Disorders of Cell Growth & Neoplasia

Lecture 4
Molecular basis of cancer

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Skin tumor in a 10-year-old Rottweiler. Considering the external appearance and color, what would be the most likely diagnosis?

**Dx: Malignant melanoma**

Post mortem examination revealed the presence of multiple well-defined, dark-brown to black masses in different areas of the brain. Assuming that these lesions are metastases from the cutaneous mass, which would be the most likely pathway of dissemination?

**Answer: Hematogenous**
Essential Alterations for Malignant Transformation

- Self-sufficiency in growth signals
- Insensitivity to growth-inhibitory signals
- Evasion of apoptosis
- Limitless replicative potential
- Sustained angiogenesis
- Ability to invade and metastasize
- Defects in DNA repair

Robbin’s Figure 7-27 Flow chart depicting a simplified scheme of the molecular basis of cancer.
1. Self-sufficiency in Growth Signals (Activation of Oncogenes)

- Tumor cells can proliferate without external stimuli (mainly via oncogene activation).

- **Oncogenes** are altered versions of normal genes (*protooncogenes*) that regulate normal cell growth and proliferation.

- A main component of neoplastic transformation is the **mutation or overexpression** (via misregulation / amplification / translocations) of protooncogenes (*oncogenes*).

- **Oncoproteins** (> 100) may function as:
  - Growth factors
  - Growth factor receptors
  - Signal transducers
  - Transcription factors
  - Cell cycle regulators
Insensitivity to Growth Inhibitory Signals (Inactivation of Tumor Suppressor Genes)

- Proteins that inhibit cell growth are the products of “tumor suppressor genes”.
- Loss of expression &/or function of TSG’s is present in most human tumors.

i) RB Gene

- RB protein is key in regulating cell proliferation at the $G_1$/$S$ transition.
- Absent &/or mutated RB \(\Rightarrow\) molecular “brakes” released and cell-cycle progression.
- RB abnormalities seen most frequently in retinoblastoma, but also in other tumors.

Robbin’s fig 7-30. Mechanism of cell-cycle regulation by RB. In a resting cell, RB is a component of the E2F/DP1/RB complex, which represses gene transcription through the recruitment of histone deacetylase, an enzyme that alters the conformation of chromatin, making it more compact. Phosphorylation of RB by cyclin D/CDK4 removes histone deacetylase from chromatin, allowing the activation of E2F transcriptional activity. E2F-mediated transcription of cyclins E & A, and of genes required for DNA replication, permit the passage through the G1 restriction point.
• RB abnormalities seen most frequently in **retinoblastoma**, but also in other tumors.
ii) **Other Genes Which Affect $G_1/S$ Cell-Cycle Transition**

- dysregulation of other genes which control $G_1/S$ transition; mimics RB dysfunction.

- mutations of p16INK4a results in inability to block cyclin D/CDK4 activation.

- several DNA viral oncogenic proteins neutralize the growth inhibitory effects of RB.

- part of p53 activity is by upregulating the CDK inhibitor p21.

Robbin’s fig 7-29 Schematic illustration of the role of cyclins, CDKs, and cyclin-dependent kinase inhibitors in regulating the $G_1/S$ cell-cycle transition.
iii) p53 Tumor Suppressor Gene ("Guardian of the Genome")

- a common mutation in many (~50%) cancers.

- most mutations of the p53 gene affect the DNA-binding activity of p53 protein.

- p53 also inactivated by upregulation of MDM2 protein or bound by viral proteins.
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 up-regulation of the cyclin-dependent kinase inhibitor p21, and the GADD45 (Growth Arrest & DNA Damage) genes, respectively. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, p53-induced activation of the BAX gene promotes apoptosis. In cells with loss or mutations of p53, DNA damage does not induce cell-cycle arrest or DNA repair, and hence genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.
Evasion of Apoptosis

- cell survival is affected by genes that promote (eg p53) or inhibit apoptosis (eg BCL-2).
- eg reduced apoptosis with overexpression of BCL-2 protein in most B-cell lymphomas.
Defects in DNA Repair

- cells are frequently exposed to DNA-damaging agents but have the ability to repair DNA or eliminate cells (via apoptosis) when DNA repair fails.

- inherited disorders of defective DNA repair genes have been identified in individuals predisposed to cancer.

- proposed that defects in DNA repair genes are the main initiating event in oncogenesis, leading to widespread mutagenesis and genetic instability ("mutator phenotype").

- Defects in any of the following DNA repair systems causes cancer in humans:
  
  i) Mismatch repair genes
  
  ii) Nucleotide excision repair genes
  
  iii) Recombination repair genes
ii) Nucleotide Excision Repair Genes.

- UV light causes cross-linking of adjacent pyrimidine nucleotides (dimer formation) preventing normal DNA replication. Such DNA damage is repaired by the nucleotide excision repair (NER) pathway.

Girl with Xeroderma pigmentosum, an inherited disorder involving defective DNA repair genes. This autosomal recessive disorder is characterized by extreme photosensitivity, and a 2000-fold increased risk of skin cancer in sun-exposed skin. The disease is caused by a mutation in one of several genes involved in NER.
Limitless replicative potential

- telomere shortening is an important component of replicative senescence.
- short telomeres result in apoptosis or cell cycle arrest.
- reactivation of telomerase (or retained activity in transformed stem cell) appears essential for unlimited proliferation of cancer cells.
- telomerase activity has been detected in most human / animal tumors.
Sustained Angiogenesis

- **Neoplastic parenchyma** = Neoplastic cells (epithelial, mesenchymal, etc)
- **Neoplastic stroma** = Connective tissue and blood vessels that support the neoplastic parenchyma (it may be disorganized but not really neoplastic)
- Tumors cannot grow larger than 1-2 mm without vascularization (O₂ / nutrients).
- Tumors stimulate host vessel growth by a process called angiogenesis.
- Angiogenic factors (esp VEGF, bFGF) may be produced by tumor cells, supporting stromal cells or inflammatory cells infiltrating the tumor.
- In contrast to normal vessels, tumor vessels are disorganized, unstable and leaky.
- Many tumors may exist *in situ* for months to years without developing a blood supply and then enlarge when angiogenic phenotypes emerge ("angiogenic switch").
Robbin’s Figure 7-41 Tumor angiogenesis. Compared to normal blood vessels (left panels), tumor vessels are tortuous and irregularly shaped. The tumor vasculature (upper right) is formed from circulating endothelial precursor cells and existing host vessels; myofibroblasts give rise to pericytes cells at the periphery of the vessels. By contrast to the stable vessel network of normal tissue, the networks formed by tumor vessels are unstable and leaky. Arterioles, capillaries, and venules are clearly distinguishable in the normal vasculature (lower left); in the tumor the vessels are disorganized and not identifiable as arterioles or venules (lower right).
Ability to Invade and Metastasize

• in a given tumor, the neoplastic cells differ widely in their ability to metastasize.
  ➢ some malignant tumors can release $10^6$ neoplastic cells in the bloodstream daily.
  ➢ yet only small proportion of malignant cells, survive to form metastases.

• metastasis is a multistep process influenced by various molecular factors.
  ➢ metastatic properties often acquired only late in the course of tumor progression.
  ➢ *neoplastic transformation* and *progression from a nonmetastatic to a metastatic tumor type* are not dependant on same oncogenes or tumor suppressor genes.
  ➢ many metastatic properties involve cell membranes, with increases or decreases in the cells ability to adhere to adjacent cells or the surrounding ECM.
Two main steps:

i) Invasion of the ECM

ii) Vascular Dissemination and Homing

Robbin’s fig 7-42. The metastatic cascade. Schematic illustration of the sequential steps involved in the hematogenous spread of a tumor.
i) Invasion of Extracellular Matrix (ECM)

Robbin’s Fig 7-44. Schematic illustration of the sequence of events in the invasion of epithelial basement membranes by tumor cells.

A. LOOSENING OF INTERCELLULAR JUNCTIONS

Tumor cells detach from each other because of reduced adhesiveness.

B. ATTACHMENT

cells then attach to the basement membrane via the laminin receptors

C. DEGRADATION

cells secrete proteolytic enzymes, including type IV collagenase and plasminogen activator

D. MIGRATION

Degradation of the basement membrane and tumor cell migration follow.
ii) Vascular Dissemination and Homing of Tumor Cells

- circulating tumor cells tend to clump with themselves &/or blood cells (esp platelets).

- clumping protects tumor cells from mechanical turbulence and immune attack.

- tumor aggregates must arrest / adhere to vessel wall & then extravasate through BM.

- the sites of metastasis are related to:
  
  ① **hemodynamic form of distribution.**
  
  - correlation between the primary tumor & lymph/blood flow to the target organ(s).

  ② **organ trophism** ("favorable vs unfavorable soil").
  
  - affinity between the neoplastic cells and specific organs (CAM’s / chemokines).
Ability to Invade and Metastasize

- tumor metastasis must be differentiated from a **multicentric tumor**.
  - feline sarcoma virus typically causes multicentric subcutaneous fibrosarcomas.
  - avian leukosis / sarcoma virus can cause multicentric soft tissue sarcomas.
  - neurofibromatosis in humans & cattle.
Neurofibromatosis type 1 in people, is an autosomal-dominant disorder characterized by multiple (or solitary) cutaneous neurofibromas (peripheral nerve sheath tumors), and cutaneous hyperpigmented macules (*café au lait* spots), among other lesions.

Sporadic neurofibromas, in various sites, are occasionally seen in cattle. Additionally, a rare cutaneous form of multicentric neurifibromatosis has been described (see photo to right) which appears to be similar to human neurofibromatosis (type 1) & caused by hereditary mutations at the bovine NF1 locus.
Molecular Basis of Multistep Carcinogenesis

- Carcinogenesis is a multistep process at both the phenotypic and genetic levels.
- Most cancers develop late in life because several cellular / genomic changes are required for malignant transformation.
- Reflects the redundancy of growth control mechanisms normally present in a cell.

Robbin’s Fig 7-46 Molecular model for evolution of colorectal cancers through the adenoma-carcinoma sequence. APC mutation is an early event & loss of p53 occurs late in the process, the timing for the other changes may show variations (note: individual tumors may not have all of the changes listed).
Dysregulation of Cancer-Associated Genes

Not on exam – should read on own (at some point)

- some classic examples of how mutations, translocations & amplifications occur and cause oncogene activation or TSG inactivation.