III. Evasion of Cell Death

- *Accumulation of neoplastic cells may result from mutations in genes regulating apoptosis and o*f these candidates, i*s the role of BCL2 in protecting tumor cells from apoptosis.*
- Approximately 85% of B cell lymphomas of the follicular type carry a characteristic t(14;18) translocation.
- 14q32, the chromosomal locus for immunoglobulin heavy-chain genes and Juxtaposition of this transcriptionally active locus with *BCL2* gene(located : at 18q21) causes overexpression of the BCL2 protein

- Because BCL2-overexpressing lymphomas arise through reduced cell death rather than explosive cell proliferation, they tend to be indolent (slow-growing) compared to other lymphomas

IV. Development of Sustained Angiogenesis

- Tumors cannot enlarge beyond 1 to 2 mm in diameter unless they are vascularized ;therefore cancer cells and large benign tumors can stimulate neovascularization that:

a. Provides Perfusion to supply needed nutrients and oxygen

b. New endothelial cells stimulate the growth of tumor cells by secreting insulin-like growth factors, and PDGF

c. for access to the vasculature and so for metastasis

NOT- The angiogenesis inducer is vascular endothelial growth factor (VEGF) and inhibitor is thrombospondin-1 (TSP-1 that is produced by stromal fibroblasts),

- Early in their growth, tumors do not induce angiogenesis, until the angiogenic switch terminates vascular quiescence by increased production of angiogenic factors and/or loss of angiogenesis inhibitors

<u>Proteases regulat the balance between angiogenic and anti-angiogenic factors:</u>

1. Many proteases can release the angiogenic basic FGF
2. conversely, the potent angiogenesis inhibitors are:-

a. angiostatin produced by cleavage of plasminogen

b endostatin, produced by proteolytic cleavage of collagen

c.vasculostatin- produced by cleavage of transthyretin

- The angiogenic switch is controlled by hypoxia which stimulates production of (VEGF), through activation of hypoxia-inducible factor-1α (HIF-1α), an oxygen-sensitive transcription factor which is

a.is continuously produced,

b. but In normoxic settings the von Hippel-Lindau protein (VHL) binds to HIF-1α, leading to ubiquitination and destruction of HIF-1α.

c- In hypoxic conditions, such as in a tumor that has reached a critical size, hypoxia prevents HIF-1α recognition by VHL, and it is not destroyed and activates VEGF.

- *VHL* is a tumor suppressor gene, and germline mutations of the *VHL* gene are associated with VHL syndrome characterized by

A. renal cell carcinoma and renal cysts

B. Hemangioblastoma and pheochromocytoma

V. Ability to invade and metastasize

1. Invasion of Extracellular Matrix (ECM): four steps

a. L*oosening* of tumor cells.: E-cadherins act as intercellular glues, and their cytoplasmic portions bind to β-catenin

- E-cadherin can transmit antigrowth signals by sequestering β-catenin

E-cadherin function is lost in epithelial cancers, either

I.. by mutational inactivation of E-cadherin genes and, by activation of β-catenin genes

II. inappropriate expression of the SNAIL and TWIST

transcription factors, which suppress E-cadherin expression.

b.Local *degradation of the basement membrane and interstitial connective tissue*

- Tumor cells may either secrete proteolytic enzymes or induce stromal cells (fibroblasts) to elaborate proteases.
- Matrix metalloproteinases (MMPs),, and plasminogen activator, have been implicated in invasion.
- MMPs regulate tumor invasion by releasing ECM-sequestered growth factors .
- Cleavage products of collagen and proteoglycans have

. chemotactic, angiogenic, and growth-promoting effects

- Benign tumors of the breast, colon, and stomach show little type IV collagenase activity, whereas their malignant counterparts overexpress this enzyme.
- The levels of metalloproteinase inhibitors are reduced so that the balance is tilted toward tissue degradation.

c. Changes in attachment of tumor cells to ECM proteins

- Integrin receptors on normal epithelial cells for basement membrane laminin and collagens are polarized at their basal surface; which help to maintain it in a resting state

- cleavage of the basement membrane proteins, collagen IV and laminin, by MMP-2 or MMP-9 generates novel sites that bind to receptors on tumor cells stimulating migration.

d. Locomotion propelling tumor cells through the degraded basement membranes and zones of matrix proteolysis.

- Migration is a multistep process potentiated and directed

1. by autocrine motility factors released from tumor cells
2. insulin-like growth factors I and II
3. Stromal cells also produce hepatocyte growth factor /scatter factor (HGF/SCF),__its concentrations are elevated at edges of the brain tumor glioblastoma

2. Dissemination and Homing of Tumor Cells

- In the circulation, tumor cells are vulnerable to destruction by immune cells but some tumor cells form emboli by adhering to leukocytes, and platelets; that afforded some protection from the antitumor host effector cells.
- Most tumor cells, however, circulate as single cells.
- The organ distribution of metastases generally can be predicted by the location of the primary tumor and its vascular or lymphatic drainage.
- But In many cases, the natural pathways of drainage do not readily explain the distribution of Metastasis,

- Some tumors (e.g., lung cancers) tend to involve the adrenals quite often but never spread to skeletal muscle.

-Such organ tropism is related to the following mechanisms

.a. Expression of adhesion molecules by tumor cells whose ligands are expressed on the endothelium of target organs

b. Expression of chemokines and their receptors

- breast cancer cells express high levels of the chemokine receptors CXCR4 and CCR7 and the ligands for these Receptors (chemokine CXCL12) are highly expressed only in organs to which breast cancer metastasize

- blockade of chemokine receptors may limit metastases

- Once the tumor cells reach a target, the tumor cells must be able to colonize the site and The mechanism of colonization may be related to the secretion of cytokines, growth factors, and proteases by tumor cells that act on the resident stromal cells, which make the metastatic site habitable for the cancer cell .

Note: it is known that after extravasation, tumor cells are dependent on a receptive stroma for growth,

- Thus, in some cases, the target tissue may be a non-permissive environment- for the growth of tumor seedlings

  such as skeletal muscles

-

Molecular Genetics of Metastasis:- An open question is whether there are genes that control metastases

- Among candidates are those encoding SNAIL and TWIST transcription factors whose primary function is to promote epithelial-to-mesenchymal transition (EMT) in which carcinoma cells downregulate (E-cadherin) and upregulate mesenchymal markers (vimentin, actin).
- These molecular changes are accompanied by morphologic change from epithelioid shape to a spindly mesenchymal shape, and increased production of proteolytic enzymes that promote migration and invasion

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Genomic Instability as an Enabler of Malignancy .

- Although humans are exposed to many mutagenic agents , cancers are relatively rare and this state results from the ability of normal cells to repair DNA damage.
- The importance of DNA repair is highlighted by several inherited disorders in which genes that encode proteins involved in DNA repair are defective and the affected persons are at *increased risk for development of cancer.*

Defects in three types of DNA repair systems include

A. Defects in genes involved in DNA mismatch repair genes

Example: Hereditary Nonpolyposis Colon Cancer Syndrome

a- Characterized by familial carcinomas of the colon

b. affecting predominantly the cecum and proximal colon

c. Due to defects in genes involved in DNA mismatch repair.

- When a strand of DNA is being repaired, these genes act as "spell checkers. so if there is an erroneous pairing of G with T, rather than normal A with T, the mismatch repair genes correct the defect and Without these "proofreaders," errors accumulate at an increased rate, a so-called mutator phenotype

- Mutations in at least four mismatch repair genes have

  been found to underlie HNPCC and each affected person inherits one defective copy of one of DNA mismatch repair genes and acquires the second hit in colonic cells.
- DNA repair genes affect cell growth indirectly by allowing mutations in other genes during process of normal cell ivision.
- A characteristic finding in the genome of patients with mismatch repair defects is microsatellite instability (MSI).

- Microsatellites are tandem repeats of one to six nucleotides found throughout the genome
- In normal people, the length of microsatellites remains constant
- and by contrast, in patients with HNPCC, these satellites are unstable and increase or decrease in length.
- MSI can be detected in about 15% of sporadic cancers
- The growth-regulating genes that are mutated in HNPCC include those encoding TGF-β receptor type II and, BAX

B. Defects in nucleotide excision repair pair

XerodermaPigmentosum

- Patients with xeroderma pigmentosum, are at increased risk for the development of cancers of sun-exposed skin.
- Ultraviolet (UV) rays in sunlight cause cross-linking of pyrimidine residues, preventing normal DNA replication.
- Such DNA damage is repaired by the nucleotide excision repair system.

C. Diseases with Defects in DNA Repair by Homologous Recombination : Three autosomal recessive disorders

a. Bloom syndrome: hypersensitivity to Ionizing radiation and d **developmental defects**

b. Ataxia-telangiectasia: hypersensitivity to Ionizing radiation and **neural symptoms :**The gene mutated is *ATM,* which encodes a protein kinase that is important in recognizing DNA damage caused by ionizing radiation and initiating p53 activation.

c. Fanconi anemia; hypersensitivity to to DNA cross-linking agents, such as nitrogen mustard and **anemia**.

Mutations in two genes, *BRCA1* and *BRCA2*, account for 50% of cases of familial breast cancer.

1- Women with *BRCA1* mutations have

a. a substantially higher risk of epithelial ovarian cancers,

b. and men have a slightly higher risk of prostate cancer

2. Mutations in the *BRCA2* gene increase the risk of breast cancer in both men and women,

- Both genes function,in the homologous recombination DNA repair pathway,. For example, BRCA1 forms a complex with proteins in the homologous recombination pathway and also is linked to the ATM kinase pathway.

- *BRCA2* was identified as one of several genes mutated in Fanconi anemia, and the BRCA2 protein has been shown to bind to RAD51, a protein required for homologous recombination.
- Both copies of *BRCA1* and *BRCA2* must be inactivated for cancer to develop.

BRCA1 and *BRCA2* are rarely inactivated in sporadic cases of breast cancer.

- In this regard, *BRCA1* and *BRCA2* are different from other tumor suppressor genes, such as *APC* and *TP53,* which are inactivated in both familial and sporadic cancers.

Carcinogens

I. Chemical Carcinogens

A. Direct-Acting Agents:

- Require no metabolic conversion to become carcinogenic and are in general weak carcinogens
- But are important because some of them are cancer chemotherapy drugs (e.g., alkylating agents) used in regimens that may cure certain types of cancer (e.g., Hodgkin lymphoma), only to evoke a subsequent, second form of cancer, usually leukemia
- The associated risk of induced cancer is low, but its existence dictates judicious use of such agents

B. Indirect-Acting Agents :refers to chemicals that require metabolic conversion to an *ultimate carcinogen*

1. polycyclic hydrocarbons, such as benzo[*a*]pyrene formed in the high-temperature combustion of tobacco in cigarette smoking and these *products are implicated in the causation of lung cancer in cigarette smokers*

- Polycyclic hydrocarbons are present in smoked meats

2. The aromatic amines and azo dyes :β-naphthylamine was responsible for increased incidence of bladder cancers in exposed workers in the aniline dye and rubber industries

3. Aflatoxin B: is a naturally occurring agent produced by

.some strains of *Aspergillus,* a mold that grows on improperly stored grains and nuts and there is a strong correlation between its level and the incidence of hepatocellular carcinoma in Africa and the Far East

4. Nitrites used as food preservatives have caused concern, since they cause nitrosylation of amines contained in the food. The nitrosamines thus formed are suspected to be carcinogenic

Mechanisms of Action of Chemical Carcinogens

- All direct and ultimate carcinogens contain highly reactive electrophile groups that form chemical adducts with DNA, as well as with proteins and RNA.
- *RAS* and *TP53,* are important targets of chemical carcinogens.

a- Carcinogenicity of some chemicals is augmented by subsequent administration of *promoters* (hormones, phenols, drugs) that by themselves are nontumorigenic.

b. Repeated exposure to the promoter must *follow* the application of the mutagenic chemical, or *initiator.* :

- **How promoters contribute to tumorigenesis?**
- Although effects of promoters are many,but mainly cause *cell proliferation*
- While the application of an initiator may cause the mutational activation of an oncogene such as *RAS,* subsequent application of promoters leads to clonal expansion of initiated (mutated) cells
- Forced to proliferate, the initiated clone of cells accumulates additional mutations, developing eventually into a malignant tumor.

II. Radiation Carcinogenesis

1. Unprotected miners of radioactive elements have a 10-fold increased incidence of lung cancers.
2. Follow-up study of survivors of the atomic bombs dropped

   on Hiroshima and Nagasaki showed increased incidence of myelogenous leukemias-after latent period of 7 years.
3. Therapeutic irradiation of the head and neck can give rise to papillary thyroid cancers years later

A. Ionizing radiation causes; Chromosome breakage translocations, and less commonly point mutations

Biologically, double-stranded DNA breaks seem to be the most important form of DNA damage caused by radiation.

B. Natural UV radiation derived from the sun

1. Can cause melanomas, squamous cell carcinomas, and basal cell carcinomas and at greatest risk are fair-skinned people who live in l Australia and New Zealand

2. Nonmelanoma skin cancers are associated with total cumulative exposure to UV radiation , whereas melanomas are associated with intense intermittent exposure-as occurs with sunbathing

- UV light caused damage DNA by forming pyrimidine dimers and this type of DNA damage is repaired by the nucleotide excision repair pathway.
- With extensive exposure to UV light, the repair systems may be overwhelmed, and skin cancer results.
- Patients with the inherited disease *xeroderma pigmentosum* have a defect in the nucleotide excision repair pathway and as expected, there is a greatly increased predisposition to skin cancers in this disorder

- HTLV-1 is associated with T cell Leukemia/lymphoma that is endemic in certain parts of Japan and the Caribbean
- HTLV-1 has tropism for CD4+ T cell and human infection requires transmission of infected T cells through sexual intercourse, blood products, or breastfeeding.
- Leukemia develops only in about 3-5% of infected persons after a long latent period of 20-50 years
- The HTLV-1 genome does not contain a viral oncogene with no consistent integration site next to a cellular oncogene has been discovered

Pathogenesis

- HTLV-1 genome contains PX region containing TAX gene

A. The *TAX* gene turns on several cytokine genes and their receptors (IL-2 , IL-2R and IL-15 and IL-15R), setting up an autocrine system that drives T cell proliferation

B. paracrine pathway is activated by increased production of granulocyte-macrophage colony-stimulating factor, which stimulates macrophages to produce other T cell mitogens

C. TAX activates cyclins and drive cell cycle progression

D. TAX can repress the function of *p16* and *TP53.*