Neoplasia

BY:
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Neoplasia

- New growth - Neoplasm
- Tumour - Swelling
- Oncology - Oncos-study of tumour neoplasms
- Cancer - Malignant tumours [crab]
Neoplasia

- Definition: Abnormal mass of tissue the growth of which exceeds & is uncoordinated with that of normal tissue & persists in the same excessive manner after cessation of stimuli.
- Heritable genetic alteration - passed to progeny of tumour cells.
- Autonomous
- Clonal
Non-neoplastic skin tissue
Neoplasia (abnormal tissue mass, Excessive growth)
Neoplasia (abnormal tissue mass, Excessive growth)
Neoplasia—Uncoordinated (Autonomous) Growth
Neoplasia persists even after initiating event (stimulus) no longer exists.
Neoplasia parasitic upon host tissues
Neoplasia

- Benign
- Malignant

- Parenchyma [proliferating neoplastic cells] - cutting edge & behaviour of tumour
- soft & fleshy
- Schirrous
Neoplasia

- Benign tumours-suffix -oma to cell of origin
  - Mesenchymal tumours- Chondroma, Fibroma
  - Epithelial-complex
    -- Adenoma-form glandular structures/arise from Glandular structures
    Renal Adenoma & Adrenal cortical adenoma
    -- Papilloma
    -- Cystadenoma
    --- Papillary Cystadenoma
    --- Polyp
Malignant tumors nomenclature:

- **Mesenchymal cancers - Sarcomas** (Fleshy)
  - Rhabdosarcoma, Liposarcoma, Fibrosarcoma, Osteosarcoma, Chondrosarcoma

- **Epithelial cancers (Epithelium of any 3 germ layers) - Carcinomas**
  (further qualified as differentiated/ site of origin)
  - Adenocarcinoma, Squamous cell carcinoma, Renal cell carcinoma, Hepatocellular carcinoma

- Undifferentiated malignant tumor
  - Cancer of undifferentiated cells of unknown tissue origin
Nomenclature of neoplasms

Tumours of Mesenchymal Tissues

<table>
<thead>
<tr>
<th>BENIGN</th>
<th>MALIGNANT</th>
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<tbody>
<tr>
<td>C.T.&amp;derivatives</td>
<td>Fibroma lipoma</td>
</tr>
<tr>
<td>Endothelial.&amp;derivatives</td>
<td>Haemangioma</td>
</tr>
<tr>
<td>Blood cells</td>
<td></td>
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<tr>
<td>Muscle</td>
<td>leiomyoma,Rhabdomyoma</td>
</tr>
</tbody>
</table>

Tumours of epithelial tissues

<table>
<thead>
<tr>
<th>Squamous cells</th>
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<tbody>
<tr>
<td>Basal cells</td>
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<tr>
<td>Epi. lining of glands</td>
<td>Adenoma,papilloma. Cystadenoma</td>
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<tr>
<td>Resp</td>
<td>Bronchogenic Ca.</td>
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<tr>
<td>Liver</td>
<td>Adenoma</td>
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<tr>
<td>Placenta</td>
<td>H.Mole.</td>
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</tbody>
</table>

Further malignant conditions:
- Leukaemia, lymphoma
- fibrosarcoma,
- Hepatocellular Ca
- Choriocarcinoma.
- **Mixed tumours** - divergent diff. of a single line of parenchymal cells into another tissue
  
  eg. Pleomorphic adenoma

- **Teratoma** - A variety of parenchymal cells from more than 1 germ layer
Neoplasia - basic concepts & definitions

Nomenclature Exceptions: Tumors
-Malignant tumors & suffix-oma

- Melanoma (cancers of Melanocytes)
- Lymphoma (cancers of Lymphoid tissue)
- Seminoma (cancers of Gonadal germ cells)
- Hepatoma (Hepatocellular carcinoma)
- Myeloma (Malignancy of Plasma cells)
Neoplasia

Choriostoma - An ectopic Rest of normal tissue
E.g. Adrenal cells under kidney capsule
Pancreatic rests - Gastric mucosa

Hamartoma - Mass of disorganised but mature cells/tissue indigenous to that site; Lungs - cartilage, b.v., bronchial structures, lymphoid tissue
Choristoma- Pancreatic acini in gastric mucosa

Lung Hamartoma
Characteristics of benign & malignant neoplasms

- Differentiation & Anaplasia
- Rate of Growth
- Local Invasion
- Metastasis
Characteristics of benign & malignant neoplasms

- Differentiation & Anaplasia - Refers to extent to which parenchymal cells resemble comparable normal cells of that tissue - morphology & function.
  - Benign - well differentiated. E.g. Leiomyoma
  - Malignant - well differentiated, Mod. diff. Poorly differentiated.
  - Anaplasia is lack of differentiation - Hallmark of MALIGNANCY
- Cancer - stem cells in specialized tissues
- Well diff. - Maturation of proliferating cells
- Poorly differentiated. Proliferation without maturation
Characteristics of benign & malignant neoplasms

- **Morphological features of Anaplasia**
  - Pleomorphism - variation in size & shape
  - Hyperchromasia
  - Nuclear shape
  - Clumped chromatin
  - Prominent nucleoli
  - Increased mitosis
  - Tu giant cells
  - Loss of orientation
  - Central necrosis
Characteristics of Benign & Malignant neoplasms
Characteristics of Benign & Malignant Neoplasms

- Dysplasia - disordered growth
  - Loss of uniformity of individual cells
  - Loss in architectural pattern
  - Mild, moderate, severe
  - Mild, moderate-reversible

- FUNCTIONAL changes - well differentiated retain function.
  - Poorly differentiated - loss of function, emergence of other fn.-fetal protein, ectopic hormone production.
- FUNCTIONAL changes - well differentiated retain function.
- Poorly differentiated - loss of function,
  --- emergence of other fn. - fetal protein
  ---- ectopic hormone production
Characteristics of Benign & Malignant neoplasms

Rate of growth: Prognosis & t/t outcome determined by 3 factors:
- Fraction of tumour cells in replicating pool
- Doubling time of tumour cells
- Rate at which cells are lost in growing lesion

Cell cycle constraints – lost in tumours but cell cycle is not shortened
Normal Cell Cycle

- **G₀**: Quiescent, stable cells (e.g., hepatocytes)
- **G₁**: Check for DNA damage (G₁/S checkpoint)
- **S**: Chromosome duplication
- **G₂**: Check for damaged or unduplicated DNA (G₂/M checkpoint)
- **M**: Mitosis
- **M**: Cell division

**CELL CYCLE**

- Centrosome duplication
- Growth in mass

**Continuous cycling tissue cells** (e.g., epidermis, GI tract epithelium)
Characteristics of Benign & Malignant neoplasms

- Proportion of cells with in tu. That are in proliferation pool - Growth fraction
- Submicroscopic level vast majority of transformed cells in proliferating pool grow, most leave cell cycle shedding
  - Decreased nutrients
  - Apoptosis
  - Differentiation
  - G₀
Characteristics of Benign & Malignant neoplasms

Rapidly proliferating tumour - G. fraction - 20%

- Cell production > cell loss
  - Rapid - leukemia/lymphoma/lung ca
  - Slow - Ca breast, colon

- G fraction determines response to chemotherapy
  - Rapid - meltaway by chemo
  - Slow (5%) - debulking – increases G fraction
Characteristics of Benign & Malignant neoplasms

Doubling time - clinically detectable tumour (approx 1gm = $10^9$ cells) requires 30 population doublings

for 1kg ($10^{12}$ cells) - only 10 more doublings

Doubling time variable
Rate of growth - not constant
Local Invasion

Infiltration of adjacent normal tissue

Nearly all benign tumors grow as cohesive expansile masses that remain localized to their site of origin.

- Next to the development of metastases, invasiveness is the most reliable feature that differentiates malignant from benign tumors.
Characteristics of Benign & Malignant neoplasms

The growth of cancers is accompanied by progressive infiltration, invasion, and destruction of the surrounding tissue.

In general, malignant tumors are poorly demarcated from the surrounding normal tissue, and a well-defined cleavage plane is lacking.
Characteristics of Benign & Malignant neoplasms

Metastasis

Metastases are tumor implants discontinuous with the primary tumor, taking up residence at some distance.

Metastasis unequivocally marks a tumor as malignant because benign neoplasms do not metastasize.

With few exceptions, all cancers can metastasize.
Characteristics of Benign & Malignant neoplasms

The major exceptions are gliomas in the CNS and basal cell carcinomas of the skin. In general, the more aggressive, the more rapidly growing, and the larger the primary neoplasm, the greater the likelihood that it will metastasize or already has metastasized.
Pathways of spread

1. **Direct seeding of body cavities & surfaces**
   - Open field
   - Pseudomyxoma peritonei

2. **Lymphatic spread**
   - Dissemination of ca, no functional lymphatics;
     - Pattern of L.N.-natural routes of lymphatics
     - CA BREAST: Upper outer quadrant-axillary L.Ns; inner quadrant – internal mammary, Lung ca-perihilar, tracheobronchial, mediastinal L.Ns.

- skip metastasis
- Prognostic marker
- Sentinel L.N.-FIRST L.N. in lymphatic drainage from pr. Tu.
- Effective barrier
- L.N. enlargement
3. Haematogenous spread

- Sarcomas
- Arteries resistant - pulmonary capillary bed
  pulmonary arteriovenous shunt
- Venous invasion follows the route of venous drainage
  lungs - caval
  liver - portal
  vertebral venous plexus - thyroid, prostate
- Carcinoma – RCC, HCC
- Gross - multiple, round lesions
Molecular basis of invasion and metastasis

INVASION AND METASTASIS

Invasion and metastasis are biologic hallmarks of malignant tumors.

They are the major cause of cancer-related morbidity and mortality.

A clear understanding of the origin of metastasis is of major importance for the management of cancer patients and the development of effective therapies to prevent tumor spread.

- Subclones of tumour cells, metastatic signature prop. Intrinsic to tu. Cells components of stroma
Molecular basis of invasion and metastasis

The metastatic cascade: Two phases

Invasion of Extracellular Matrix

Vascular Dissemination and Homing of Tumor cells
Molecular basis of invasion and metastasis

- **TUMOR STROMA**
- **COMPOSITION OF THE STROMA**
  EC connective tissue consisting of collagen, proteoglycans, blood vessels, stromal fibroblasts, inflammatory and immune cells, etc.
- **TUMOR-STROMAL INTERACTIONS**
Molecular basis of invasion and metastasis

Steps of Invasion
- loss of tumor cell-cell adhesion
- invasion of basement membrane and extracellular matrix
- adherence to matrix
- secretion of proteolytic enzymes
- cell locomotion
- invasion of blood vessels and lymphatics
Molecular basis of invasion and metastasis

Invasion of Extracellular Matrix [active process]
Detachment ("loosening up") of the tumor cells from each other
Attachment to matrix components
Degradation of ECM
Migration of tumor cells
Molecular basis of invasion and metastasis

- Normal cells-cadherins [E-cadherins], catenins
- Receptor mediated attachment of tumor cells to laminin, collagen IV [integrins, Ig family]
- Increase integrins, laminin receptors
- Different integrins [α4β1 integrins]
Molecular basis of invasion and metastasis

Invasion of ECM:

- Enzymatic Degradation of Matrix: Invasion of the ECM is not merely due to passive growth pressure but requires active enzymatic degradation of the ECM components.
- Tumor cells secrete proteolytic enzymes themselves or induce host cells (e.g., stromal fibroblasts and infiltrating macrophages) to elaborate proteases such as plasminogen activators, collagenases, etc.
- Serine, cystine, matrix metalloproteinases (MMP-9, MMP-2)
- Inhibitors of MMPs
Molecular basis of invasion and metastasis

- While the most obvious effect of matrix destruction is to create a path for invasion by tumor cells
- Cleavage products of matrix components, derived from collagen and proteoglycans, also have growth promoting, angiogenic, and chemotactic activities
- Degradation of collagen IV exposes cryptic domains
  - Angiogenesis
- Antiangiogenesis-endostatin, tumsttin
Molecular basis of invasion and metastasis

Tumor Metastasis

Steps of Extravasation

- circulating tumor cells
- formation of tumor clumps
- adhesion to endothelium
- penetration of basement membrane
Once in the circulation, tumor cells are particularly vulnerable to destruction by innate and adaptive immune defenses. Within the circulation, tumor cells tend to aggregate in clumps. This is favored by homotypic adhesions among tumor cells as well as heterotypic adhesion between tumor cells and blood cells, particularly platelets. Formation of platelet-tumor aggregates may enhance tumor cell survival and implantability.
Molecular basis of invasion and metastasis

Vascular Dissemination and Homing of Tumor Cells

Arrest and extravasation of tumor emboli at distant sites involve adhesion to the endothelium, followed by egress through the basement membrane.

Involved in these processes are adhesion molecules (integrins, laminin receptors) and proteolytic enzymes.
Molecular basis of invasion and metastasis

- Sites of Tumor Metastasis
  Proximity to tumor site
  --regional lymph nodes
  --Tumor specific targets e.g. prostate carcinoma mets to bone
  ✓ Organ-specific adhesion molecules?
  ✓ Growth factor secretion
  ✓ Suitable environment
Molecular basis of invasion and metastasis

Mechanism of Organ Tropism

- Because the first step in extravasation is adhesion to the endothelium, tumor cells may have **adhesion molecules** whose ligands are expressed preferentially on the endothelial cells of the target organ.
- The endothelial cells of the vascular beds of various tissues differ in their expression of ligands for adhesion molecules.
Molecular basis of invasion and metastasis

- **Chemokines** have a very important role in determining the target tissues for metastasis. e.g. Some breast cancer cells express the chemokine receptors CXCR4 and CCR7. The chemokines that bind to these receptors are highly expressed in tissues to which breast cancers commonly metastasize.

- Some target organs may liberate chemoattractants that tend to recruit tumor cells to the site. Examples include insulin-like growth factors I and II.
Molecular basis of invasion and metastasis

Mechanism of Organ Tropism

In some cases, the target tissue may be an unpermissive environment-unfavorable soil, for the growth of tumor seedlings. e.g., although well vascularized, skeletal muscles are rarely the site of metastases.
Staging of Malignant Neoplasms

**TNM Staging [UICC] Union int. contre cancer**

- **T**<sub>0</sub> In situ, non-invasive (confined to epithelium)
- **T**<sub>1</sub> Small, minimally invasive within primary organ site
- **T**<sub>2</sub> Larger, more invasive within the primary organ site
- **T**<sub>3</sub> Larger and/or invasive beyond margins of primary organ site
- **T**<sub>4</sub> Very large and/or very invasive, spread to adjacent organs

- **N**<sub>0</sub> No lymph node involvement
- **N**<sub>1</sub> Regional lymph node involvement
- **N**<sub>2</sub> Extensive regional lymph node involvement
- **N**<sub>3</sub> More distant lymph node involvement
- **M**<sub>0</sub> No distant metastases
- **M**<sub>1</sub> Distant metastases present
Staging

- AJC staging
- Stage O-IV
- All 3 components of UICC
Grading

- Grading schemes are based upon the microscopic appearance of a neoplasm with H&E staining.
- Degree of differentiation and no. of mitosis
- Most grading systems have three or four grades (designated with numbers or roman numerals).
- Broders’ for sq. cell ca
  Grade I- Well diff.- less than 25% anaplastic cells
  Grade II- Mod. Diff.- 25%-50% anaplastic cells
  Grade III- Mod. Diff.- 50%-75% anaplastic cells
  Grade IV- Poorly diff.- more than 75% anaplastic cells
- In the diagram utilizing an adenocarcinoma as an example, the principles of grading are illustrated:
Grading

G1  G2  G3  G4
Prognostic Markers

- **Clinical Prognostic Markers**
  - Size, grade, vascular invasion, nodal involvement

- **Molecular markers**
  - Expression of oncogene C-met, c-ras
  - CD44
  - Estrogen receptors
  - EGF receptor
  - Angiogenesis factors
Epidemiology and predisposing factors to Neoplasia

- Incidence-20-23% of all mortality
- Most common ca in males-lung, prostate, colorectal
- Most common ca in females-breast, lung, colorectal
- **Age**- males-6-8 decade
  females-4 6 decade
  children [less than 15]-leukaemia, lymphoma, wilms Tu., retinoblastoma, neuroblastoma, medulloblastoma, rhabdomyosarcoma, hepatoblastoma
Predisposing factors

Racial and geographical factors

- Japanese—ca stomach<7-8 fold>
- Europeans and Americans—Ca lung, Breast, Colon
- Black Africans—Ca skin, cervix, penis
- South east Asians—Nasopharyngeal
- Indians—oral cavity, upper aero digestive
Predisposing factors

Environmental and cultural

- Smoking, Alcohol, Betel nut, Diet
- Carcinoma cervix—early marriage, parity, and multiple partners
- Penile Cancer – Rare in Jews and Muslims, smegma-carcinogenic
- **arsenic compounds**
  --Lung, skin, hemangiosarcoma
  Byproduct of metal smelting. Component of alloys, electrical and semiconductor devices, medications and herbicides, fungicides, and animal dips
- **Asbestos**
  --Lung, mesothelioma; gastrointestinal tract (esophagus, stomach, large intestine)
  --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)
• **Benzene**
  --Leukemia, Hodgkin lymphoma
  --Principal component of light oil.
  Although use as solvent is discouraged, many applications exist in printing and lithography, paint, rubber, dry cleaning, adhesives and coatings, and detergents, fumigant

• **Beryllium and beryllium compounds**
  --Lung
  --Missile fuel and space vehicles.
  • Cadmium and cadmium compounds
    --Prostate
    --Used in batteries and as alloy and in metal platings and coatings
- Cadmium and cadmium compounds
  --Prostate
  --. Used in batteries and as alloy and in metal platings and coatings
- Chromium compounds
  --Lung
  --Component of metal alloys, paints, pigments, and preservatives
- Ethylene oxide
  ---Leukemia
  ---Ripening agent for fruits and nuts. Used in rocket propellant and chemical synthesis, in fumigants for foodstuffs and textiles, and in sterilants for hospital equipme
- Nickel compounds
  ---Nose, lung
  ---Nickel plating. Component of ferrous alloys, ceramics, and batteries.
• Radon and its decay products
  --Lung
  --From decay of minerals--
  containing uranium. Can be serious hazard in quarries and mines

• Vinyl chloride
  --Angiosarcoma, liver
Hormones And Cancer

- Estrogen—Endometrial ca, Vaginal Ca
- Contraceptive pills-ca breast, Liver Adenomas
- Anabolic Steroids ---liver tumors
Heredity

Hereditary forms of cancer can be divided into three categories

- Inherited Cancer Syndromes
- Familial Cancers
- Inherited Autosomal Recessive Syndromes of Defective DNA Repair
Inherited cancer syndromes

- Inherited cancer syndromes include several well-defined cancers in which inheritance of a single mutant gene greatly increases the risk of developing a tumor.
- The predisposition to these tumors shows an autosomal dominant pattern of inheritance.
- ``Childhood retinoblastoma -- Approximately 40% of retinoblastomas are familial.
- Carriers of this gene have a 10,000-fold increased risk of developing retinoblastoma, usually bilaterally.
- greatly increased risk of developing a second cancer, particularly osteogenic sarcoma.
Familial adenomatous polyposis --- an extraordinarily high risk of cancer.

Individuals who inherit the autosomal dominant mutation have, at birth or soon thereafter, innumerable polypoid adenomas of the colon, and virtually 100% of patients develop a carcinoma of the colon by age 50 p53 --- Li-Fraumeni syndrome (various tumors)
- Tumors within this group often are associated with a specific marker phenotype.
- There may be multiple benign tumors in the affected tissue, as occurs in familial polyposis of the colon and in multiple endocrine neoplasia.
- Sometimes, there are abnormalities in tissue that are not the target of transformation (e.g., Lisch nodules and café-au-lait spots in neurofibromatosis)
Familial Cancers

- Familial clustering of cases, but role of inherited predisposition not clear for each individual
- Breast cancer (not linked to BRCA1 or BRCA2)
- Ovarian cancer
- Pancreatic cancer
Familial cancers

- Virtually all the common types of cancers that occur sporadically have been reported to occur in familial forms.
- Features that characterize familial cancers include:
  - early age at onset
  - tumors arising in two or more close relatives of the index case
  - sometimes multiple or bilateral tumors.
Familial cancers are not associated with specific marker phenotypes. For example, in contrast to the familial adenomatous polyposis syndrome, familial colonic cancers do not arise in preexisting benign polyps.

The transmission pattern of familial cancers is not clear. In general, siblings have a relative risk between 2 and 3 times.

Segregation analysis of large families usually reveals that predisposition to the tumors is dominant, but multifactorial inheritance cannot be easily ruled out.
Inherited Autosomal Recessive Syndromes

- Inherited Autosomal Recessive Syndromes of Defective DNA Repair
- Xeroderma pigmentos
- Ataxia-telangiectasia
- Bloom syndrome
- Fanconi anemia
Acquired Preneoplastic Disorders

- certain clinical conditions have well-recognized predispositions to the development of malignant neoplasia and are referred to as preneoplastic disorders.

*This designation is unfortunate*

- Persistent regenerative cell replication
  - (e.g., squamous cell carcinoma in the margins of a chronic skin fistula or in a long-unhealed skin wound
  - hepatocellular carcinoma in cirrhosis of the liver)
Hyperplastic and dysplastic proliferations

- endometrial carcinoma in atypical endometrial hyperplasia
- bronchogenic carcinoma in the dysplastic bronchial mucosa of habitual cigarette smokers
- Chronic atrophic gastritis (e.g., gastric carcinoma in pernicious anemia or following long-standing Helicobacter pylori infection)
- an increased incidence of colorectal carcinoma in long-standing disease Chronic ulcerative colitis)
• increased risk of squamous cell carcinoma following Leukoplakia of the oral cavity, vulva, or penis
• Villous adenomas of the colon (e.g., high risk of transformation to colorectal carcinoma)

• ALL BENIGN TU—INHERENT RISK: NEVER TO FREQUENT
MOLECULAR BASIS OF CANCER

- Nonlethal genetic damage (or mutation) lies at the heart of carcinogenesis. Cancer is a genetic disease,
  - Envt. Damage
  - Germ Line

- A tumor is formed by the clonal expansion of a single precursor cell that has incurred the genetic damage (i.e., tumors are monoclonal)
  -- Clonality of tumors is assessed readily in women who are heterozygous for polymorphic X-linked markers, such as the enzyme glucose-6-phosphate dehydrogenase
  --HUMARA
Sex chromosomes

Female zygote

Blastocyst—
inactivation of one
X chromosome

Neoplasms

Polyclonal neoplasm
(two isoenzymes)

Monoclonal neoplasm
(one isoenzyme)
- Four classes of normal regulatory genes – proto-oncogenes
  - tumor suppressor genes
  - apoptosis-regulating genes
  - genes involved in DNA repair
- the principal targets of genetic damage
Mutant alleles of proto-oncogenes are called oncogenes. They are considered dominant because mutation of a single allele can lead to cellular transformation.

typically both normal alleles of tumor suppressor genes must be damaged for transformation to occur, so this family of genes is sometimes referred to as recessive oncogenes.

in some cases, loss of a single allele of a tumor suppressor gene can promote transformation (haploinsufficiency).

Genes that regulate apoptosis may be dominant, as are proto-oncogenes, or they may behave as tumor suppressor genes.
DNA repair genes affect cell proliferation or survival indirectly by influencing the ability of the organism to repair nonlethal damage in other genes, including protooncogenes, tumor suppressor genes, and genes that regulate apoptosis.

Carcinogenesis is a multistep process at both the phenotypic and the genetic levels.

- TUMOUR PROGRESSION
Principal Targets of Genetic Damage: 4 Classes of Normal Regulatory Genes

- Growth-promoting protooncogenes
- Growth-inhibiting tumor suppressor genes
- Apoptosis-regulating genes; genes that regulate the programmed cell death
- DNA repair genes
Essential Alterations for Malignant Transformation

- Self-sufficiency in growth signals
- Insensitivity to growth-inhibitory signals
- Evasion of apoptosis
- Defects in DNA repair
- Limitless replicative potential
- Sustained angiogenesis
- Ability to invade and metastasize
Simplified flow chart of the molecular basis of cancer
Normal Cell Cycle

- It is important to understand the molecular regulation of the normal cell cycle, since cell cycle abnormalities are fundamental to cancer growth and many of the genes that cause cancer perturb the cell cycle.
- The orderly progression of cells through the various phases of cell cycle is orchestrated by cyclins and cyclin-dependent kinases (CDKs), and by their inhibitors.
Main Cell-Cycle Components and Their Inhibitors

- Cyclin-Dependent Kinases (CDK)
- CDK Inhibitors
- Checkpoint Components
Cell-Cycle Inhibitors

- The activity of cyclin-CDK complexes is tightly regulated by inhibitors, called CDK inhibitors.
- Two main classes of CDK inhibitors: the Cip/Kip
- These inhibitors function as tumor suppressors and the INK4/ARF families are frequently altered in tumors
  - Cip/Kip-p21 p27 p57
  - INK4/ARF- p16INK4, p14ARF
Cell-Cycle Checkpoints

- The cell cycle has its own internal controls, called checkpoints.
- Two main checkpoints: One at the G1/S transition and another at the G2/M.
- To function properly, cell-cycle checkpoints require sensors of DNA damage, signal transducers, and effector molecules.
G1/S Checkpoint

- The S phase is the point of no return in the cell cycle, and before a cell makes the final commitment to replicate, the G1/S checkpoint checks for DNA damage.
- If DNA damage is present, the DNA repair machinery and mechanisms that arrest the cell cycle are put in motion.
- The delay in cell-cycle progression provides the time needed for DNA repair; if the damage is not repairable, apoptotic pathways are activated to kill the cell.
- Thus, the G1/S checkpoint prevents the replication of cells that have defects in DNA, which would be perpetuated as mutations or chromosomal breaks in the progeny of the cell.
- DNA damaged after its replication can still be repaired as long as the chromatids have not separated.
G2/M Checkpoint

- The G2/M checkpoint monitors the completion of DNA replication and checks whether the cell can safely initiate mitosis and separate sister chromatids.
- This checkpoint is particularly important in cells exposed to ionizing radiation.
- Cells damaged by ionizing radiation activate the G2/M checkpoint and arrest in G2.
- Defects in this checkpoint give rise to chromosomal abnormalities.
Checkpoint Components

- The **sensors** and **transducers** of DNA damage appear to be similar for the G1/S and G2/M checkpoints.
- Sensors - the RAD family and ataxia telangiectasia mutated (ATM)
- Transducers - the CHK kinase families
- The checkpoint **effector** molecules differ, depending on the cell-cycle stage at which they act.
- In the G1/S checkpoint, cell-cycle arrest is mostly mediated through p53, which induces the cell-cycle inhibitor p21.
- Arrest of the cell cycle by the G2/M checkpoint involves both p53-dependent and independent mechanisms. Defect in cell-cycle checkpoint components is a major cause of genetic instability in cancer cells.
Mechanisms regulating cell populations
Steps of Cell Proliferation under physiologic conditions

1. The binding of a growth factor to its specific receptor generally located on the cell membrane
2. Transient and limited activation of the growth factor receptor, which, in turn, activates several signal transducing proteins on the inner leaflet of the plasma membrane
3. Transmission of the transduced signal across the cytosol to the nucleus via second messengers or by signal transduction molecules that directly activate transcription
4. Induction and activation of nuclear regulatory factors that initiate DNA transcription
5. Entry and progression of the cell into the cell cycle, ultimately resulting in cell division
Self-Sufficiency in Growth Signals

- **Oncogenes** - genes that promote autonomous cell growth in cancer cells
- **Protooncogenes** - their normal cellular counterparts; genes in normal cells which encode proteins that have normal function in cell
Protooncogenes and Oncogenes

- Protooncogenes are physiologic regulators of cell proliferation and differentiation.
- Oncogenes are characterized by the ability to promote cell growth in the absence of normal mitogenic.
Protooncogenes and Oncogenes

- Oncogene products, called **oncoproteins**, resemble the normal products of protooncogenes with the exception that oncoproteins are devoid of important regulatory elements.
- Their production in the transformed cells becomes constitutive, that is, not dependent on growth factors or other external signals.
- Because oncoproteins are constitutively expressed, they endow the cell with self-sufficiency in growth.
- ‘Enemies with in’
Cellular Oncogenes (c-onc)

- MUTATED FORM
- V-onc
- V-FES, v-SIS
- Some cancer-causing RNA viruses (i.e., leukemia virus) have no viral oncogenes
- Proviral DNA insertion near a protooncogene induces a structural change in the cellular gene, converting it into a cellular oncogene (conc, or onc) – **insertional mutagenesis**
- Also retroviral promoters inserted in the vicinity of the protooncogenes lead to dysregulated expression of the cellular gene
Where Do Oncogenes Come From?

Proto-oncogenes-- Oncogenes via:

- mutations causing altered properties of the protooncogene product, inhibiting its normal activity
- mutation of regulatory sequences leading to overexpression of the protooncogene
- incorporation of foreign DNA causing altered expression of altered protooncogene product
- some may arise from chromosomal translocations
Requirements for Altered Cell Growth

Oncogenes act dominantly, needing only one gene copy to generate the altered phenotype. Loss of cell growth control therefore requires:

- Mutations in both copies of tumor-suppressor genes
- Mutation of one copy of the proto-oncogene
Functional Category of Oncogenes

- Growth Factors
- Growth Factor Receptors
- Proteins Involved in Signal Transduction
- Nuclear Regulatory Proteins
- Cell-Cycle Regulators
FIGURE 7-31 Subcellular localization and functions of major classes of cancer-associated genes. The protooncogenes are colored red, cancer suppressor genes blue, DNA repair genes green, and genes that regulate apoptosis purple.
# Selected Oncogenes, Their Mode of Activation, and Associated Human Tumors

<table>
<thead>
<tr>
<th>Category</th>
<th>Protooncogene</th>
<th>Mode of Activation</th>
<th>Associated Human Tumor</th>
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<tbody>
<tr>
<td><strong>Growth Factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF-β chain</td>
<td>SIS</td>
<td>Overexpression</td>
<td>Astrocytoma</td>
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<td>FGFR3 (HST-1)</td>
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<td>INT2</td>
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<td>EGFR receptor family</td>
<td>EGFR (ERB-B1)</td>
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<td>ERBB2 (ECFR)</td>
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<td>RET</td>
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<td>KIT</td>
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<td>K-RAS</td>
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<td>Colon, lung, and pancreatic tumors</td>
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<td>H-RAS</td>
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<td>N-RAS</td>
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<td>Chronic myeloid leukemia</td>
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<td>RAS signal transduction</td>
<td>BRAF</td>
<td>Point mutation</td>
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<td>Cyclin-dependent kinase</td>
<td></td>
<td>Amplification or point mutation</td>
<td>Glioblastoma, melanoma, sarcoma</td>
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</table>
RAS Oncogene

- 15-20% of all human cancers have a RAS mutation
- Normally, RAS is activated by receptors to exchange GDP for GTP
- Activated RAS returns to ground state by its intrinsic GTPase activity
- GTPase activating proteins (GAPs) augment this process [1000 fold]
- Mutant forms of RAS bind GAP but their GTPase activity is not augmented
- K-ras - colon, pancreatic, cholangioca
- H-ras - bladder ca
- N-ras – haematological tus
- Cell cycle regulation – cyclins
- Anti – ras therapy
Oncogenes, Oncoproteins: Nonreceptor tyrosine kinase

Tyrosine kinase in the signal transduction pathways regulating cell growth
ABL protooncogene product with TK action
Reciprocal translocation of Chr 9 (c-ABL) to chr 22 (BCR), BCR-ABL protein with unrestricted tyrosine kinase activity
Chronic myeloid leukemia, ALL
Oncogenes, Oncoproteins: Transcription factors

C-MYC, C-FOS, C-JUN
MYC is most common mutated
C-MYC (chr 8) translocated to chr 14 (Ig gene) increased MYC protein
Burkitt’s lymphoma
C-MYC amplified in colon, breast CA; L-MYC amplification small cell CA lung

[Diagram showing the translocation of MYC from chr 8 to chr 14 with Ig-MYC hybrid gene, leading to increased MYC protein]
Oncogenes, Oncoproteins: Transcription factors

N-MYC amplification in Neuroblastoma

MYC gene amplification

Chr 2
Homogenous Staining region

Extra-chr
Double minutes
Oncogenes, Oncoproteins: cyclins, CDKs

- Cyclin D gene overexpressed
  - Breast, esophagus & liver CA
- Cyclin E gene overexpressed in breast CA
- CDK4 gene amplification in GBM & sarcomas
- Lymphomas
  - Mantle cell lymphoma

- Chr 11 with Cyclin D1
- Ig gene
- Chr 14 with Ig-Cyclin D1 hybrid Gene
- Increased CyclinD1
Insensitivity to Growth Inhibitory Signals:

Tumor Suppressor Genes

- Failure of growth inhibition is one of the fundamental alterations in the process of carcinogenesis.
- The proteins that apply brakes to cell proliferation are the products of tumor suppressor genes.
- Misnomer
<table>
<thead>
<tr>
<th>Subcellular Location</th>
<th>Gene</th>
<th>Function</th>
<th>Tumors Associated with Somatic Mutations</th>
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<td>Inhibition of RAS signal transduction and of p21 cell-cycle inhibitor</td>
<td>Neuroblastomas</td>
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<td>Cytosol</td>
<td>APC/β-catenin</td>
<td>Inhibition of signal transduction</td>
<td>Carcinomas of stomach, colon, pancreas, melanoma</td>
<td>Familial adenomatous polyposis col/colon cancer</td>
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<td>PI-3 kinase signal transduction</td>
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<td>Nucleus</td>
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<td>Regulation of cell cycle</td>
<td>Retinoblastoma; osteosarcoma; carcinomas of breast, colon, lung</td>
<td>Retinoblastomas, osteosarcoma</td>
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<td>Most human cancers</td>
<td>Li-Fraumeni syndrome; multiple carcinomas and sarcomas</td>
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<td>Nuclear transcription</td>
<td>Wilms tumor</td>
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<td>p16 (INK4a)</td>
<td>Regulation of cell cycle by inhibition of cyclin-dependent kinases</td>
<td>Pancreatic, breast, and esophageal cancers</td>
<td>Malignant melanoma</td>
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<td>BRCA-1 and</td>
<td>DNA repair</td>
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<td>KLF6</td>
<td>Transcription factor</td>
<td>Prostate</td>
<td>Unknown</td>
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</table>
Tumor suppressor genes

- Normally serve to inhibit cell proliferation
- First recognized in retinoblastoma, rare pediatric tumor of the eye
- RB ---tumor suppressor gene is a nuclear phosphoprotein that regulates cell cycle

- Knudson [Two-hit] Hypothesis of oncogenesis
  - Loss of heterozygosity [LOH]
  - Products of Tumor suppressor genes—cell cycle control, regulation of apoptosis, growth and survival
  - Functions—transcription factors, cell cycle inhibitors, signal transduction molecules, cell surface receptors, regulators of cell response to DNA damage
Tumor suppressor genes

- RB protein hypoPO4 binds E2F
- Mutation---RB absent or deficient binding E2F
- Unrestrained E2F function;
- Unchecked entry of cell from G1-S phase
- Retinoblastoma, osteosarcoma, Other tumors
- 40% familial (inherit AD one defective (mutated) copy of RB gene at chr13q14), LOH for this locus---both RB genes mutated & so lack of any functional RB protein---unrestrained E2F function
- 60% sporadic (both copies/alleles of RB locus acquired mutation)
- Other tumors have mutation of genes controlling the RBPO4 & most of cancers have at least one of (cyclin, CDK, RB & p16INK4a) genes mutated
- DNA viruses-HPV
p53: Guardian of Genome

- Both copies of genes mutated--- acquired
- >50% cancers have p53 mutation
- One copy of mutated p53 gene may be inherited (Li-Fraumeni syndrome) & other mutation acquired. 25 fold increased risk by 50 yrs
  - Sarcomas, breast CA, leukemia, brain tumor

  - **Gatekeeper**
  - **Molecular policeman**
• Major function: Cell-cycle arrest and initiation of apoptosis in response to DNA damage.
• p53-induced cell-cycle arrest occurs late in the G1 phase and is caused by the p53-dependent transcription of the CDK inhibitor p21.
• If the DNA damage can not be successfully repaired, normal p53, as a last effort, sends the cell to the graveyard by inducing the activation of apoptosis-inducing genes, such as BAX.
• Loss of p53 results in DNA damage unrepaired, mutations fixed in dividing cells, and eventually malignant transformation.
Tumor suppressor genes: P53

- DNA damage---DNA dependent Kinases & Ataxia telangiectasia Mutated (ATM) proteins activated---PO4 to P53—unfolds---binds DNA---active transcription factor----p21(CDK inhibitor ---
- GADD45 (DNA repair)
- unrepairable damage ---BAX (apoptosis promotion by inhibition of the apoptosis inhibitor)
- Mutation---abnormal p53---no binding DNA---binding/blocking normal p53
- Homozygous loss of p53-- DNA damage unrepaird, mutations getting fixed, cell cycle goes unchecked—malignancy
Role of p53 in Maintaining the Integrity of Genome
Tumor suppressor genes: P53

- E6 HPV protein of HPV binds and degrades p53
- MDM2 levels (Degrades p53) increased in sarcomas & leukemias
- Radiotherapy & chemotherapy damage DNA induce apoptosis
- Tumor cells with defective p53 less likely respond
- Tumor cells with normal p53 likely respond
- Therapeutic enhancing p53 levels or killing p53 deficient tumor cells
- Partially homologous p63 & p73 proteins
Tumor suppressor genes

APC gene both copies inactivated for adenoma formation & in other colon tumors HNPCC

Thousands of adenomatous polyps late teens/20s (familial adenomatous polyposis-one mutation inherited); one or more malignant degeneration CA by additional mutations

β-Catenin: hepatoblastoma (50%) & HCC (20%)
Tumor suppressor genes

APC (adenomatous polyposis coli) gene product

Wnt           APC       catenin

no TCF

no cell cycle proteins

TCF
cell cycle proteins
Tumor suppressor genes

**TGF** B: HNPCC-Ca colon Gastric ca, sporadic colon ca

**NF1[17q]**: benign neurofibromas (plexiform), MPNST
Neurofibromin-GTPase activating protein (GAP) for RAS deactivation

**NF-2[22q]**: Merlin—R.B.C. memb. Protein 4.1—actin, CD44

**VHL[3p]**: von hippel lindau protein (ubiquitin ligases)---acts on HIF—transcription of VEGF
Lack degradation of HIF, VEGF
RCC, Pheochromocytoma, Hemangioblastoma, Retinal angiomas, renal cysts
WT1[11p13]: involved in renal and gonadal genesis---- wilms tumor

PTEN[10q]( Phophatase & Tensin homologue)-cell cycle arrest and apoptosis  p27
Endometrial CA, GBM

INK4a/ARF: 20%melanomas, SCC, PANCREATIC Adenoca
cervical cancer--hypermethylation
KLF-6— T.F.—target genes-TGF &TGFR . INHIBITS PROLIFERATION
by p21
Prostatic ca

PATCH[Patched] --Patched hedgehog family 20%Gorlin syndrome
[naevoid basal cell ca]- TRF

CADHERINS
- PATCH[PATCHED] -- Patched hedgehog family
  TGF-β, PDGF-R
  Gorlin syndrome [naevoid basal cell ca]- TRF
  - 20-50% SPORADIC basal cell ca

- CADHERINS
  - Ca oesophagus, breast, ovary, colon, prostate
  - Familial gastric ca
Apoptosis associated genes

BCL2 increased expression inhibits apoptosis
P53 decreased expression decreases BAX protein (BAX promotes apoptosis)
PTEN decreased expression decreases apoptosis.
Follicular lymphoma
BCL2--- decreased apoptosis--- reduced cell death--- B lymphocyte accumulation-
lymphadenopathy
Increased cell life but not explosive cell proliferation.

Apoptosis associated genes

Ig gene
Chr 14

Chr 18
BCL2

Ig gene
Chr 14

Chr 14 with
Ig-BCL2 hybrid
Gene

Increased BCL2
DNA Repair genes

Mismatch repair genes
NER genes
Homologous recombination

Why the patients of Hereditary non polyposis colon carcinoma (HNPCC) syndrome develop early colon cancers and why genetic study of Microsatellites is significant in such patients.
DNA Repair genes

DNA replication mistakes (A—G, C---T),
Checked & corrected by Mismatch repair proteins
(MMR genes: MSH2, MLH1, PMS & PMS2 defective in HNPCC syndrome; one defective copy inherited other copy acquired)
Defective repair--- accumulation of DNA changes, mutations in the protooncogenes & tumor suppressor genes
DNA Repair genes

Microsatellites are the tandem repeats of 1-6 nucleotides in the genome.

Due to accumulation of the DNA mistakes the microsatellites vary in length markedly (microsatellite instability) which is marker for the defective mismatch repair genes.

Endometrial cancer, ovarian CA increased in the HNPCC.

TGF-β receptor, β-catenin pathway, BAX gene usually mutated in HNPCC.

Mismatch repair genes usually mutated in sporadic cancers (15% colon CA).
DNA Repair genes

NER genes

Why patients with xeroderma pigmentosum have increased predisposition of developing the skin cancers
DNA Repair genes

UV-A (320-400nm)
UV-B (280-320nm)
UV-C (200-280nm)

Radiation damage mutation in p53 & RAS genes (skin CA)

Type of radiation: UV-C highly mutagenic but filtered out by ozone

Intensity of exposure (duration)
Quantity of light-absorbing melanin
DNA Repair genes

Pyrimidine dimers formation due to UV-B rays (280-320nm) repaired by the Nucleotide excision repair NER proteins

In XP NER genes are defective DNA changes accumulate

skin degenerative changes

cancers result from the accumulation of the mutated protooncogenes & tumor suppressor genes
DNA Repair genes

Homologous recombination repair

Why the patients with ataxia-telangiectasia, Bloom syndrome are prone to develop neoplasia
DNA Repair genes

Proteins responsible for sensing & repairing the homologous recombination of DNA when it is damaged by the Ionizing radiations (x-rays, radioactive radiations/ particulate) or reactive oxygen species (ROS) are defective.

Ataxia telangiectasia (ATM-protein kinase---p53 stimulation does not occur & DNA damage/ changes accumulates---mutated oncogenes)

ATM heterozygous 1% population at risk of radiation damage

Bloom syndrome (BLM-helicase)
DNA Repair genes

BRCA-1, BRCA-2 genes defective & mutations in 80% familial breast CA & Ovarian CA
BRCA-1: breast & Ovarian CA, prostatic ca, colon ca
BRCA-2 male breast ca, melanomas, pancreatic ca
Fanconi anemia FANCD1 gene
Proteins usually involved in the repair of DNA damage (radiation/ROS) by homologous recombination.
Limitless Replicative Potential: Telomerase

Telomerase enzyme is detected in 90% of tumors

*explain the significance of this finding*
Replicative Senescence

- Normal cells, after a fixed number of divisions, become arrested in a terminally nondividing state known as replicative senescence.
- With each cell division there is some shortening of specialized structures, called telomeres, at the ends of chromosomes.
  Once the telomeres are shortened beyond a certain point, the loss of telomere function leads to activation of p53-dependent cell-cycle checkpoints, causing proliferative arrest or apoptosis. Thus, telomere shortening functions as a clock that counts cell divisions.
- Cancer cells must find a way to prevent telomere shortening.
Replicative Senescence
Limitless Replicative Potential

- Telomerase activity and maintenance of telomere length are essential for the maintenance of replicative potential in cancer cells.
- Transformed cells may have defects in cell-cycle checkpoints, allowing for critical telomere shortening in dividing cells. These cells may die by apoptosis or survive with chromosome defects that cause genomic instability.
Replicative Senescence

- Multiple rounds of cell replication
  - Short telomeres
    - Checkpoint intact
      - Proliferative arrest
    - Checkpoint bypassed: additional telomere shortening
      - Apoptosis
      - Chromosome fusion
      - Non-reciprocal recombination
        - Genomic instability
          - Reactivation of telomerase
            - CANCER
          - CELL DEATH

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Cancer is associated with a progressive accumulation of genetic and epigenetic abnormalities. These defects lead to disruption of cell growth, cell death, apoptosis, differentiation, DNA repair, and other critical pathways. The molecular events in the development of familial adenomatous polyposis provide a good example of the genetic evolution that underlies progressive morphologic changes.
Multiple steps
Sequence not sure in many cancers
Step for Initiation of cancer may be different from the step of cancer progression
Gatekeeper genes: directly regulate the cell growth—protooncogenes (MYC, RAS) & tumor suppressor genes p53 (related p14ARF, MDM2 genes) & RB (related INK4, cyclinD & kinases) the principal two pathways
Caretaker genes: regulate the DNA repair
Germline mutation of either predisposes to cancer
More with gatekeeper genes
Hypothesis: DNA repair defect is initial event in very early stage of cancer development
Normal epithelium

Hyperproliferative epithelium
Dysplastic foci

Early adenoma

Mutation of RAS gene on chromosome 12p

Intermediate adenoma

Loss of tumor suppressor on chromosome 18q

Late adenoma

Loss of p53 gene on chromosome 17p

Carcinoma
Gatekeeper genes: directly regulate the cell growth—protooncogenes (MYC, RAS) & tumor suppressor genes p53 (related p14ARF, MDM2 genes) & RB (related INK4, cyclinD & kinases) the principal two pathways

Caretaker genes: regulate the DNA repair

Germline mutation of either predisposes to cancer

More with gatekeeper gene

--- widespread mutation & genetic instability in neoplastic cells further susceptible to additional mutations (cancer cells with mutator profile)

Tumor grows—aggressive—greater malignant potential (Tumor progression)
MECHANISMS OF CARCINOGENESIS

Chemical carcinogenesis
Radiation carcinogenesis
Viral oncogenesis
Chemical Carcinogenesis

Initiation
Promotion
Chemical carcinogens INITIATION

- Initiation results from exposure of cells to sufficient dose of a carcinogenic agent (initiator); an initiated cell is altered, making it potentially capable of giving rise to a tumor. Initiation alone, however, is not sufficient for tumor formation.

- Initiation causes permanent DNA damage (mutations). It is therefore rapid, irreversible and has “memory”. Tumors are produced even if the application of the promoting agent is delayed for several months after a single application of the initiator.
Chemical Carcinogens PROMOTION

- Promoters can induce tumors in initiated cells, but they are nontumorigenic by themselves.
- Tumors do not result when the promoting agent is applied before, rather than after, the initiating agent.
- In contrast to the effects of initiators, the cellular changes resulting from the application of promoters do not affect DNA directly & reversible.
1. Initiation:

- Initiation involves irreversible changes in DNA - a heritable change.
- Initiated cells (altered cells) may reproduce faster, or fail to undergo apoptosis normally.
- Initiation may involve more than one genetic alteration before a cell has the potential to become malignant.
- Initiation itself is believed to involve at least 2 steps.
- Molecular lesion in a target cell.
- The fixation of such lesions by a round of cell proliferation.
2. Promotion:
Expansion of precursor populations

- The process by which an initiated tissue is induced to develop focal proliferative lesion
- Promotion alone does not lead to malignant transformation.
- Promotion must occur after initiation, or during stepwise initiation.
- Promotion is reversible.
- Promotion most likely does not involve genetic mutation. It can be viewed as an epigenetic change involving altered gene expression without a change in the DNA sequence
Because malignant transformation results from mutations---most chemical carcinogens are mutagenic.

Indeed, all direct and ultimate carcinogens contain highly reactive electrophile groups that form chemical adducts with DNA, as well as with proteins and RNA.
**electrophile**---reacts with DNA (nucleophile)---**Adduct** (addition formation)---DNA damage---Mutation
---?cell death? DNA repair ? **Tumor Initiation**
This initiated cell must divide once for the DNA change to get fixed (Irreversible) in the cell genome

Further proliferation of the initiated cell is augmented by acetaldehyde & phenol (Promoters) in the cigarette smoke (Tumor Promotion) also by the toxic damage & reactive proliferation

Proliferation of the Initiated cell with mutation is accompanied by further predisposition & accumulation of mutations (Tumor Promotion)
Events in chemical carcinogenesis
Chemical Carcinogens

Carcinogen (Initiator) directly acting on DNA or metabolized first from procarcinogen to active form.

- Most carcinogens are metabolic products & predisposition to cancer also depend upon the metabolizing enzyme activity.
- 10% population highly inducible form of p-450 CYP1A1 (metabolizes benzopyrene) 7x increased risk of lung CA from light smoking.
- 50% population have deleted gene for glutathione S transferase (enzyme for detoxifying benzopyrene) increased risk of lung CA.

Few of chemical carcinogens alkylating agents (cyclophosphamide, chlorambucil, Busulphan), & Acylating agents do not require metabolic activation.
Although any gene may be the target of chemical carcinogens, the commonly mutated oncogenes and tumor suppressors, such as RAS and p53, are important targets of chemical carcinogens.

**MOLECULAR FINGERPRINT**

- Initiators usually attack the DNA at specific sites (aflatoxin b1 mutation at codon G:C—T:A 249Ser p53 gene)
- METABOLIC INACTIVATION
- Ames TEST—Mutagenic potential
Chemical Carcinogens

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Chemical Carcinogens

- Direct-acting compounds – do not require chemical transformation for their carcinogenicity

- Indirect-acting compounds (procarcinogens) – require metabolic conversion in vivo to produce ultimate carcinogens capable of transforming cells
Chemical Carcinogens

**Direct-Acting Carcinogens**

*Alkylating Agents*
- Diepoxybutane
- Dimethyl sulfate
  - Propiolactone
- Anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, and others)

*Acylating Agents*
- Dimethylcarbamyl chloride
- 1-Acetyl-imidazole
Chemical Carcinogens

*Procarcinogens That Require Metabolic Activation*

- **Polycyclic and Heterocyclic Aromatic Hydrocarbons**
  - Benz(a)anthracene
  - 3-Methylcholanthrene
  - Dibenz(a,h)anthracene
  - Benzo(a)pyrene
  - 7,12-Dimethylbenz(a)anthracene
Chemical Carcinogens

- *Aromatic Amines, Amides, Azo Dyes*
- 2-Naphthylamine (-naphthylamine)
- Benzidine
- 2-acetylaminofluorene
- Dimethylaminoazobenzene (butter yellow)
Chemical Carcinogens

- *Natural Plant and Microbial Products*
  - Griseofulvin
  - Aflatoxin B
  - Betel nuts
- *Others*
  - Insecticides, fungicides
  - Vinyl chloride, nickel, chromium
  - Nitrosamine and amides
Some of the most potent indirect chemical carcinogens—the polycyclic hydrocarbons—are present in fossil fuels. For example, benzo[a]pyrene and other carcinogens are formed in the high-temperature combustion of tobacco in cigarette smoking. These products are implicated in the causation of lung cancer in cigarette smokers.

Polycyclic hydrocarbons may also be produced from animal fats during the process of broiling meats and are present in smoked meats and fish.
Few of chemical carcinogens alkylation agents (cyclophosphamide, chlorambucil, Busulphan), & Acylating agents do not require metabolic activation.
Chemical Carcinogens

Other examples:
- Polycyclic aromatic hydrocarbons in broiled & smoked animal meat/fish GIT CA
- Dye industry (amines /azo dyes-2-naphthylamine) bladder Ca
- Aspergillus infection (certain strains) of stored food grains in China & south Africa Aflatoxin B1---hepatocellular carcinoma
- Nitrosamines formed by gut bacteria from nitrates & nitrosable amines in food preservatives---Gastric CA
- Asbestos---Mesothelioma & Bronchogenic CA
- Vinyl chloride (PVC)---liver angiosarcoma
- Arsenic---skin CA
Viral carcinogenesis

- HPV
- EBV
- KSHV
- HBV
Among 70 genetic types of Human papilloma virus (HPV)
1,2,4,7 types are associated with the benign tumors (squamous papillomas of skin) atypes 16 & 18 are associated with the squamous cell carcinomas esp.

cervix
anogenital
Oral cavity
larynx

benign warts&low grade lesion—6&8
high grade lesion&invasive ca---16,18,31,33,35,51
- Benign tumors the viral DNA remains in episomal form in the cytoplasm.
- Malignant tumors the viral DNA interrupted specifically at the E1/E2 of viral genome & is integrated with the host’s DNA.
- E2 normally represses transcription of E6 & E7 early viral protein; the E1/E2 disruption releases such repression & E6 & E7 proteins are formed in the host cells infected with HPV16 & 18.
• E6 binds p53 & degrades it
• also activate Telomerase
• E7 binds RB protein & degrades it
• also inhibits p53 transcription
• also inhibits p21
• Cell cycle breaks released
• DNA damage is not repaired goes unchecked-----
cancer
HPV16, 18 & 31 E6 bind p53 with high affinity & degrade it
HPV 6 & 11 bind p53 poorly & do not degrade it

P53 polymorphic at aminoacid 72—arginine & proline
HPV infection initiating event for Tumor initiation with more mutations---cancer

Majority of HPV infections are cleared by the immune response only in few the infection causes the tumor initiation.

(additional cofactors: smoking
microbiological infections
hormonal changes
dietary factors)
Epstein-Barr virus (EBV) infection is associated with B-cell lymphomas
Burkitt’s lymphoma
B-cell NHL
Hodgkin’s lymphoma
Nasopharyngeal CA

what mechanisms are involved in such causation?
What are the evidences of such association?
EBV—B lymphocytes (CD21)
Episomal infection,
Immortal B lymphocyte
Latent Membrane protein (LMP-1)---
---signaling pathway (NFkB & JAK/STAT)-----Promotes B cell proliferation & survival
EBNA-2
---Activates the LMP-1 expression
   & Cyclin D
--EBV ubiquitous infection
--Mostly asymptomatic
--Few Infectious mononucleosis (self limited)
Endemic Burkitt’s lymphoma (central Africa/ New Guinea) 90 % of tumors contain EBV genome
100% pts have antibodies agst EBV
Such antibody titers are correlated with risk of BL.
In the endemic areas cofactors (chronic malaria) favor sustained proliferation Immortal B-lymphocyte (EBV infected)---

---actively dividing cells----increased risk of mutation----
---t (8;14)---c-myc activation---other mutations----
release growth regulation----Burkitt’s lymphoma
Multiple CNS lymphomas

Initially polyclonal population---Monoclonal population---tumors (lack of host immunity)

Nasopharyngeal Ca

100% contain clonal EBV genome integration in tumor cells, China, Arctic, Africa

EBV infection one step in the CA development other geographic factors? Genetic, ?environmetal as cofactors in development of CA
HBV

- Hepatitis B virus strongly linked to hepatocellular carcinoma (China & Africa)
- Chronic tissue injury, regenerative Hepatic cells------larger pool of dividing cells----prone to mutation (?spontaneous/?environmental agents)
- HBx protein
  - binds p53 (inhibits)
  - Promotes transcription of Growth factors (ILGF)
Human T-cell leukemia virus type 1 (HTLV-1)

- T-cell Lymphoma / Leukemia (Japan/ Caribbean)
- Infects T-lymphocytes & integrates with host’s genome
- RNA virus (gag, pol, env & LTR regions +TAX gene)
- TAX protein promotes cell proliferation:
  - c-FOS gene
  - IL-2/ IL-2 receptor
  - myeloid CSF
  - inactivates p16INK4a
  - enhances cyclin D activation
- TAX protein leads to genomic instability:
  Interfere DNA repair, ATM checkpoints
Helicobacter pylori

- Helicobacter pylori infection—90%
- Chronic gastritis, atrophy, metaplasia—sustained epithelial proliferation (risk of mutation)
  --Gastric ca, Lymphoma (MALTOMA)
- H pylori protein immunogenic—T cells & B cells, lymphoid follicles in gastric mucosa
- CagA, VacA
- ----in few cases B cell Lymphoma (continuous proliferation t (11;18) MALT Lymphoma
effects of a tumor on host
What are the local & hormonal effects of a tumor

Site specific mass effect (mechanical & functional compromise)
- GIT tumors: obstruction, rupture, Intussusception
- Respiratory tract tumors: obstruction, respiratory function loss,
- Oral cavity & upper aerodigestive tract tumors
--erosive destructive malignant growth/ pressure
growth by benign tumor-----ulceration, secondary
infection, bleeding

-Endocrine gland tumors
  - Non-functional loss of endocrine function
  - Metastases
  - functional endocrine tumors (usually well
differentiated/ benign)
  - Acromegaly, hyperthyroidism, Hyperparathyroidism,
hypoglycemia, Diabetes, Cushing syndrome
Many cancer patients suffer progressive loss of body fat and lean body mass, accompanied by profound weakness, anorexia, and anemia, referred to as cachexia.

There is some correlation between the size and extent of spread of the cancer and the severity of the cachexia. Cachexia is not caused by the nutritional demands of the tumor. Current evidence indicates that cachexia results from the action of soluble factors such as cytokines produced by the tumor and the host rather than reduced food intake.
In patients with cancer, calorie expenditure remains high, and basal metabolic rate is increased, despite reduced food intake. This is in contrast to the lower metabolic rate that occurs as an adaptational response in starvation.

- TNF produced by macrophages in response to tumor cells or by the tumor cells themselves mediates cachexia.
- TNF suppresses appetite
  - inhibits the action of lipoprotein lipase, inhibiting the release of free fatty acids from lipoproteins.
  - a protein-mobilizing factor called proteolysis-inducing factor, which causes breakdown of skeletal muscle proteins by the ubiquitin-proteosome pathway,
paraneoplastic syndrome

Symptom complex not attributable to local/distant tumor spread or indigenous site hormone production
--10% of malignant tumors
-may be earliest tumor manifestation
-significant clinical problem & may be lethal
-mimic other disease (management problem)
Endocrinopathies

- Cushing syndrome
  - ACTH or ACTH-like substance, POMC
  - Small-cell carcinoma of lung
  - Pancreatic cancer
  - Neural tumors
• Syndrome of inappropriate antidiuretic hormone secretion
  
  Small-cell carcinoma of lung
  intracranial neoplasms
  Antidiuretic hormone or atrial natriuretic hormone
- Hypercalcemia
  - Parathyroid hormone-related protein, TGF-α, TNF, IL-1
    - Squamous cell carcinoma of lung
    - Breast carcinoma
    - Renal carcinoma
      - Adult T-cell leukemia/lymphoma
    - Ovarian carcinoma
- Hypoglycemia
  - Fibrosarcoma
  - Insulin or insulin-like substance
- Other mesenchymal sarcomas
- Hepatocellular carcinoma
● Hypoglycemia
  Insulin or insulin-like substance
    Fibrosarcoma
    Other mesenchymal sarcomas
    Hepatocellular carcinoma
- Carcinoid syndrome
- Serotonin, bradykin
- Bronchial adenoma (carcinoid)
- Pancreatic carcinoma
- Gastric carcinoma
- Polycythemia  Erythropoietin
- Renal carcinoma
- Cerebellar hemangioma
- Hepatocellular carcinoma
Nerve and Muscle disorders

- Myasthenia
- Bronchogenic carcinoma -- Immunological
- Disorders of the central and peripheral nervous systems Breast carcinoma

Dermatologic Disorder

- Acanthosis nigricans Gastric carcinoma
  Immunologic; secretion of epidermal growth fac
- Lung carcinoma
- Uterine carcinoma
- Dermatomyositis Bronchogenic, breast carcinoma
  Immunologic
- **Osseous, Articular, and Soft-Tissue Changes**
  - Hypertrophic osteoarthropathy and clubbing of the fingers
    - Bronchogenic carcinoma
- **Vascular and Hematologic Changes**
  - Venous thrombosis (Trousseau phenomenon)
    - Pancreatic carcinoma
    - Bronchogenic carcinoma
  - Tumor products (mucins that activate clotting)
  - Nonbacterial thrombotic endocarditis
  - Advanced cancers
    - Hypercoagulability
  - Anemia
- **Nephrotic syndrome**
  - Tumor antigens, immune complex
Tumour immunity
TUMOUR Ags

- Products of altered oncogene/ tumor suppressor gene (altered p53, BCR-ABL, RAS proteins)
- Products of other mutated genes (esp Radiation & Chemicals)
- Overexpressed or aberrantly expressed proteins (Tyrosinase in melanoma cells & cancer-testis family antigens-MAGE in melanoma cells); GAGE, RAGE, BAGE
- Proteins of oncogenic viruses (HPV & EBV)
- Oncofetal antigens (AFP & CEA)
- Altered cell surface glycolipids & glycoproteins (Ganglioside GM2 GD2 GD3, CA125, CA 19.9, MUC-1)
- Cell type specific differentiation antigens (CD10, CD20, idiotypic determinants of surface immunoglobulin)
<table>
<thead>
<tr>
<th>Normal host cell displaying multiple MHC-associated self antigens</th>
<th>Tumor cells expressing different types of tumor antigens</th>
<th>EXAMPLES</th>
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<tr>
<td>Normal self proteins</td>
<td>Oncogene products: mutated RAS, BCR/ABL fusion proteins</td>
<td>Tumor suppressor gene products: mutated p53 protein</td>
</tr>
<tr>
<td>MHC Class I</td>
<td>Various mutant proteins in carcinogen or radiation, induced animal tumors; various mutated proteins in melanomas</td>
<td>Overexpressed: tyrosinase, gp100, MART in melanomas</td>
</tr>
<tr>
<td>No T cell response</td>
<td>Overexpressed or aberrantly expressed self protein</td>
<td>Human papilloma virus E6, E7 proteins in cervical carcinoma; EBNA proteins in EBV-induced lymphoma</td>
</tr>
</tbody>
</table>
What type of antitumor effector mechanisms exist
- **Cytotoxic lymphocytes** CD8+ T cells
  - MHC 1 antigen, wide variety Tumors
  - Esp. Viral induced Tumors (EBV & HPV)
  - Harvesting & expansion (in vitro) reinfusion

- **Natural killer lymphocytes** kill the tumor cells without prior sensitization, esp tumor cells with low MHC expression (In some tumors) IL2 activates; immunotherapy with in vitro expansion

- **Macrophages** activated by IFN γ from T cells & Natural killer cells

- **Antibodies** kill tumor cells by complement activation & antibody mediated cytotoxicity (by macrophages & NK cells)
What is immune surveillance, what are the evidences that it exists, how tumor cells evade it
Normal function of immune system is to survey the body for emerging malignant cells & to destroy them.

Increased cancer incidence in immunodeficient.

But mainly lymphoma (B cell immunoblastic).

Lymphocytic infiltrate in the tumors.

Tumors mainly in immunocompetent.

(likely many must have been curbed & destroyed & the successful tumors develop ways to evade the immune system.)
Mech. To evade immunity

- Selective outgrowth of antigenic negative Nonimmunogenic tumor cell variants
- Loss or reduced MHC molecule expression
  (evades killing by CTL but not by NK cells)
- Lack of costimulation
  MHC 1 with tumor antigen but no costimulatory molecule---
  t cells not stimulated or anergy or apoptosis
  (Autologous tumor cells with gene for costimulatory molecule---B7 or autologous APC wth tumor antigen expanded)
- Immunosuppression: chemicals, radiations, tumor products (TGFβ)
- Antigen masking by glycocalyx mucopolysaccharides
- Apoptosis of cytotoxic Lymphocytes
  (Fas ligand on tumor cells causes apoptosis of Fas receptor on T cells)
Lab. Diagnosis of cancer
1. What are the histologic methods used for the tumor diagnosis.
Incisional biopsy (Diagnosis of Tumor & Typing)

(part of tumor is cut & examined, before any definite tumor management planned)

- Skin biopsy, mucosal biopsy (Oronasal, genital)
- Laryngoscopic/bronchoscopic biopsy for tumors of respiratory tract, transbronchial biopsy for tumors in lung parenchyma
- Endoscopic biopsy GIT tumors
- Cystoscopic biopsy urinary bladder
- Trans urethral resection of prostate (TURP)
Core Needle biopsy (Trucut needle biopsy)
Breast, prostate, liver, chest/ mediastinal, brain (burr hole), retroperitoneum

Excision specimen (biopsy) (Grading & Staging)
Skin & Soft tissue tumors
  Appendicectomy
  Cholecystectomy,
  Colectomy
  Mastectomy
  Hysterectomy
  Lobectomy
- Fixation 10% formalin (Formaldehyde sol.)
- Tissue Processing (Tissue treated with reagents for impregnation with paraffin wax)
- Paraffin wax block of tissue
- Thin sections (3-5um) Microtome—on glass slides
- Stained with Dyes (Hematoxylin & Eosin)
- Microscopic evaluation
- Clinical correlation, Benign/Malignant criteria & tumor type differentiation & grading
- Histologic Sections & wax blocks can be stored for very long periods (many years—indefinitely)
- ~Two days (fixation, Processing & slide preparation)
Frozen sections:
Fresh tissue obtained from lesion during operation & sections prepared by freezing tissue at Cryostat, diagnosis in minutes conveyed to surgeon for intra-operative decision making
- Breast tumors
- intraabdominal tumors
- Brain tumors
- excision margins
2. What are the cytological methods of tumor diagnosis
Exfoliative cytology (Cancer cells loose adhesions)

- Urine cytology
- Sputum, bronchial washing, BAL, Ascitic fluid, pleural fluid, CSF cytology
- Vaginal, cervical cytology (Papanicolaou (PAP) smear test)

Most cost effective cancer reduction program
- **George N Papanicolaou**
- 70-80 % reduction of mortality from cervical Ca
- Early detection of precancer & treatable CA
- Reduction depends intensity of screening---screening highest Scandinavian countries
1930 -- USA Cervical CA most common cause of cancer death
2/3 rd mortality reduction to 8th position
89% women USA had screening
In developing countries < 5% women screening, cervical CA leading cause of cancer mortality
Evaluating microscopy: Nuclear atypia & Cytoplasmic, Architecture features
Fine Needle Aspiration Cytology

Fine Needle Aspiration Cytology (22G-25G)

- Cellular material aspirated by thin needle usage & smeared on glass slide---stained (MGG & H & E, PAP) & microscopically evaluated
- Tumors or other pathologies diagnosed, with experience High sensitivity & specificity
- **Superficial Palpable masses** (Thyroid, breast, lymph nodes, salivary glands, soft tissue)
- **Image guided** (visceral --kidney, liver, lung, retroperitoneum, ovarian ; deep seated masses) CT scan, USG
- OPD procedure, economical, no anesthesia, can be repeated
- Architecture not completely evaluated, Invasiveness evaluation not as specific as that of biopsy evaluation
- Blood cells & Lymphoid tissue tumors evaluated: Peripheral blood smear—stain, Bone marrow aspiration (Iliac crest)---smear---stained; Bone marrow trephine biopsy
- Lymph node biopsy
3. What is Immunohistochemistry, how it is applied to the tumor diagnosis?
Monoclonal antibodies tag the type specific tumor antigens, such bound antibodies in sections are marked by anti globulins/or Avidin biotin complex tagged with stain markers. Positivity in histology sections is seen as colored product with specific locations.
Categorization of the poorly differentiated/undifferentiated tumors

- Undifferentiated Ca (Cytokeratin)
- Sarcoma (Desmin, vimenetin)
- Melanoma (HMB-45)
- Lymphoma (LCA, CD3/CD19-20)

Categorization of Leukemia & Lymphoma

Specific lineage differentiation correlate with prognosis & tumor biology (T vs. B vs Myeloid)
Metastatic deposit origin PSA, Thyroglobulin

Prognostic Therapeutically significant molecules
ER/PR better prognosis breast CA Anti estrogen therapy
Her 2neu Poor prognosis but respond to Herceptin
4. How the molecular techniques are changing the ways of cancer patient’s management
Diagnosis of neoplasms:

- Clonal arrangement of antigen receptor gene (T cell receptor/ immunoglobulin receptor rearrangement)
- Detection of specific translocation
- (routine cytogenetic analysis or Fluorescent in situ hybridization (FISH))
  -- Burkitt’s lymphoma t 8; 14
  -- Follicular lymphoma t 14;18
  -- Mantle cell lymphoma t 11; 14
  -- Chronic myeloid leukemia t 9;22
Detection of hybrid gene or specific DNA segment or its transcript by PCR Polymerase chain reaction

Sarcomas

- T ---Ewings sarcoma,
- t 12; 22 ---clear cell sarcoma
- , t 8; 22 ---epithelioid sarcoma,
- t x; 18--- synovial sarcoma,
- t 8; 11--- embroynal rhabdomyosarcoma
- t 3;12; ring (3) chromosome ----Atypical lipoma
Prognosis

- N-MYC amplification & 1p deletion bad prognosis for neuroblastoma
- T 9;22 in ALL bad prognosis
- t 15;17 in AML good prognosis

Minimum residual disease

Routine histology/ cytology/ radio-imaging methods not sensitive/reliable to pick residual neoplastic disease after the anti-neoplastic treatment

PCR technique---amplify even minute DNA of tumor & specific sequences for the tumors can be identified by this method
BCR-ABL residual leukemia cells, K-RAS in stool samples colon cancer recurrence

- Hereditary predisposition to Cancer

BRCA 1/ BRCA2 RET oncogenes high association with Breast CA & MEN
5. Flowcytometry

- Cell/ nuclear size
- Cytoplasmic granules
- DNA content of cells
- Cell surface antigens markers
  - (for diagnosis, typing of Hematolymphoid neoplasms)
  - Aneuploidy bad prognosis
What are tumor markers, how are they useful in diagnosis & monitoring of tumors
Support to the diagnosis in clinical context
Need to investigate
Prognosis Levels indicate tumor burden of tumor
Response to therapy (levels decline)
Residual disease (> 6 wks CEA high levels residual disease)
Relapse in the follow up period
(PSA, HCG, AFP, CEA, CA125, Calcitonin)
Tumor substances detectable in body fluids

- **Human chorionic gonadotropin (HCG)**----
  Trophoblastic tumors, NSeminnomatous testicular T
- **Calcitonin**---Medullary CA thyroid
- **Catecholamine/ metabolites**---Pheochromocytoma
- **α-Fetoprotein**---HCC, NSGCTT (Yolk sac, fetal liver)
  git (liver injury, cirrhosis, fetal distress/ neural tube
  defect, pregnancy) CA colon, pancreas lung
- **Carcinoembryonic antigen CEA** (embryonic liver git
  pancreas) (IBD-CD/UC, alcoholic cirrhosis, hepatitis)
  colorectal CA 60-90%, pancreatic 50-80%, gastric/
  breast CA 25-50%
- Prostate specific antigen PSA---Prostate CA (Prostatitis)
- Neuron specific enolase---neuroblastoma, small cell CA
- Immunoglobulins (M Protein)---Myeloma
- CA-125---Ovarian Ca
- CA19-9----Pancreatic colon Ca
- CA15-3----Breast Ca
Molecular Tumor markers

- P53, APC, RAS mutation in stool/ serum colon Ca
- p53 & RAS (stool & serum) -- Pancreatic Ca
- p53 & RAS (sputum & serum) --- Lung Ca
- p53 (urine) --- Urinary bladder Ca