Disorders of Cell Growth & Neoplasia

Lecture 4
Molecular basis of cancer

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Molecular Basis of Cancer

Fundamental principles

i) Nonlethal genetic damage

• damage may be inherited or acquired (radiation, chemical, viral, spontaneous).

ii) Clonal expansion of single, genetically damaged cell.

• become heterogeneous in genotype & phenotype
• clones can vary in morphology, karyotype, metastatic capacity, etc.

Robbin's 7-12. Clonal evolution of tumors and generation of tumor cell heterogeneity. New subclones arise from the descendants of the original transformed cell, and with progressive growth the tumor mass becomes enriched for those variants that are more adept at evading host defenses & are likely to be more aggressive.
Fundamental principles (cont’d)

iii) Normal regulatory genes are the targets of genetic damage
    • growth-promoting protooncogenes.
    • tumor suppressor genes.
    • genes that regulate apoptosis.

iv) Dysfunction of DNA repair genes
    • Affected cells develop a mutator phenotype

v) Neoplastic transformation is a multistep process
    • at both the genetic and phenotypic levels.
The Normal Cell Cycle

The Cell-Cycle and Cellular Proliferation

- C-C: a highly controlled sequence that permits cell division

- cell-cycle phases = $G_0$ (quiescent),
  $G_1$ (presynthetic),
  $S$ (DNA synthesis),
  $G_2$ (premitotic)
  $M$ (mitotic)

- checkpoints present to monitor for DNA damage

- progression through the cycle regulated by cyclins & CDK’s (regulated by inhibitors)

- growth factors stimulate proliferation by upregulating cyclins or downregulating inhibitors.
The Normal Cell Cycle

- checkpoints present to monitor for DNA damage

Robbin’s Fig 3-3. Cell-cycle landmarks: cell-cycle phases (G0, G1,G2, S, and M), the location of the G1 restriction point, and the G1/S and G2/M cell-cycle checkpoints. Cells from labile tissues such as the epidermis and the gastrointestinal tract may cycle continuously; stable cells such as hepatocytes are quiescent but can enter the cell cycle; permanent cells such as neurons and cardiac myocytes have lost the capacity to proliferate.
The Normal Cell Cycle

- Progression through the cycle regulated by cyclins & CDK’s (regulated by inhibitors)

Robbin’s Fig 7-28. Expression of cyclin-cyclin-dependent kinase (CDK) complexes during the various stages of the cell cycle.

- Growth factors stimulate proliferation by upregulating cyclins or downregulating inhibitors

Robbin’s fig 7-29 Schematic illustration of the role of cyclins, CDKs, and cyclin-dependent kinase inhibitors in regulating the G1/S cell-cycle transition.
### Growth Factors

- extracellular polypeptide signal molecules that stimulate a cell to grow or proliferate.

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Symbol</th>
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<tbody>
<tr>
<td>Epidermal growth factor</td>
<td>EGF</td>
</tr>
<tr>
<td>Transforming growth factor alpha</td>
<td>TGF-α</td>
</tr>
<tr>
<td>Hepatocyte growth factor</td>
<td>HGF</td>
</tr>
<tr>
<td>Vascular endothelial cell growth factor</td>
<td>VEGF</td>
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<tr>
<td>Platelet-derived growth factor</td>
<td>PDGF</td>
</tr>
<tr>
<td>Fibroblastic growth factor</td>
<td>FGF</td>
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<tr>
<td>Keratinocyte growth factor</td>
<td>KGF</td>
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<tr>
<td>Insulin-like growth factor</td>
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<table>
<thead>
<tr>
<th>Cytokine</th>
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<tr>
<td>Interleukins</td>
<td>IL-1, etc</td>
</tr>
<tr>
<td>Interferons</td>
<td>IFN-α, etc</td>
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Signaling Mechanisms in Cell Proliferation

- GF-receptor binding ➔ “receptor-initiated signal transduction” ➔ gene transcription ➔ activation or inhibition of growth promoter or growth inhibitor genes

Robbin’s Fig 3-9 Examples of signal transduction systems that require cell-surface receptors. Shown are receptors with intrinsic tyrosine kinase activity, seven transmembrane G-protein-coupled receptors, and receptors without intrinsic tyrosine kinase activity. The figure also shows important signaling pathways transduced by the activation of these receptors through ligand binding.
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**

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

- tumor cells can proliferate without external stimuli (via oncogene activation)

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

- oncogenes $\Rightarrow$ overexpression of their protein products (oncoproteins)

- 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)

- “tumor suppressor genes” inhibit cell growth
- loss of expression of TSG’s is present in most human tumors.

i) RB (retinoblastoma) Gene

- RB protein is key in regulating cell proliferation at the G\(_1\)/S transition.
- absent /mutated RB $\Rightarrow$ molecular “brakes” released and cell-cycle progression.

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.
iii) *p53 Tumor Suppressor Gene* ("Guardian of the Genome")

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

Robbin's Fig 7-37 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).
- overexpression of BCL-2 protein occurs in most B-cell lymphomas.
Defects in DNA Repair

- cells are frequently exposed to DNA-damaging agents but have DNA repair genes/mechanisms

- inherited disorders of defective DNA repair genes predispose to cancer.

- defects in DNA repair genes lead to widespread mutagenesis and genetic instability (“mutator phenotype”).

- Defects in any of the following DNA repair systems cause cancer:
  
i) Mismatch repair genes
  
ii) Nucleotide excision repair genes
  
iii) Recombination repair genes
Defects in DNA Repair

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

- the replicative capacity of a cell depends on telomere length / telomerase activity (related to age).

- short telomeres (DNA damage) result in cell cycle arrest or apoptosis.

- reactivation of telomerase (or retained activity in transformed stem cells) causes unlimited proliferation of cancer cells.

- telomerase activity has been detected in many human / animal tumors.
Sustained Angiogenesis

- *Neoplastic parenchyma* = Neoplastic cells (epithelial, mesenchymal, etc)

- *stroma* = Connective tissue and blood vessels that support the neoplastic parenchyma (this is non-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 (VEGF, bFGF) produced by tumor, stromal or inflammatory cells

- Tumor vessels are disorganized, unstable and leaky.
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).
• 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 are acquired late in the course of tumor progression.

➢ neoplastic transformation and progression to a metastatic tumor type are not dependant on same genes

➢ metastatic properties involve cell membranes
Two main phases:

i) Invasion of the ECM

ii) Vascular dissemination and homing of tumor cells

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)

Tumor cells detach from each other because of reduced adhesiveness.

Cells then attach to the basement membrane via the laminin receptors.

Cells secrete proteolytic enzymes, including type IV collagenase and plasminogen activator.

Degradation of the basement membrane and tumor cell migration follow.

Robbin's Fig 7-44. Schematic illustration of the sequence of events in the invasion of epithelial basement membranes by tumor cells.
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 in the target organ(s).
  2. **organ trophism**
     - affinity between the neoplastic cells and specific organs (CAM’s / chemokines).
7 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 neurofibromatosis has been described (right) which appears to be similar to human neurofibromatosis (type 1) & caused by hereditary mutations at the bovine NF1 locus.
Summary of the Essential Alterations for Malignant Transformation (*)

“The seven cellular sins”

Robbin’s Figure 7-27 Flow chart depicting a simplified scheme of the molecular basis of cancer.
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).