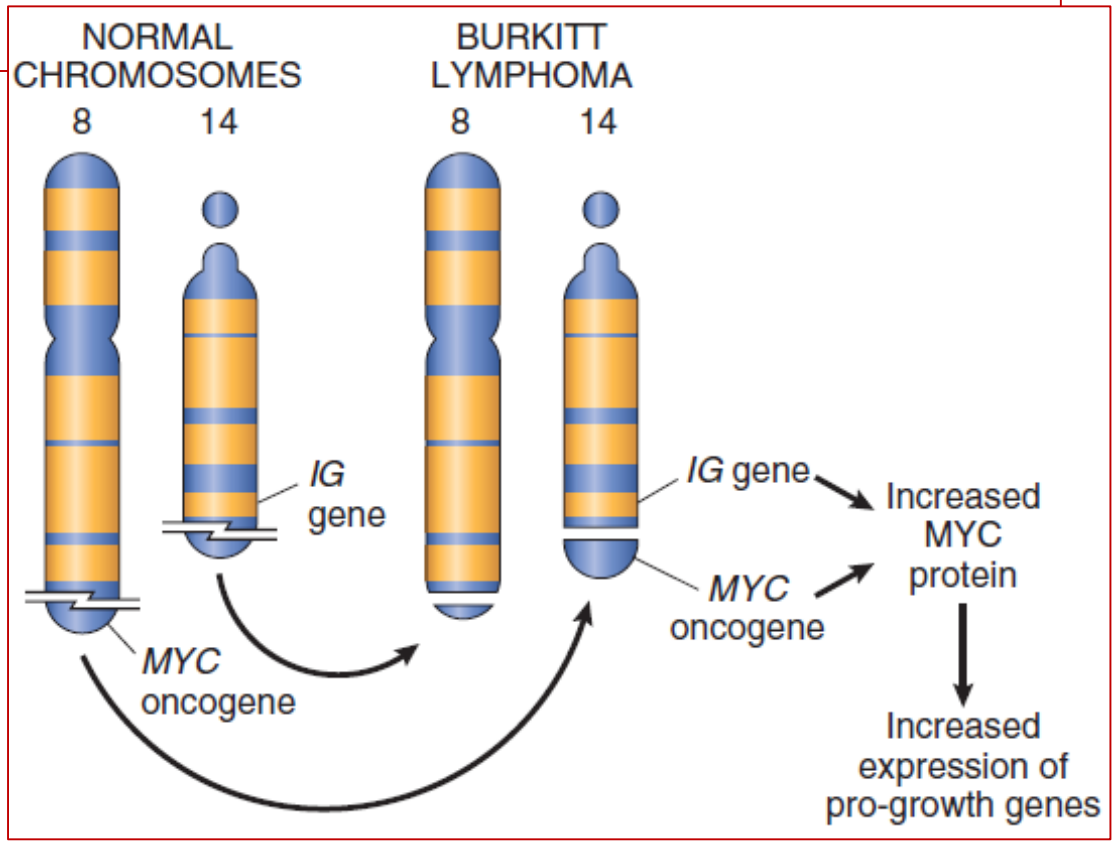
The tumor suppressor gene that is most commonly affected by point mutations in cancer is *TP53*, a prototypical "guardian" type tumor suppressor gene.

Gene Rearrangements

- Gene rearrangements may be produced by chromosomal translocations or inversions. These rearrangements can activate proto-oncogenes in two ways:

1- Some gene rearrangements result in overexpression of proto-oncogenes by removing them from their normal regulatory elements and placing them under control of an inappropriate, highly active promoter or enhancer.

Two different kinds of B cell lymphoma provide illustrative examples of this mechanism. In more than 90% of cases of *Burkitt lymphoma,* the cells have a translocation, usually between chromosomes 8 and 14, that leads to overexpression of the *MYC* gene on chromosome 8 by juxtaposition with immunoglobulin heavy chain gene regulatory elements on chromosome 14 (Fig. 6.14). In *follicular lymphoma,* a reciprocal translocation between chromosomes 14 and 18 leads to overexpression of the anti-apoptotic gene, *BCL2,* on chromosome 18, also driven by immunoglobulin gene regulatory elements.

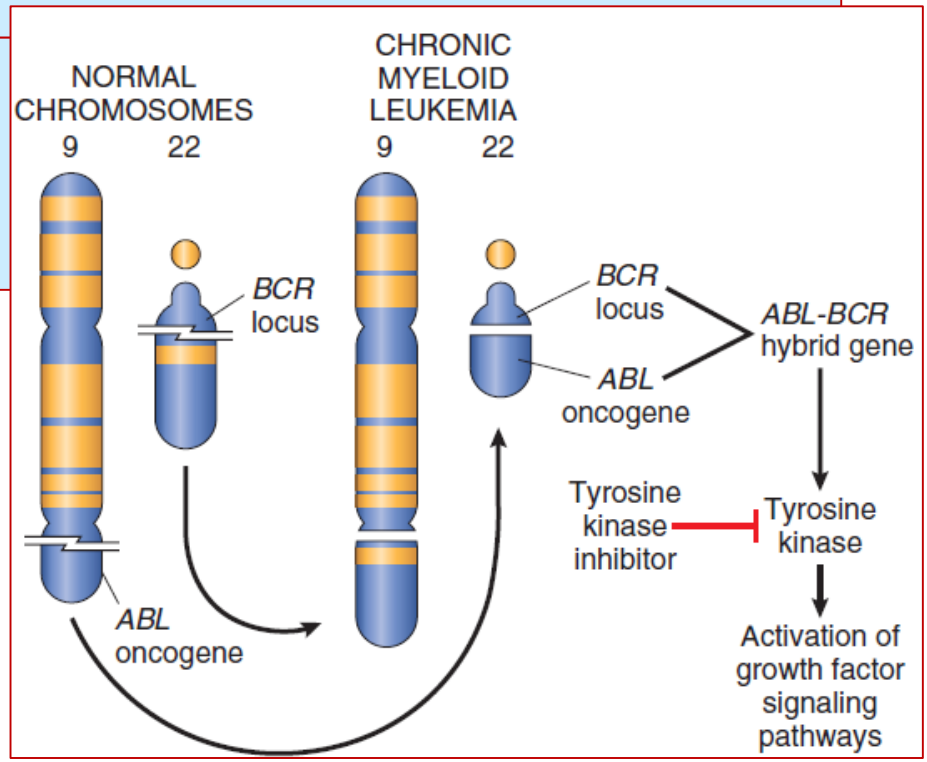


Other oncogenic gene rearrangements create fusion genes encoding novel chimeric proteins.

Most notable is the Philadelphia (Ph) chromosome in chronic myeloid leukemia, consisting of a balanced reciprocal translocation between chromosomes 9 and 22 (see Fig. 6.14).

As a consequence, the derivative chromosome 22 (the Philadelphia chromosome) appears smaller than normal.

This cytogenetic change is seen in more than 90% of cases of chronic myeloid leukemia and results in the fusion of portions of the *BCR* gene on chromosome 22 and the *ABL* gene on chromosome 9.



Deletions

Deletions are another prevalent abnormality in tumor cells.
Deletion of specific regions of chromosomes may result in the loss of particular tumor suppressor genes.

Tumor suppressors generally require inactivation of both alleles in order for them to contribute to carcinogenesis.

A common mechanism for this is an inactivating point mutation in one allele, followed by deletion of the other, nonmutated allele.

Gene Amplifications

Proto-oncogenes may be converted to oncogenes by gene amplification, with consequent overexpression and hyperactivity of otherwise normal proteins. Such amplification may produce several hundred copies of the gene, a change in copy number that can be readily detected by molecular hybridization with appropriate DNA probes.

Two clinically important examples of amplification involve the *NMYC* gene in neuroblastoma and the *HER2* gene in breast cancers. *NMYC* is amplified in 25% to 30% of neuroblastomas, and the amplification is associated with poor prognosis (Fig. 6.15). *HER2* (also known as *ERBB2*) amplification occurs in about 20% of breast cancers, and antibody therapy directed against the receptor encoded by the *HER2* gene has proved effective in this subset of tumors.

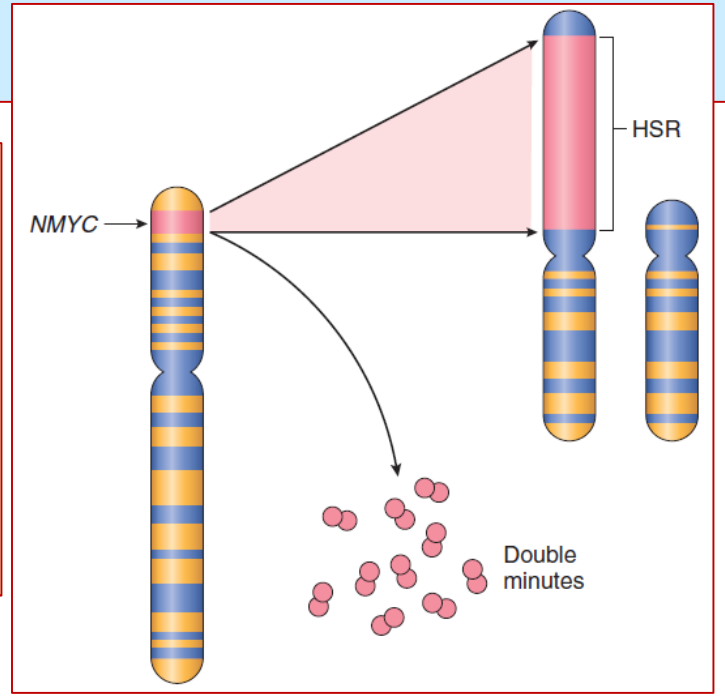


Fig. 6.15 Amplification of the *NMYC* gene in human neuroblastoma. The *NMYC* gene, present normally on chromosome 2p, becomes amplified and is seen either as extrachromosomal double minutes or as a chromosomally integrated homogeneous-staining region (HSR). The integration involves other autosomes, such as 4, 9, or 13.

(Modified from Brodeur GM, Seeger RC, Sather H, et al: Clinical implications of oncogene activation in human neuroblastomas. Cancer *58:541, 1986. Reprinted by permission of Wiley-Liss, Inc, a subsidiary of John Wiley & Sons, Inc.)*

Aneuploidy

Is defined as a number of chromosomes that is not a multiple of the haploid state; for humans, that is a chromosome number that is not a multiple of 23.

Aneuploidy is remarkably common in cancers, particularly carcinomas, and was proposed as a cause of carcinogenesis over 100 years ago. Aneuploidy frequently results from errors of the mitotic checkpoint, the major cell cycle control mechanism that acts to prevent mistakes in chromosome segregation. The mitotic checkpoint prevents aneuploidy by inhibiting the irreversible transition to anaphase until all of the replicated chromosomes have made productive attachments to spindle microtubules.

Aneuploidy tends to increase the copy number of key oncogenes and decrease the copy number of potent tumor suppressors.

For example, chromosome 8, which almost never is lost and often is present in increased copies in tumor cells, is where the *MYC* oncogene is located. By contrast, portions of chromosome 17, where the *TP53* gene is located, are often lost and are infrequently gained.

MicroRNAs and Cancer

microRNAs (miRNAs) are function as negative regulators of genes. They inhibit gene expression posttranscriptionally by repressing translation or, in some cases, by messenger RNA (mRNA) cleavage. In view of their important functions in control of cell growth, differentiation, and survival **miRNAs** also can contribute to carcinogenesis. Specifically, if the target of a miRNA is a tumor suppressor gene, then overactivity of the miRNA can **reduce** the tumor suppressor protein. Such miRNAs are sometimes referred to as *oncomIRs.*

Conversely, if an miRNA **inhibits** the translation of an oncogene, a reduction in the quantity or function of that miRNA will lead to **overproduction** of the oncogene product. Such relationships have already been established by miRNA profiling of several human tumors.

For example, downregulation or deletion of certain miRNAs in some leukemias and lymphomas results in increased expression of *BCL2,* an anti-apoptotic gene. Thus, by negatively regulating *BCL2,* such miRNAs behave as tumor suppressor genes.

Epigenetic Modifications and Cancer

Epigenetics refers to reversible, heritable changes in gene expression that occur without mutation. Such changes involve posttranslational modifications of histones and DNA methylation, both of which affect gene expression.

In normal, differentiated cells, the major portion of the genome is not expressed.

These regions of the genome are silenced by DNA methylation and histone modifications. On the other hand, cancer cells are characterized by a global DNA hypomethylation and selective promoter-localized hypermethylation.

Genome-wide hypomethylation has been shown to cause chromosomal instability and can induce tumors in mice.

Deep sequencing of cancer genomes has identified mutations in genes that regulate epigenetic modifications in many cancers.

The epigenetic state of particular cell types—a feature described as the epigenetic context—also dictates their response to signals that control growth and differentiation.

As mentioned earlier, epigenetic modifications regulate gene expression, allowing cells with the same genetic makeup (e.g., a neuron and a keratinocyte) to have completely different appearances and functions. In some instances, the epigenetic state of a cell dramatically affects its response to otherwise identical signals. For example, the *NOTCH1* gene has an oncogenic role in T-cell leukemia, yet acts as a tumor suppressor in squamous cell carcinomas.

As would be expected, this dichotomy exists because activated *NOTCH1* turns on pro-growth genes in T-cell progenitors and tumor suppressor genes in keratinocytes.

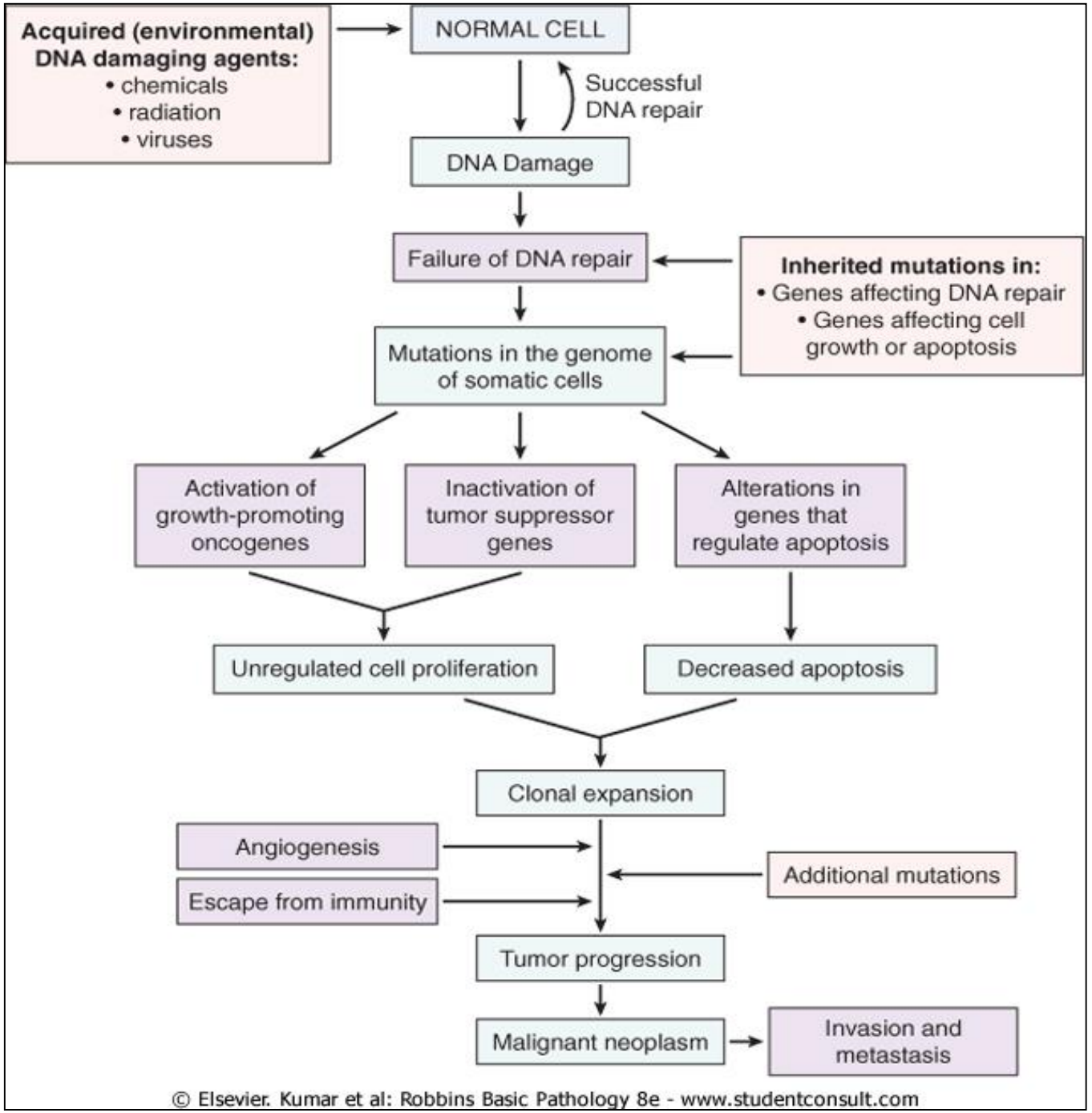
Carcinogenesis: The Molecular Basis of Cancer

Four fundamental principles of carcinogenesis: {very well-illustrated in next figure, a flow chart showing a simplified scheme of the molecular basis of cancer}.

(I) Nonlethal (non-killing) genetic damage (or mutation) lies at the heart of carcinogenesis. Such mutation may be: **Inherited** in the germ line, or it may be **Acquired** by the action of environmental agents, such as chemicals, radiation, or viruses.

The genetic hypothesis of cancer implies that a tumor mass result from the clonal *expansion of a **single*** **progenitor** cell that has incurred (suffered) the genetic damage, i.e., **Tumor result from monoclonal proliferation of single mutant cell.**

This expectation has been **proven** in most Tumors that have been analyzed.

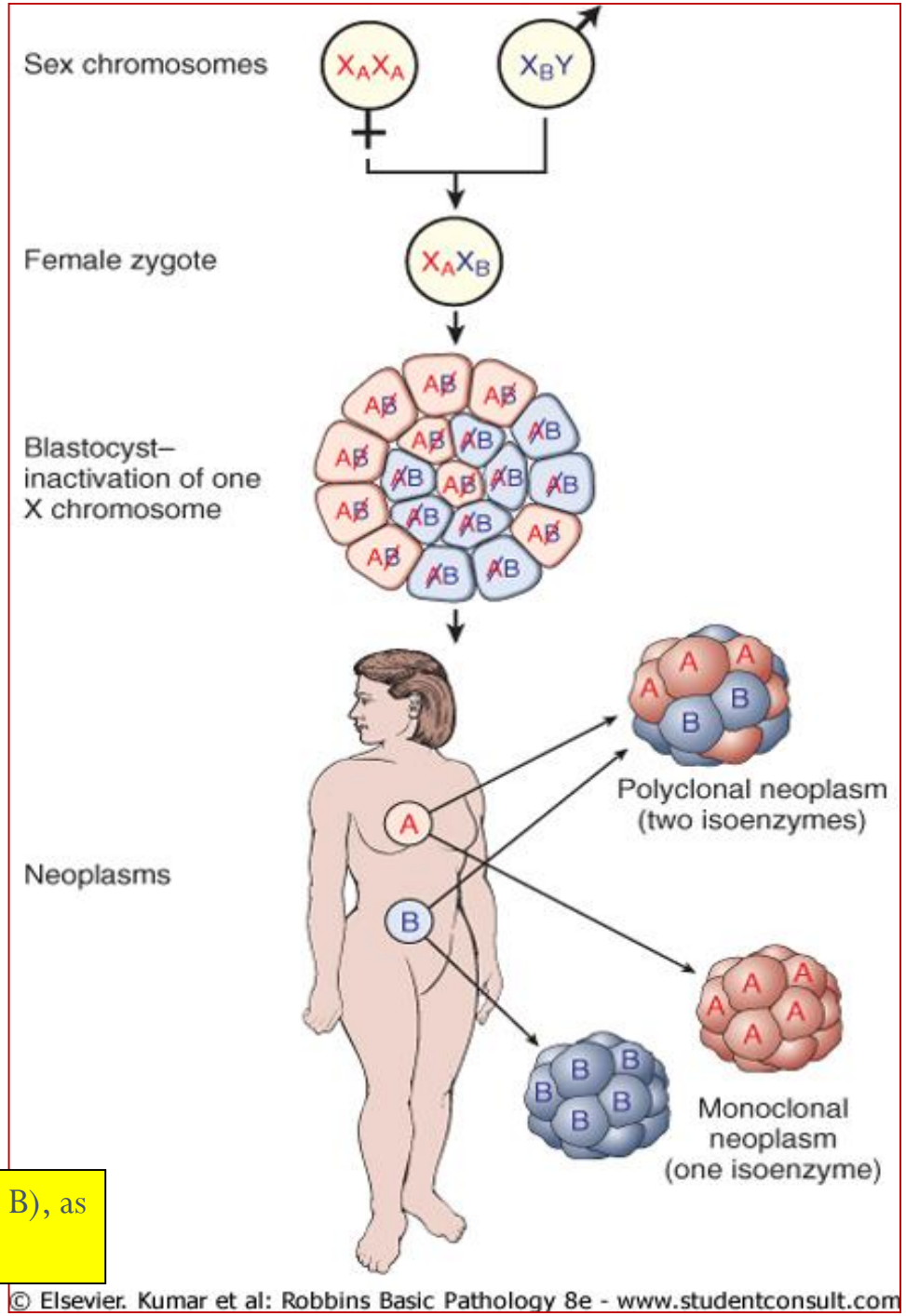


Flow chart depicting a simplified scheme of the molecular basis of cancer.

Best example to explain monoclonity of tumors, is in all women, who are heterozygous for polymorphic X-linked markers, such as the enzyme glucose-6-phosphate dehydrogenase (G6PD)

All females are mosaics, with two cell populations in their normal body: one population contains G6PD isoenzyme A in its cells, & the second contains G6PD isoenzyme B in its cells.

When a Tumor, whether Benign or Malignant, arises in such heterozygous female analyzed, then, all the Tumor cells contain either the A or the B isoenzyme, but not both. This means that the tumor cells are monoclonal (while the rest of the female cells except the tumor cells, remain heterozygous, with both A & B cells!).



The use of X- linked isoenzyme cell markers (G6PD isoenzyme A or B), as evidence of the monoclonality of neoplasm.

(II) Three classes of normal regulatory genes;

(1) growth-promoting ***protooncogenes****;*

(2) growth-inhibiting ***cancer suppressor genes,***

(3) genes that regulate ***apoptosis,*** are the main **targets of genetic damage**.

- **Mutant alleles of protooncogenes** are called **oncogenes.**

They are considered **Dominant** because they transform cells despite the presence of their normal counterpart.

- **In contrast**, both normal alleles of tumor suppressor genes must be damaged for transformation to occur, so these **suppressor** genes are considered **Recessive oncogenes**

- **Apoptosis** regulating genes may be either dominant, as are protooncogenes, or they may be recessive.

(III) A 4th category of genes that regulate **repair** of damaged DNA is important in carcinogenesis, e.g. *Nucleotide Excision Repair genes* (defective in *xerodrema pigmentosum*).

- DNA repair genes affect, **indirectly** the cell proliferation or survival by influencing the ability of the organism to **repair** nonlethal damage in other genes, including protooncogenes, tumor suppressor genes, & genes that regulate apoptosis.
- A disability in the DNA repair genes can predispose to widespread mutations in the genome & to neoplasia.

(IV) Carcinogenesis is a multistep process at both the genetic & the phenotypic levels.Cancer, or **MT** has several phenotypic characteristics, such as excessive growth, invasion & metastasis.

These characteristics are acquired in a stepwise fashion, a phenomenon called **tumor progression**.

Carcinogenesis: a Multistep process

Fortunately, in most if not all instances, no single mutation is sufficient to transform a normal cell into a cancer cell.

Carcinogenesis is thus a multistep process resulting from the accumulation of multiple genetic alterations that collectively give rise to the transformed phenotype and all of its associated hallmarks.

Beyond tumor initiation from a single founding cell, it is important to recognize that cancers continue to undergo Darwinian selection and therefore continue to evolve (Fig. 6.16).

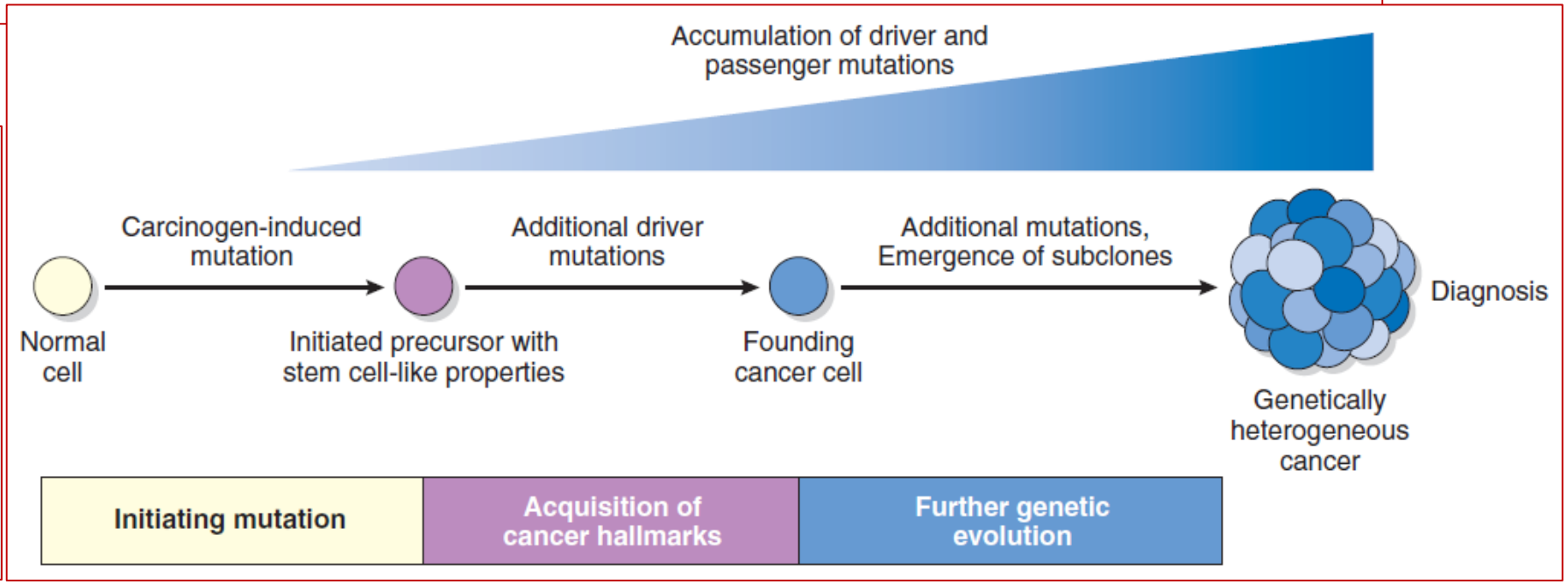


Fig. 6.16 Development of cancer through stepwise accumulation of complementary driver mutations. The order in which various driver mutations occur is usually unknown and may vary from tumor to tumor.

As a result of continuing mutation and Darwinian selection, even though malignant tumors are monoclonal in origin they are typically genetically heterogeneous by the time of their clinical presentation.

In advanced tumors exhibiting genetic instability, the extent of genetic heterogeneity may be enormous.

Genetic evolution shaped by Darwinian selection can explain the two most pernicious properties of cancers: the tendency over time for cancers to become both more aggressive and less responsive to therapy. Thus, genetic heterogeneity has implications not only for cancer progression but also for its response to therapy.

Experience has shown that when tumors recur after chemotherapy, the recurrent tumor is almost always resistant to the original drug regimen if it is given again.

Hallmarks of Cancer

It appears that **all cancers display eight fundamental changes in cell physiology, which are considered the hallmarks of cancer.**

These changes are illustrated in Fig. 6.17 and consist of the following:

- *Self-sufficiency in growth signals*
- *Insensitivity to growth-inhibitory signals*
- *Altered cellular metabolism*
- *Evasion of apoptosis*
- *Limitless replicative potential (immortality)*
- *Sustained angiogenesis*
- *Invasion and metastasis*
- *Evasion of immune surveillance*

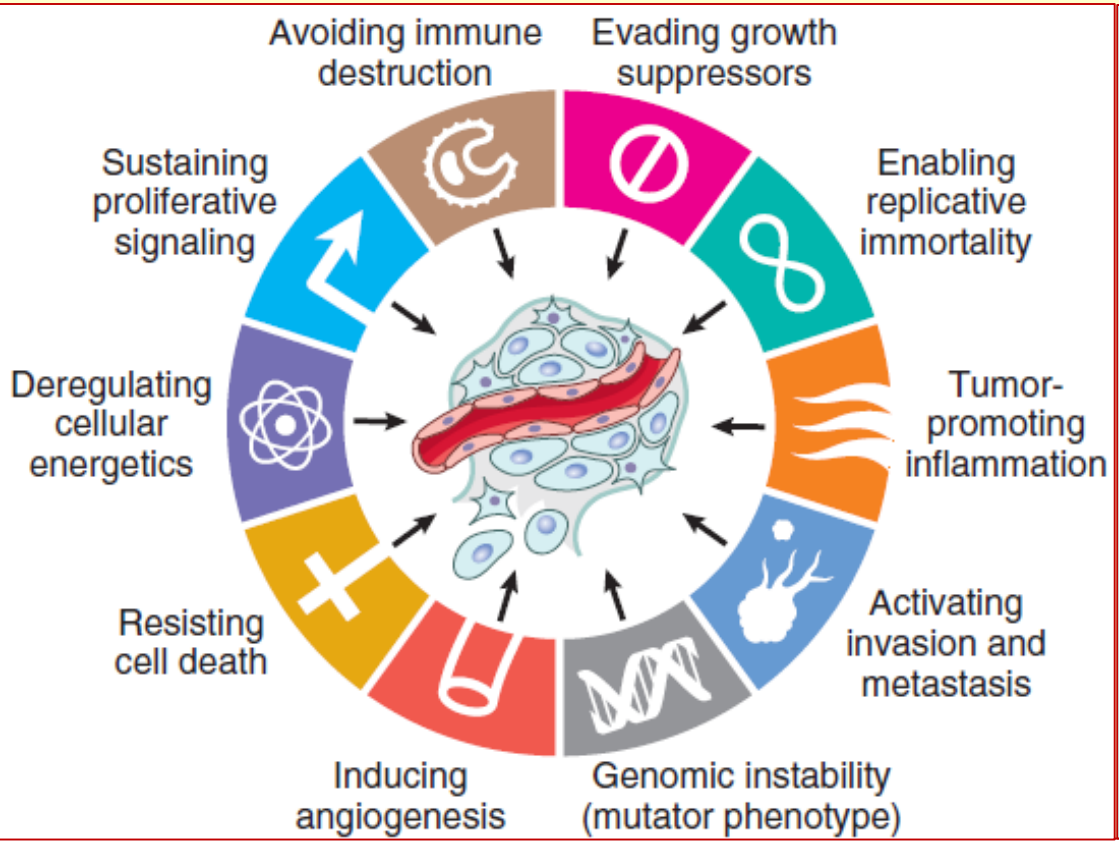


Fig. 6.17 Eight cancer hallmarks and two enabling factors (genomic instability and tumor-promoting inflammation). Most cancer cells acquire these properties during their development, typically due to mutations in critical genes. *(From Hanahan D, Weinberg RA: Hallmarks of cancer: the next generation.* Cell *144:646, 2011.)*

Self-Sufficiency in Growth Signals

Mutant alleles of protooncogenes (i.e., growth-promoting genes) are called **oncogenes** which are ***characterized*** *by the ability to promote cell growth in the absence of normal growth-promoting signals resulting in the autonomous cell growth in cancer cells.*

Oncogenes products called (**oncoproteins)**, ***resemble*** the normal products of protooncogenes (**protooncoproteins**), except that oncoproteins are devoid of important regulatory elements, & *their production in the transformed cells does not depend on the GFs or other external signals.*

To understand the nature & functions of oncoproteins, it is necessary to review the sequence of events characterize cell proliferation under **normal physiological** conditions, which can **be readily resolved into the following steps:**

(1) *The binding of a* **GF** *to its specific receptor* ***GFR*** *on the cell membrane.*

(2) *Transient & limited activation of the* **GFR**, which in turn activates several (**signal-transducing**) proteins on the inner leaflet of the plasma cell membrane

(3) *Transmission of the transduced signal across the cytosol to the* nucleus via second messengers

(4) Induction & activation of nuclear regulatory factors that *initiate* **DNA transcription,**

(5) Entry & progression of the cell into the cell cycle, resulting ultimately in **cell division**.

Now, ***we can identify the strategies*** *(ways) used by cancer cells to acquire self-sufficiency in growth signals.*

They can be grouped on the basis of their role in the signal transduction cascade & cell cycle regulation into:

(1) GF

(2) GFR

(3) Signal-transducing proteins

(4) Nuclear transcription factors,

(5) Cycling & CDK (Cyclin-dependent kinases) .

Growth Factors (GFs)

All normal cells require **GFs** stimulation to proliferate.

Most soluble GFs stimulate cell proliferation through **paracrine action** (they are made by one cell type & act on a neighboring cell).

Many cancer cells **acquire** growth self-sufficiency *by acquiring the ability to synthesize the same GF to which they are responsive, e.g.*

(a) **PDGF** secretion by **glioblastomas.**

(b) **TGF-α** produce by **sarcomas.**

(c) genes encode **FGF-3** have been detected in several **GIT & breast** tumors.

Growth Factor Receptors (GFR)

Mutation & pathological overexpression of normal forms of GFR have been detected in several tumors.

Mutant GFR proteins deliver continuous mitogenic signals to cells, even in the absence of GF in the environment.

Overexpression of GFR is more common than mutations, it render cancer cells hyper responsive to normal levels of the GF, *a level that would* ***not normally trigger proliferation***.

Best-documented examples of overexpression involve the: epidermal growth factor receptor (EGFR) family are:

(a) ERBB-1 is overexpressed in 80% of SCCa of the lung,

(b) ERBB-2, commonly called **HER2**, is overexpressed in 25% to 30% of breast cancers, & adenocarcinoma of ovary, lung & salivary glands. These tumors are very sensitive to the mitogenic effects of small amounts of GFs, &, a **high level of HER2 protein in breast cancer** cells (common test in clinical practice) is a **harbinger** (indication) of **poor prognosis.**

The significance of HER2 in the pathogenesis of breast caners is illustrated by the **clinical benefit** *derived from blocking the extracellular domain (area) of this receptor with anti-HER2 antibodies*. Treatment of breast cancer with anti-HER2 antibody is **elegant example** of "bench to bedside" medicine.

Downstream Signal-Transducing Proteins

A relatively common mechanism by which cancer cells acquire growth autonomy is by mutations in genes that encode various components of the signaling pathways.

RAS & *ABL* genes are the 2 important signaling members.

(I) ***RAS*** gene mutation is the **most common oncogene abnormality in human tumors**, seen in about **30% of all human tumors** & even higher in **colonic & pancreatic ca.**

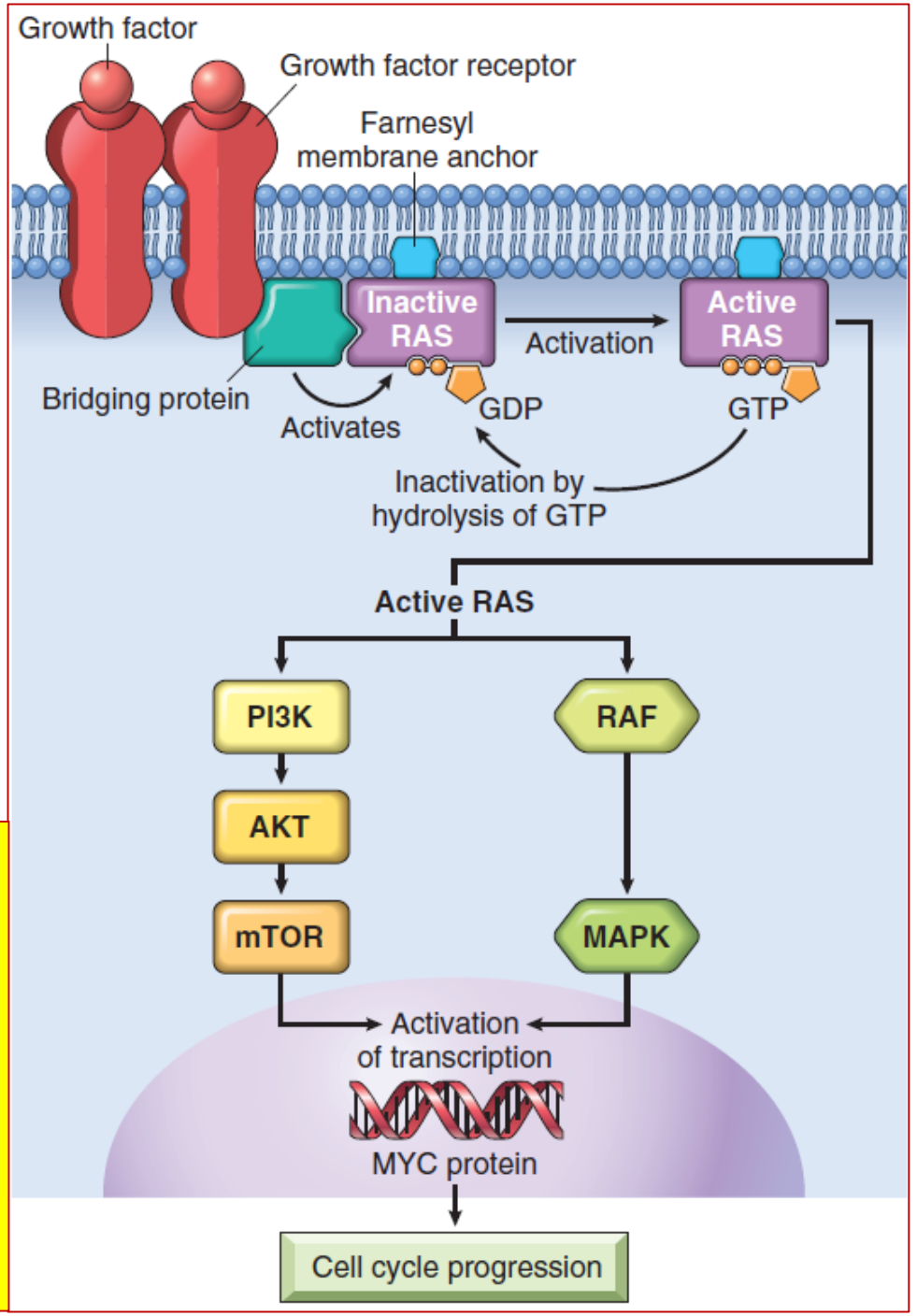


Fig. 6.18 Model for action of RAS.

Normally When a normal cell is stimulated through a GFR, the *inactive (GDP-Bound) RAS* is *activated* (phosphorylated) *to a GTP-bound state, which* recruits RAF-1 & stimulates the MPK-kinase mitogenic pathway to transmit growth-promoting signals to the nucleus for cell proliferation. Ending its function, the active GTP is inactivated by its intrinsic GTPase (which hydrolyzes GTP to GDP, releasing a phosphate group) & *returning the RAS to its inactive*, quiescent ground state (GDP-bound).

In mutant RAS protein failure of the GTPase to inactivate GTP to GDP (as in **familial neurofibromatosis type 1**) results in permanent activation & continuous stimulation of the cells without any external trigger.

The RAS gene is commonly activated by ***point mutations*** in hot spots centered around codons 12, 13, & 61.

{**Point mutation** is a type of mutation occurring at hot points along the chromosome, these are unstable points that are easily changed. By changing one nucleotide to another, one gets a whole new protein, i.e., mutant protein}.

In addition to *RAS,* several ***non-receptor-associated tyrosine kinases*** also function in signal transduction pathways. In this group, ***ABL*** is the most well defined with respect to carcinogenesis.

Normally, the ***ABL*** protooncogene has tyrosine kinase activity that is dampened (repressed) by negative regulatory domains {in addition, ABL protein localizes in the nucleus, where its role is to promote apoptosis of cells that suffer DNA damage, similar to TP53 protein}.

Pathologically, *ABL* translocation from its normal abode (house) on Chromosome 9 to Chromosome 22, t(9;22) where it fuses with part of the breakpoint cluster region *(BCR)* gene, the resultant *BCR-ABL* hybrid (mixture) gene has potent tyrosine kinase activity, & it activates several pathways, including the *RAS-RAF* cascade just described, {in addition, the *BCR-ABL* hybrid gene cannot perform the function of promoting apoptosis because it is retained in the cytoplasm}.

The *crucial double role* of ***BCR-ABL*** in transformation (the abnormal tyrosine kinase activity leading to growth autonomy & the impaired apoptosis) has been confirmed by the *dramatic clinical response of patients with* ***chronic myeloid leukemia (CML)*** *after therapy with* ***an*** **inhibitor of ABL kinase called STI 571 (Gleevec); another example** of rational **drug** design emerging from an understanding of the molecular basis of cancer.

Nuclear Transcription Factors

Ultimately, all signal transduction pathways enter the nucleus, & have an impact on the mitotic cycle. Growth autonomy may occur as a consequence of **mutation** affecting genes that regulate transcription of DNA.

A number of oncoproteins have been localized to nucleus, the ***MYC*** gene is the commonest one involved.

Normally, the MYC protein (protooncogene) is expressed in all normal cells, **It** is induced rapidly when quiescent cells receive a signal to divide. **It** binds to the DNA; causing transcriptional activation of several growth-related genes, including CDKs **(Cyclin-Dependent Kinases),** whose products derives cells into the cell cycle, ***its*** *levels* ***decline*** *to near basal level when the cell cycle begin*, **BUT**;

In *contrast*, *mutant (oncogenic version) of the MYC gene,* *are associated with* ***persistent*** *expression or overexpression*, leading to sustained proliferation.

Dysregulation of the *MYC* gene resulting from **t(8;14)** occurs in **Burkitt lymphoma.**

MYC gene is **amplified in colon, lung , breast** & many other cancers; The related *N-MYC* & *L-MYC genes* are amplified in **neuroblastomas** & **SCCL** respective.

Cyclins & Cyclin-Dependent Kinases (CDKs)

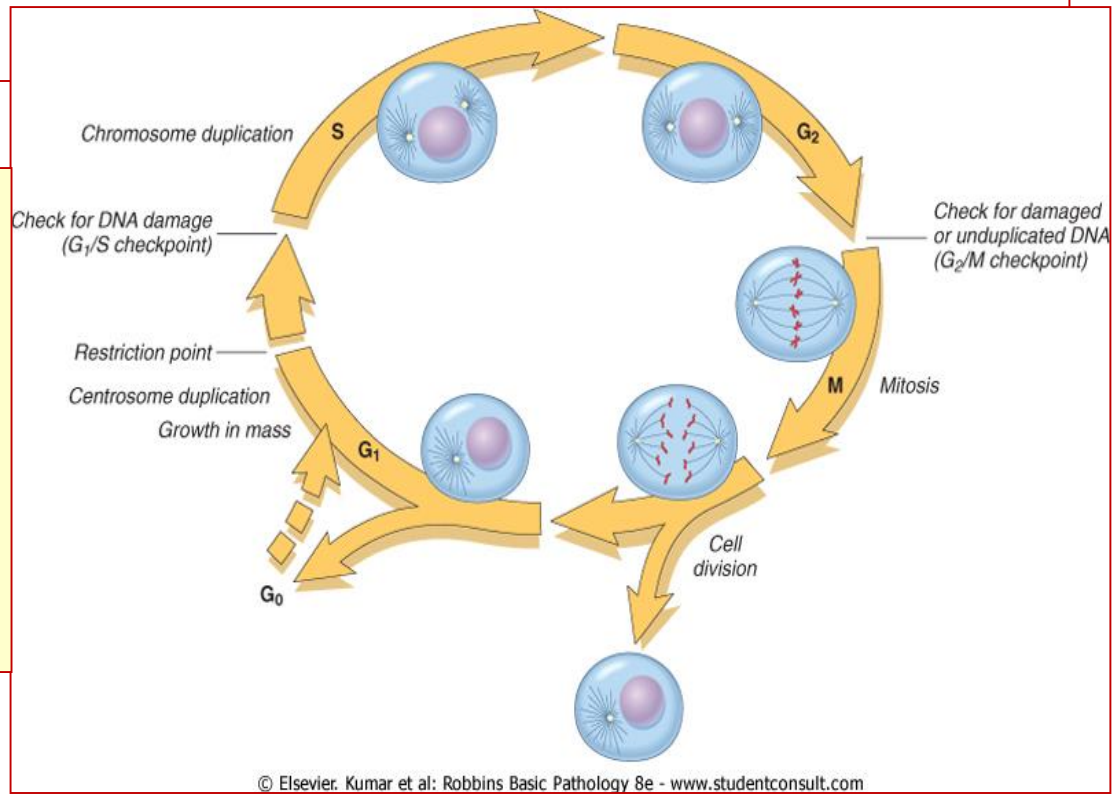
The ultimate outcome of all growth-promoting stimuli is the entry of quiescent cells into the cell cycle (from G0 to⟶ G1).

Cells may become autonomous if the genes that drive the cell cycle become dysregulated by amplification or mutations.

Normally, various **cyclins (proteins)** are synthesized during specific phases of the cell cycle & their function is to activate the CDKs by binding to them.

After completing this task, the cyclin levels decline rapidly (called cyclins because of their cyclic production & degradation).

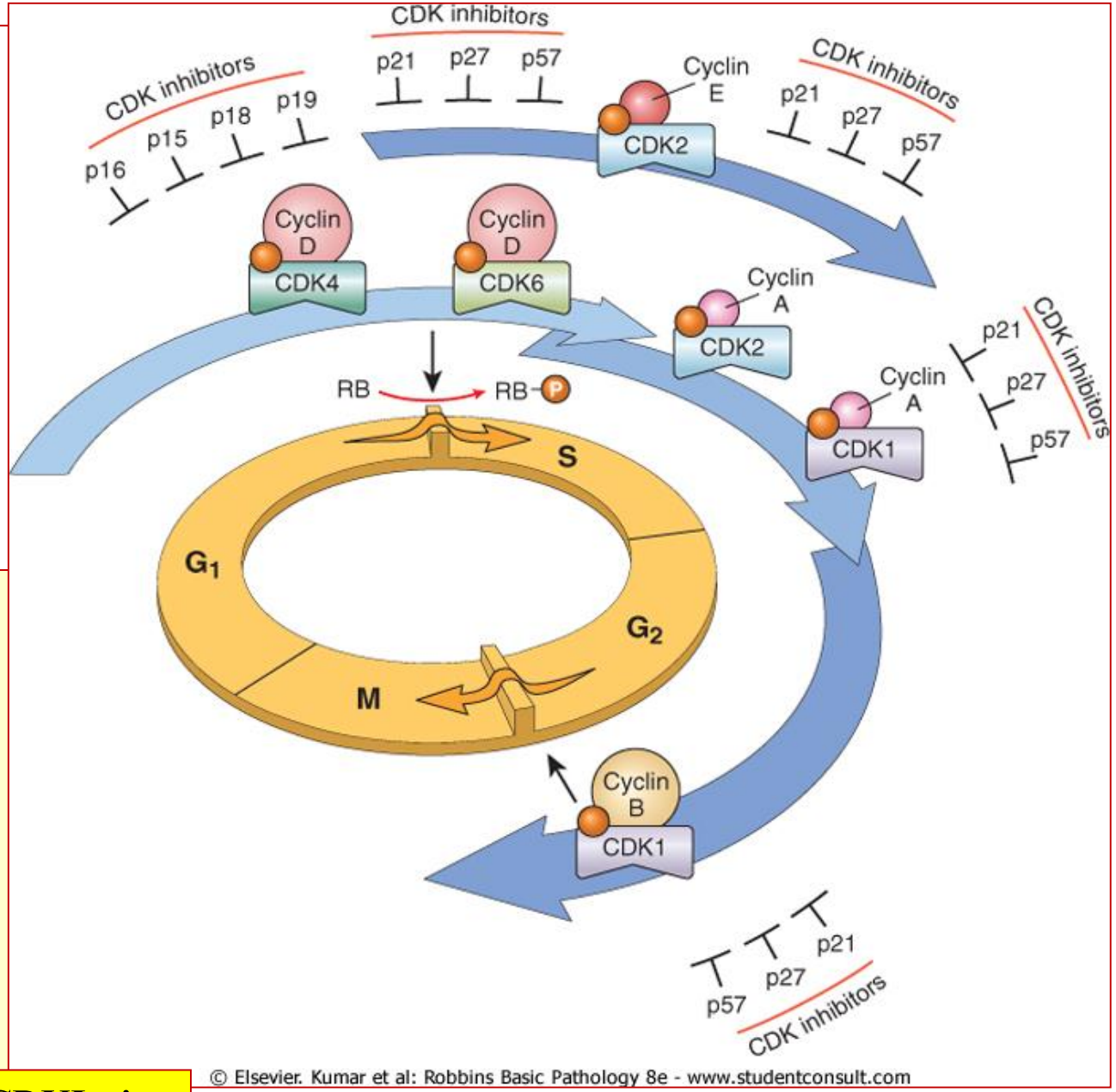
While cyclins activate CDKs, many inhibitors (CDKIs) silence the CDKs & exert negative control over the cell cycle.



Normally, although each phase of the cell cycle is monitored carefully, the transition from G1 to S is believed to be an *extremely important checkpoint in the cell cycle clock:*

when a cell encounters growth-promoting signals, cyclins D levels go up & CDK4 & CDK6 are activated.

The G1 to S checkpoint is guarded by a retinoblastoma protein pRB, & phosphorylation of pRB by the cyclin D/CDK4 & cyclin D/CDK6 overcomes the G1 to S obstacle & allows entry of cells into the DNA synthetic phase. S to G2 progress is facilitated by the up regulation of cyclin A, which binds to CDK2 & to CDK1, G2 to M progress is helped by the complexes cyclin B/CDK1.



Role of cyclins, CDKs, & CDKIs, in regulating the cell cycle.

Normally, the activity of CDKs is regulated by two families of ***CDK inhibitors (CDKIs***), one family, called CDKN1A inhibits the CDKs broadly, whereas the other family of CDKIs has selective effects on the cyclin D/CDK4 & cyclin D/CDK6, this second family is called sometimes called **INK4**.

Pathologically, mutations that dysregulated the activity of cyclins & CDKs would favor cell proliferation, & particularly those affecting cyclin D or CDK4 seem to be a common event in neoplastic transformation.

The *cyclin D genes are overexpressed in many cancers, including those of breast, esophagus, liver & lymphomas*.

Amplification of the *CDK4 gene occurs in melanomas, sarcomas, & glioblastomas*.

Mutations affecting cyclin B, cyclin E, & other CDKs, are much less frequent than those affecting cyclin D/CDK4

Insensitivity to Growth Inhibitory Signals: Tumor Suppressor Genes

Isaac Newton predicted that **every action has an equal & opposite reaction,** a formulation holds true for cell growth**.**

Although **oncogenes** encode (instruct, programmed) proteins that **promote** cell growth, the products of **tumor suppresser genes** apply breaks to cell proliferation.

Disruption of such genes mimics the growth-promoting effects of oncogenes.

Cancer suppressor genes are 4:

(1) Retinoblastoma (RB) gene,

(2) TGF-β,

(3) Adenomatous Polyposis Coli (APC) gene – β Catenin pathway,

(4) TP53 gene.

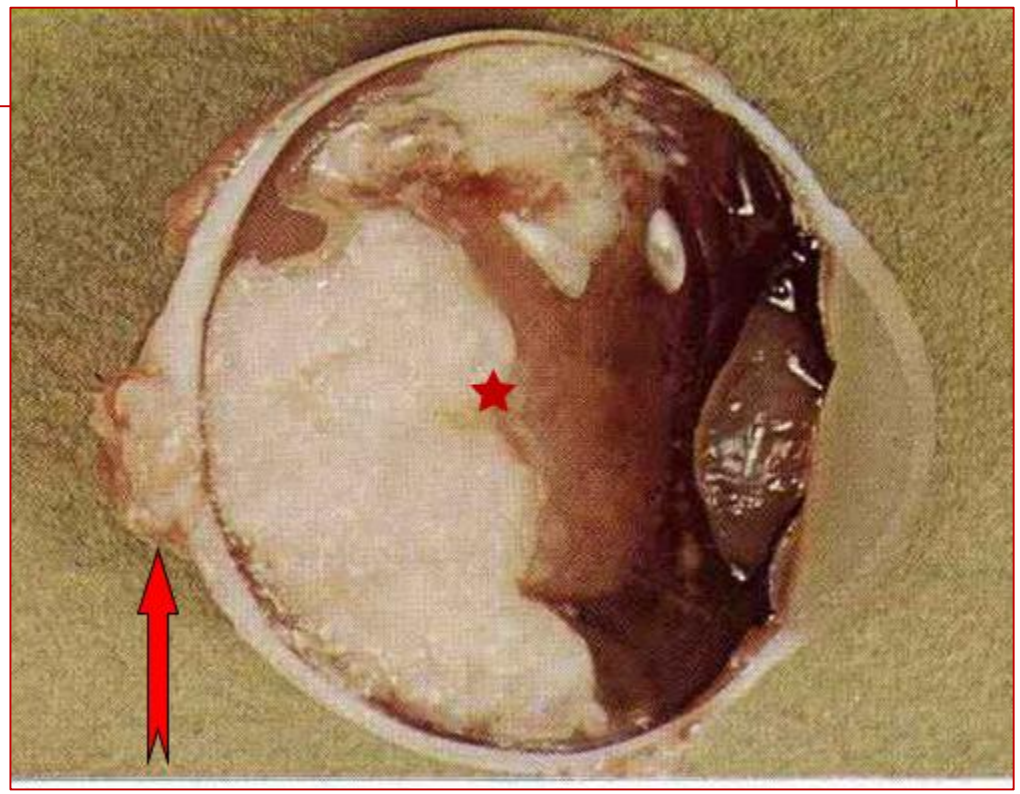
Retinoblastoma (*RB*) gene

The first cancer suppressor gene to be discovered**.**

Retinoblastoma, uncommon childhood cancer, 60% of cases are sporadic, & 40% are familial, the predisposition to this cancer being **transmitted as an autosomal dominant trait,** *but the action of the gene is recessive!*. One must have double mutation of both gene alleles to be abnormal, for the disease to present. How?

Retinoblastoma: eye. The vitreous (posterior chamber of the eye) is infiltrated extensively by a ★ gelatinous greyish-white tumor.

The tumor arises from retinal cell incurred homozygous loss of both normal *RB* genes. Tumor extends down into the optic nerve (left, arrow) with eventual direct intracranial spread.



Knudson (1974) proposed his famous ***two-hit hypothesis:*** {Two mutations (*hits*) are required to produce retinoblastoma. These involve the *RB* gene, located on Ch13q14. Both of the normal alleles of the *RB* locus must be inactivated (two hits) for the development of retinoblastoma.

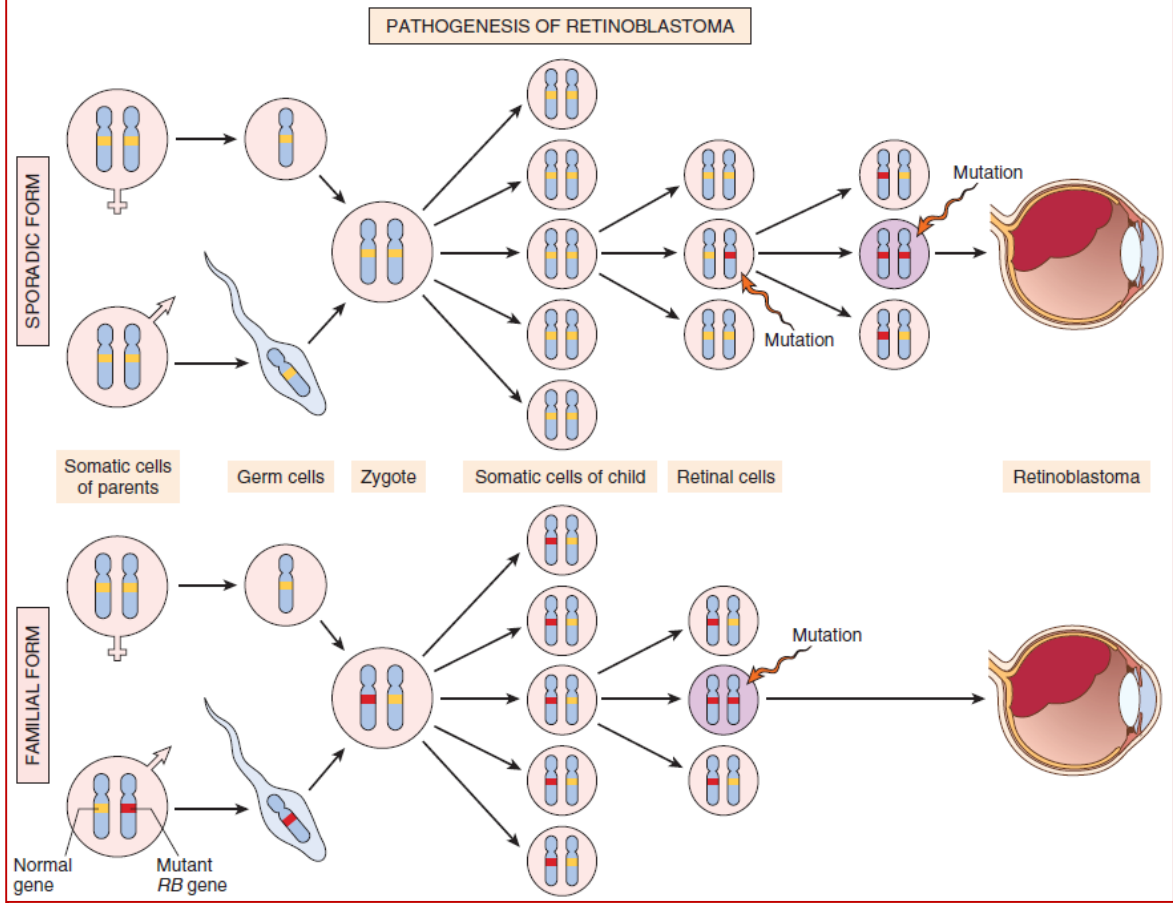
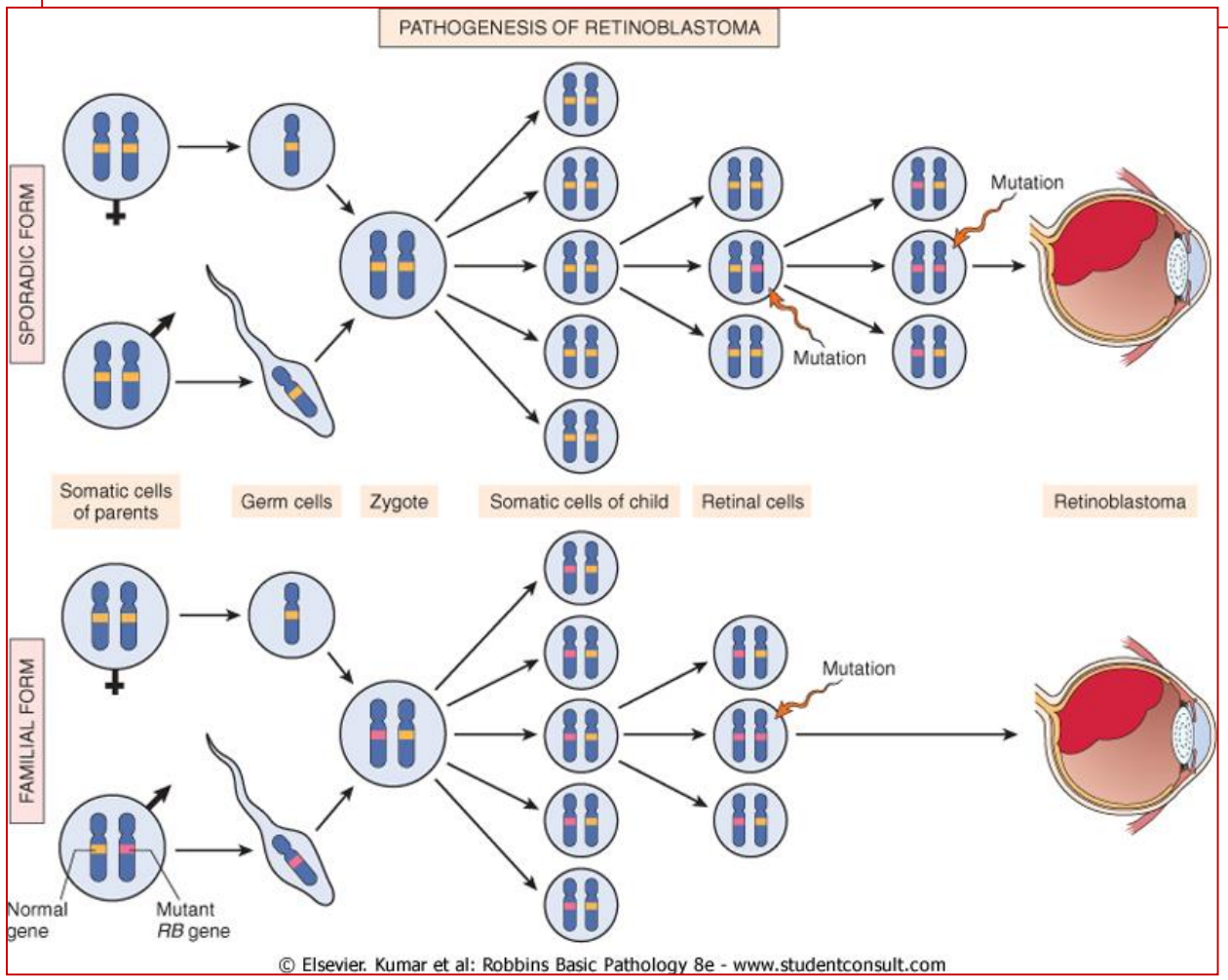


Fig. 6.19 Pathogenesis of retinoblastoma. Two mutations of the *RB* chromosomal locus, on 13q14, lead to neoplastic proliferation of the retinal cells. In the sporadic form, both *RB* mutations in the tumor-founding retinal cell are acquired. In the familial form, all somatic cells inherit one mutant *RB* gene from a carrier parent, and as a result only one additional *RB* mutation in a retinal cell is required for complete loss of *RB* function.

In the familial cases: Children inherit one defective copy of the RB gene from a carrier parent in the germ line (zygote) & thereafter *in all their somatic cells.* The other copy is normal. **If after birth**, a second somatic mutation or hit affects the RB locus of the retinal cells (retinoblasts), retinoblastoma develops. **Because in retinoblastoma** families only a single somatic mutation is required for the expression of the cancer the familial transmission follows an autosomal dominant inheritance pattern

Patients with familial retinoblastoma are, also, at greatly increased risk of developing osteogenic & soft tissue sarcomas.

In sporadic cases, *both normal RB alleles are lost by somatic mutation in one of the retinoblasts.* The **end result** is the same: A retinal cell that has lost both of the normal copies of the *RB* gene develops retinoblastoma.



Although homozygous loss of normal *RB* genes was discovered initially in retinoblastomas, it is now evident that such a loss is a fairly common event in several cancers, including breast, bladder, & SCLC.

Because neoplastic transformation is associated with loss of both of the normal copies of the RB gene, this & other cancer suppressor genes also are called ***recessive cancer genes.***

In principle, **antigrowth** signals can prevent cell proliferation by two mechanisms;

(1) It may cause dividing cells **to go into G0** (quiescence), where they remain until external stimulus their reentry into the proliferative pool, or

(2) the cell may enter a **postmitotic** differentiated pool & lose replicative potential.

RB Gene & Cell Cycle

Much is known about the *RB* gene because this was the first Tumor suppressor gene discovered.

Normally *,the RB gene product is a DNA-binding protein that is expressed in every cell type examined, where it exists in* ***an active hypophosphorylated & an inactive hyperphosphrylated state.***

In its active state, **RB gene serve as a brake**, in the advancement of cells from G1 to S phase of the cell cycle.

When the cells are stimulated by GFs, the RB protein is inactivated by phosphorylation, the brake is released & the cells transverse & cross the G1 ⟶ S checkpoint.

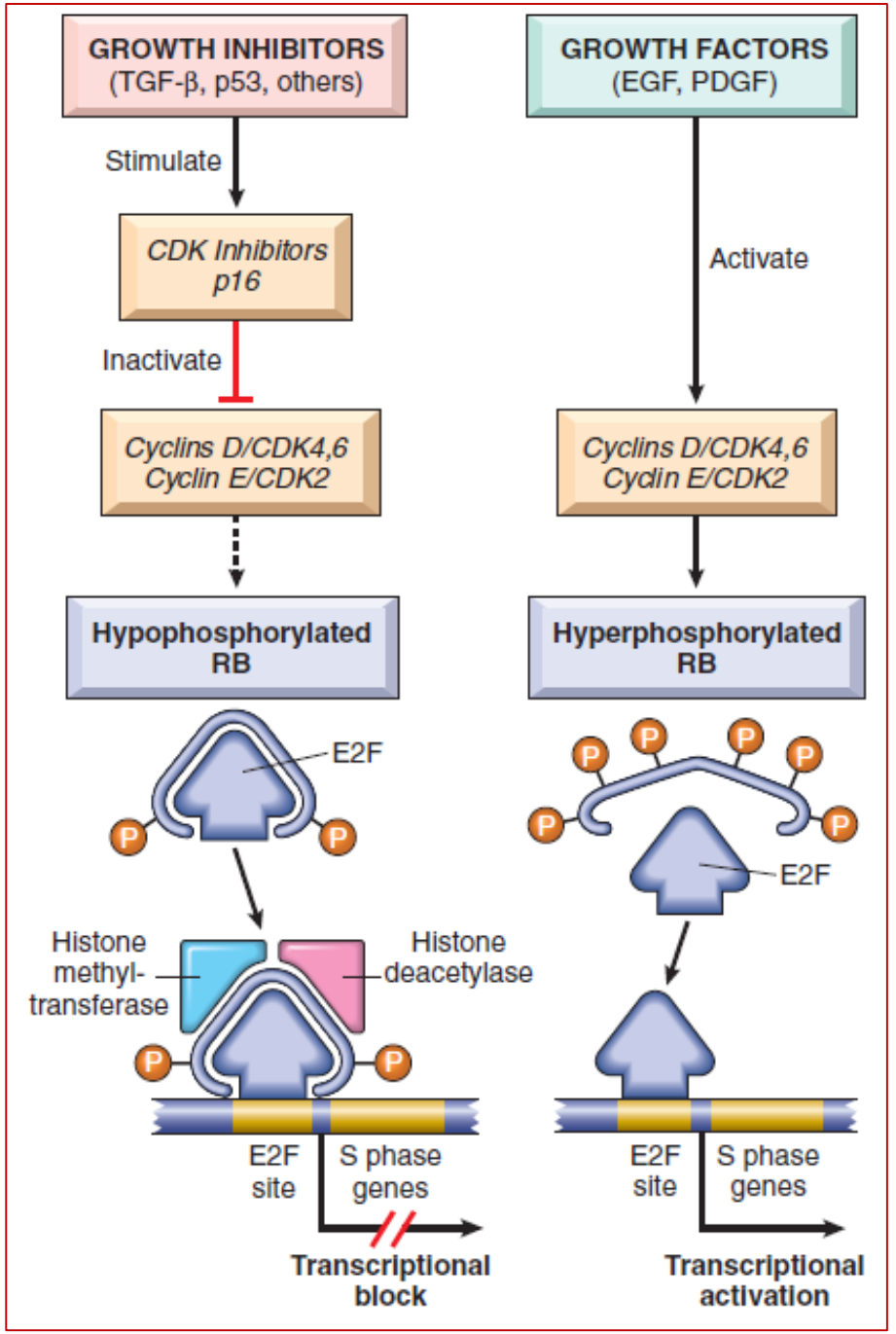
When the cells enter S phase, they are committed (forced) to divide without additional growth factor stimulation.

Fig. 6.20 The role of RB in regulating the G1–S checkpoint of the cell cycle. Hypophosphorylated RB in complex with the E2F transcription factors binds to DNA, recruits chromatin remodeling factors (histone deacetylases and histone methyltransferases), and inhibits transcription of genes whose products are required for the S phase of the cell cycle. When RB is phosphorylated by the cyclin D–CDK4, cyclin D–CDK6, and cyclin E–CDK2 complexes, it releases E2F.

The latter then activates transcription of S-phase genes.

The phosphorylation of RB is inhibited by CDKIs, because they inactivate cyclin-CDK complexes. Virtually all cancer cells show dysregulation of the G1–S checkpoint as a result of mutation in one of four genes that regulate the phosphorylation of RB; these genes are *RB, CDK4, cyclin D, and CDKN2A [p16].*

EGF, Epidermal growth factor; *PDGF,* platelet-derived growth factor.



When the quiescent cells are stimulated by GF, the concentrations of D & E cyclins go up & the resultant activation of cycling, D/CDK4, D/CDK6,& E/CDK2 leads to phosphorylation of RB, this hyperphosphorylated form of RB releases the E2F transcription factors & activates the transcription of several target genes.

If the RB protein is **absent,** or if it is **mutated**, e.g., through **binding with HPV oncogenic protein**, (so that it is unable to sequester E2F transcription factors, & is functionally deleted), the result is the same, the molecular brakes on the cell cycle are released, & the cells move to S phase.

Mutations in other genes that control RB phosphorylation can mimic the effect of RB loss; such genes are mutated in many cancers that seem to have normal *RB* genes.

Examples: **mutational activation** of cyclin D or CDK4 would favor RB phosphorylation & cell proliferation.

Cyclin D is overexpressed in many tumors because of gene amplification or translocation.

Mutational inactivation of the inhibitors {CDKIs} also would drive the cell cycle by removing the inhibition over the activity of cyclins & CDKs. Deletion or mutational inactivation of *CDKN2A gene* is **extremely common** in human cancers, e.g., it is seen in 75% of pancreatic ca, 40%-70% of glioblastomas; 50% of esophageal ca; & 20% of non-SCCL, bladder ca, & soft tissue sarcomas.

In summary: loss of normal cell cycle control is central to malignant transformation &, at least one of the 4 key regulators of cell cycle (Cyclin D, CDK4, CDKN2A, & *RB*) is mutated in most human cancers.

In cells that harbor mutational activation of cyclin D, CDK4, or mutational inactivation of CDKN2A, the function of the *RB* gene is disrupted (stopped, or becomes functionally inactive) even if the *RB* gene is normal or not mutated!

TP53 Gene: Guardian of The Genome

The TP53 tumor suppressor gene is one of the most commonly mutated genes in human cancers.

TP53 can exert:

(1) Antiproliferative effects, but equally important it,

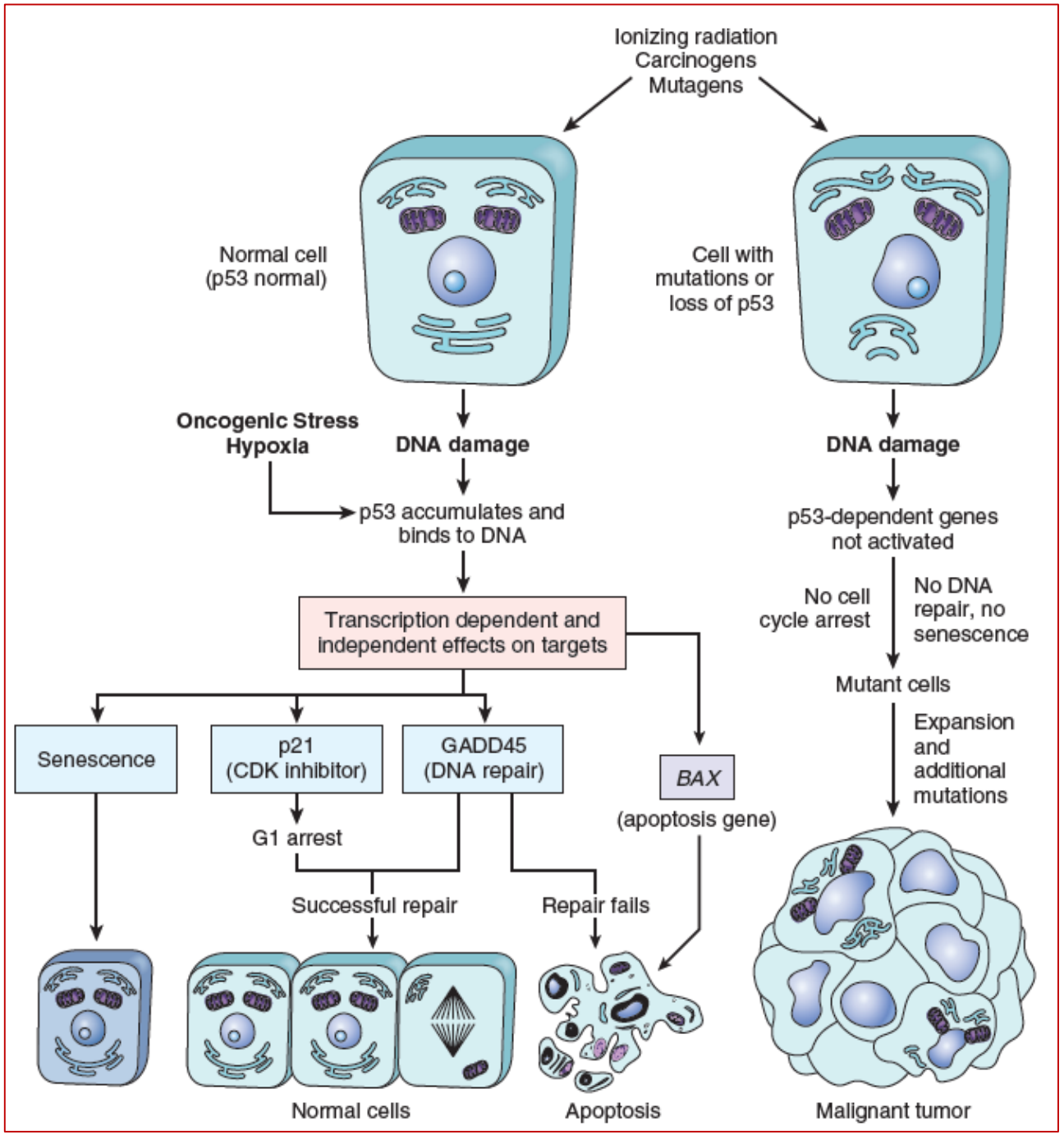
(2) Regulates apoptosis.

NORMALLY, TP53 in non-stressed cells has a short half-life (20 minutes). This short half-life is due to an association with MDM2, a protein that targets it for destruction. When the cell is stressed, such as by an assault on DNA (e.g. ionizing radiation, carcinogen, or mutagens), TP53 undergoes modification that **(1)** release it from MDM2 & increase its half-life, & **(2)** activating it as a transcriptional factor for dozens of genes, grouped in into two categories:

Fig. 6.21 The role of p53 in maintaining the integrity of the genome.

Activation of normal p53 by DNA-damaging agents or by hypoxia leads to cell cycle arrest in G1 and induction of DNA repair, by transcriptional upregulation of the cyclin-dependent kinase inhibitor CDKN1A (p21) and the *GADD45* genes.

Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, p53 triggers either apoptosis or senescence. In cells with loss or mutations of TP53, DNA damage does not induce cell cycle arrest or DNA repair, and genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.



Cell cycle arrest occurs late in the G1 phase & is caused mainly by ***TP53***-dependent transcription of the *CDKN1A (p21), also called INK4* which **inhibits** cyclin D/CDK complexes & prevents phosphorylation of RB essential for cells to enter G1 phase. Such a pause in cell cycling is welcome because it gives the cell "breathing time" to repair DNA damage.

If DNA damage is repaired successfully, *TP*53 up-regulates transcription of MDM2, which then down-regulates TP53 (ending it life), relieving cell cycle block.

If during the pause DNA damage cannot be successfully repaired, normal TP53 directs the cell to the graveyard by triggering apoptosis, by inducing apoptosis- inducing genes such as BAX & BAK.

How *TP*53 senses DNA damage? & how it determines the adequacy of DNA repair?

is unknown! {*TP53* → to DNA damaged cell → Stop → Repair your DNA → If you can, then O.K.; go on through your cycle,
BUT → if you cannot, → Kill yourself } = Stop--Repair- or Die.

In view of these activities, TP53 has been rightfully called a "GUARDIAN OF THE GENOME".

With homozygous loss of TP53: DNA damage goes unrepaired, mutations become fixed in dividing cells, & the cell turns onto a one-way street leading to cancer.

More than **70% of human cancers have a defect *TP53* gene**, & the remaining have defects in genes up-stream or down-stream of *TP53*. *Homozygous loss of the TP53 gene is found in virtually every type of cancer, including the three leading causes of cancer deaths;* ***Ca. of the lung, colon, & breast.***

In most cases, the inactivating mutations affecting both *TP53* alleles are **acquired** in somatic cells.

Less commonly, **some individuals inherit a mutant TP53 allele**. Such inheritance predisposes individuals to develop cancers because only one additional ***hit*** is needed to inactivate the 2nd normal allele {**Knudson 2-*hit hypothesis***}.

Such individuals, said to have the ***Li-Fraumeni syndrome***, *have a X* ***25-fold*** *greater chance of developing a cancer by age 50* compared with the general population.

In contrast to patients who inherit a mutant *RB* allele, the spectrum of Tumors that develop in patients with Li-Fraumeni syndrome is varied; the most common types of Tumors are Ca. of breast,& adrenal cortex, sarcomas, leukemia,& brain tumors.

As with RB protein, normal TP53 also can be rendered nonfunctional by certain DNA viruses. Proteins encoded by oncogenic **HPVs, HBV**, & possibly **EBV** can bind to normal TP53 proteins & nullify their protective functions.

Thus, DNA viruses can subvert (destroy or damage) two of the best –understood tumor suppressor genes, RB & TP53.

Transforming Growth Factor β Pathway

TGF-β is a potent inhibitor of cell proliferation, mainly of most normal epithelial, endothelial, & hematopoietic cells, through three receptors, called types I, II, & III.

The anti-proliferative effects of TGF-β are mediated in large part by regulating the RB pathways.

TGF-β arrest cells in the G1 phase of the cell cycle by stimulating production of the CDKI p15 & by inhibiting the transcription of CDK2, CDK4, cyclins A & E.

These changes result in decrease phosphorylation (activation) of RB protein & cell cycle arrest.

All pancreatic cancers & 83% of **colonic** cancers, at least one of the three types of the TGF-β pathway is mutated.

Contact Inhibition, NF2, and APC

When cancer cells are grown in the laboratory, their proliferation fails to be inhibited when they come in contact with each other.

Cell–cell contacts in many tissues are mediated by homodimeric interactions between transmembrane proteins called *cadherins*. E-cadherin (E for *epithelial*) mediates cell–cell contact in epithelial layers. Two mechanisms have been proposed to explain how E-cadherin maintains contact inhibition:

One mechanism is mediated by the tumor suppressor gene *NF2*. Its product, neurofibromin-2, more commonly called *merlin,* acts downstream of E-cadherin in a signling pathway that helps for maintain contact inhibition. Homozygous loss of *NF2* is known to cause certain neural tumors, and germ line mutations in *NF2* are associated with a tumor-prone hereditary condition called *neurofibromatosis type 2.*

A second mechanism by which E-cadherin may regulate contact inhibition involves its ability to bind β-catenin, another signaling protein.

β-catenin is a key component of the WNT signaling pathway, which has broad but as of yet incompletely understood roles in regulating the morphology and organization of epithelial cells lining structures such as the gut.

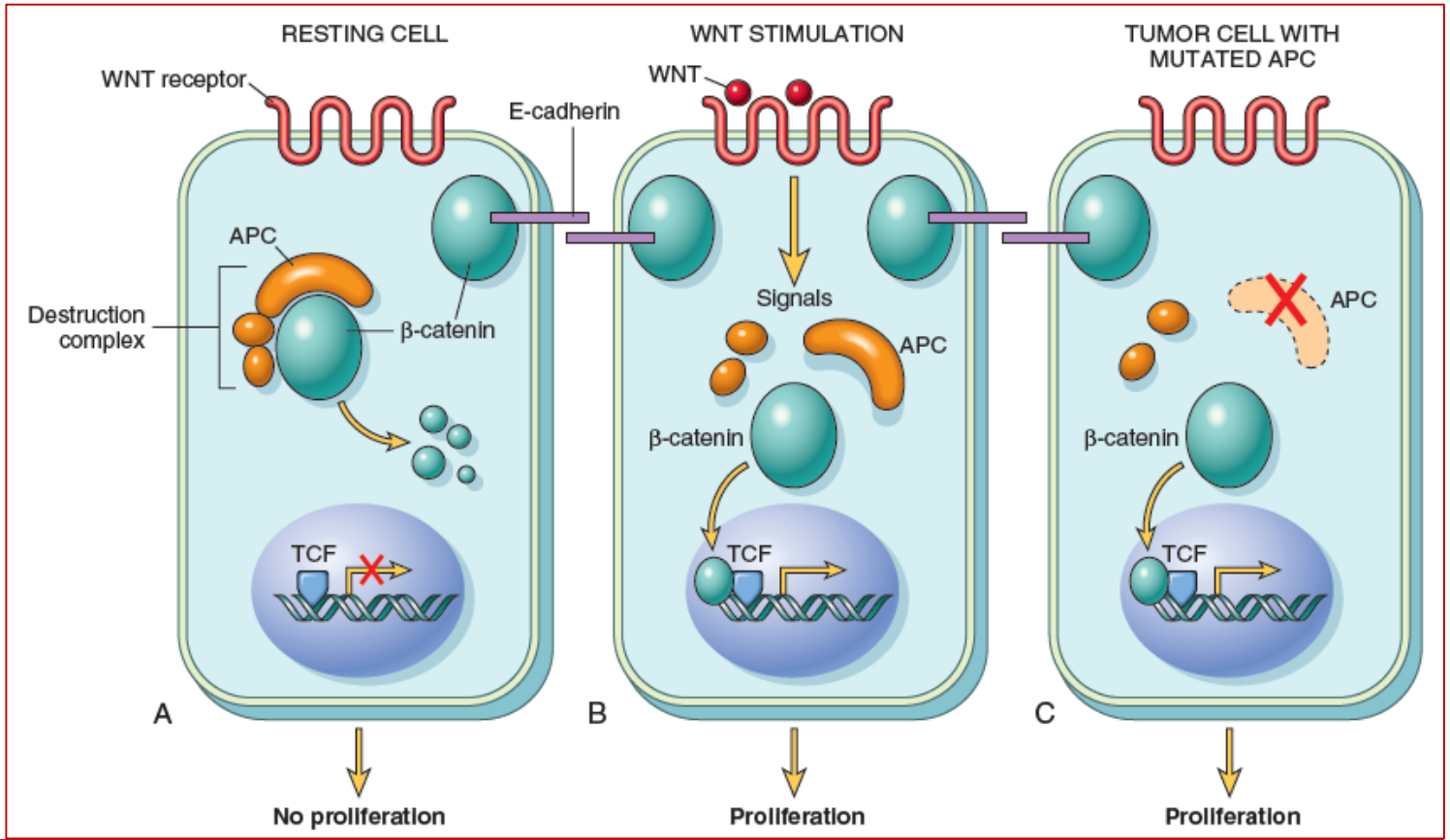
A further clue to the important of E-cadherin and β-catenin in epithelial cancers is illustrated by the rare hereditary disease ***adenomatous polyposis coli* (APC).**

This disorder is characterized by the development of numerous adenomatous polyps in the colon that have a very high incidence of transformation into colonic cancers.

The *APC* gene, the loss of which is common in colonic Ca. (commonest cancer in Jordanian males & 2nd commonest in Jordanian females, after breast cancer), exerts anti-proliferative effects in **an unusual** manner.

APC is a cytoplasmic protein whose dominant function is to regulate the intracellular levels of β-catenin, whose functions: **(1)** β-catenin binds to the cytoplasmic portion of E-cadherin, a cell surface protein that maintains intracellular adhesiveness, **(2)** β-catenin can translocate to the nucleus, & **activate cell proliferation.** Catenin is an important component of the WNT signaling pathway.

Fig. 6.22 The role of APC in regulating the stability and function of β-catenin. APC and β-catenin are components of the WNT (Wingless/Integrated) signaling pathway. **(A)** In resting cells (not exposed to WNT), β-catenin forms a macromolecular complex containing the APC protein. This complex leads to the destruction of β-catenin, and intracellular levels of β-catenin are low.



(B) When cells are stimulated by secreted WNT molecules, the destruction complex is deactivated, β-catenin degradation does not occur, and cytoplasmic levels increase. β-Catenin translocates to the nucleus, where it binds to TCF, a transcription factor that activates several genes involved in the cell cycle.

(C) When APC is mutated or absent, the destruction of β-catenin cannot occur. β-Catenin translocates to the nucleus and coactivates genes that promote the cell cycle, and cells behave as if they are under constant stimulation by the WNT pathway.

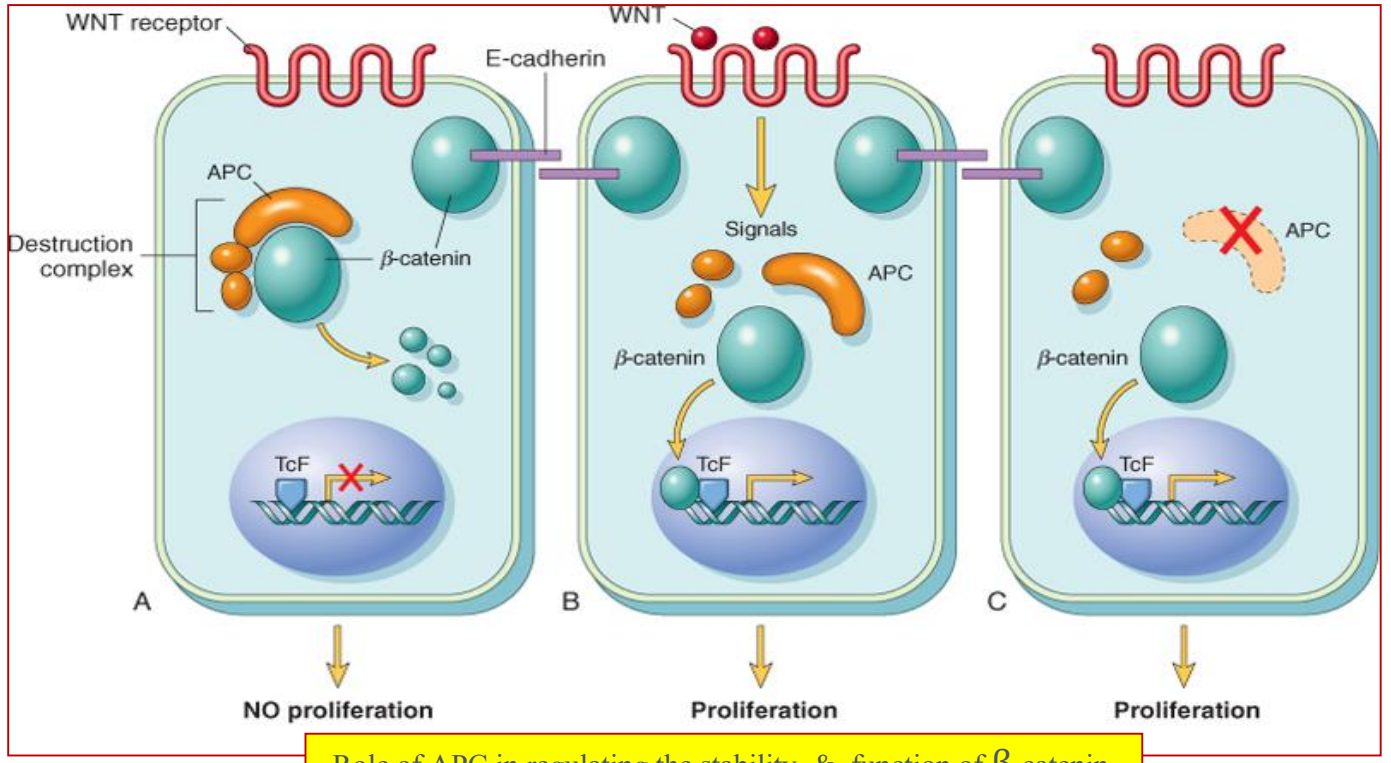
WNT is a soluble factor that can induce cellular proliferation.

It does so by:

(a) binding to its receptor, &

(b) transmitting signals that prevent the degradation of β-catenin, allowing it to translocate to the nucleus, where

(c) it acts as a transcriptional activator in conjunction with other molecule, called TcF.



Role of APC in regulating the stability & function of β-catenin.

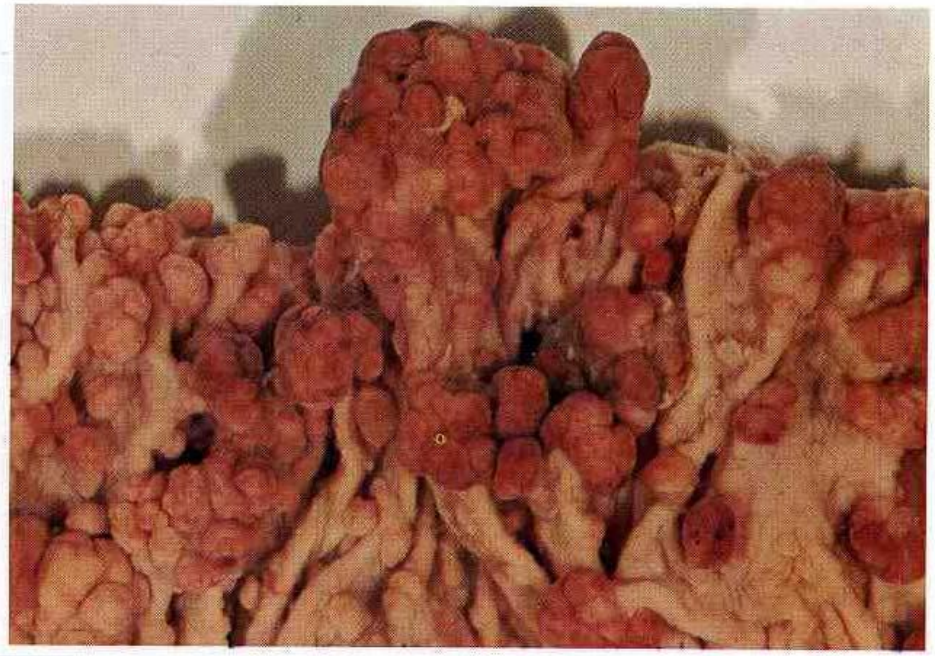
Normally, in quiescent cells, which are not exposed to WNT, cytoplasmic β-catenin is degraded by a ***destruction complex,*** of which **APC** is an **integral part**. In resting normal cells, **APC** prevents β-catenin signaling by favoring its destruction (A).

In malignant cells, with loss of **APC, β-catenin degradation is prevented (C)** , the WNT signaling response is **continually activated**. This leads to transcription of growth-promoting genes, such as cyclin D1 & *MYC*.

APC behaves as a typical T suppressor gene. Individuals born with one mutant allele develop hundreds to thousands (at least 200) of adenomatous polyps in the colon during their teens or 20s (Multiple Familial Adenomatous Polyposis Coli).

In All patients (100%), one or more polyps develop carcinoma.

Multiple Familial Adenomatous Polyposis Coli: Colon. The colonic mucosa is covered with large number of polypoid adenomas of various sizes. The large pedunculated polyp (top center, arrow) has undergone carcinomatous change.



Familial adenomatous polyposis: colon

As with other Tumor suppressor genes, both copies of the **APC** gene must be lost for T development.

Remember *additional mutations must occur for colonic Ca. to develop.*

APC mutations are seen in 70% to 80% of sporadic colon carcinomas. Colonic cancers that have normal **APC** genes, show activating mutation of β-catenin that render them refractory to the degrading action of **APC.**

Altered Cellular Metabolism

Even in the presence of ample oxygen, cancer cells demonstrate a distinctive form of cellular metabolism characterized by high levels of glucose uptake and increased conversion of glucose to lactose (fermentation) via the glycolytic pathway. This phenomenon, called the *Warburg effect* and also known as *aerobic glycolysis*.

Why is it advantageous for a cancer cell to rely on seemingly inefficient glycolysis (which generates two molecules of ATP per molecule of glucose) instead of oxidative phosphorylation (which generates up to 36 molecules of ATP per molecule of glucose)?

Aerobic glycolysis provides rapidly dividing tumor cells with metabolic intermediates that are needed for the synthesis of cellular components, whereas mitochondrial oxidative phosphorylation does not.

A growing cell has a strict biosynthetic requirement; it must duplicate all of its cellular components (DNA, RNA, proteins, lipid, and organelles) before it can divide and produce two daughter cells.

While oxidative phosphorylation yields abundant ATP, it fails to produce any carbon moieties that can be used to build the cellular components needed for growth (proteins, lipids, and nucleic acids).

By contrast, in actively growing cells only a small fraction of the cellular glucose is shunted through the oxidative phosphorylation pathway, such that on average each molecule of glucose metabolized produces approximately four molecules of ATP.

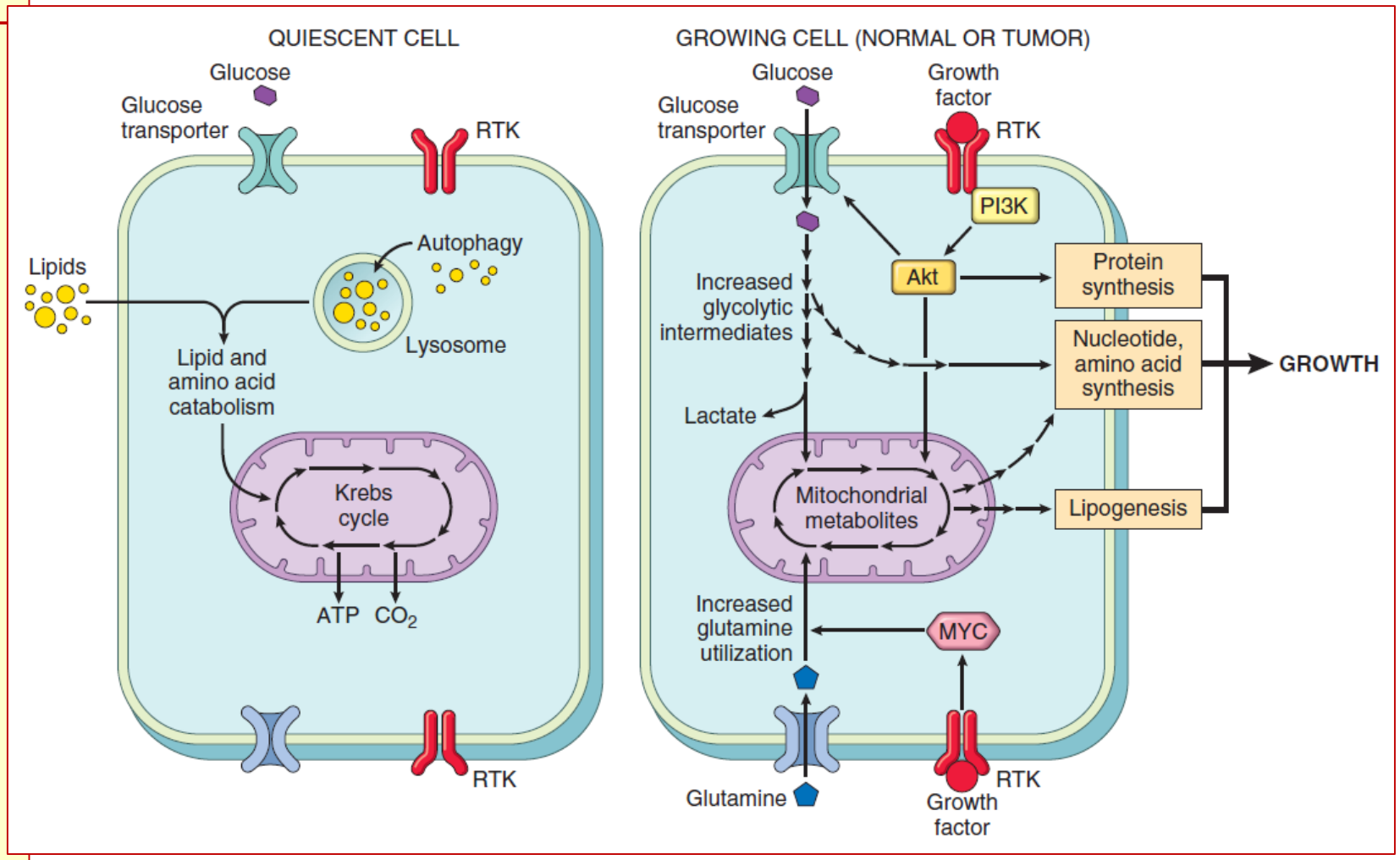
So how is this profound reprogramming of metabolism, the Warburg effect, triggered in growing normal and malignant cells?

Metabolic reprogramming is produced by signaling cascades downstream of growth factor receptors, the very same pathways that are deregulated by mutations in oncogenes and tumors suppressor genes in cancers.

Thus, whereas in rapidly dividing normal cells aerobic glycolysis ceases when the tissue is no longer growing, in cancer cells this reprogramming persists due to the action of oncogenes and the loss of tumor suppressor gene function.

Fig. 6.23 Metabolism and cell growth.

Quiescent cells rely mainly on the Krebs cycle for ATP production; if starved, autophagy (self-eating) is induced to provide a source of fuel. When stimulated by growth factors, normal cells markedly upregulate glucose and glutamine uptake, which provide carbon sources for synthesis of nucleotides, proteins, and lipids. In cancers, oncogenic mutations involving growth factor signaling pathways and other key factors such as MYC deregulate these metabolic pathways, an alteration known as the *Warburg effect.*



Tumor suppressors often inhibit metabolic pathways that support growth (the “braking” effect of the tumor suppressors NF1 and PTEN on signals downstream of growth factor receptors and RAS, allowing them to oppose the Warburg effect.).

Indeed, it may be that many (and perhaps all) tumor suppressors that induce growth arrest suppress the Warburg effect. For example, p53, arguably the most important tumor suppressor, upregulates target genes that collectively inhibit glucose uptake, glycolysis, lipogenesis, and the generation of NADPH (a key cofactor needed for the biosynthesis of macromolecules)

Autophagy **is a state of severe nutrient deficiency in which cells not only arrest their growth, but also cannibalize their own organelles, proteins, and membranes as carbon sources for energy production**

Oncometabolism

Another group of genetic alterations are mutations in enzymes that participate in the Krebs cycle. Of these, mutations in **isocitrate dehydrogenase (IDH)** (Fig. 6.24).

The proposed steps in the oncogenic pathway involving IDH are as follows:

- IDH acquires a mutation that leads to a specific amino acid substitution involving residues in the active site of the enzyme. As a result, the mutated protein loses it ability to function as an isocitrate dehydrogenase and instead acquires a new enzymatic activity that catalyzes the production of 2-hydroxglutarate (2-HG).
- 2-HG in turn acts as an inhibitor of several other enzymes that are members of the TET family, including TET2.
- TET2 is one of several factors that regulate DNA methylation, which you will recall is an epigenetic modification that controls normal gene expression and often goes awry in cancer. According to the model, loss of TET2 activity leads to abnormal patterns of DNA methylation.
- Abnormal DNA methylation in turn leads to misexpression of currently unknown cancer genes, which drive cellular transformation and oncogenesis.

Evasion of Cell Death (avoidance of Apoptosis)

Tumor cells frequently contain mutations in genes that regulate apoptosis, making the cells resistant to cell death.

There are two pathways that lead to apoptosis: the extrinsic pathway, triggered by the death receptors FAS and FAS-ligand; and the intrinsic pathway (also known as the *mitochondrial pathway*), initiated by perturbations such as loss of growth factors and DNA damage. Cancer cells are subject to a number of intrinsic stresses that can initiate apoptosis, particularly DNA damage, but also metabolic disturbances stemming from dysregulated growth as well as hypoxia caused by insufficient blood supply.

Evasion of apoptosis by cancer cells occurs mainly by way of acquired mutations and changes in gene expression that disable key components of the intrinsic pathway, or that reset the balance of regulatory factors so as to favor cell survival in the face of intrinsic stresses (Figure 6.25).

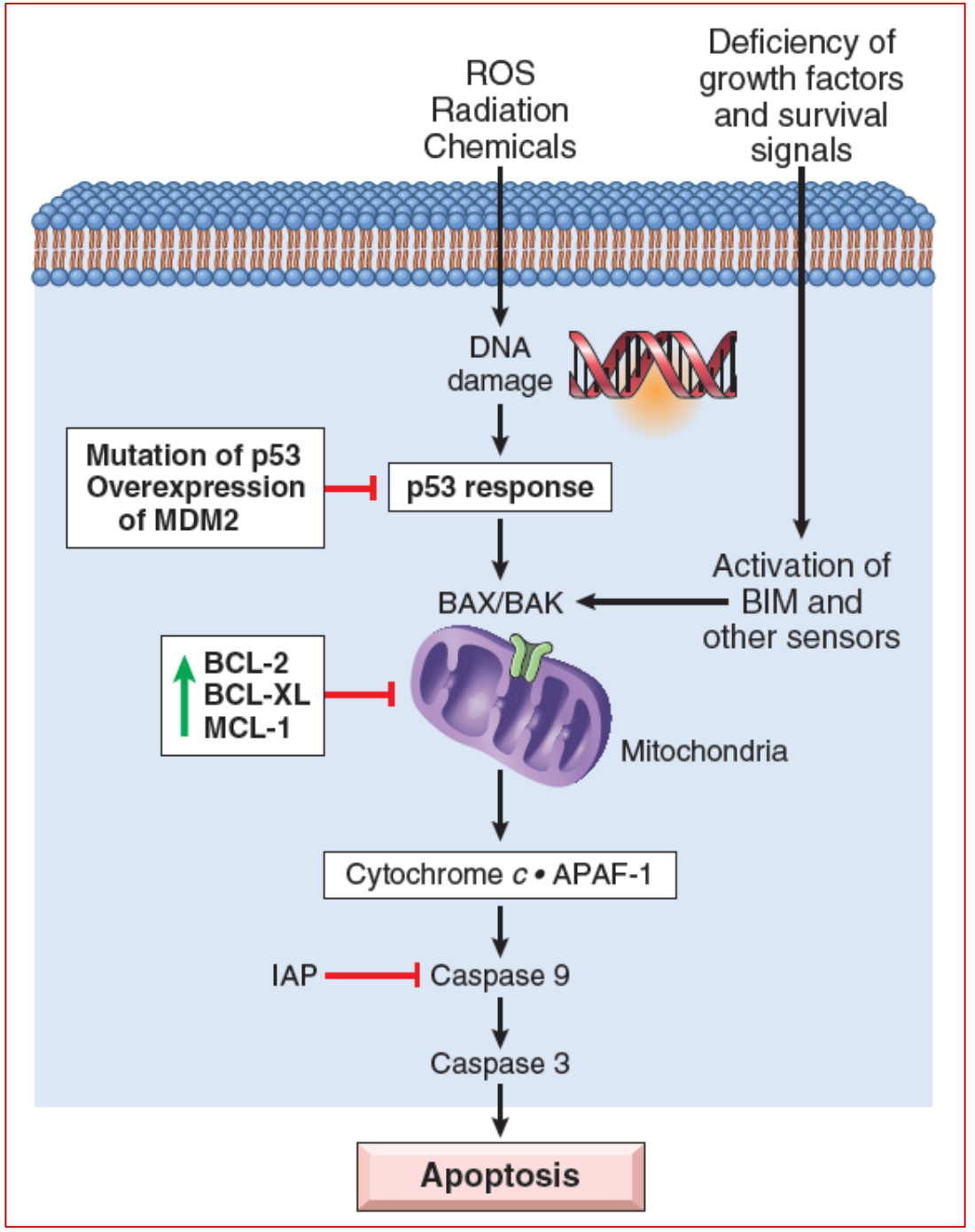


Fig. 6.25 Intrinsic pathway of apoptosis and major mechanisms used by tumor cells to evade cell death.
(1) Loss of p53, either through mutation or through antagonism by MDM2.
(2) Reduced egress of cytochrome *c* from mitochondria as a result of upregulation of anti-apoptotic factors such as BCL2, BCL-XL, and MCL-1. IAP, Inhibitor of apoptosis.

Accumulation of neoplastic cells may result not only from:

(1) Activation of growth-promoting oncogenes, or **(2)** Inactivation of tumor suppressor genes, **but also**,

(3) from mutations in the genes that regulate apoptosis.

Two biochemical pathways leads to apoptosis: (A) *DNA damage* (e.g. radiation) or by GF deprivation, (B) By *signaling through the death receptor (Fas)*. Both pathways act through the genes of the BCL2 family, on the mitochondria to release cytochrome C.

Some members of this family (e.g. ***BCL2, BCL2-XL***) inhibit apoptosis by preventing cytochrome C release, while others, ***BAX & BAK*** promote apoptosis by favoring cytochrome C release.

* **The proapoptotic effects of TP53** triggered by DNA damage, is mediated by up-regulation of **BAX.**

Best example of {apoptosis inhibiting genes} **is *BCL2***, which is overexpressed in 85% of **follicular type** B-cell lymphomas, resulting from characteristic t(14;18) (q32; q21).

Overexpression of BCL2 **protects lymphocytes from apoptosis, & allows them to survive for long periods**; this result in a study accumulation of B lymphocytes, resulting in lymhadenopathy (LN enlargement) & marrow infiltration.

Because BCL2-overexpressing lymphomas arise in large part from reduced cell death, rather than explosive cell proliferation, they tend to be slowly growing, compared with most other lymphomas.

***TP53* is an important proapoptotic gene** that induces apoptosis in cells with irreparable DNA damage.

Its actions are mediated by transcriptional activation of ***BAX***.

Loss of ***TP53*** action leads to decreased apoptosis, allowing DNA damaged cells to continue their proliferation, & increase their chance of transformation.

Limitless Replicative Potential (Immortality)

Most normal human cells have a capacity of 60 to 70 doublings, after which the cells lose the capacity to divide & enter a non-proliferative senescence.

This phenomenon is due to the **progressive shortening of telomeres** at the ends of chromosomes.

With each cell division, telomeres are shortened, & beyond a certain point, loss of telomeres leads to massive chromosomal abnormalities & death.

For tumors to grow indefinitely, loss of growth restraints (limits) is not sufficient.

Tumor cells also must avoid cellular senescence; *this is acquired by* ***activation*** *of the enzyme* ***telomerase****, which can maintain normal telomere length*.

Telomerase (which prevents the shortening of the chromosome in successive cell division) *is active in normal germ & stem cells*, but is absent from most somatic cells.

By contrast, telomere maintenance is seen in virtually all types of cancers.

In 85% to 90% of cancers, this is due to up-regulation of the enzyme telomerase, which allows tumor cells to divide indefinitely.

A few tumors use other mechanisms, termed *alternative lengthening of telomeres,* which depend on DNA recombination.

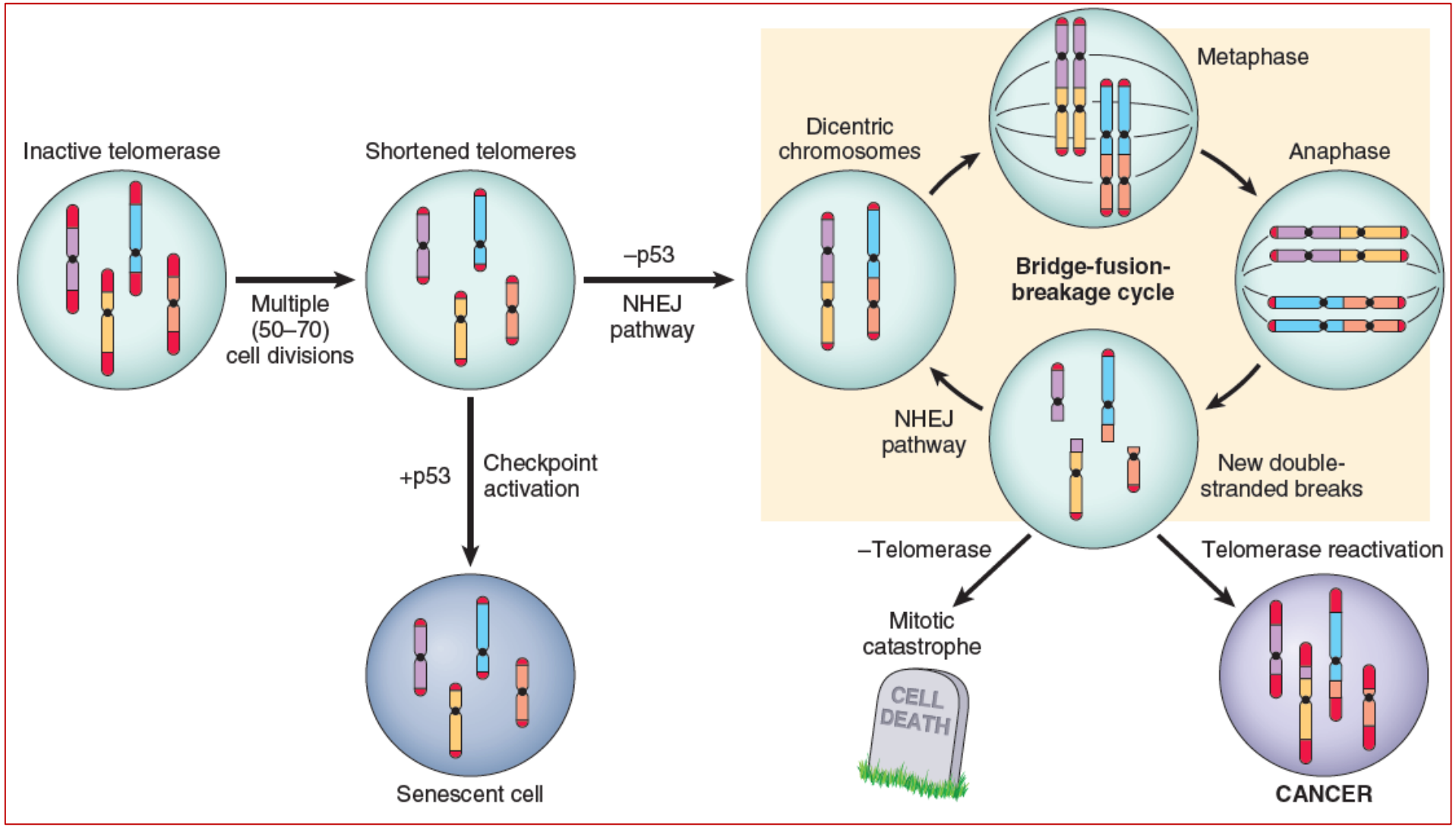


Fig. 6.26 Escape of cells from replicative senescence and mitotic catastrophe caused by telomere shortening.

Development of Sustained Angiogenesis

Even with all the genetic abnormalities discussed above, tumors cannot enlarge more than 1 to 2 mm in diameter or thickness (Which represents the maximal distance across which oxygen & nutrients can diffuse from Blood Vessel) **unless they are vascularized.**

*Beyond this size, the tumor **fails** to enlarge* without vascularization, because hypoxia induces apoptosis by activation of TP53

Neovascularization has a two effects on tumor growth:

(1) Continued tumor growth: perfusion supplies oxygen & nutrients, & the newly formed EC stimulate the growth of adjacent T cells by secreting polypeptides, such as: IL-1, PDGF, insulin-like GF, granulocyte-macrophage colony-stimulating factor (GM-CSF).

(2) Angiogenesis is required for metastasis. Without access to vasculature, the tumor cells cannot metastasize.

Q: How do growing tumors develop a blood supply?

Tumors contain angiogenic factors produced by: **Tumor cells**, the important 2 out of 12 are **VEGF** & **bFGF, & Inflammatory cells:** macrophages infiltrating the **Tumor** .

Tumor cells NOT only produce angiogenic factors, **BUT** also induce anti-angiogenesis molecules.

Therefore, **Tumor growth** is controlled by the **balance** between angiogenic factors & factors that inhibit angiogenesis.

An important anti-angiogenesis factors are**:**

(1) Thrombospondin-1, produced by the Tumor cells themselves under the influence of *TP53*

(2) Angiostatin, endostatin, & vasculostatin; 3 potent angiogenesis inhibitors derived by proteolytic cleavage of plasminogen, collagen, & transthyretin, respectively.

Clinically, due to the crucial role of angiogenesis in T growth much interest is focused on antiangiogenesis therapy. Results of ongoing clinical trials with several angiogenesis inhibitors seem promising.

Early in their growth**,** most tumors do not induce angiogenesis, & they remain small or in situ for years, until the angiogenic switch terminate this stage.

The molecular basis of the angiogenic switch is not entirely clear, but may involve increased production of angiogenic factors OR loss of angiogenesis inhibitors.

The *TP53* inhibit angiogenesis by inducing thrombospondin-1.

With **mutational inactivation of both *TP53* alleles,** commonly seen in cancers, the levels of thrombospondin-1 drop, tilting the balance in favor of elevating angiogenic factors.

Both factors, hypoxia within growing tumor by releasing hypoxia-inducible factor-1(HIF-1), & ***RAS*** oncogene, both factors stimulate the production of **VEGF.**

- **Proteases** can release **bFGF** stored in the ECM.

Conversely, plasmin cleavage gives rise to angiostatin, a potent angiogenesis inhibitor.

Ability to Invade & Metastasize (Invasion and Metastasis)

Tumor spread is a complex process involving a series of sequential steps which may be interrupted at any stage by either host- or tumor- related factors.

Cells within a tumor are heterogeneous in their metastatic potential. Only **certain subclones** possess right combination of gene products to complete all the steps outlined in this figure.

The metastatic cascade can be subdivided into two phases:

(1) Invasion of ECM, &

(2) Vascular dissemination & homing of tumor cells.

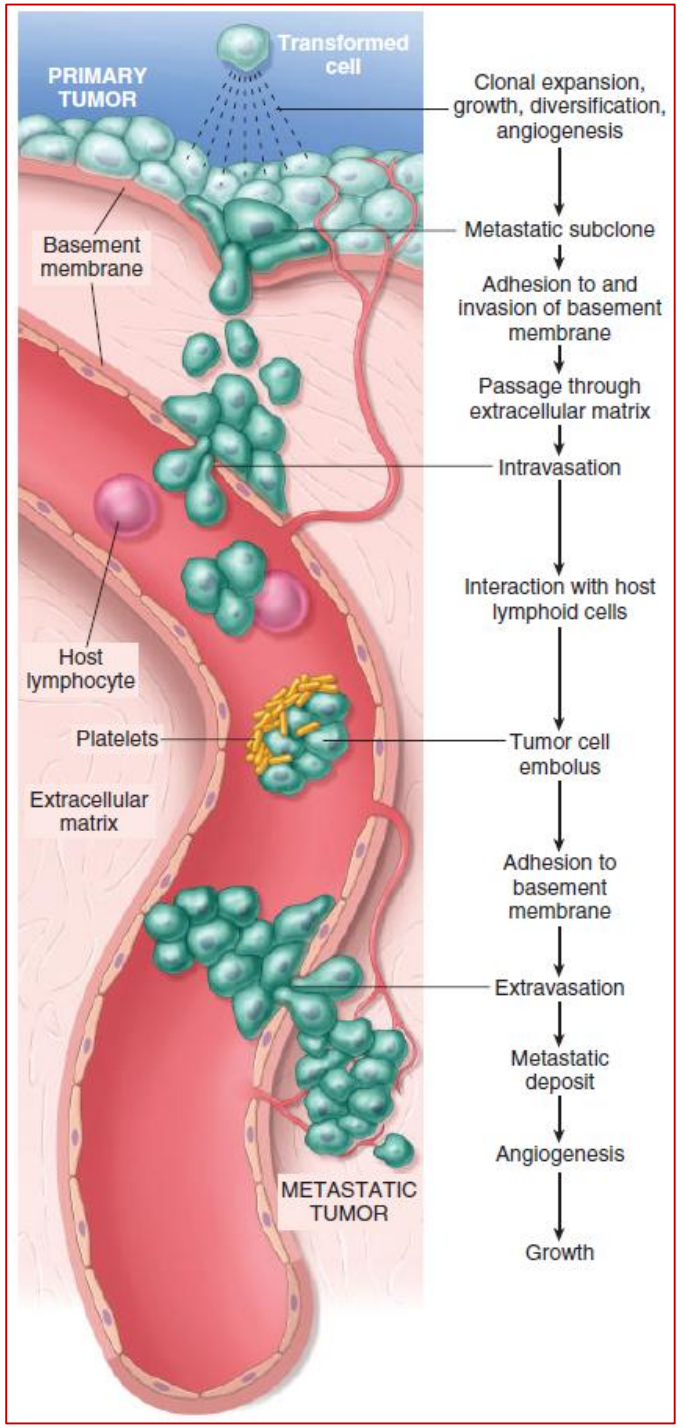


Fig. 6.27 The metastatic cascade: The sequential steps involved in the hematogenous spread of a tumor.

Invasion of ECM

Normally, human tissue are organized into a series of compartments separated from each other by two types of ECM: **BM, & interstitial connective tissue.**

A carcinoma must first:

(1) breach (break) the underlying BM, then

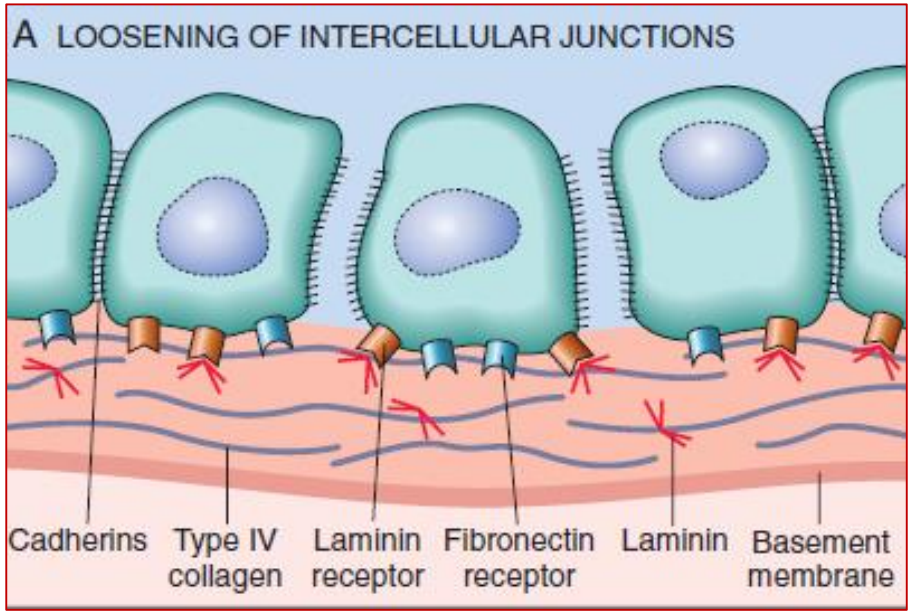
(2) traverse the interstitial connective,& then

(3) penetrate the Blood Vessel Basement Membrane to enter the circulation.

The cycle is repeated (in opposite manner) when tumor cell emboli extravasate (move out the Blood Vessel) at a distant site.

Invasion of the ECM is an active process that is accomplished in four steps:

I. Detachment of T cell from each other, II. Degradation of ECM, III. Attachment to novel ECM components, & IV. Migration of T cells.



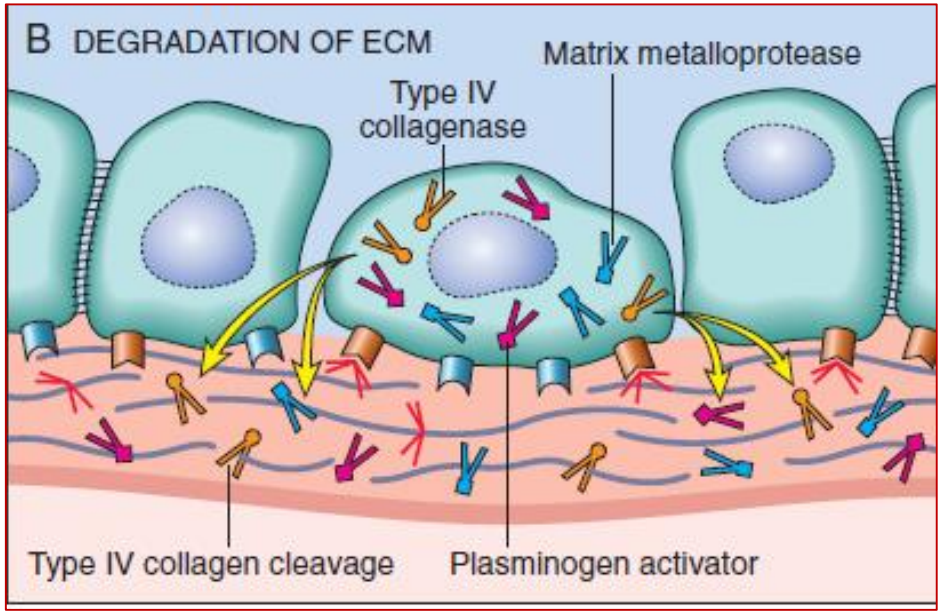
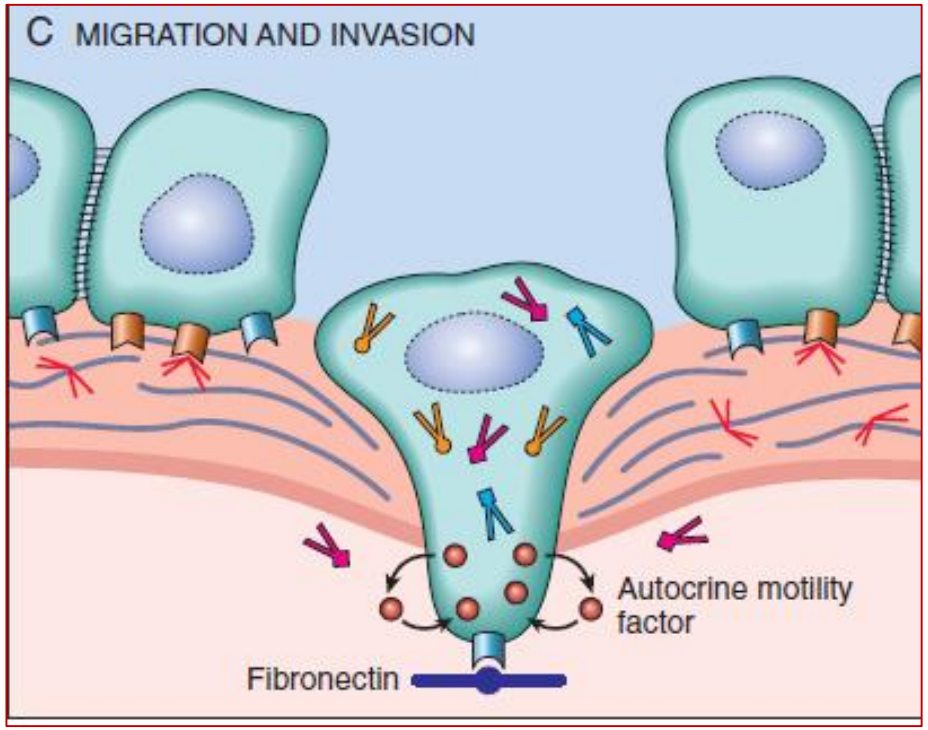


Fig. 6.28 Sequence of events in the invasion of epithelial basement membranes by tumor cells.

Tumor cells detach from each other because of reduced adhesiveness and attract inflammatory cells. Proteases secreted from tumor cells and inflammatory cells degrade the basement membrane.

Binding of tumor cells to proteolytically generated binding sites and tumor cell migration follow.



(I) Detachment or loosening of Tumor cells from each other.

Normally, *E-cadherins* act as intercellular glues, & their cytoplasmic portions bind to β-catenin

(1) **Adjacent E-cadherin** molecules keep the cells together,

(2) Adhesions mediated by E-cadherin transmit antigrowth signals via β-catenin. **Free β- catenin** can activate transcription of growth-promoting genes.

E-cadherin function is lost in almost all epithelial cancers, either by mutational inactivation of E-cadherin genes or by activation of β-catenin genes.

Recall: that the *reduced adhesiveness* of T cells & their detachment from each other form the basis for the **cytological examination of the exfoliated cancer cells.**

(II) The second step in invasion **is local degradation of the BM & interstitial connective tissue**. *Tumor cells may either: **(1)** themselves, secrete **proteolytic** enzymes*, or they **(2)** *induce the stromal cells (e.g. fibroblasts & inflammatory cells) to elaborate proteases*.

Multiple different families of proteases, such as: **(1) MMPs**, **(2) Cathepsin D**, & **(3) Urokinase plasminogen activator,** have been implicated in Tumor cell invasion.

MMPs regulate Tumor invasion, by: **(a)** remodeling insoluble components of the BM & interstitial matrix, & by **(b)** releasing ECM-sequestered GFs.

Indeed, cleavage products of collagen & proteoglycans also have chemotactic, angiogenic, & growth-promoting effects.

E.g. MMP-9 is a gelatinase that cleaves type IV collagen of the epithelial & vascular BM & also, stimulates the release of VEGF from ECM-sequestered pools.

Benign Tumors of **breast, colon & stomach** show **little type IV collagenase activity**, **whereas their malignant counterparts overexpresse this enzyme.**

Concurrently, the levels of MMP inhibitors are reduced, so the balance is tilted greatly toward tissue degradation.

Indeed, overexpression of MMPs & other proteases have been reported for many tumors.

Clinically, because of these observations, attempts is made to use **protease inhibitors as therapeutic agents.**

(III) Invasion 3rd step involves **changes in attachment of T cells to ECM proteins.**

Normally, epithelial cells have receptors, such as **integrins,** for BM laminin & collagens that are polarized at their basal surface; these R help to maintain the cells in a resting, differentiated state.

Loss of adhesion in normal cells leads to induction of apoptosis, while, not surprisingly, T cells are resistant to this form of cell death.

Additionally, the matrix itself is modified in ways that promote invasion & metastasis, E.g., cleavage of the BM proteins collagen IV & laminin by PPP-2 or MMP 9 generates novel sites that bind to receptors on T cells & stimulate migration.

(IV) The fourth & final step of invasion is locomotion, motility of tumor cells through degraded BM & zones of matrix proteolysis.

(a) Migration seems to be mediated by tumor cell-derived cytokines, such as **autocrine motility factors.**

(b) In addition, cleavage products of matrix components (eg collagen & laminin) & some GFs

(e.g. insulin-like GF I & II) have **chemotactic activity for tumor cells.**

Stromal cells also produce paracrine effectors of cell motility, e.g. hepatocytes GF/scatter factor **(HGF/SCF),** which bind to receptors on T cells. Concentrations of **HGF/SCF** are elevated at the advancing edges of the highly invasive brain tumor **glioblastoma multiforme**, supporting their role in motility.

Vascular Dissemination & Homing of Tumor Cells

Following vascular invasion (**intravasation**), tumor cells are liable to destruction by the host immune cells (e.g., in hepatic cell carcinoma, the presence gross malignant secondaries in other organs is much less than expected as a result of the extensive & constant venous invasion of the Ca.!).

Most Tumor cells circulate as:

(1) singles, or **(2)** as **emboli** by aggregating & adhering to circulating WBC & platelets.

Both forms **extravasate from the Blood Vessel** by:

(1) first **adhering to EC** followed, **(2)** by **passage** through the BM of the BV to form metastatic deposit.

- Mostly, the site of extravasation & the organ distribution of metastases can be **predicted** by the **location of the primary Tumor,** its **vascular or lymphatic drainage & the tropism of particular tumors for specific tissues.**

Q: what is (are) the site (s) of metastases of cancer of the: Bones? Colon? Lung? Stomach? Breast? Prostate? Thyroid? Skin? Liver? Ovary? Bladder?

However, *in many cases*, the *natural pathways of drainage do not readily explain the distribution of* metastases, e.g.:

(1) lung Ca. involve the **adrenal** glands with some regularity.

(2) *Neuroblastomas* *tend to spread to the* ***liver & bones*****,**

(3) Melanomas & SCCL can metastasize almost to any site in the body, except the skeletal muscles (which almost never, involved by any malignant secondaries!).

Such organ tropism (affinity) may be related to the:

(1) *expression of* ***adhesion molecules by tumor cells*** *whose ligands are expressed preferentially on the EC of target sites OR,*

(2) chemokines & their receptors: e.g. breast ca cells express high levels of the chemokines Receptor genes *CXCR4* & *CCR7*. The ligands for these Receptors are highly expressed only in those organs where breast ca cells metastasize.

On the basis of this fact, it is speculated that blockade of chemokines Receptors may limit metastases.

Despite foregoing considerations, the **precise localization of metastases** cannot be predicted in any form of cancer!

Metastasis

Of central importance in oncology is the question, **why do only some tumors metastasize?**

It is sobering to realize that satisfying answers are still lacking. Some variation in metastasis clearly relates to inherent differences in the behavior of particular tumors; for example, small cell carcinoma of the lung virtually always metastasizes to distant sites, whereas with some tumors, such as basal cell carcinoma, metastasis is the exception rather than the rule.

However, identification of metastasis-specific mutations and metastasis-specific patterns of gene expression has proven to be difficult.

There is evidence that the makeup of the stroma, the presence of infiltrating immune cells, and the degree and quality of angiogenesis also influence metastasis.

Another open question is whether there are genes whose principal or sole contribution is to control programs of gene expression that promote metastasis. This question is of more than academic interest, because if altered forms of certain genes promote or suppress the metastatic phenotype, their detection in a primary tumor would have both prognostic and therapeutic implications.

Among candidates for such metastasis oncogenes are those encoding SNAIL and TWIST, transcription factors whose primary function is to promote epithelial-to-mesenchymal transition (EMT).

In EMT, carcinoma cells downregulate certain epithelial markers (e.g., E-cadherin) and upregulate certain mesenchymal markers (e.g., vimentin, smooth muscle actin).

Loss of E-cadherin expression seems to be a key event in EMT, and SNAIL and TWIST are transcriptional repressors that downregulate E-cadherin expression.

Evasion of Immune Surveillance

Normal function of the immune system is to constantly "scan" the body for emerging malignant cells and destroy them.

- Cancer cells express a variety of **antigens** that stimulate the host immune system, which appears to have an important role in preventing the emergence of cancers.
- Despite the antigenicity of cancer cells, the immune response to established tumors is ineffective, and in some instances may actually promote cancer growth, due to acquired changes that allow cancer cells to evade anti-tumor responses and foster pro-tumor responses.
- Defining mechanisms of immune evasion and "immunomanipulation" by cancer cells has led to effective new immunotherapies that work by reactivating latent host immune responses.

Tumor Antigens.

In some instances, unmutated proteins expressed by tumor cells also can stimulate the host immune response.

• One such antigen is *tyrosinase*, an enzyme involved in melanin biosynthesis that is expressed only in normal melanocytes and melanomas.

• Another group of tumor antigens, the *cancer-testis antigens*, are encoded by genes that are silent in all adult tissues except germ cells in the testis—hence their name.

Thus, for all practical purposes **these antigens are tumor specific** and are therefore capable of stimulating anti-tumor immune responses.

An additional important class of tumor antigens consists of **viral proteins** that are expressed in cancer cells transformed by **oncogenic viruses** {the most important of which are human papilloma virus (HPV) and Epstein-Barr virus (EBV).}.

There is abundant evidence that **cytotoxic T lymphocytes (CTLs)** recognize viral antigens and play important roles in surveillance against virus-induced tumors through their ability to recognize and kill virus-infected cells.

Effective Immune Responses to Tumor Antigens.

What are the key components of an effective host immune response?

Immune reactions to cancers are initiated by the death of individual cancer cells, which occurs due to dysregulated growth, metabolic stresses, and hypoxia due to insufficient blood supply.

When tumor cells die they release "danger signals" that stimulate innate immune cells, including resident phagocytes and antigen presenting cells. It is believed that **some of the dead cells are phagocytosed by dendritic cells**, which migrate to draining **lymph nodes** and present tumor neoantigens in the context of MHC class I molecules, a process termed cross-presentation.

The displayed tumor antigens are recognized by antigen-specific **CD8+ T cells**, which become activated, proliferate, differentiate into active **CTLs**, and home to the site of the tumor, where they recognize and kill tumor cells presenting neoantigens in the context of their own MHC class I molecules (Fig. 6.29).

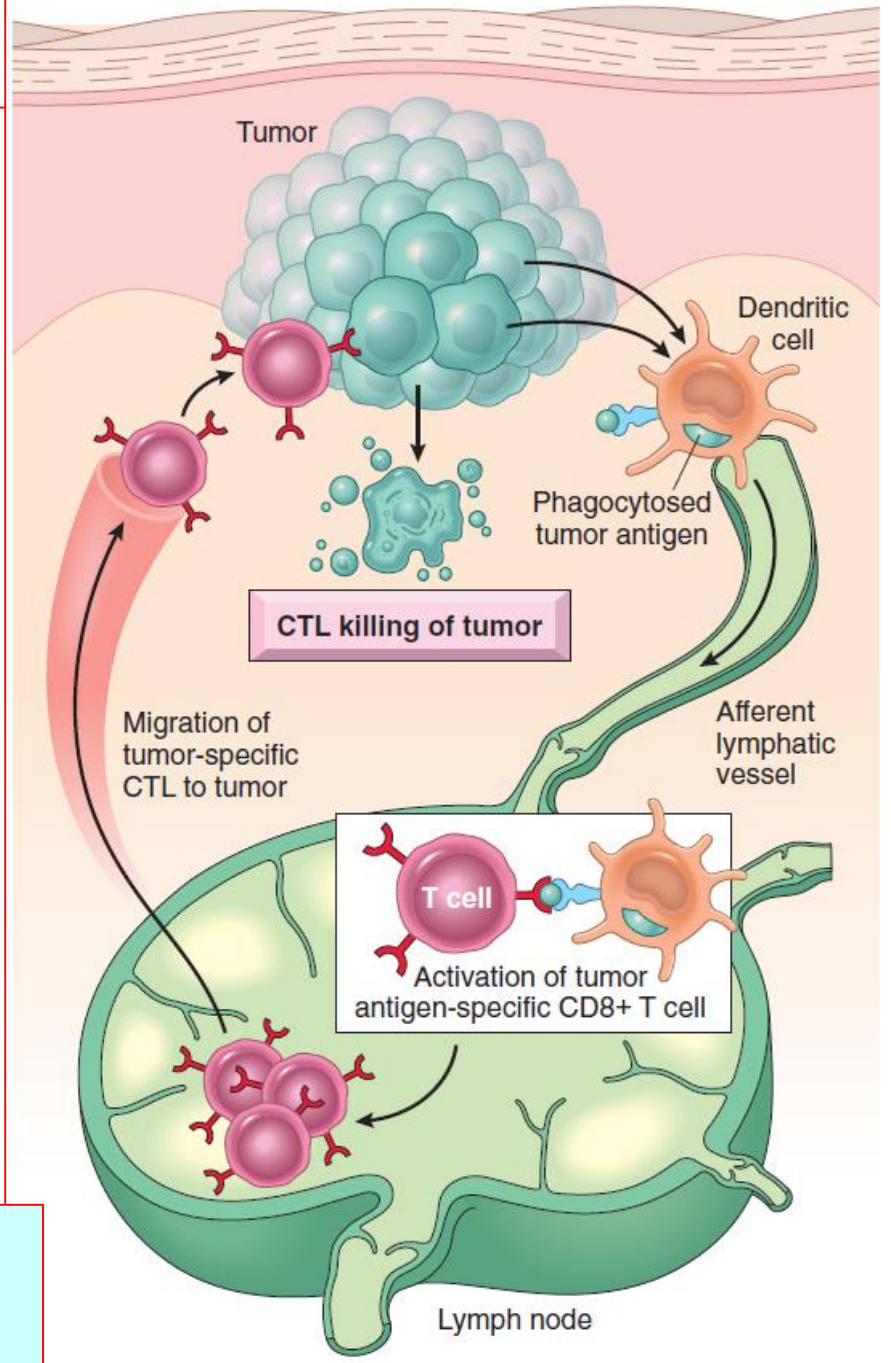


Fig. 6.29 Cross-presentation of tumor antigens and induction of CD8+ cytotoxic T cell antitumor response. *(Modified from Abbas AK, Lichtman AH, Pillai S: Cellular and molecular immunology, ed 9, Philadelphia, 2018, Elsevier.)*

Immune Evasion by Cancers.

The term ***cancer immunoediting*** has been used to describe the ability of the immune system to promote the Darwinian selection of the tumor subclones that are most able to avoid host immunity or even manipulate the immune system for their own malignant purposes.

Tumor cells show a variety of **alterations** that abrogate **CTL** responses. These include acquired **mutations** in **β2-microglobulin** that prevent the assembly of functional MHC class I molecules, and increased expression of a variety of proteins that inhibit CTL function.

These proteins work by activating what is referred to as ***immune checkpoints*****,** inhibitory pathways that normally are crucial for maintaining self-tolerance and controlling the size and duration of immune responses so as to minimize collateral tissue damage.

One of the best-characterized immune checkpoints involves a protein called **PD-L1** (programmed cell death ligand 1), which is often expressed on the surface of tumor cells (Figure 6.30).

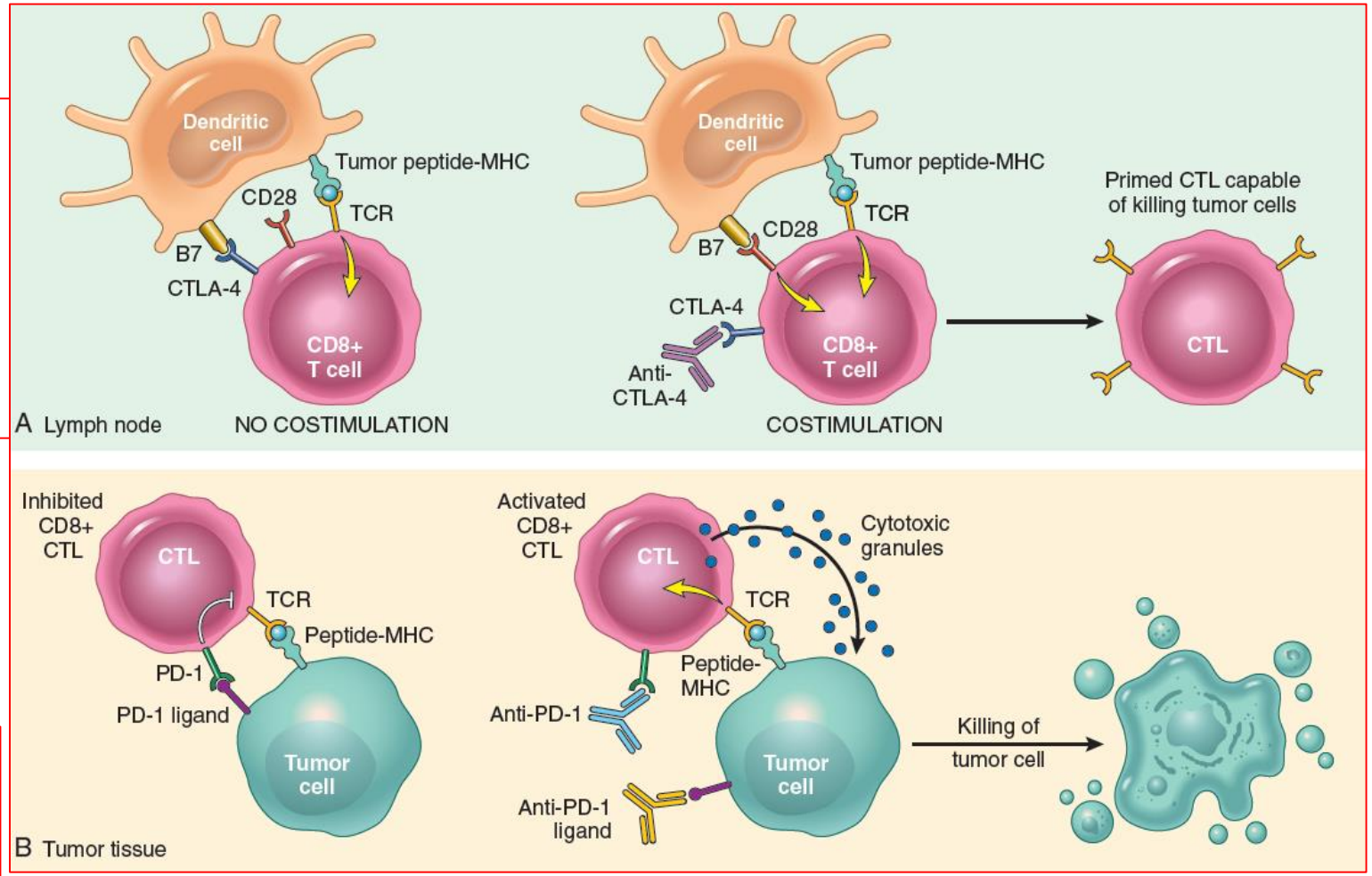


Fig. 6.30 Activation of host antitumor immunity by checkpoint inhibitors.

(A) Blockade of the CTLA4 surface molecule with an inhibitor antibody allows cytolytic CD8+ T cells (CTLs) to engage B7 family coreceptors, leading to T cell activation.

(B) Blockade of PD-1 receptor or PD-1 ligand by inhibitory antibodies abrogates inhibitory signals transmitted by PD-1, again leading to activation of CTLs.

(Reprinted from Abbas AK, Lichtman AH, Pillai S: Cellular and molecular immunology, *ed 9, Philadelphia, 2018, Elsevier.)*

The discovery of checkpoints that shut off anti-tumor immunity has led to the development of antibodies that block these checkpoints and release the brakes on the immune response.

Current checkpoint blockade therapies have resulted in response rates of 10–30% in a variety of solid tumors (melanoma, lung cancer, bladder cancer, and others), and even higher rates in some hematologic malignancies such as Hodgkin lymphoma.

The remarkable response of advanced cancers to immune checkpoint inhibitors has energized other work focused on harnessing the immune system to combat cancer. These include efforts to develop personalized tumor vaccines as well as new kinds of adoptive immunotherapy.

The most advanced of the latter are patient-derived CTLs that are engineered to express **chimeric antigen receptors (CARs).** CARs have extracellular domains consisting of antibodies that bind tumor antigens and intracellular domains that delivered signals that activate CTLs following their engagement with antigen on the surface of tumor cells.

Genomic Instability- Eabler of Malignancy

Although human literally swim in environmental mutagenic agents (sunlight, chemicals & others) **cancers are relatively rare,** with an overall incidence of about **1 to 3 new cancer cases per 1000 individuals per year (world wide).**

Q: WHY?

Answer: The ability of normal cells to repair DNA damage.

The importance of DNA repair in maintaining genome integrity is showing by several inherited disorders in which there is mutation in DNA repair genes.

Individuals born with such inherited mutations are at a greatly high risk of developing cancer,

Four Examples of these disorders:

(I) Xeroderma pigmentosum.

These patients are at *high risk for the development of cancers of the skin exposed to the UV* light *contained in sun rays, an exposure which* ***usually*** causes cross-linking of pyrimidine residues, preventing normal DNA replication, this damage is ***normally*** repaired by the **Nucleotide Excision Repair system**, this system is controlled by several genes, & loss of any one of them give rise to this disease.

(II) Hereditary nonpolyposis colon carcinoma syndrome

HNPCC characterized by **familial colonic ca**, affecting mainly the caecum & proximal colon, resulting from defects in genes involved in **"DNA mismatch repair".**

Normally, when a strand of DNA is being repaired, these genes act as "spell checkers". E.g. if there is an erroneous pairing of G with T rather than the normal A with T, the **mismatch repair genes correct the defect**. *Without these proofreaders,* ***errors*** *slowly accumulate in many genes including protooncogenes & cancer suppressor genes.*

Mutations *in at least 5 mismatch repair genes* *have been found to underlie* ***HNPCC***. Each affected individual:

(a) **inherits one** defective copy of one of several DNA mismatch repair genes, &

(b) **Acquires the 2nd** hit in colonic epithelial cells. Thus, *DNA repair genes behave similar to T suppressor genes in their mode of inheritance,* but in contrast to them (& to oncogenes), they affect cell growth **indirectly**-by allowing mutations in other genes during the process of normal cell division.

Ataxia-telangiectasia, Bloom syndrome, & Fanconi anemia, a group of an **autosomal recessive disorders** characterized by hypersensitivity to DNA-damaging agents, like ionizing radiation (the first two), or DNA cross-linking agents, e.g. nitrogen mustard (Fanconi anemia).

All three disorders predispose to cancer.

(III) *Diseases With Defects in DNA Repair by Homologous Recombination*

(Mutations in 2 DNA repair genes, *BRCA1 & BRCA2 is established cause of 80% of cases of familial breast Ca*.)

(1) In addition to breast ca, women with *BRCA1* mutation have a substantially higher risk of **ovarian Ca.**, & men have a slightly higher risk of prostate Ca.,

(2) mutations in the *BRCA2* gene increases the risk of breast Ca. in both men & women, & cancer of the bile ducts, ovary, prostate, pancreas, stomach, & melanocytes.

Both copies of BRCA1 & BRCA2 must be inactivated for cancer to develop. They *are rarely inactivated in sporadic, non familial cases of breast ca.* In this regard, *BRCA1 & 2* genes are **different** from other T suppressor genes, like APC & TP53 which are inactivated in familial & in sporadic cancers.

(IV) Cancers Resulting From Mutations Induced by Regulated Genomic Instability: Lymphoid Neoplasms.

A special type of DNA damage plays a central role in the Pathogenesis of tumors of B cells and T lymphocytes.

Adaptive immunity relies on the ability of **B** cells and **T** cells to diversify their antigen receptor genes. Immature B cells and T progenitors both express a pair of gene products, RAG1 and RAG2, that carry out V(D)J segment recombination, permitting the assembly of functional immunoglobulin and T-cell receptor genes. In addition, after encountering antigen, **mature B cells** express a specialized enzyme called *activation-induced cytosine deaminase (AID),* which catalyzes both immunoglobulin gene class switch recombination and immunoglobulin diversification through somatic hypermutation

Tumor-Promoting Inflammation as an Enabler of Malignancy

Infiltrating cancers provoke a chronic inflammatory reaction.

In patients with advanced cancers, this inflammatory reaction can be so extensive as to cause systemic signs and symptoms, such as anemia (the so-called “anemia of chronic disease”), fatigue, and cachexia.

Proposed cancer-enabling effects of inflammatory cells and resident stromal cells include the following:

- ***Release of factors that promote proliferation.***
- ***Removal of growth suppressors***.
- ***Enhanced resistance to cell death.***
- ***Angiogenesis.***
- ***Invasion and metastasis.***
- ***Evasion of immune destruction.***

Etiology of Cancer: Carcinogenic agents

Carcinogenic agents inflict genetic damage, which lies at the heart of carcinogenesis. Three classes of carcinogenic agents have been identified:

(1) chemicals, **(2) radiant energy**,

both of the above are well documented causes of cancer in humans, and

(3) microbial products. oncogenic viruses are involved in the pathogenesis of Tumors in several animal models & at least, in some human tumors.

It is important to note that **2 or all 3 classes of carcinogenic agents may act in concert** (together), or ***sequentially***, to produce the multiple genetic abnormalities characteristic of neoplastic cells.

Chemical Carcinogens

It has been over 200 years since the London surgeon, Sir Pott, correctly **attributed scrotal skin cancer in chimney sweeps (cleaners) to chronic exposure to soot (dust).**

A few years later, based on this observation the Danish Chimney Sweeps Guild (Association) ruled that its members must bathe daily.

No public health measure since that time has achieved so much in the control of a form of cancer.

Since that time, **hundreds** of chemicals have been shown to be carcinogenic in animals. The following observations have emerged from the study of **chemical carcinogens**:

(1) They are of extremely **diverse structure,** include **natural & synthetic products**.

(2) **Few** *are direct reacting* & require no chemical transformation to induce carcinogenicity, but **Most** *are indirect reacting* (referred to as ***procarcinogens)*** & *become active only after metabolic conversion to their active end products*, which are called (***ultimate carcinogens***).

(3) All, direct-reacting & ultimate carcinogens chemicals, are ***highly reactive electrophiles*** *(i.e., have electron-deficient atoms) that* react with the electron-rich atoms in the DNA, RNA, & cellular proteins.

(4) The carcinogenicity of some chemicals is **augmented** *by agents that by themselves have little, if any, transforming activity. Such* augmenting agents are called **promoters**; however, many carcinogens have no requirement for promoting agents.

(5) Several chemical carcinogens may **act in concert** with other types of carcinogenic influences (e.g. viruses or radiation) to induce neoplasia.

Major Chemical Carcinogens

Direct-Acting Carcinogens:

Alkylating agents: Anticancer drugs (cyclophosphamide, chlorambucil, Nitrosoureas)

Acylating agents: 1-Acetyl-imidazole & Dimethylcarbamyl chloride

Procarcinogens That Require Metabolic Activation: Polycyclic & heterocyclic aromatic hydrocarbons:

Benz [*a*] anthracene, Benzo [*a*] pyrene, Dibenz [*a,h*] anthracene, 3-Methylcholanthrene

Aromatic amines, amides, azo dyes: 2-Naphthylamine (β-naphthylamine), Benzidine, 2-Acetylaminoflurene

Dimethylaminoazobenzene (butter yellow)

Natural plant & microbial products: Aflatoxin B1+ Betel nuts + Griseofulvin.

Others:

Nitrosamine & amides, Vinyl chloride, Asbestos Nickel, Chromium, Arsenic, Insecticides, Fungicides, Polychlorinated Biphenyls (PCBs).

Direct-Reacting Agents

These agents *require no metabolic conversion to become carcinogenic*; they are in general ***weak carcinogens*** but are important.

Cancer chemotherapeutic drugs (Alkylating agents: cyclophosphamide, chlorambucil, nitrosoureas, & others) that have ***successfully cured***, controlled, or delayed recurrence of certain types of cancer (e.g. leukemia, lymphomas & ovarian Ca.), ***only to evoke later a 2nd cancer, usually leukemia!***

This situation is even more tragic when their initial use has been for non-neoplastic disorders, such as rheumatoid arthritis (RA). The risk of inducing cancer is **low,** but the fact that it exists **dictates careful use of such drugs!**

Indirect Acting Agents or **Procarcinogens**

are chemicals that require metabolic conversion before they become active i.e., ultimate carcinogen.

Some of the most potent indirect chemical carcinogens, **the polycyclic hydrocarbons** are present in fossil (rock) fuels**.**

(a) **Benz [*a*] anthracene** ***produces cancer wherever it is applied:*** *When painted on the skin, it induces* ***skin ca****; when injected subcutaneously, it induces* ***fibrosarcoma****.*

(b) **Benzo [*a*] pyrene**, + & other carcinogens, are formed in the high-temperature combustion of tobacco in **cigarette smoking,** products implicated in the causation of **lung cancer in cigarette smokers**.

(c) Polycyclic hydrocarbons also may be produced from animal fats during the process ***of broiling (roasting) meats & are present in smoked meats & fish.***

Aromatic amines & azo dyes

Before its carcinogenicity was recognized,

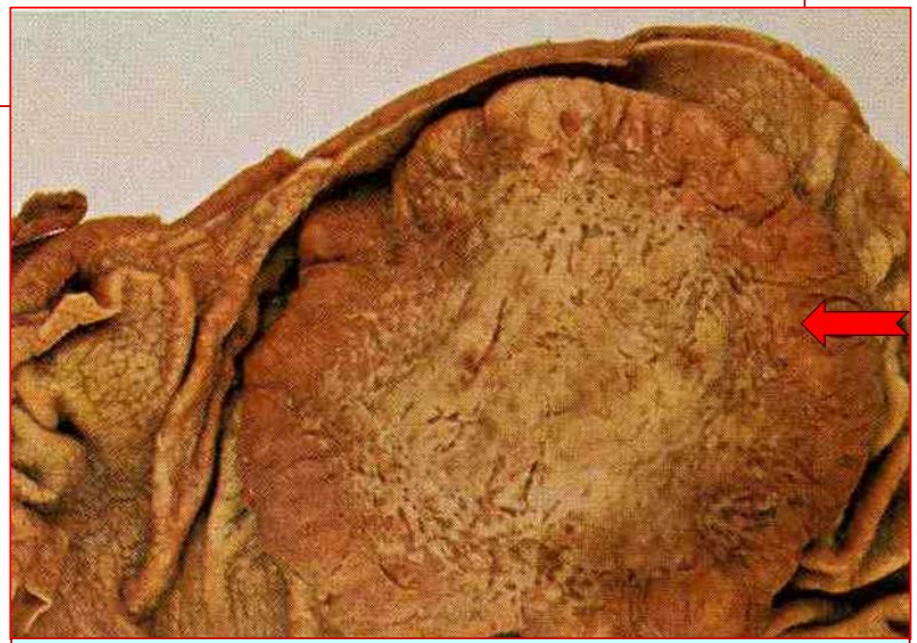
β-naphthylamine was responsible for a X **50-fold increase incidence of bladder cancers** in heavily exposed *workers in the aniline dye rubber industries*.

Some of the **azo dyes** ***were developed to color food (e.g. butter-yellow*** *to make margarine more enticing (provocative) & scarlet-red for maraschino cherries.*

Nitrosamines & nitosamides: that can be formed endogenously in the stomach,increase the risk of gastric Ca.

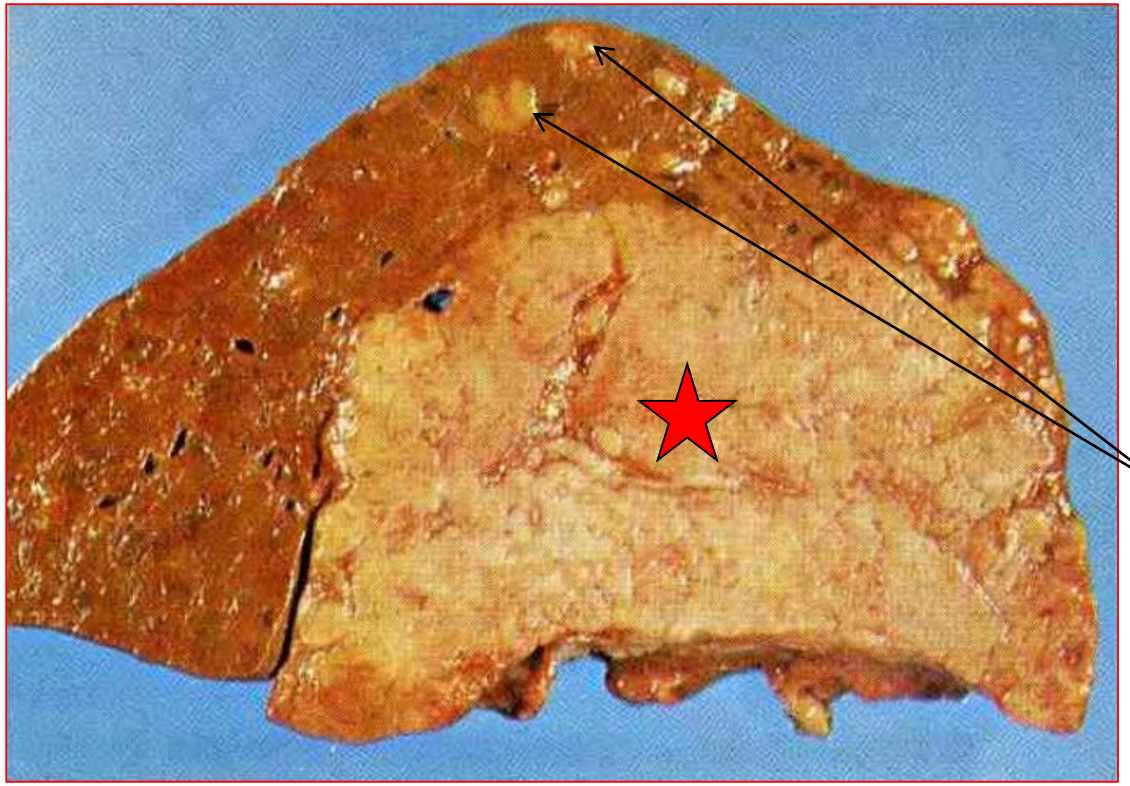
Amines (derived from food) + nitrites derived from nitrates (found in food, drinking water), & preservatives for meat = Nitrosamines compounds.

Nitrosamines are present in **tobacco smoke** & after absorption (through mucosa into the blood) could lead to cancers in a variety of organs (e.g. bladder & cervix).



Carcinoma; stomach. Large fungating flat polypoidal tumor arising from the body of the stomach.

Aflatoxin B1 is a naturally occurring agent, produced by some strains of ***Aspergillus*** mold that grows on improperly stored grains & nuts. There is **strong correlation** between the dietary level of this food contaminant & the incidence of **hepatocellular Ca.** in parts of Africa & Far East**.**



Hepatocellular carcinoma: liver.

★ Single large tumor replacing most of the right liver lobe with several small satellite nodules in the surrounding liver. There is no cirrhosis

Also**,** there is a **correlation** between the prevalence of infection with ***HBV*** *& hepatocellular ca*. **Aflatoxin & HBV** may act in **concert** to produce hepatic cancer.

Potential carcinogens in the house & workplace:

Arsenic (component of alloys, medications, herbicides, fungicides,& animal dips),

Asbestos (heat + fire + friction resistance & insulation, brake lining),

Vinyl chloride (refrigerant, plastics adhesive),

Ethylene oxide (fruits & nuts ripening agent, fumigants for foodstuffs & in sterilizing hospital equipments),

Insecticides, fungicides, Polychlorinated biphenyls (PCBs), nickel, Chromium, Cadmium & Benzene solvent

Because indirect-acting carcinogens **require metabolic** activation for their conversion to DNA-damaging agents**,** much interest is focused on the enzymatic pathways that are involved, such as the **cytochrome P-450.**

The genes that encode these enzymes are **polymorphic**, & enzyme activity varies among different individuals, therefore it is widely believed that the **susceptibility** to chemical carcinogenesis (e.g. cigarette smoke) depends, at least in part on the specific allelic form of the enzyme inherited.

Q: why {some} smokers develop lung cancer, while {others} are not?

Persons with specific genetic polymorphism involving P-450 genes have an ⇧ capacity to metabolize procarcinogens derived from cigarette smoke &, clearly, incur the greatest risk of developing lung cancer.

This may explain why some smokers develop lung cancer, while others are not.

Mechanisms of Action of Chemical Carcinogens

Because malignant transformation results from mutations that affect protooncogenes & cancer suppressor genes, it is no surprise that **most chemical carcinogens are mutagenic**.

Although any gene may be the target of chemical carcinogens, ***RAS*** (family of related proteins) ***gene mutations*** *are* particularly **common** in several chemically induced cancers in **(rodents).**

Among Tumor suppressor genes, ***TP53*** is an important target.

Specific chemical carcinogens, e.g. **aflatoxin B1,** produce characteristic (*signature mutation)* in the *TP53* gene sufficiently strong to incriminate the Aflatoxin.

These associations are proving useful tools in **epidemiological** studies of chemical carcinogenesis.

Promotors: Carcinogenicity of some chemicals is **augmented** by subsequent administration of promoters (e.g. phorbol esters, hormones, phenols, & some drugs) that by themselves are **nontumorigenic**.

To be effective, repeated or sustained exposure to the ***promoter must follow*** the *application of the mutagenic chemical, or initiator.*

The **initiation-promotion sequence** of chemical carcinogenesis raises an important *question.*

Q: Since promoters are not mutagenic, how do they contribute to tumorigenesis?

Answer: **(1)** induction of cell proliferation is a *sine qua non* (basic requirement) of Tumor promotion. The best studied tumor promoter Tetradecanoylphorbol 13-acetate TPA, (**a phorbol ester)** is **(a)** a powerful activator of protein kinase C, an enzyme that is a crucial component of several signal transduction pathways, including those activated by GFs, & **(b)** in addition, TPA also causes GF secretion by some cells.

(2) It seems most likely that while the application of an ***initiator may cause the mutational*** *activation* of an oncogene such as *RAS,* the subsequent *application of* ***promoters force or leads to clonal expansion*** *of the initiated (mutated) cells.*

Such cells (especially after *RAS* activation) have decrease GF requirements. Forced to proliferate, these initiated mutated clone of cells, suffers additional mutations, developing eventually into cancer.

The concept that **sustained cell proliferation increases the risk of mutagenesis, & hence neoplastic transformation**, is also *applicable to* ***human*** *carcinogenesis*.

Example:

(1) ***Atypical endometrial hyperplasia*** *&*

(2) **Increased regenerative activity** accompanies ***chronic liver cell injury*** are associated with the development of ***endometrial & liver cell Ca.*** *respectively.*

(3) ***Estrogens*** influence *on the occurrence of* ***breast Ca.*** may relate in part to the proliferative effect of estrogen on mammary duct epithelium. The clinical fact that some breast Ca. express estrogen receptors (ER) & benefit from ER antagonists supports a role for estrogen in breast Ca.

It must be emphasized that carcinogen-induced damage to DNA does not necessarily lead to initiation of cancer, Q: Why?

Answer:

Several forms of **DNA damage** (incurred spontaneously or through the action of carcinogens) **can be repaired by cellular enzymes**. Were this not the case, the incidence of environmentally induced cancer in all likelihood would be much higher than the current rate of approximately 1 to 3 new cancer cases/ per1000 individuals/ per year, World wide!

This is **best exemplified** by the rare hereditary disorders of DNA repair, including **xeroderma pigmentosum**, which is associated with defective DNA repair & a greatly elevated risk of cancers induced by UV light & certain chemicals.

Radiation Carcinogenesis

Radiation is an established carcinogen whatever its source -UV rays of sunlight, X-rays, radioactive isotopes, & nuclear fission (Bomb or reactors).

(1) Many of the **pioneers** in the development of X-ray developed **skin cancers.**

(2) **Miners of radioactive** elements (e.g. uranium) have suffered a X ten-fold increased incidence of **lung Ca.**

(3) Follow-up of survivors of the atomic bombs dropped on **Hiroshima & Nagasaki** (**1945**) disclosed a markedly High incidence of myelocytic **leukemia** after an average latent period of about 6 years.

Decades later, the leukemia risk is still above the level for control populations, as is the mortality rate from thyroid, breast, colon, & lung Ca.

(4) The nuclear power accident at **Chernobyl (1986)** in the former Soviet Union continues to cause higher than normal cancer incidence in the surrounding areas.

(5) **Even therapeutic irradiation** has been documented to be carcinogenic. *Scandinavian follow-up study during 1945-1980 period shows that* **papillary thyroid ca** had developed *in approximately 9% of individuals exposed during their infancy & childhood to head & neck irradiation.*

It is very clear that ionizing radiation is strongly oncogenic. This is related to its mutagenic effects, radiation causes:
(1) chromosome breakage,
(2) translocations, &, less frequently,
(3) point mutations.

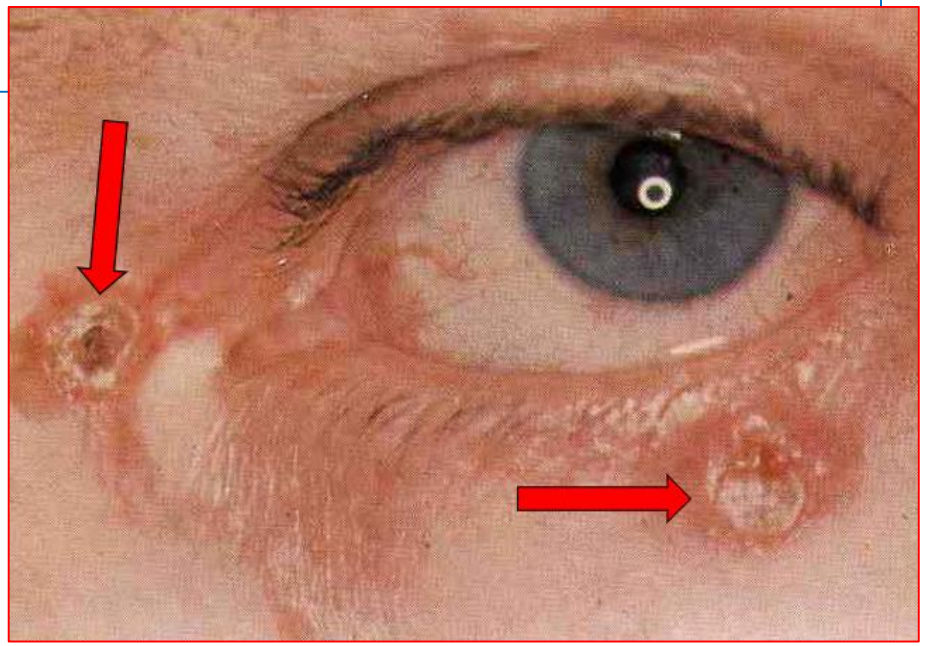
Biologically, double-stranded ***DNA breaks seem to be the most important for radiation carcinogenesis***.

Because the latent period of irradiation-associated cancers is **extremely long**, it seems that cancers emerge only after the progeny of initially damaged cells **accumulate additional mutations**, induced possibility by other environmental factors.

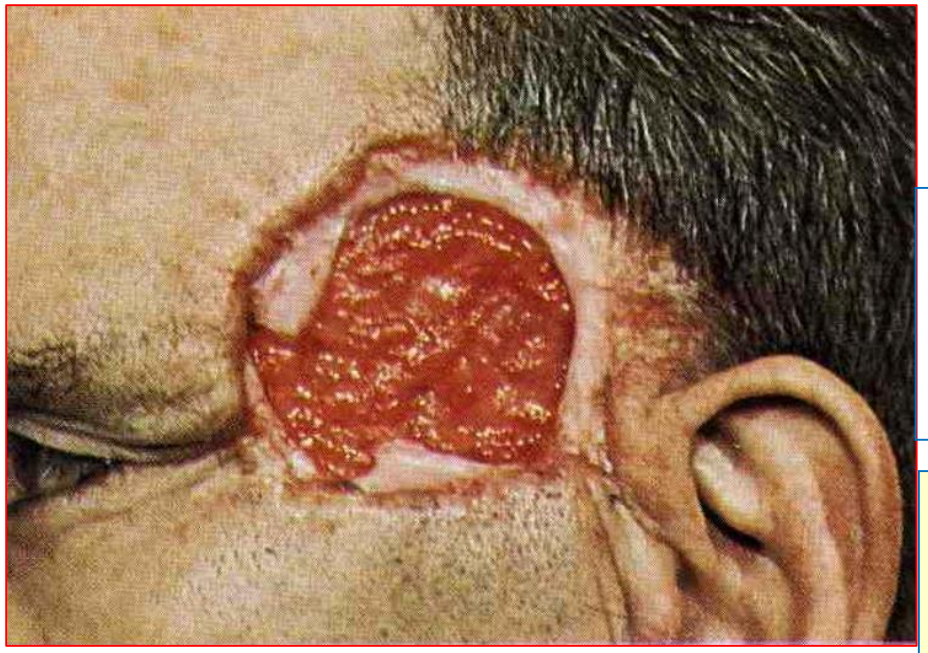
The oncogenic effect of UV rays is important because it shows the importance of DNA repair in carcinogenesis.

Natural UV radiation derived from the sun *can cause 3 skin cancers* ⇨ BCCa, SCCa, melanomas.

Basal cell carcinoma: Two smooth ulcerated BCCa nodules with characteristic **"rolled"** red border, 1st in the inner canthus of the left eye & 2nd on the lower eyelid.



BCCa, the most common human cancer in the world. A locally invasive, slowly growing cancer, occurs predominantly in fair- skinned people (as in this patient), in the part of the face bounded by the hairline, ears & upper lip

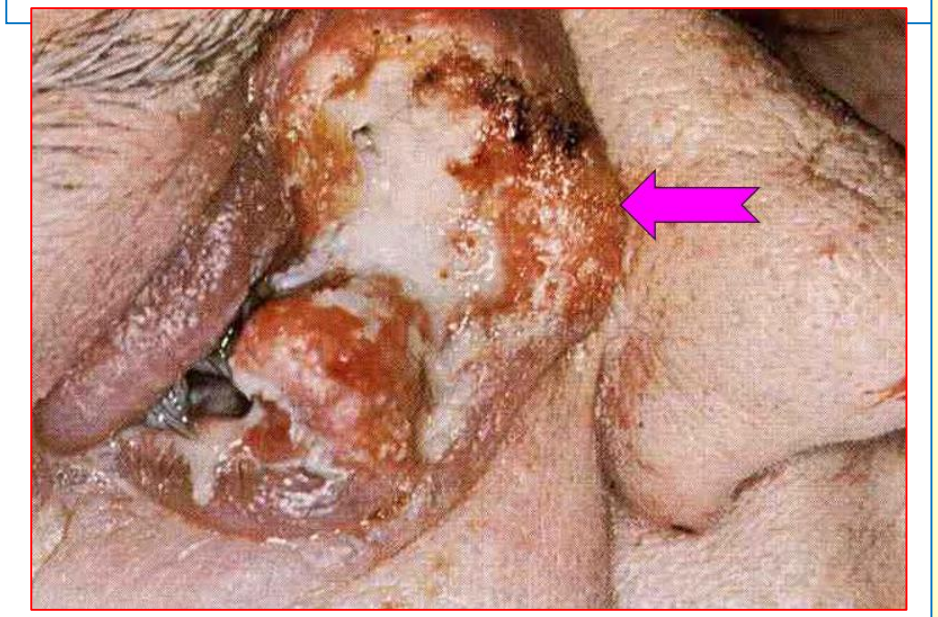


Basal cell Ca. ('Rodent ulcer').
Advance large ulcerating BCCa of the left temporal region, with bright red granular base & a smooth, white, rolled border.

This is the cicatricial type of BCCa which is characterized by:
(1) Superficial peripheral spread with ulceration &
(2) Subsequent central scarring (so-called **fire in the field**).

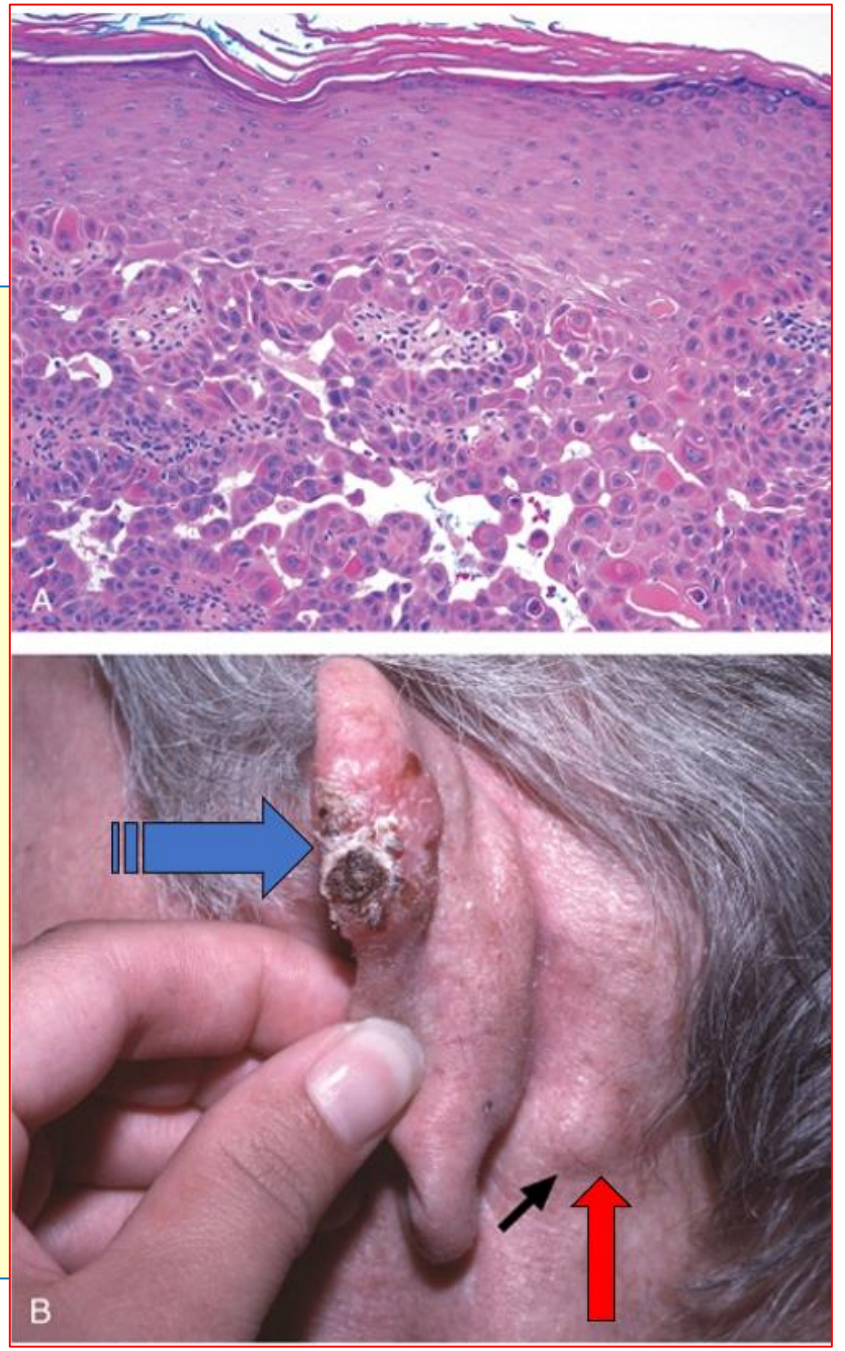
Squamous cell carcinoma: Skin.

Advanced SCCa of the inner canthus of the right eye. The exuberant papillary growth forms a heaped-up mass, as well as areas of ulceration covered with a thick creamy-white slough.

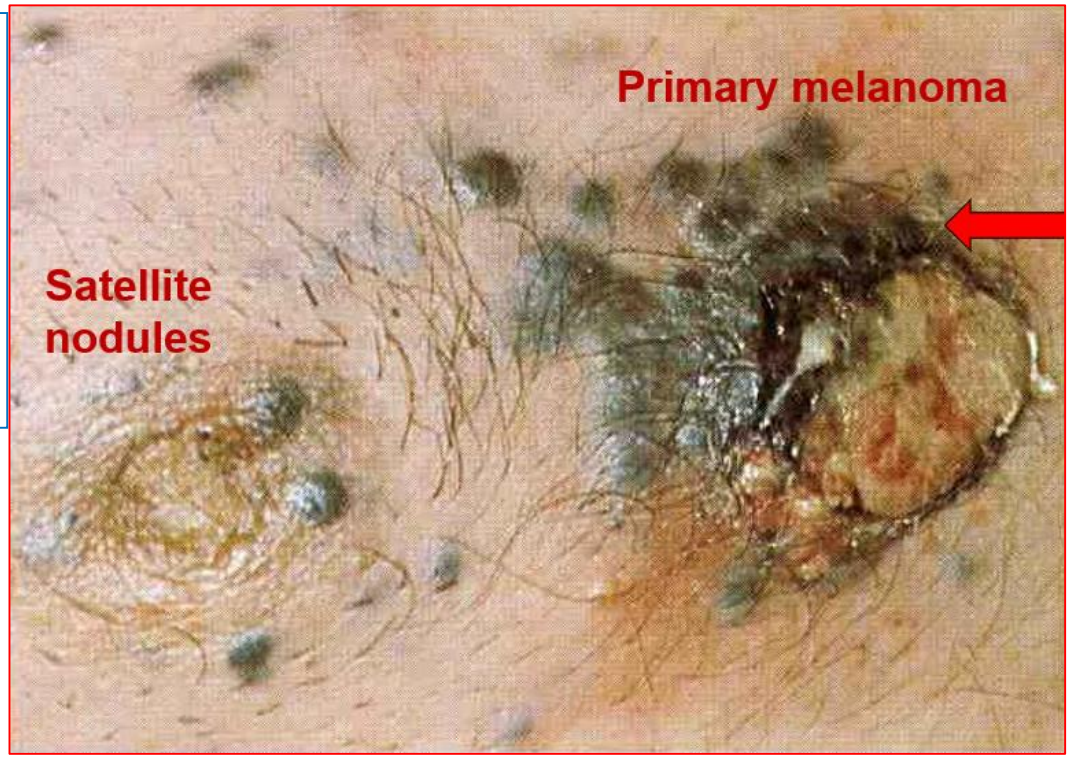


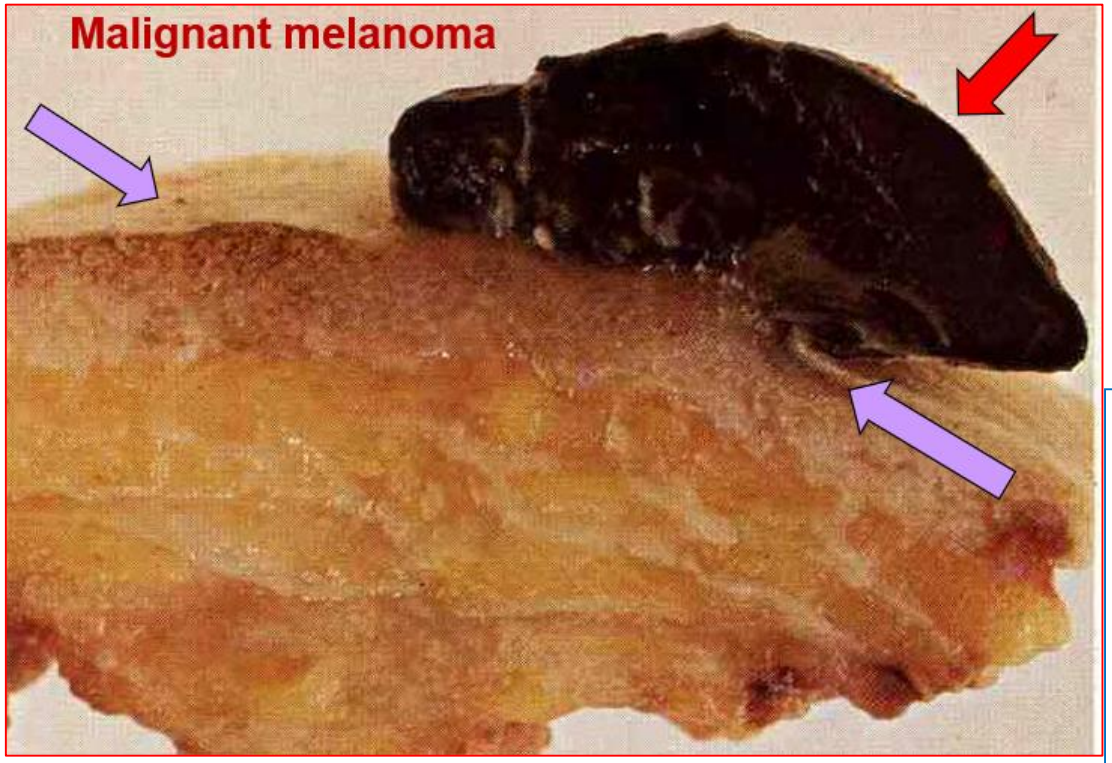
Invasive squamous cell carcinoma. A, The SCCa invades the dermis as irregular projections of atypical squamous epithelium; this particular case is acantholytic (poorly cohesive squamous cells).

B, A nodular hyperkeratotic **SCCa** occurring on the left ear, unfortunately with early metastasis to a prominent **postauricular lymph node** (Red arrow).



Malignant melanoma. Local spread.
Many small metastatic satellite nodules have formed in the tissue around the ulcerated & pigmented primary melanoma (right) which was situated on the **chest wall.**





Malignant melanoma. Deeply pigmented, elevated tumor 2X2X1cm situated on the **skin of the back**.
Note that in the dermis, beneath & to the left of the tumor, there is a diffuse flat spreading brownish-colored lesion, suggesting that the melanoma arose in a previously benign nevous.

Fair-skinned people who live in areas that receive a **great deal of sunlight** are at greatest risk & cancers of their exposed skin are particularly common in Australia & New Zealand.

UV light cause **DNA damage** **by forming pyrimidine dimers,** which is ***repaired*** *by a complex set of proteins that effect* **{nucleotide excision repair, NER}**. **Thus,** with extensive exposure to UV light, this NER system may be overwhelmed & skin cancer result.

The *importance* of NER is illustrated in an autosomal recessive inherited disease **xeroderma pigmentosum,**, in which NER mechanism is **defective or deficient**, resulting in a greatly increased predisposition to all above three **skin cancers.**

UV light characteristically **causes *TP53* gene mutations**

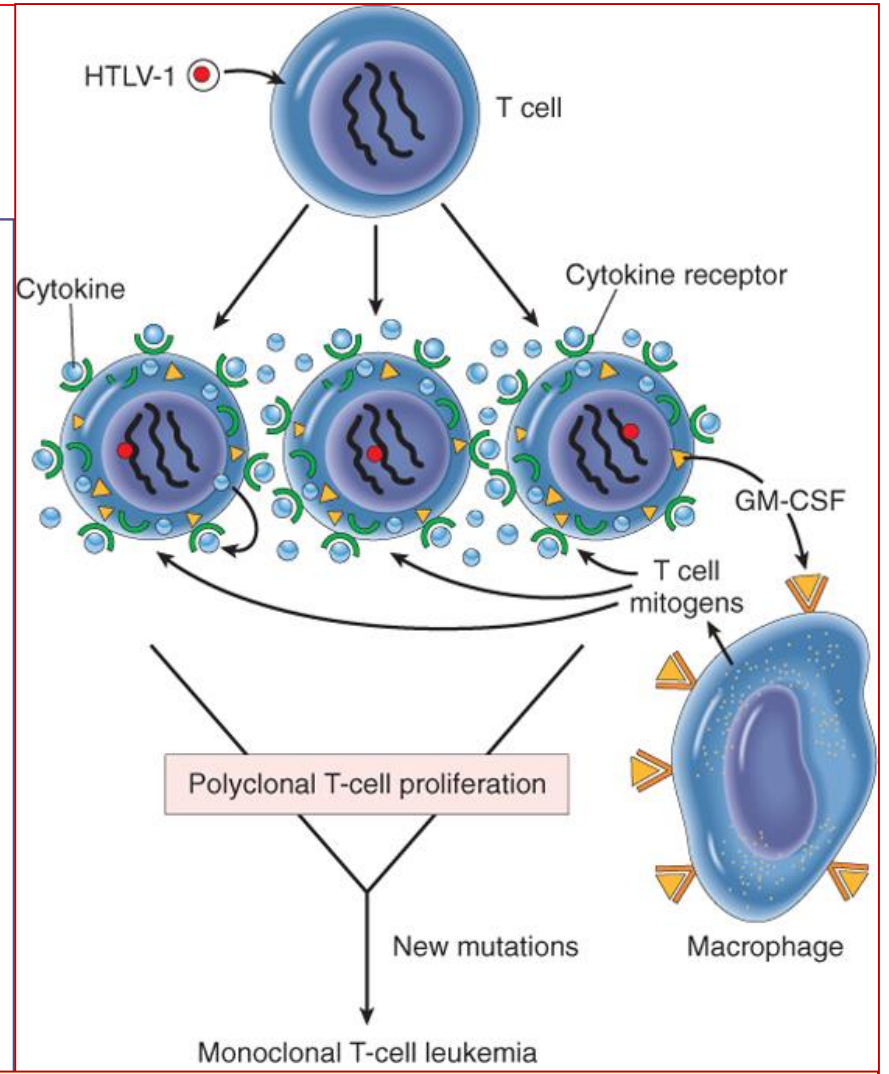
Viral & Microbial Oncogenesis

RNA Oncogenic Virus, the only known one is:

Human T-Cell Leukemia Virus Type 1 (HTLV-1) is associated with a form of T-cell leukemia/ lymphoma, endemic in certain parts of Japan, but is found sporadically elsewhere, including USA.

- **Similar to the AIDS virus**, **(I)** HTLV-1 has tropism for CD+4 T cells, & this subset of Tumor cells is the major target for neoplastic transformation. **(II)** Infection requires transmission of infected Tumor cells via sexual intercourse, blood products, or breast-feeding.
- Leukemia develops in only about 1% of infected individuals after a latent period of 20 to 30 years.

The molecular mechanisms of transformation are not clear, but from many observations, the following *scenario* is emerging:



HTLV-1 infects many T cells ⟶ the viral *TAX* gene *trigger* auto- & para-crine **polyclonal T cell proliferation** initially, & at the same time *TAX inhibit* the function of several Tumor suppressor genes that control the cell cycle, including the TP53 & CDKN2A/p16.

Ultimately, **when one proliferating T cell suffers** additional **mutations** a monoclonal T cell leukemia/lymphoma result.

DNA Oncogenic Viruses

4 DNA viruses, **HPV**, **EBV**, **HBV** (discussed below), & **HHV-8** (Kaposi sarcoma herpesvirus) are strongly associated with human cancer.

Human Papillomavirus (HPV)

HPV genetic types 1, 2, 4, & 7 definitely cause benign SC papillomas.

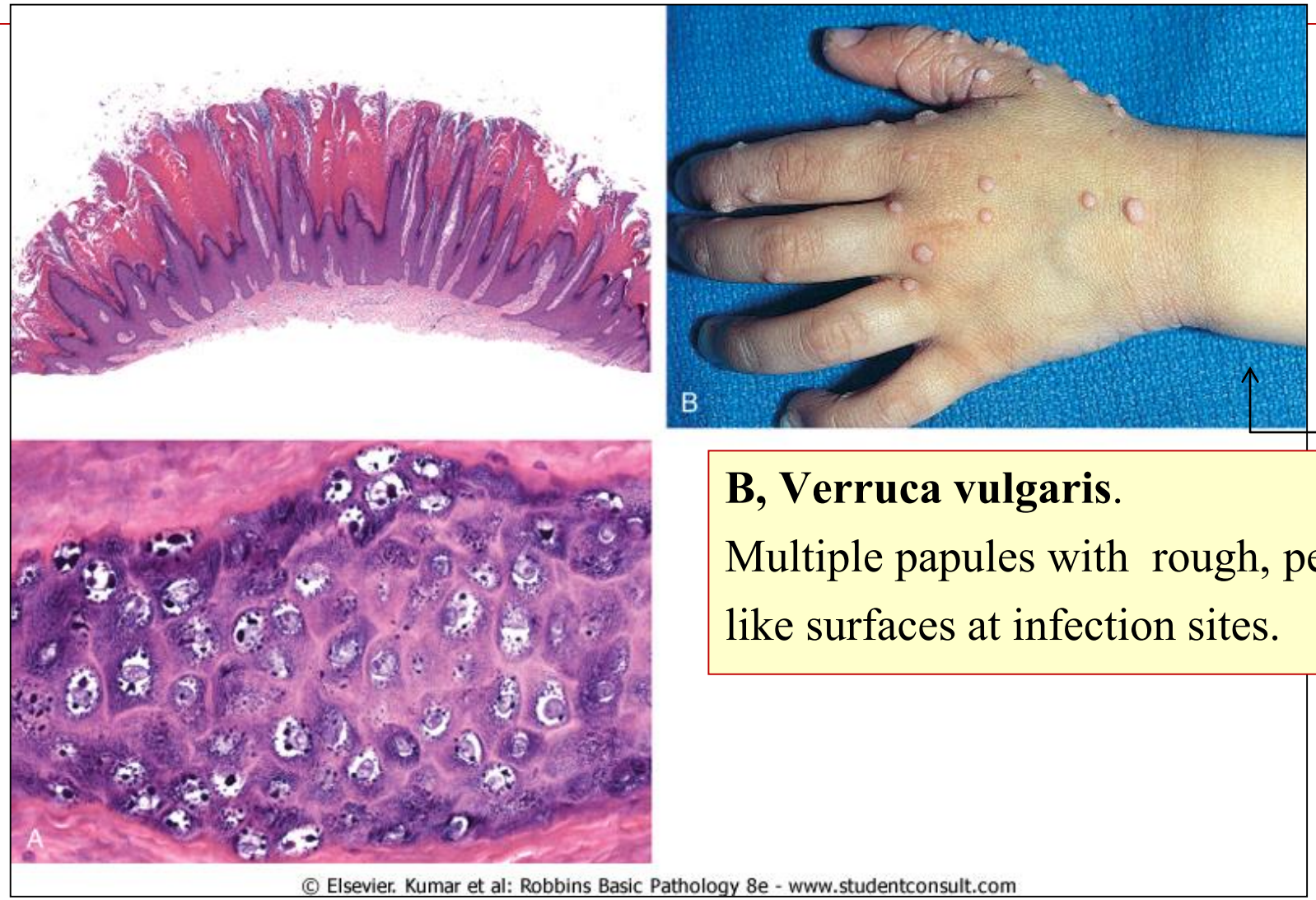
HPV genetic types 16,18, & 31 are implicated in the genesis of SCCa of cervix; and anal, perianal, vulvar, & penile cancers.

20% of oropharyngeal Ca. are HPV-associated.

A, Verruca vulgaris.

A, symmetrical papillary epidermal proliferation radiating like the points of a crown (top) .

Histology shows nuclear pallor, prominent keratohyalin granules of HPV 1,2,4, or 7 infection.



B, Verruca vulgaris.

Multiple papules with rough, pebble-like surfaces at infection sites.

Epidemiologic studies suggest that **cervical ca** is caused by a **sexually transmitted agent & HPV** is strongly linked to this Tumor HPV types **16,18, & 31** are found in 75% to 100% of cervical severe **dysplasia**, SCCa **in situ**, & **invasive** SCCa.

In contrast to cervical Ca., benign genital warts are associated with distinct HPV types, predominantly **HPV types 6 &11**.

To summarize, infection with high-risk HPV types stimulates the loss of Tumor suppressor genes + activates cyclins + inhibits apoptosis + & combats cellular senescence = thus, HPV drives many hallmarks of cancer.

Epstein-Barr Virus (EBV)

EBV has been implicated in the pathogenesis of several Tumors:

(A) Tumor of the **B-cell origin**, including:

(1) **Hodgkin** lymphoma described in1830 by Hodgkin,

(2) **Burkitt** lymphoma described in 1958 by Burkitt,

(3) **AIDS** related lymphomas {reported after1981},

(4) **Post-transplant** lympho-proliferative disease,

(B) Nasopharyngeal carcinoma.

Burkitt lymphoma is endemic in part of Africa & is sporadic elsewhere**.**

In endemic areas*, Tumor cells in virtually all patients carry the* ***EBV genome****.* EBV show strong tropism for B cells & infects many B cells, causing polyclonal B cell proliferate.

In immunologically normal individuals, EBV-driven polyclonal B-cell proliferation in vivo is readily controlled, & the individual either remains asymptomatic or develops a self-limited episode of **infectious mononucleosis**.

In Africa, where Burkitt lymphoma is endemic, concomitant endemic **malarial infection** impairs immune competence, **allowing sustained B-cell proliferation**, these B-cells do not express cell surface antigenes that can be recognized by host T-cells, therefore, they are relieved from immunoregulation.

Such B-cells are at high **risk** of acquiring mutations, such as the **t (8:14)** tanslocation, which **activates the *MYC*** oncogene & is a consistent (fixed) feature of **Burkitt lymphoma.**

Activation of *MYC* causes further loss of growth control, leading to additional gene damage, ultimately leads to the development of monoclonal Burkitt lymphoma.

N.B: in non-endemic areas, 80% of Burkitt lymphomas do not harbor the EBV genome, but all of them possess the specific t (8:14) translocation.

To summarize: EBV or any other ? carcinogenic factor, may trigger B-cells, to suffer **t (8:14)** translocation which **activate** the ***MYC*** oncogene, resulting in Burkitt lymphoma.

In immunosuppressed patients (eg AIDS & organ-transplant recipients), EBV-infected B-cells undergo polyclonal expansion, & may end up with a potentially lethal proliferation, which, **fortunately, can be arrested**, if the immunologic status of the host improves, as may occur with withdrawal of immunosuppressive drugs in transplant recipients.

Nasopharyngeal ca is endemic in southern China & some other locals, & the **EBV genome** is found in all tumors.

As in Burkitt lymphoma, EBV acts in concert with other? unidentified factors.

Hepatitis B Virus (HBV)

There is strong epidemiological evidence linking chronic HBV infection with hepatocellular ca.

The oncogenic effect of HBV seems to be:

(1) First, by causing chronic liver cell injury & accompanying **regeneration,** HBV predisposes the cells to mutations, caused possibly by environmental agents (dietary Aflatoxin?)

(2) Second**,** virus-induced **gene damage** in regenerating liver cells may set the stage for multistep carcinogenesis.

Although not a DNA virus, ***hepatitis C virus (HCV)*** *also is strongly linked to hepatocellular ca* with mechanism of tumor production similar to that described for HBV.

Helicobacter Pylori (*H. pylori*):

Known cause of **peptic ulcer**, now ? a cause of **gastric lymphoma & carcinoma?**

The gastric lymphomas are of B-cell origin, & because the transformed B-cells normally reside (present usually) in the marginal zones of lymphoid follicles, these tumors are called **MALTomas** (Marginal zone-Associated Lymphomas).

Their pathogenesis involves:

initial chronic gastritis, causes lymphoid follicles to develop in the gastric mucosa, *H. pylori* infection leads to the formation of *H. pylori* - reactive T-cell, which in turn cause { polyclonal B-cell proliferation}, in time, a { monoclonal B-cell tumor} emerges in the proliferating B-cells, perhaps as a result of *accumulation of mutations*.

In keeping with this, early in the course of disease, *eradication of H. pylori "cures" the lymphoma by removing antigenic stimulus for T- cells*.

In addition, *H. pylori* has now been linked strongly to the pathogenesis of gastric Ca.

Here is the scenario seems to be:

An initial development of → chronic gastritis, followed by → gastric atrophy → intestinal metaplasia of the lining cells → dysplasia → carcinoma.

This sequence takes decades to complete & occurs in only 3% of infected patients.

Although *H. pylori* causes 3 gastric lesions (peptic ulcer, gastric Ca., & gastric lymphoma), these conditions do not occur in the same patient**.**

 # Strangely, & for unknown reasons, patients who have **duodenal peptic ulcer**, **Never** develop **gastric carcinoma** (up till now)**!**

Clinical Aspects of Neoplasia

Importance of tumors lies in their effects. *Any Tumor*, even a benign one, may cause *morbidity* (functional inability) & *mortality* (death).

Every new growth requires correct diagnosis, as to whether it **is malignant or not**?

Best e*xample,* ***lump in a female breast*** (a very common lesion). Benign lesions of the breast are more common than cancers, but, every mass should be consider suspicious, until proved otherwise (as in the thyroid).

The only way to confirm the exact nature of the lesion is by doing histological examination of the excised tumor.

This is equally true of all tumors.

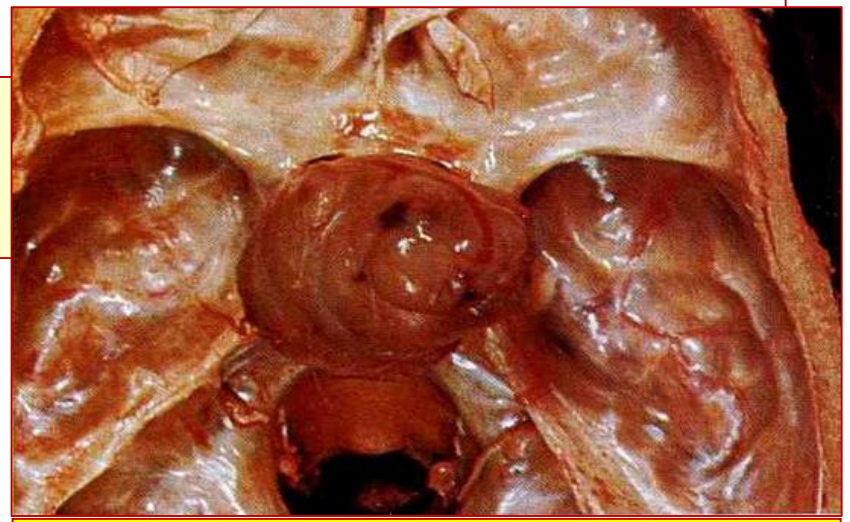
Rule: **ALL MASSES** require histological examination to exclude the possibility of cancer.

Effects of Tumors on Host

Malignant Tumors are far more threatening to the host than benign Tumors. However, *both BT & MT may cause problems due to their:* ***(1)*** *Location,* **(2)** *Hormone production,* **(3)** *Ulceration & bleeding,* **(4)** *cachexia, &* **(5)** *paraneoplastic syndromes.*

(1) **Location is crucial in both B & M T.** A **0.5 cm** tumor within the: ***ureter, common bile duct,*** *or in the* ***wall of the renal artery*** may induce: *unilateral hydronephrosis, fatal biliary tract obstruction, or renal ischemia & serious hypertension respectively.*

- A *1 cm pituitary adenoma* can compress & destroy the surrounding normal gland & give rise to *hypopituitarism*.



Pituitary adenoma. Large pink fleshy, lobulated mass, causes enlargement of the sella turcica, & bulges out upwards through the diaphragma sellae as a suprasellar extension into the cranial cavity, hypothalamus or midbrain, causing ↑ ICP.

(2) Hormone production: by benign, or well-differentiated ca arising in endocrine gland, e.g.

(a) in **β *cells of the islets*** *of the pancreas often produce* ***hyperinsulinism,*** **(b)** *in* ***adrenal cortex,*** *elaborating aldosteron causing Conn's syndrome with* ***hypertension.***

(3) Ulceration, bleeding or secondary infection are common in Tumors, e.g. gastric, colonic, & bladder Ca.

A pedunculated (projecting) Tumor of the intestine, whether benign like lipoma, or malignant like lymphoma, may be (trapped) in the peristaltic movement, to telescope the tumor & its site of origin, into the down-stream (next) segment of gut, resulting in **(intussusception)**, which may cause mucosal ulceration, obstruction, infarction, or gangrene of the **intestine**.

(4) Cancer Cachexia (wasting) refers to:

(a) loss of body weight, due to loss of body fat & lean body mass**, (b) severe weakness, (c) anorexia (loss of appetite), & (d) anemia.**

Usually, an inter current infection brings an end (death) to the slow deterioration of the patient.

There is in general some *correlation between the size & extent of the spread of the cancer & the severity of the cachexia*.

Small localized cancers are generally silent & produce no cachexia, but there are many exceptions.

The causes of cachexia are multiple

(1) Anorexia {loss of appetite}, a common problem in cancer patients, but reduced calorie intake is not sufficient to explain the cachexia of malignancy,

(2) The BMR {basal metabolic rate} **is increased in patients with cancer**, despite reduced food intake, in contrast to the lower BMR that occurs as an adaptational response in **starvation.** The reason for the increaseing BMR is not clear, but, perhaps circulating TNF & IL-1, released from activated macrophages, are involved.

TNF suppresses appetite & inhibits the action of lipoprotein lipase, inhibiting the release of free fatty acids from lipoproteins

(3) A protein- mobilizing factor that causes breakdown of skeletal muscle protein by the ubiquitin-proteosome pathway has been detected in the serum of cancer patients. In healthy animals, injection of this factor causes acute weight loss without causing anorexia.

There is no satisfactory treatment for cancer cachexia other than removal of the underlying cause, the cancer.

(5) Paraneoplastic Syndromes:

Are symptom complexes, other than cachexia, that occur in patients with cancer, & that cannot be readily explained by **(1)** local or distant spread of the tumor, or by **(2)** the elaboration of hormones indigenous to the tissue of origin of the tumor.

Frequency: appear in 10% to 15% of patients with cancer.

Importance: it is important to recognize these syndromes,

(1) Because they may represent the ***earliest manifestation of an occult T.***

(2) In the affected patients, they ***may represent significant clinical problem, & may even be fatal.***

(3) They may ***mimic (show similarity to) metastatic cancer***, leading to inappropriate treatment plans.

Commonest 3 of more than 11 syndromes reported are:, **Cushing syndrome** + **Thrombotic endocarditis** + **Hypercalcemia**. The most often tumors associated with these are: ***lung, breast, & pancreatic cancers.***

- **Cushing syndrome** as a paraneoplastic phenomenon is usually related to ectopic production by the cancer cells of ACTH or ACTH-like polypeptides.
- **Thrombotic endocarditis**, seen in many cases of advanced cancers is usually caused by hypercoagulability of blood**.**
- **Hypercalcemia etiology** is multifactorial:

(1) synthesis of parathyroid hormone-related protein {**PTHrP that** resembles parathyroid hormone, but can be distinguished from it by specific assay} by Tumor cells, especially SCCa of lung,

(2) Synthesis by Tumor cells of:

(a) TGF-α that activates osteoclasts; & **(b)** the active form of vitamin D.

Another mechanism for **Hypercalcemia** is widespread osteolytic metastatic tumor **(secondaries)** in bones, but such Hypercalcemia, **is not consider a paraneoplastic syndrome.**

One Tumor may induce several paraneoplastic syndromes at the same time, **e.g. lung Ca.** may elaborate: ACTH, ADH, HCG, Parathyroid Hormone, serotonin, etc.

Grading & Staging of Cancer *(not for benign Tumors)*

Grading of a cancer: is an estimate of *cancer level of malignancy* based on: cytologic differentiation of Tumor cells & the number of mitoses within it.

Cancer may be classified as **grade I, II, III, or IV** in order of ⇧ anaplasia, **or, as a, well, moderately, poorly differentiated or undifferentiated** Ca. or sarcoma, respectively, with/without lymphatic or vascular invasion.

Staging of cancer is an **assessment of cancer** based on the:

(1) *size of the primary Tumor*, its *extent of* ***(2)*** *spread to regional LN, & the* ***(3)*** *presence or absence of metastases*.

*A*ssessment is based on clinical, radiographic, CT & MRI &, in some cases surgical exploration.

Two methods of staging are currently in use:

The **TNM system** (**T**, primary T; **N**, regional LN involvement; **M**, metastases) & the **AJM (American Joint Committee) system.**

In the TNM system, T1, T2, T3, & T4 describe the increasing size of the primary tumor;

N0, N1, N2 & N3 indicate progressively advancing LN involvement; &

M0 & M1 reflect the absence or presence of distant metastases respectively.

In the AJM method, the cancers are divided into stages **0 to IV**, incorporating the size of primary tumor & the presence of LN spread & of distant metastases**.**

It is important to note that when compared with grading; staging has proved to be of greater clinical value.

Laboratory Diagnosis of Cancer

Morphologic Methods

Requests for histopathological examination should be accompanied by sufficient **clinical data**, which is invaluable for optimal pathological ex. Name, age, sex, the exact anatomical **site** from which the biopsy was taken, & the **provisional (suspected) clinical diagnosis should be mentioned.**

Example:

{Patient name: X, Age 45y, Sex: female. P/ W with left upper outer quarter breast mass, hard, approx. 2 cm./D,

of one month duration, painless. Biopsy of the mass, to exclude malignancy. Dr. Name, signature & date.}

Biopsy specimen **must** be **adequate**, **representative**, & properly **preserved** in fixative like formalin.

Several sampling approaches are available:

Excisional biopsy (of the **entire lesion**), or **Incisional biopsy** (of a representative **part of the lesion** with avoidance the necrotic part) if the excision of entire lesion is not possible for any reason.

Endoscopic biopsies should be **multiple & representative**.

Frozen-section: a quick method, in which the sample is quick-frozen (e.g. by CO2 gas), sectioned (by cryostat), stained by H&E, allowing histopathological evaluation within 20 minutes (while the usual paraffin-embedded, H&E stained sections require 3 days or more).

frozen- section diagnosis is sometimes desirable, as for example:

(1) in determining the nature of a **breast mass**, is it Benign or Malignant? &, therefore, to deal with it accordingly at the time of operation, either by **lumpectomy, if it is Benign** or by **mastectomy,** (removal of the breast with/without axillary LN) if it is Malignant

(2) in evaluating the ***margins of an excised cancer*** to ascertain that the entire cancer has been removed.

The patient is spared the expense & the trauma of a 2^{nd} operation.

In experienced competent hands, frozen–section diagnosis is accurate in most instances.

In some difficult cases, it should be postpones, till the H&E sections are available.

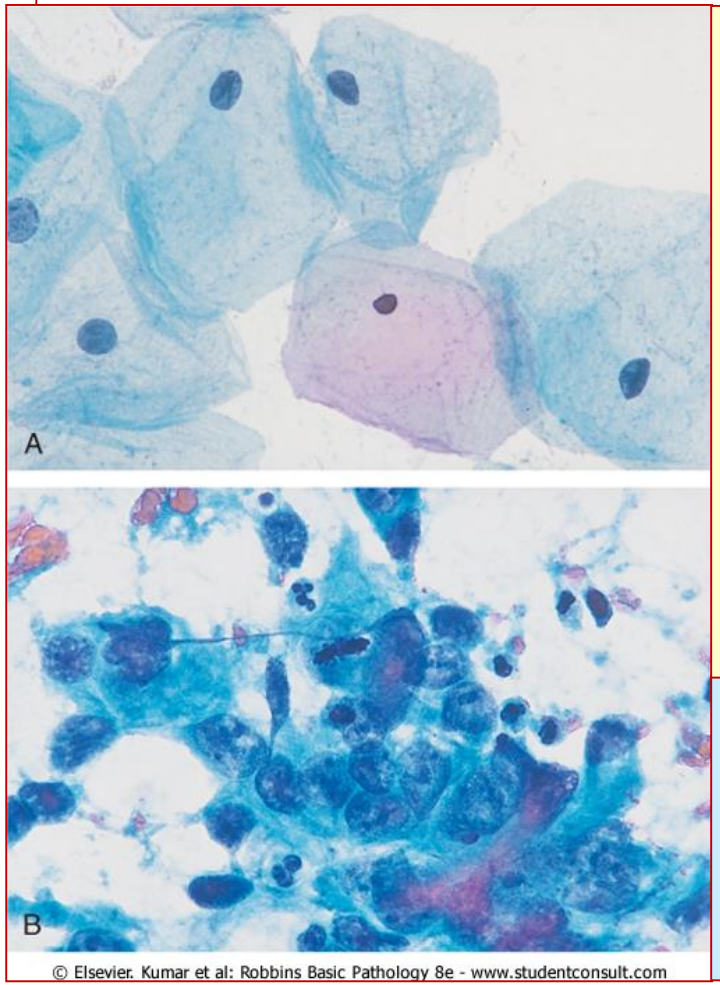
 # Fine-needle aspiration (FNA) of Tumor is another approach that is growing in popularity.

It involves aspiration of cells from a mass, followed by cytological examination of the smear.

This method is most commonly used with **palpable masses** in the breast, thyroid, LN, salivary glands, bone, or skin, either directly or giddied in deep structures by CT Scan.

Modern imaging techniques enable the method to be extended to deeper structures, such as the liver, pancreas, pelvic LN. It avoids the need for surgery & its attendant risks. Although it involves some difficulties, such as small sample size & sampling errors, in experienced hands it can be extremely reliable, rapid, & useful.

Cytological examination invented by Papanicolaou in 1920 used widely, for the early detection of cervical Ca..



Cytological examination is also used with many other forms of suspected malignancies, such as bronchogenic, bladder & gastric Ca. as well as in the identification of tumor cells in the peritoneal, pleural, pericardial, & CSF **fluids**.

The basis for cytological examination is that *neoplastic cells are less cohesive than normal cells* & so they are shed (drop) into fluids or secretions. The shed cells are smeared on slide, fixed, stained, & examined for features of dysplasia or anaplasia; this study is called **(cytology).** The *big advance in the control of cervical cancer is the best evidence to the value of the cytologic method.*

A- Normal Papanicolaou smear from the uterine cervix. Six normal large, flat, squamous cells with small nuclei.

B- Abnormal smear containing a sheet of malignant cells, with large hyperchromatic nuclei. There is nuclear pleomorphism, & one cell is in mitosis

Immunocytochemistry offers a powerful adjunct to routine histology.

(1) Detection of **cytokeratin** by specific monoclonal antibodies labeled with peroxidase points to a diagnosis of undifferentiated carcinoma rather than large cell lymphoma.

(2) Detection of **prostate-specific antigen (PAS)** in metastatic deposits by immunohistochemistry allows definitive diagnosis of a primary carcinoma in the prostate.

(3) Immunohistochemical detection of **estrogen receptors (ER) & HER-2** allows prognostication & directs treatment in **breast ca.**

Flow cytometry: Now is used routinely in the **classification of leukemias & lymphomas**.

In this method, fluorescent antibodies against cell surface molecules and differentiation antigens are employed to obtain the phenotype of malignant cells.

Flow cytometry also is **useful in assessing DNA** contents of the tumor cells.

In many tumors, DNA content (ploidy) has a bearing on prognosis.

Biochemical Assays

Biochemical assays for Tumor-associated enzymes, hormones, & other Tumor markers in the blood **cannot be consider as methods for diagnosing cancer**; however, they:

(a) may help in the finding cases, **(b)** may help in determining the **effectiveness of therapy**, & **(c)** may help in the **detection of tumor recurrence** after treatment,

Examples:

(I) Elevated levels of Prostatic Specific Antigen (PAS) in the blood can occur in **Prostatic Ca.** but it may also occur in benign prostatic hyperplasia, therefore, although it is helpful, elevations in PAS are not diagnostic of prostatic cancer.

(II) Radioimmunoassays for circulating **hormones** may point to the presence of

(1) Tumor in the endocrine system, & in some instances

(2) to the ectopic production of hormones, by nonendocrine Tumor.

(III) One of the best established circulating Tumor markers is **carcinoembryonic antigen (CEA).**

CEA, normally produced in embryonic tissue of the gut, pancreas, & liver, is a complex glycoprotein that is elaborated by many different tumors.

CEA levels is **elevated** (positive) in up to: 90% of **colorectal Ca.**, 80% of **pancreatic Ca.**, & 50% of **gastric & breast cancers.**

In almost all types of Tumors, the level of elevation is correlated with the body burden (load) of Tumor, so that the **highest levels** are found in patients with **advanced metastatic cancers.**

CEA elevations also have been reported in **many benign** disorders, however, such as alcoholic cirrhosis, hepatitis, ulcerative colitis, & Crohn's disease.

Occasionally, levels of this antigen are elevated in **apparently healthy smokers**. **Therefore, CEA assays lack specificity & sensitivity required for the detection of early cancers.**

 # CEA importance is in: (1) Providing presumptive evidence of the **possibility of colorectal Ca.** as this cancer yields the highest CEA levels; & **(2) Detecting recurrences of the Tumor after excision.** With successful resection of the Tumor, **CEA** disappears from the serum; its reappearance almost always signifies the appearance of **cancer recurrence.**

(IV) The other well-established tumor marker is **α-fetoprotein:** Elevated circulating levels are encountered in adults with cancers arising principally in the **(1) liver**, & **(2)** from the **yolk sac remnants in the gonads**. Less regularly, it is elevated in **(3) malignant teratomas** of the ovary, testis, & extragonadal sites.

As with CEA, benign conditions, including cirrhosis, hepatitis, & pregnancy (especially with fetal distress or death), may cause modest elevation of **α-fetoprotein**.

Thus, it lack specificity & sensitivity, but the marker may provide **presumptive evidence of e.g. a hepatocellular Ca.** & is of value in the follow-up of therapeutic intervention.

Molecular Diagnosis

an increased number of molecular techniques is being used for the diagnosis of Tumors & for predicting their behavior.

Diagnosis of malignancy:

Because each T & B cell has unique rearrangement of its antigen receptor gene, polymerase chain reaction **(PCR)-based detection** of T-cell receptor or immunoglobulin genes ***allows distinction between monoclonal (neoplastic) & polyclonal (reactive) proliferations.***

PCR –based detection of *BCR-ABL* transcripts provides a molecular signature of chronic myeloid leukemia.

Fluorescent in situ hybridization (FISH) technique is useful in detecting translocation characteristic of many Tumors, including Ewing sarcoma, several leukemias, & lymphomas.

Prognosis and behavior*:* FISH & PCR methods can also be used for showing amplification of oncogenes such as *HER-2 & N-MYC*. These oncogenes provide prognostic information for breast Ca. & Neuroblastomas respectively.

Detection of minimal residual disease. For example, detection of *BCR-ABL* transcripts by PCR assay gives a measure of residual disease in patients treated for chronic myeloid leukaemia.

Diagnosis of hereditary predisposition to cancer. Germ line mutation of several tumor suppressor genes, such as *BRCA1,* increases a patient's risk for developing certain types of cancer.

Therapeutic decision-making. Therapies that directly target specific mutations are increasingly being developed, and thus detection of such mutations in a tumor can guide the development of targeted therapy.

Molecular Profiling of Tumors: The Future of Cancer Diagnostics

The recent new technologies can rapidly sequence an entire genome; assess epigenetic modifications genome-wide (the epigenome); quantify all of the RNAs expressed in a cell population (the transcriptome); measure many proteins simultaneously (the proteome); and take a snapshot of all of the cell's metabolites (the metabolome). Thus, the diagnosis, management and study of cancer has entered the age of "omics!"

However, RNA is prone to degradation and is a more difficult analyte to work with than DNA in clinical practice. Furthermore, DNA sequencing is technically simpler than RNA sequencing, permitting the development of methods that rely on massively parallel sequencing (so-called “next-generation [NextGen] sequencing”) that can be readily performed on virtually any tissue specimen.

These advances have enabled the systematic sequencing and cataloging of genomic alterations in various human Cancers.

Thank you.
Next lecture is
Amyloidosis