Oncogenes and Tumor Suppressor Genes NOTES

Cancer Introduction

- Cancer can be defined as the uncontrolled growth and spread of cells throughout the body.
- Cancer is not a single disease, but is comprised of over 100 different diseases, defined on the basis of site of origin, specific characteristics, and in some cases causative molecular events.
- The human body contains trillions of cells, and the growth and survival of these cells must be controlled to maintain normal homeostasis.
- Cancer is a breakdown of the normal mechanisms controlling cell division.
  - The GAS PEDAL: Positive signals induce cell division.
  - The BRAKES: Negative signals inhibit cell division and/or promote apoptosis (programmed cell death).

The basics of cancer genetics

- Tumors arise through the combined effects of mutations in genes that are oncogenes and genes that are tumor suppressor genes.
- Oncogenes (the “GAS PEDALS”) encode proteins that promote tumor formation by stimulating cell division through positive signals.
- Cancer-causing mutations in oncogenes usually result in a gain-of-function such that the oncogene is always ON, and the GAS PEDAL is always pushed, driving continuous cell division.
- The term proto-oncogene refers to the normal (non-mutated) properly functioning form of such genes present in non-cancerous, normally dividing cells. The term oncogene refers to the mutated versions of these genes that cause tumor formation in cancerous cells.
- Tumor suppressor genes (the “BRAKES”) encode proteins that inhibit tumor formation by inhibiting cell division or causing programmed cell death (apoptosis) through negative signals.
- Cancer-causing mutations in tumor suppressor genes usually result in loss-of-function mutations, such that the BRAKES don’t work anymore and the cell loses the ability to exit the cell cycle.
- Both oncogenes and tumor suppressor genes generally regulate only a few processes, including:
  - Proliferative signals and the cell cycle
  - Apoptosis (programmed cell death)
  - Genomic integrity (meaning in the DNA intact and undamaged)
- Other genetic changes found in cancer cells relate to other cellular processes that are not as well-understood:
  - Angiogenesis (meaning the creation of blood vessels)
  - Metastasis (meaning the ability of cancer cells to break off from a tumor, spread throughout the body, and start new-tumors in different locations)
  - The interactions between tumors and the surrounding body tissues
  - Immune surveillance (meaning the ability of cancer cells to escape detection and destruction by your own immune system)

Oncogenes in detail

Oncogene Function

- Obviously, proto-oncogenes did not evolve for the purpose of causing cancer. Under normal conditions, they are vital to cell growth and organism development by promoting completion of the normal cell cycle and driving cell division.
- Oncogenes are proto-oncogenes whose DNA sequence has been mutated or whose gene expression has been modified in a way that promotes tumor formation.
- 2.9% of human genes are classified as proto-oncogenes.
• Many proto-oncogenes are involved in the regulation of proliferation (cell division), differentiation (cell specialization), and cell survival.
• Known proto-oncogenes include cyclins and cyclin dependent kinases (CDKs) that normally act to promote the cell cycle when appropriate conditions are met, for example when growth factors in the environment signal to activate cell division.

Oncogenes and Growth Factor Signaling
• Normal cells are dependent on external signals to promote cell division and cell survival.
• The signals are provided by growth factors, by contact with other cells, and other signals.
• Many tumor cells have a reduced or absent requirement for these external signals.
• Proto-oncogenes commonly encode genes involved in growth factor signaling.
• Growth factors are extracellular proteins (meaning proteins outside the cell) that bind to specific proteins within a cell’s plasma membrane (such proteins are known as “growth factor receptors”) to trigger a series of signals within the cell that direct cell division or cell survival.
• Some proto-oncogenes are growth factors, mutations of which allow some tumors to produce their own growth factors.
• Other proto-oncogenes are growth factor receptors, which can be mutated to an always active form.

Summary of Oncogenes
• Proto-oncogenes are present in our genome, and are involved in growth, differentiation, and survival regulation.
• Proto-oncogenes can become activated into oncogenes by a variety of mutations (point mutation, deletion, insertion, and translocation) or by the insertion of certain viruses into the genome.
• This activation leads to elevated expression and/or modification of the sequence and encoded protein's activity, and can then promote tumor formation.

Tumor Suppressor Genes in detail
Tumor Suppressor Gene Function
• Tumor suppressor genes are genes that act to control cell division or cause programmed cell death.
• Tumor suppressor genes inhibit tumor formation.
• They differ from oncogenes in that tumor suppressor genes inhibit cell division if proper conditions for growth are not met.
• Conditions that would trigger the tumor suppressor gene 'brakes' of the cell include DNA damage, a lack of growth factors, or defects in the division apparatus (remember the three cell cycle checkpoints...)

The genetics of Tumor Suppressor Genes
• A key to understanding tumor suppressor genes is that it is the LOSS OF FUNCTION of these genes that leads to problems. This is in contrast to oncogenes which often have gained functions (or lost the ability to be controlled) in their mutant form.
• Unlike oncogenes, tumor suppressor genes generally follow the "two-hit hypothesis", meaning that both alleles that code for a particular protein must be affected before an cancer-causing effect is seen. This is because if only one allele for the gene is damaged, the second can still produce the correct protein.
• In other words, mutant tumor suppressor gene alleles are usually recessive whereas mutant oncogene alleles are typically dominant.
• Known tumor suppressor genes include p53 and Retinoblastoma (Rb).
• The product of the normal Rb gene inhibits DNA replication until appropriate signals (such as growth factor signals) are present. If both alleles of Rb are mutated, DNA replication becomes uncontrolled, promoting continuous cell division.
The product of the normal p53 gene functions to monitor the integrity of a cell’s DNA and prevents the progression through the $G_1 \rightarrow S$ checkpoint when the DNA is damaged. If both alleles of p53 are mutated, DNA replication will proceed even if the cell’s DNA is damaged.

- p53 gene mutations have been identified in OVER HALF (>50%) of all human cancer types.

**Objective:** Explain **HOW mutations in oncogenes and tumor suppressor genes** lead to cancer.

Explain/contrast the role of **proto-oncogenes and tumor suppressor genes** in control of the $G_1$ to $S$ checkpoint.
**FOR SUPER EXTRA BONUS AWESOMENESS...**
(MEANING NOT REQUIRED INFORMATION, BUT POTENTIALLY HELPFUL)

Specific examples of proto-oncogenes and oncogenes

**Non-Receptor Tyrosine Kinase**
- The first example of an oncogenic activation by gene fusion was discovered by cloning of the breakpoint region of the Philadelphia chromosome in chronic myeloid leukemia.
- Generates a reciprocal translocation t(9:22) between the bcr and abl genes.
- The chimeric protein has enhanced tyrosine kinase activity, and confers growth factor independence on the cell. The abl gene can also be activated by fusion with the TCL gene or the gag gene of the Abelson leukemia virus.
- This kinase has been specifically targeted with an inhibitor (Imatinib, or Gleevec), and is used to treat CML patients.

**Ras**
- Small GTP-binding proteins are membrane-bound signaling molecules.
- Ras proteins are critical points of convergence of RTK signaling involved in regulation proliferation, differentiation and survival.
- Ras is a molecular switch active when GTP-bound.
- In response to RTK signaling, a guanine nucleotide exchange factor (GNEF) is recruited to the membrane, promoting the release of Ras-GDP. This is replaced by Ras-GTP (active form).
- Ras is also regulated by GTPase activating proteins (GAPs) the promote GTP to GDP hydrolysis.
- There are two viral Ras genes from Harvey and Kirsten sarcoma viruses, with cellular versions H-ras and K-ras.
- Ras is mutated in over fifteen percent of human cancers, and in certain types, the frequency is much higher: in pancreatic cancer, the Ras mutation rate is over ninety percent.
- Point mutations of these genes at certain residues occur in cancer, mainly because most other Ras mutations increase the rate of intrinsic GTPase activity. These oncogenic mutations tend to increase the rate of GTPase activity.
- Chemical carcinogens which trigger point mutations can trigger Ras oncogenesis.
- Ras activation triggers signal transduction pathways with diverse effects dependent on cell type and genetic background, including proliferation, survival, and morphological transformation.

**Oncogenes and the Cell Cycle**
- E2F activity is controlled by Retinoblastoma (Rb) family proteins.
- E2F activity inhibits the S phase (DNA synthesis) of the cell cycle by sequestering certain transcription factors.
- Growth factor signaling results in activation of G1 cyclin-CDK complexes.
- These complexes phosphorylate RB family members.
- Hyperphosphorylated RB is unable to interact with E2F, allowing these transcription factors to drive transcription of S-phase genes.
- Overexpression of E2F1 is also sufficient to drive cells into the S phase.

**Oncogenes and Apoptosis**
- Oncogenes can also promote tumor formation by the inhibition of apoptosis.
- Follicular lymphoma is associated with a reciprocal translocation which brings bcl2, an apoptosis inhibitor, under the control of the Ig locus.
- Bcl2 is over-expressed in a number of tumor types.
Transgenic studies have demonstrated that bcl2 cooperates with proliferative oncogenes such as myc in tumor formation.

Components of Signal Transduction Pathways
- Most oncogenes that influence apoptosis modulate signal transduction pathways rather than functioning as direct components of the death effector machinery.
- Many of these oncogenes can have pleiotropic effects on cells, stimulating both proliferation and survival.
- Growth factor pathways can function to promote proliferation, survival, or both.

Ras and Akt Apoptosis Inhibition
- Loss of integrin-mediated cell-matrix contact induces apoptosis (anoikis) in certain epithelial cells, but Ras activation can inhibit this.
- Activated Ras can directly associate with PI3K, activating this lipid kinase, which produces a second messenger that activates many other proteins, including Akt, a powerful kinase apoptosis inhibitor.

Differentiation and Cancer
- Cancer cells usually display an undifferentiated phenotype, since fully-differentiated cells are unlikely to become cancerous.
- In principle, blocking differentiation could lock a cell into a proliferation-competent state indefinitely.
- A mutant retinoic acid receptor, or RAR (retinoic acid is a pro-differentiation signal), has lower ligand affinity (loss of function) than native RAR. Blood progenitor cells can then remain undifferentiated and cause leukemia. When treated with high levels of retinoic acid, such leukemias are often observed to go into remission as their cells begin to differentiate.

Specific examples of tumor suppressor genes

Retinoblastoma
- Retinoblastoma is present in a familial form, and is inherited in a dominant fashion.
- Knudson proposed a "two-hit hypothesis" which suggested that in the inherited form, the germ line contained a mutation in one allele of the tumor suppressor gene RB1. For cancer to occur, only one more mutation needs to occur, increasing the likelihood of tumor development.
- In its sporadic form it is rare, due to the unlikelihood of two "hits" to the same gene.
- The second "hit" to tumor suppressor genes can occur by a number of mechanisms:
  - Non-disjunction and reduplication, resulting in loss of the normal chromosome and reduplication of the RB1-mutant chromosome,
  - Non-disjunction, leaving only the mutant copy in the tumor cell,
  - Mitotic recombination leading to loss of the normal allele.
- RB1 functions as a G1/S cell cycle checkpoint protein, sequestering E2F. If RB1 is non-functional, cells will be able to indiscriminately enter S phase.

INK4a
- Loss of heterozygosity of chromosome 9p was found in many melanomas.
- Linkages studies of families with inherited melanoma suggested the presence of a tumor suppressor gene at this site.
- The gene involved was identified and termed INK4a. Deletion of this gene in mice results in a high incidence of tumor formation; and such mutations are found in melanomas, gliomas, leukemia, and pancreatic and bladder cancers.
• INK4a encodes a cyclin-dependent kinase (CDK) inhibitor which induces cell cycle arrest. When inactivated, an important regulatory element is missing.

p53
• p53 is the most commonly-mutated gene in human cancer: around half of all cancers have mutations in p53.
• p53 knockout mice contract some form of cancer within six months.
• p53 promotes cell cycle arrest and apoptosis, and as such, its loss decreases genome stability.

Alternative Reading Frame (ARF)
• Detailed studies of the INK4a locus revealed the presence of a novel transcript containing sequences identical to p16INK4a.
• Termed p19ARF or p14ARF (mouse or human, respectively). The Arf transcript results as a consequence of a different first exon, followed by common exons 2 and 3. However, these exons are read in different reading frames in p16 and ARF, so although related at the nucleotide level, they have no homology in terms of amino acids.
  o The odds of such a construct arising by chance are orders of magnitude lower than the simple odds of the initial gene arising by chance. Probability dictates that alternative-reading-frame transcripts of genes are highly unlikely to produce native (properly-folding) proteins, much less functional ones.
• ARF functions as a tumor suppressor acting to inhibit mdm2-mediated degradation of p53.

PTEN
• Akt genes are amplified in a number of human malignancies including breast and ovarian cancers.
• More common mechanisms of modulating Akt activity is loss of the tumor suppressor PTEN (phosphatase and tensin homolog).
• This lipid phosphatase partially-degrades PIP3, the activator for Akt, preventing Akt activity.
• Since Akt is tumor-suppressive, PTEN's inactivation of Akt is tumorifacultative.