Chapter 4: Oncogenes

Oncogenes

- Discovered from parts of genomes of transforming retroviruses
- Consist of host proto-oncogenes that are activated by insertion of retrovirus
- Activation of proto-oncogenes by:
  - Translocation, gene amplification, point mutations
- May act as:
  - Extracellular growth factors
  - As receptors for growth factors
  - As signaling proteins
  - Protein kinases
- Generally a single oncogene NOT sufficient for cancer

Oncogenes & Protooncogenes

TABLE 8–4. ONCOGENES AND PROTO-ONCOGENES

Oncogene	Virus	Protein Encoded by Oncogene	Protein Encoded by Proto-oncogene
Peripheral Membrane Proteins with Tyrosine-specific Protein Kinase Activity			
v-src	Rous sarcoma virus, B77 ASV	$pp60^{v\text{-}src}$	$pp60^{c\text{-}src}$
v-abl	Abelson murine leukemia virus	$pp160^{v\text{-}gag\text{-}abl}$	$pp145^{c\text{-}abl}$
v-fes/fps	Snyder-Theilen FeSV; class II ASVs including Fujinami sarcoma virus	$pp85^{v\text{-}gag\text{-}fes}$ $pp130^{v\text{-}gag\text{-}fps}$	$pp92^{c\text{-}fes/fps}$
v-yes/fgr	Y73 Esh sarcoma virus Gardner-Rasheed FeSV	$pp80^{v\text{-}gag\text{-}yes}$ $pp70^{v\text{-}gag\text{-}fgr}$	$p55^{c\text{-}yes/fgr}$
v-kit	Hardy-Zuckerman FeSV	$pp80^{v\text{-}gag\text{-}kit}$	?
Transmembrane Receptors with Tyrosine-specific Protein Kinase Activity			
v-erbB	Avian erythroblastosis virus (AEV)	$P45^{v\text{-}erbB}$	EGF receptor: 150K
v-fms	McDonough FeSV	$P140^{v\text{-}gag\text{-}fms}$	Macrophase colony stimulating factor (CSF1) receptor: 170K
v-ros	UR2 ASV	$P68^{v\text{-}gag\text{-}ros}$	$P56^{c\text{-}ros}$
v-erbA	AEV	$P75^{v\text{-}gag\text{-}erbA}$	Thyroid hormone receptor: 70K
Cytoplasmic Serine/Threonine-specific Protein Kinases			
v-mos	Moloney murine sarcoma virus (Mo-MSV)	$P37^{v\text{-}mos}$	$P41^{c\text{-}mos}$
v-mil-raf	MH2 ASV; Moloney murine sarcoma virus strain	$P100^{v\text{-}gag\text{-}mil}$ $P75^{v\text{-}gag\text{-}raf}$ and $P90^{v\text{-}gag\text{-}raf}$	$P72^{c\text{-}mil/raf}$
Plasma Membrane-associated GTP-Binding Proteins with GTPase Activity			
v-ras	Harvey and Kirsten murine sarcoma virus (Ha- and Ki-MSV)	$P21^{v\text{-}ras}$	$P21^{c\text{-}ras}$
Growth Factor			
v-sis	Simian sarcoma virus (SiSV)	$P28^{v\text{-}sis}$	$P32^{c\text{-}sis}$; PDGF
Nuclear DNA-binding Proteins			
v-myc	Avian myelocytomatosis virus (MC29)	$P110^{v\text{-}gag\text{-}myc}$	$P62^{c\text{-}myc}$
v-myb	Avian myeloblastosis virus (AMV)	$P45^{v\text{-}myb}$	$P75^{c\text{-}myb}$
v-fos	FBJ murine osteosarcoma virus	$P55^{v\text{-}fos}$	$P55^{c\text{-}fos}$
v-ets	E26 avian acute leukemia virus	$P135^{v\text{-}gag\text{-}myb\text{-}ets}$	$P54^{c\text{-}ets}$, $P75^{c\text{-}myb}$
v-ski	SKV770		

Retroviral Oncogenes

- Only HTLV1 (human T lymphotrophic virus) & HIV known retroviruses to cause cancer in humans
  - In lower animals several retroviruses known to cause cancer
    - Acute vs slow
    - Transducing vs cis-acting

- Acute Transfoming retroviruses
  - These **carry genes** that cause cell transformation
    - ALV (avian leukosis virus) and RSV (Rous Sarcoma virus)
      - Cause leukemias, lymphomas, sarcomas
      - Rapid appearance after infection
      - RSV carries *v-src* (characterized in 1970's)
        - » Protein kinase- located at inside of plasma membrane
        - » Phosphorylates tyrosine
        - » Oncogenes generally expressed as fusion protein with gag protein (pol and part of gag replaced by oncogene and virus is no longer able to express viral progeny)virus. Fusion protein expressed at HIGH levels and leads to growth of cell.
    - >24 oncogenes have been isolated from retroviruses and biochemically characterized (table 4.1)

- Insert fig 4.1

- Slow-Acting Transforming Retroviruses
  - Replication competent
  - Cause transformation by integrating within or in the vicinity of cellular genes and altering their expression
    - » Convert proto-oncogenes to oncogenes
  - – Act by activation of cellular gene promoter by the enhancer in the retroviral LTR
    - – Most effective if retrovirus integrates in inverse orientation upstream from the promoter
    - – The Long Terminal Repeat is something which is often found in strands of RNA or DNA. At each end of the string is the same sequence of code.
  - There are two important functions for the LTR:
    - – They are "sticky ends" which the integrase protein uses to insert the HIV genome into host DNA.
    - – Secondly, they act as promoter/enhancers - when integrated into the host genome, they influence the cell machinery which transcribes DNA, to alter the amount of transcription which occurs. Protein binding sites in the LTR are involved with RNA initiation.

- c-myc
  - Cellular proto-oncogene
    - – Consists of 3 exons and contains some negative regulatory elements upstream of the 2 transcriptional START sites
      - » Transcription from 1st start site goes into 1st intron and waits until next signal
      - » Retroviral insertion into the c-myc gene disrupts this negative control and causes transcription from the normally inactive gene- becomes independent of normal control signals

- v-myc found in myelocytomatosis virus
  - c-myc picked up by recombination with viral mRNA (viral genome is +RNA)
    - – When picked up c-myc transformed to v-myc by mutation that changes regulation of normal gene when integrated and gene is overexpressed.
- Acutely transforming retrovirus over expresses an altered cellular protein
- Slowly transforming retrovirus deregulates the endogenous protein

- Retroviral oncogenes almost always altered compared to their “normal” counterparts (proto-oncogenes)
  - Altered by:
    - Truncation
    - Mutation
    - Fusion to viral genes (proteins)
  - Alterations cause changes in regulation
    - v-erbB encodes a growth factor receptor EGFR
    - Composed of 3 domains (extracellular (binds GF), transmembrane and cytoplasmic TK domain)- viral product lacks most of extracellular domain and contains a point mutation
      - » These alterations lead to an overexpression of a constitutively active protein

- Acutely transforming retroviruses transduce the activated oncogene into each cell they infect and create a large pool of potentially transformed cells
- Slowly transforming retroviruses integrate into many different sites in different infected cells and only rarely hit a cellular proto-oncogene, yielding only a few potentially transformed cells

- Transduction of an activated oncogene into a large number of cells will…
  - Alter levels of autocrine/paracrine cytokines that may overcome immune response
  - A larger pool of cells that have this one alteration may be more likely to have one of these cells get a 2nd alteration/mutation that will lead to cancer (2nd retrovirus? Spontaneous mutation?)
- There is a cellular homologue for each retroviral oncogene found so far

Genes Activated by Retroviral Insertion

- Table 4.3

Identification of Human Oncogenes

- No acutely transforming retroviruses have been observed in humans – However-
  - Several “orthologs” in animal models are over-expressed or mutated in human cancers
- 3T3 Cell Transformation Assay
  - 3T3 Fibroblast cell line
  - Immortalized cell line infected with oncogene carrying retrovirus and FOCI of transformed cells obtained
  - Isolate gene that causes altered phenotype from foci
    - Found v-ras, Ki-ras, and Ha-ras
      - » Each carry a point mutation a specific site (codons 12, 13 or 61)
  - This assay has limited use since it does not identify other altered phenotypes

- REF Assay
  - Use of primary **r**at **e**mbryo **f**ibroblasts infected with retrovirus
  - In this assay two oncogenes are required for focus formation
    - A “ras-type” AND A “myc-type” – shows oncogene cooperativity
  - “myc-type” oncogenes cause immortality and stimulate proliferation while “ras-type” genes elicit overgrowth and altered morphology

- Gene Amplification
  - Gene overexpression occurs in many cancers and may be due to recurrent amplifications of specific chromosomal regions
  - Detected by cytogenetic techniques
    - MYCN overexpressed in some neuroblastomas
    - MYCL overexpressed in some lung cancers
    - ERBB1 overexpressed in breast cancers (now known as ERBB2, HER-2 or NEU)

- Chromosomal Translocations
  - Activation of genes at the translocation sites to become oncogene
  - myc is the best studied of the translocation genes
  - CCND1 encodes for Cyclin D1- regulator or cell cycle progression- translocation and amplification
  - BCL2 gene (breakpoint cluster gene) found translocated in follicular lymphoma-- a direct regulator of apoptosis at the mitochondrion

Functions of Human Oncogenes

- Cell proliferates in response to extracelluar signals (ligand and ligand receptor interaction)
  - Peptide growth factors
    - TGFα, FGF1- mitogens for epithelial cells
    - Bind to and activate receptors at cell membrane
      - » Receptor molecules may be over expressed
      - » Receptor may be mutated to be active constitutively
      - » RTK= receptor tyrosine kinase- biggest class
  - Peptide growth factor RECEPTORS
    - » EGFR- epidermal growth factor receptor- product of the ERBB1 gene
    - » FGFR- amplified & point mutations (bladder & cervix)

- Receptor Tyrosine Kinases (RTK's)
  - One of biggest classes of oncogenes
    - Binding activates tyrosine kinase and receptor subunits phosphorylate each other
    - Over expression of RTKs in tumor cells favors dimer formation and sensitizes cells to lower concentrations of ligand growth factors
      - » May lead to ligand INDEPENDENT growth by having too many receptors that become active whether ligand is present or not
      - » Point mutations typically occur in inhibitory loop of protein RTK and cause constitutive activation of TK activity
  - One receptor may bind to many different proteins and thus one receptor can activate several different signaling pathways

- When activated the RTKs will ….
  - Have their C- terminus post-translationally modified
  - Bind alternatively to GTP or GDP:
    - In the active state GTP is bound
    - Hydrolysis of GTP to GDP by the combined action of RAS proteins (Ha, Ki) and GTPase activator protein (GAP) restores the basal inactive state
  - Activation of RAS involves-
    - Interaction with SOS which acts as a guanine nucleotide exchange factor (GEF) loading the RAS with GTP
    - The activated state of normal RAS is short-lived because of GTP hydrolysis
    - Mutations in RAS amino acids 12, 13 and 61 (which surround the GTP binding site) block the access for the GAP protein and prolong the active state
  - Activated RAS affects protein synthesis, cytoskeleton and cell survival

- The main manner that RAS proteins transmit their signal is through RAF proteins
  - RAF is a mitogen-stimulated protein kinase that functions as a component of the signaling cascade that leads to the stimulation of mitogen-activated protein kinase
  - There are 3 RAF proteins- they are all serine/threonine protein kinases
    - In the inactive state the RAF proteins are in the cytoplasm and the kinase activity is blocked
    - Activated RAS translocates to the membrane and regains ability to act as kinase
      - » RAF activity is regulated by phosphorylation of one of its domains by SRC kinases and PKC isozymes (activated by PLC→ IP_3 and DAG)

- RAF is the first of many in a cascade of protein kinases
  - Mitogen activated protein kinase = MAPK
  - MAPK activation increase protein synthesis and cytoskeletal changes, cell growth and migration
  - MAPK stimulates “early response” genes (those seen when quiescent cells are stimulated to grow by growth factors or serum
    - These include FOS, JUN and MYB
    - These induce cell cycle progression
    - MAPK induces CYCLIN D1 and upregulates the cell cycle progression