Chapter 5: Tumor Suppressor Genes

- If TS genes lose function then tumor formation progresses
- Many hereditary cancers result from germ-line mutations in TS genes
  - One allele may be inherited and then chances of 2nd hit on other allele increases risk of cancer
  - LOH (loss of heterozygosity)- when a tumor suppressor gene (allele is lost by deletion or recombination)

- Genetics of Cancer: Loss of Heterozygosity Is Driven by Genetic Similarity
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- ***Background:*** **Tumor suppressor genes, which code for proteins that inhibit uncontrolled cell proliferation, are frequently mutated in human cancer. Heterozygous cells, cells with a single copy of a particular tumor suppressor gene, will not undergo aberrant cell proliferation leading to tumor formation. However, if the normal copy of the gene is lost, the cell becomes predisposed to abnormal growth and if enough mutations accumulate, a tumor may form. Losing the gene is known as loss of heterozygosity (LOH) the mechanisms of which are not fully understood.**
- ***Advance*****: LOH can occur during cell division when chromosomes exchange DNA also known as mitotic recombination . The mechanism of mitotic recombination is not well understood; however, this team of researchers has demonstrated that the level of mitotic recombination in mice is affected by the degree of similarity between the two sets of chromosomes, one of which is inherited from each parent. When two unrelated strains of mice were crossed, the mitotic recombination and the subsequent loss of heterozygooisty were diminished. However, when back crosses of the progeny were performed causing a higher degree of chromosomal similarity, the mitotic recombination and subsequent chromosomal exchange were restored making these animals more susceptible to developing genetic diseases.**
- ***Implication*****: This finding may have implications in the development of human cancer. Genetic differences between individuals is probably sufficient to reduce the risk of mitotic recombination and therefore, reduce the risk of cancer. These data suggest, however, that if a high degree of genetic similarity exists between parents, their offspring may be more susceptible to diseases, like cancer, that are associated with mitotic recombination and loss of heterozygosity.**
- ***Publication*****: Shao C, Stambrook PJ, Tischfield JA. Mitotic recombination is suppressed by chromosomal divergence in hybrids of distantly related mouse strains. Nat Genet. 2001 Jun;28(2):169-72.**

- Retinoblastoma
  - Most studied of TS genes and used as example
  - RB1 inactivated- codes for a regulator of the cell cycle (G1→S transition) and also important for proper mitotic segregation. Also regulated apoptosis
  - Acts by binding to and regulating (inhibiting) E2F transcriptional factors when in the non-phosphorylated state. When phosphorylated cannot bind to E2F (phosphoryalted by CDKs)
    - E2Fs activate genes required for entry into the S phase and DNA synthesis

- TP53 tumor suppressor genes
  - Most altered gene in human cancers
  - TP53 acts to activate p21, which when active will inhibit cell cycle progression
  - Inactivation of P53 effects the ability of the cell to react to DNA damage (UV damage, viral integration). Inactivation of P53 prevents the cell from undergoing apoptosis and allows the cell to continue to proliferate and accumulate more mutations
  - BRCA1 and BRCA2 act in similar ways
  - RB1 acts as “gatekeeper” and monitors cell cylcing while TP53 acts as “caretakers” looking for mutations and inducing apoptosis
  - DNA tumor viruses contain proteins that inactivate both TP53 and RB1

Tumor Suppressor Genes in Hereditary Cancers

- Patients with familial cancers usually develop these cancers at a significantly lower age than cancers found with somatic mutations
  - Most sporadic cancers of the colon and breast occur in 60s-70s while familial cancers arise in the 20s-30s
  - Patients with familial cancers may also demonstrate development of more than one cancer of the same type or multiple cancers of different types (multifocality or bilaterality occurs in paired organs)

- Retinoblastoma
  - Rare tumor/ occurs in young children
  - Undifferentiated retinocyte precursors form in cell mass in retina
  - Incidence is ~ 1:20,000 live births, but familial inheritance may be as high as 1:2 (typical autosomal dominant inheritance with high penetrance)
  - Patients with RB also develop other tumors later in life (bone (osteosarcoma)
  - RB requires two “hits” within one cell
    - Could be two alleles of same gene or one allele each of different genes

RB1 and the Cell Cycle

- Rb1 gene product- pp110
  - Central regulator of the cell cycle
  - Controls transition from G1→ S
    - Binds to E2F proteins and represses the promoters of genes needed for the entrance into S (inhibits E2F when bound to it)
    - Suppression released when Rb1 protein is HYPERphosphorylated (always phosphorylated to some extent thus there is no state in which it is not phosphorylated)- RB1 is inactive when hyperphosphorylated
    - 1st phosphorylation done by the CDK2/Cyclin molecule
    - Following mitosis, RB1 is dephosphorylated
  - Loss of RB1 function leads to unregulated proliferation
    - i.e. in retinoblastoma- cells grow so fast they do not differentiate
  - Overactivity of E2F may lead to apoptosis

Protein Inhibitors of CDKs

- Two Classes
  - CIP/KIP
    - p21 = CIP1, p27 = KIP1 and p57 = KIP2
    - CIP/KIP proteins are CDK/kinase inhibitory proteins
      - » They block the CDK/Cyclin complex (holoenzyme)
  - INK
    - p15 = INK^{4B}, p16 = INK^{4A}
    - INK4 proteins compete with D-Cyclins for binding to CDK4 and block activity
- The different CDK inhibitors respond to different signals that lead to cell arrest and allow a fine-tuned cellular response to different signals

- RB1 controls cell cycle progression that ensures that cell proliferation occurs only in response to proper sets of signals *(i.e., following stimulation by growth factors via the MAPK)*
  - Considered a tumor suppressor gene because loss of function of RB1 leads to unregulated growth
  - Does not actually suppress tumor growth but allows coordinated proliferation and differentiation during development of normal tissues- if lack RB1 animals are not viable and show defects in development of tissues
  - Over-expression of D-Cyclins or CDK4 (as consequence of gene amplification) or mutations in CDK4 that make it unresponsive to CDK4 inhibitors exert similar effects on growth dysregulation

TP53 as Tumor Suppressor

- Animals can live without TP53
  - Mice lacking TP53 are more susceptible to tumors and die within a few months of life
  - In Li-Fraumeni- syndrome one defective allele is inherited
    - A variety of tumors develop in different tissues
    - These tumor cells demonstrate a 2nd inactivation of other allele or exhibit LOH at chromosome 17p
  - TP53- 20kb on chromosome 17p13.1
    - Encodes a 53kDa phosphoprotein
    - Has DNA binding capability and acts as a transcriptional activator at a few hundred different genes
    - Can also bind to damaged DNA and functions as an exonuclease and may act to regulate protein synthesis at ribosomal level

- TP53 coordinates cellular response to many types of damage to genome
  - Ds DNA breaks induced by ionizing radiation, guanine nucleotide imbalance, viral infection and oncogene-induced hyperproliferation
  - Also involved in regulation of replicative senescence
  - Regulated by post-translational phosphorylation which alter stability and activity of protein
  - ½ life only 10-20 minutes (short)- MDM2/HDM2 protein blocks transcriptional activity and adds ubiquitin to TP53 to mark for degradation

- TP53 phosphorylated by PKC and CDK2 at a variety of serine AA's
- RAS induced cell proliferation activates TP53
- P53 induces multiple cellular responses:
  - Cell cycle arrest
  - DNA repair
  - Altered secretion of growth factors (angiogenic factors)
  - Apoptosis and
  - Inactivation of MDM2/HDM2
- Which response induced determined by:
  - Cell type
  - Extent of DNA damage or cellular stress
  - Basal and induced pattern of phosphorylation of TP53
  - Competing signals
  - Which response occurs first (competition)
  - Several hundred genes activated or inactivated by TP53

- Arrest of the cell cycle by TP53 mediated:
  - By p21 and 14-3-3σ – these block cell cycle in G1 or G2
- Induction of apoptosis by TP53 mediated:
  - Different factors in different cells
    - Induction of BAX- an antagonistic homolog of BCL2 and induces apoptosis in mitochondria
    - Induces FAS (death receptor) which binds to FASL on activated $T_{cytotoxic}$ cells
- Communication to other cells by TP53:
  - TP53 induces thrombospondin (TSP1) which inhibits the proliferation of endothelial cells and blocks angiogenesis
- Induces feedback mechanism to limit own activity
  - Accumulating MDM2/HDM2 blocks transcriptional activation by TP53 and causes its degradation
    - » Mice lackingTP53 are viable (but die of tumors at early age), but in mice lacking MDM2 they die *in utero* from widespread apoptosis- knockout of the TP53 gene corrects this defect

Summary of TP53

- Regulates cellular response to most kinds of genetic damage and cellular stress
- Defects in TP53 found in many tumors
- Cancers lacking TP53 tolerate more DNA damage (strand breaks and aneuploidy)
- Will not enter replicative senescence as easily with defective TP53

Mechanisms of TP53 Inactivation

- Missense mutations plus LOH
  - Most common mechanism
    - Missense mutation in one allele and loss of 2nd allele by deletion or recombination
    - Most mutation affect the DNA binding region
- Non-sense and splice mutations
  - More rare
    - Mutations may act by making “dominant negatives” with more advantage???? Under investigation
- MDM2 overexpression
  - Amplifications and overproduction causes less function of P53

Mechanisms of TP53 Inactivation

- Insert table 5.3

Classification of TS Genes

- Vogelstein & Kinzler proposed:
  - Caretakers- cancer arises when cell cycle regulators not sensing DNA damage
  - Gatekeepers- implies that a cancer can only arise if the function of this tumor suppressor is abolished
- Genes may be irrelevant for growth but loss is essential for metastasis (metastasis suppressor genes?)
- Knudson Model
  - All genes that lead to cancers are inherited in an autosomal-dominant fashion, but behave in a recessive mode at the cellular level- i.e., one mutant functionally inactive allele is inherited and the second one is inactivated in the tumors by point mutation, deletion, insertion, recombination or promoter hypermethylation.