Chapter 6: Cancer Pathways

- Limited number of pathways control proliferation and differentiation
  - Transmit signals from growth factors, hormones, cell-to-cell communications/interactions
  - Pathways turn into cancer pathways by deregulation (activation or inactivation of oncogenes & tumor suppressor genes)
  - MAP kinase pathways transduce signals from membrane to nucleus as well as to translational machinery and cytoskeletal components
    - MAPK pathway activated by receptor tyrosine kinases (RTKs)
      - » RAS, RAF, MEK, ERK protein kinases (ERK most imp't)

Other Pathways

- Phospholipase C (PLC)
  - Activates PKC isozymes that stimulate MAPK
    - » Formation of IP_3 and DAG (inositol trisphosphate and Diacylglycerol)
    - » Translocation of inactive PKC to membrane and activation by proteases and Ca^{++} and Phosphotidyl serine (PS)
    - » Phorbol esters act as long-lasting membrane activators (act like DAG which is short-lived)
- Growth factors through RTKs & RAS proteins
  - » Activate PI3K and trigger protein kinase cascade that increases cell survival, protein synthesis and proliferation
- MAPK promotes progression of cell cycle
  - » Leads to phosphorylation and temporary inactivation of RB1 by CDKs

Cancer Pathways

- Over 250 genes causally involved in cancer progression- many can be associated with specific pathways
  - "Cancer pathway is a cellular regulatory system whose activation or inactivation by a genetic or epigenetic mutation is essential for the development of at least one human cancer".
    - MAPK pathway
    - TP53 system
    - Cell cycle regulatory pathway centered around RB1
    - Also WNT and Hedgehog and NOTCH pathways
      - » These are essential in regulating the shaping and differentiation of tissues during fetal development and tissue homeostasis
  - All these pathways interact with each other

- The **hedgehog signaling pathway** is one of the key regulators of animal development conserved from flies to humans. The pathway takes its name from its polypeptide ligand, an intercellular signaling molecule called Hedgehog (*Hh*) found in fruit flies of the genus Drosophila. *Hh* is one of Drosophila's segment polarity gene products, involved in establishing the basis of the fly body plan. The molecule remains important during later stages of embryogenesis and metamorphosis.
- Mammals have three Hedgehog homologues, of which Sonic hedgehog is the best studied. The pathway is equally important during vertabrate embryonic development. In knockout mice lacking components of the pathway, the brain, skeleton, musculature, gastrointestinal tract and lungs fail to develop correctly. Recent studies point to the role of hedgehog signaling in regulating adult stem cells involved in maintenance and regeneration of adult tissues. The pathway has also been implicated in the development of some cancers. Drugs that specifically target hedgehog signaling to fight this disease are being actively developed by a number of pharmaceutical companies.

- In molecular biology, ***Ras*** is the name of a protein, the gene that encodes it, and the family and superfamily of proteins to which it belongs. Proteins in the Ras family are very important molecular switches for a wide variety of signal pathways that control such processes as cytoskeletal integrity, proliferation, cell adhesion, apoptosis, and cell migration. Ras and ras related proteins are often deregulated in cancers, leading to increased invasion and metastasis, and decreased apoptosis. The Ras superfamily includes the Ras, Rho, and Rab families.
- RAS is a G protein (specifically a small GTPase): a regulatory GTP hydrolase that cycles between two conformations – an activated or inactivated form, respectively RAS-GTP and RAS-GDP. It is activated by guanine exchange factors (GEFs, eg. CDC25, SOS1 and SOS2, SDC25 in yeast), which are themselves activated by mitogenic signals and through feedback from Ras itself. It is inactivated by GTPase-activating proteins (GAPs, the most frequently cited one being RasGAP), which increase the rate of GTP hydrolysis, returning RAS to its GDP-bound form, simultaneously releasing an inorganic phosphate. RAS is attached to the cell membrane by prenylation, and in health is a key component in many pathways which couple growth factor receptors to downstream mitogenic effectors involved in cell proliferation or differentiation (Reuter et al., 2000). RAS activates a number of pathways but an especially important one seems to be the mitogen-activated protein (MAP) kinases, which themselves transmit signals downstream to other protein kinases and gene regulatory proteins (Lodish et al., 2000).

- Mutations in the RAS family of proto-oncogenes (comprising H-RAS, N-RAS and K-RAS) are very common, being found in 20% to 30% of all human tumours (Bos JL, 1989).
- Inappropriate activation of the gene has been shown to play a key role in signal transduction, proliferation and malignant transformation (Lodish et al., 2000). Mutations in a number of different genes as well as RAS itself can have this effect. Oncogenes such as p210BCR-ABL or the growth receptor erbB are upstream of RAS, so if they are constitutively activated their signals will transduce through RAS. The tumour suppressor gene NF1 encodes a RAS-GAP – its mutation in neurofibromatosis will mean that RAS is less likely to be inactivated. RAS can also be amplified, although this only occurs occasionally in tumours. Finally, RAS oncogenes can be activated by point mutations so that its GTPase reaction can no longer be stimulated by GAP – this increases the half life of active RAS-GTP mutants (Reuter et al., 2000).
- Because it is central in so many pathways, and prominent in so many tumours it would be extremely useful if a drug was found which could reintroduce regulation in to the RAS system, or kill cells with uncontrolled RAS pathways. Ideally a drug targeting RAS would be able to distinguish between its oncogene and the normal homolog - simply targeting all cells with RAS would also affect normal cells, producing toxic side effects. However the differences between these molecules is very slight (resulting from single amino acid changes) and this might prove a very difficult task. Instead, other approaches have been investigated, including targeting the processes responsible for prenylating RAS with the farnesyltransferase inhibitors.

- *Constitutively active Ras* (*RasD*) is one which contains mutations that prevent GTP hydrolysis, thus locking Ras in a permanently 'On' state. The most common mutations are found at residue 12 and residue 61. The glycine to valine mutation at residue 12 renders Ras insensitive to inactivation by GAP and thus stuck in the "on state". Ras requires a GAP for inactivation as it is a relatively poor catalyst on its own, as opposed to other G-domain-containing proteins such as the alpha subunit of heterotrimeric G proteins. Residue 61 is responsible for stabilizing the transition state for GTP hydrolysis. Because enzyme catalysis in general is achieved by lowering the energy barrier between substrate and product, mutation of Q61 necessarily reduces the rate of intrinsic Ras GTP hydrolysis to physiologically meaningless levels.

- **mitogen-activated protein (MAP) kinases** (EC 2.7.11.24) are serine/threonine-specific protein kinase that respond to extracellular stimuli (mitogens) and regulate various cellular activities, such as gene expression, mitosis, differentiation, and cell survival/apoptosis [1]. Extracellular stimuli lead to activation of a MAP kinase via a signaling cascade ("MAPK cascade") composed of MAP kinase, MAP kinase kinase (MKK or MAP2K), and MAP kinase kinase kinase (MKKK or MAP3K, EC 2.7.11.25)[1]. A MAP3K that is activated by extracellular stimuli phosphorylates a MAP2K on its serine and threonine residues, and then this MAP2K activates a MAP kinase through phosphorylation on its serine and tyrosine residues. This MAP kinase signaling cascade has been evolutionarily well-conserved from yeast to mammals.

- To date, four distinct groups of MAPKs have been characterized in mammals:
  - extracellular signal-regulated kinases (ERKs)
  - c-Jun N-terminal kinases (JNKs)
  - p38 isoforms
  - ERK5
- MAPK is involved in the action of most nonnuclear oncogenes. It is responsible for cell response to growth factors such as BDFN or nerve growth factor. The ERKs (also known as classical MAP kinases) signaling pathway is preferentially activated in response to growth factors and phorbol ester (a tumor promoter), and regulates cell proliferation and cell differentiation. The JNKs (also known as stress-activated protein kinases; SAPKs) and p38 signaling pathways are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in cell differentiation and apoptosis. And ERK5, which has been found recently, is activated both by growth factors and by stress stimuli, and it participates in cell proliferation.

MAPK Signaling

- Composed of RAF, MEK and ERK proteins
  - Required for normal cell proliferation
    - Relays signals for RTKs activated by growth factors to the nucleus and activates gene expression
  - Cancer cells
    - Pathway enhanced in cancer (by oncogneic activation of RTKs?)
      - Leads to phosphorylation of the transcriptional activator **JUN**.
        - » In response to stress caused by UV irradiation and heat
  - Activation of MAPK usually results in **STOP** responses and can induce apoptosis

PI3K Pathway (Phosphatidyl inositol phosphate kinase)

- PI3K functions in …
  - The control of cellular metabolism (esp. glucose transport and utilization)
  - Regulation of cell growth (esp. in protein synthesis)
  - Prevents apoptosis
  - Pathway is stimulated by RAS proteins and by active RTKs
  - The major inhibitory component is called **PTEN** and this is a tumor suppressor
    - 2 other proteins (TSC1 and TSC2) modulate intermediate steps in pathway
    - PI3K may be an oncogenic protein (amplified in ovarian cancer)

- Several different isozymes
  - PI3Kα has protein kinase activity
  - Heterodimer consisting of a p110 catalytic subunit and a p85 regulatory subunit (in basal [resting] state the regulatory subunit inhibits the activity of the catalytic subunit)
  - RTK activation and autophosphorylation of the RTK, the heterodimer binds to tyrosine phosphate through the p85 subunit and this leads to activation of the catalytic subunit

- Following this activation…
  - **Lipid phosphorylation accurs and generates PIP at the inner face of the plasma membrane**
  - **This creates binding sites for proteins containing a pleckstrin homology domain and allows for translocation to the membrane of a variety of proteins (i.e., PKC)**
  - *Pleckstrin is the major substrate of protein kinase C in platelets, although its precise function is not known. This protein contains two pleckstrin homology (PH) domains. This particular domain has the same name as the protein because it was first detected in pleckstrin. The PH domain is usually made up of 100 amino acid residues, and is found in many proteins that are involved in intracellular signaling. The true domain function is also not known, but it has been shown to bind to the following: the beta/gamma subunit of heterotrimeric G proteins, phosphatidylinositol-4,5-bisphosphate (or PIP2, e.g. lipids), phosphorylated Serine/Threonine residues, and membranes. The N terminal PH domain of pleckstrin is 113 amino acid residues long. Its structure consists of an up-and-down beta-barrel made of 7 antiparallel beta-strands and a C terminal amphiphilic alpha-helix which caps one end of the barrel.*

- One outcome of this pathway is to …
  - Phosphorylate a variety of proteins, including…
    - mTOR – a protein kinase controlling the initiation of translation at the EIF4 step
    - This activity leads to an increase in protein synthesis
      - » Important for cell proliferation and growth of tumor cells
  - mTOR= mammalian target of rapamycin (rapamycin inhibits this kinase specifically)
  - There are TSC genes and proteins and AKT genes and proteins that aid in these responses and the generation of cancer
    - » TSC genes act as tumor suppressors (persons inherit one altered allele and 2nd allele is hit and causes bladder and renal carcinomas
    - » AKT protein kinase stimulates protein synthesis and cell survival and proliferation

Summary

- During normal cell proliferation the PI3K pathway may be essential to complement the MAPK pathway
  - MAPK pathway stimulates cell proliferation (DNA synthesis and mitosis), but requires additional signals through the PI3K pathway which provides for stimulated protein synthesis and counteracts the apoptotic effects caused by MAPK alone
  - Certain Growth Factors (IGF1 and IGF2) stimulate the PI3K pathway and this acts as a “survival” event, leading to constitutive activation of the MAPK pathway

Regulation of Cell Cycle by the MAPK and PI3K Pathways

- Coordination of these pathways is necessary for normal cell proliferation
  - Induce increased metabolism, enhanced protein synthesis, reorganization of the cytoskeletal system, inhibition of apoptotic signals and stimulation of cell cycle progression
  - Ultimate action is to activate ERK protein kinases, and these phosphorylate several transcriptional activators involved in cell cycle progression (extracellular signal-regulated kinases) (AP1)
  - Cyclin D transcription enhanced and accumulation of cyclin D causes cell cycle progression
- This begins to set up a positive feedback loop that causes commitment of S-phase and then transition to G2

- These alterations are counteracted by "fail-safe mechanisms"
  - Activation of TP53 is caused by the hyperproliferation signals and there is also accumulation of CDK inhibitors (like $p16^{INK}$ and $p21^{CIP1}$)
  - These mechanisms lead to apoptosis or to replicative senescence
- At some point in cancer development these inhibitory mechanisms need to be inactivated
  - By loss of RB1 and/or TP53

Modulators of the MAPK and PI3K Pathways

- MAPK and PI3K have multiple effects on the cell, and they are activated by a variety of signals from RTKs
  - MAPK and PI3K are also regulated by a variety of other receptor activations, kinases (substrate level specificity of phosphorylation to different AAs)
- Most RTKs and some G-protein coupled receptors (GPCR) activate the MAPK cascade through RAS activation
  - Phopholipases are used in this activation process to generate IP_3 and DAG

- DAG and Ca^{++}
  - Activates 10 isozymes of PKC (serine/threonine protein kinase)
    - Have different tissue distribution with regards to isozyme levels
    - Have different calcium requirements (some are activated independent of calcium)
      - » PKC has regulatory (binds to DAG and Ca^{++}) subunit and a catalytic subunit (binds to substrate proteins)
      - » Regulatory site also binds phorbol esters (TPA-tetradecanoyl phorbol acetate) [tumor promotor NOT tumor initiator]
    - PKC activated at inner face of plasma membrane

- PKC used in the cell to relay signals from one activated pathway to others – "cross-talk"
  - Inhibition of PKC activity does block the proliferation of some cancer cells and TPA stimulates the growth of transformed cells
  - PKCs are mediators of several other oncogenes

TP53 Network

- Phosphorylation of TP53 activates protein to:
  - Increase transcriptional activity
  - decrease sensitivity to inhibition by HDM2
  - Increases ½ life
- Cell stress (hypoxia, imbalances of nucleotide metabolism, …) activate TP53
- Phosphorylation at C-terminal (or N-terminal??) by CDKs in G1 and G2 phases and by casein kinase II
  - TP53 accumulation in nucleus prevented by transport out and degradation by HDM2
  - Phosphorylation of TP53 as a response to cell stress (DNA damage) overcomes these inhibitory constraints

- Principal mechanisms governing the activity of p53 occur at the protein level. These include post-translational modifications, regulation of the stability of p53 protein, and control of its sub-cellular localization (for a review, see Woods & Vousden 2001). Post-translational modifications of the protein take place in response to stress, and different agents elicit diverse responses (reviewed by Appella & Anderson 2001). The human p53 protein has been shown to be modified at least at 17 different sites (for a review, see Appella & Anderson 2000). Of the post-translational modifications of p53, the most widely studied and best-known so far is phosphorylation. After DNA damage induced by ionizing radiation or UV light, phosphorylation takes place mostly at the N-terminal domain of p53 (reviewed by Appella & Anderson 2001). Another important modification is acetylation, which has been shown to occur in response to chemically induced DNA damage and hypoxia (Ito *et al.* 2001). In response to DNA damage, the p53 protein is also modified by conjugation to SUMO-1, a ubiquitin-like protein (Gostissa *et al.* 1999). Many proteins able to interact with p53 may also play a role in p53 regulation (reviewed by Vousden & Lu 2002). (Link to whole article CLICK)

- TP53 activation induces:
  - Cell cycle inhibitors ($p21^{CIP1}$ in G1 and 14-3-3σ in G2)
  - Cell cycle arrest and apoptosis are in competition with each other. If one wins out the other does not occur.
    - » Cells with strong expression of apoptotic inhibitors like BCL2 may undergo cell cycle arrest, while others that are exposed to ligands of TNFRSF (induced by p53) may undergo apoptosis
  - Increased activity of ERK and MAPK pathways leads to p53 activation
  - Activation of PI3K and NFκB pathways counteract the effects of p53
    - » PI3K inhibits p53 due to the activation of HDM2/MDM2 by AKT and increased "survival signaling"
    - » Overactivity of NFκB more selectively prevents apoptosis induced by p53

Signaling by TGFβ Factors

- About 30 growth factors in this "superfamily" with different functions
  - TGF β (**transforming growth factor beta**) is a growth inhibitory factor for epithelial cells
  - Mutations in this pathway common in tumors
  - Inhibits cell response within immune system
  - promotes proliferation of mesenchymal cells and helps to stimulate anchorage-dependent groath of cells in culture (transformed cell characteristic)

- TGF β may be produced by tumor cells
  - Helps escape from immune surveillance
  - Stimulates cell growth
- Pathway of TGFβ
  - GF binds with receptor type II on membrane and forms trimeric complex with receptor type I
  - Receptor type I becomes phosphorylated as becomes a serine/threonine protein kinase
  - Phosphorylates other proteins (SMADs) and these are released from the membrane and are transported to the nucleus where they activate or inactivate a variety of gene transcriptional events
    - There are inhibitory SMAD proteins that help to regulate the pathway

STAT Factors

- >7 different STAT factors and distributed differentially in different cell types
  - STAT proteins promotes cell proliferation in hematopoetic cells in response to interleukins and other growth factors
  - Constitutive activation of STAT signaling is a common finding in hematological cancers
  - STATs are not strong transcriptional activators and need to combine with other factors

NFκB Pathway

- “Nuclear Factor regulating the expression of the Ig kappa chain in B cells”
- Functions in the regulation of activation of lymphoid cells, inflammation and apoptosis
  - NFκB factors are heterodimeric transcription factors composed of a large REL (p65) subunit and a smaller p50 (or p52) subunit
    - Heterodimers held in cytoplasm by I κB which needs to be released before transcriptional activation occurs

- Activation of NF κB induced by reactive oxygen species, and other stresses, or by cytokine receptor activation

– 4 kinds of target genes
  - 1. feedback inhibitors (i.e., IκB)
  - 2. regulators of cell proliferation myc and cyclin D
  - 3. modulators of apoptosis BCL-X and FLIP are anti-apoptotic
  - 4. proteins that related to immune function- cell-cell communication, adhesion, chemokines, …
    - NF κB main mediators of induction of iNOS and COX2 (inflammatory responses)

– Factors that block NFκB can reduce inflammation and may prevent certain cancers