MOLECULAR BIOLOGY OF CANCER

- Gain-of-function (dominant) mutations can activate oncogenes, which are positive effectors of transformation.

- Loss of function (recessive) mutations can inactivate tumor suppressor genes, products which are negative growth regulators.

FUNCTIONS OF ONCOGENES & TUMOR SUPPRESSOR GENES

- Cells are chronically faced with decisions to:
  - Divide
  - Differentiate
  - Undergo programmed cell death (apoptosis).

- All three outcomes affect the net cell number.

- These decision pathways are primary targets for action of oncogenes and tumor suppressor genes.

ONCOGENES IN RNA TUMOUR RETROVIRUSES

- Acutely transforming retroviruses can form tumors in animals within weeks to months.

- This type of retrovirus carries within its genome an oncogenic gene (v-ONC) that was captured (transduced) from the genetic material of a host cell in an earlier cycle of infection.
  - v-ONC: first oncogenes to be identified
  - v-ONC: placed under altered regulatory (viral) control
  - v-ONC: mutated alleles of normal cellular genes (proto-oncogenes) with remarkable conservation between species.

Transforming Viruses Carry Oncogenes

Transformation may result from tumor virus infection thus "oncogenes":

- Polyomavirus/dsDNA/6kb/T antigen/Early viral gene/Inactivate tumor suppressor gene
- Human papillomavirus/dsDNA/8kb/E6 & E7 genes/Early viral gene/Inactivate tumor suppressor gene
- Adenovirus/dsDNA/37kb/E1A & E1B genes/Early viral genes/Inactivate tumor suppressor gene
- Retrovirus/retRNA/6-9kb/Individual genes/Cellular origin/Activate oncogenic pathway

Transformation occurs in non-permissive infection (vs. productive infection in permissive hosts) [Fig 28-4].
### ONCOGENES

- Protooncogenes are important **regulators of biologic processes**
- Despite their name, they do not reside in the genome for the sole purpose of promoting the neoplastic phenotype
- They are essential to normal biologic processes (more than 100 identified)
- They play diverse roles in the control of cellular growth, including proliferation, apoptosis, genome stability, and differentiation

### ONCOGENE ACTIVATION

- Genetic damage: **activation of protooncogenes**
- Qualitative or quantitative changes
- Mechanisms:
  - Retroviral insertion mutagenesis
  - Point mutation
  - Gene amplification
  - Gene translocation

### ONCOGENES: Mechanism of action

- Four major biochemical mechanisms of action:
  - Abnormal signaling: structurally abnormal **cytokine/growth factor**
  - Aberrant phosphorylation of proteins: altered receptors and other **signal transducer kinases**
  - Abnormal transmission of signals: **G proteins**
  - Disturbed regulation of gene transcription: abnormal **transcription factors**
ONCOGENES & SIGNAL TRANSDUCTION PATHWAYS

- Oncogene products can override growth factor dependency by functioning as:
  - constitutively active ligands
  - constitutively active receptors
  - constitutively active downstream elements

ONCOGENES AS EXTRACELLULAR GROWTH FACTORS

- A number of other growth factor genes have been activated through promoter insertion in experimental viral systems (e.g. interleukins 2 and 3 and granulocyte/macrophage colony-stimulating factor)

ONCOGENES AS RECEPTOR TYROSINE KINASES

- Receptor oncogenes are activated in human cancers by gene amplification (which leads to over-expression), rearrangements, and point mutations
  - Both N- and C-terminal deletions can partly activate the transforming potential of receptor tyrosine kinases

ONCOGENES AS EXTRACELLULAR GROWTH FACTORS

- The first oncogene product with an explicit function was the v-SIS protein, a modified form of platelet-derived growth factor (PDGF)
  - Infection of cells with simian sarcoma virus, which harbors v-SIS, results in production of functional PDGF
  - This autocrine stimulation generates a chronic growth stimulus for PDGF-responsive cells

ONCOGENES & SIGNAL TRANSDUCTION PATHWAYS

- Each control point can be the target of deregulation by oncoproteins:
  - over-expression
  - ectopic expression (no alteration in their normal structure)
  - point mutations or truncations

- By causing deregulation of the signaling, oncoproteins can force a cell into uncontrolled cell division or invasive growth
Mutations in the RET gene: responsible for inherited cancer syndromes:
- MEN IIA and familial medullary thyroid cancer mutations
  - elimination of conserved cysteins in the extracellular domain
  - formation of disulfide-linked receptor dimers
- MEN IIB is associated with mutation of the tyrosine kinase domain

RAS functions analogously to other G proteins that cycle between inactive guanine diphosphate (GDP)-bound states and active GTP-bound forms.
The GTP-bound forms activate downstream signaling proteins until GTP hydrolysis which is mediated by the intrinsic activity of RAS returns the system to the basal state.

Transforming mutants of RAS are resistant to the GTPase.

Activations of one of the three human RAS genes Ha-, Ki-, or N-RAS are the most common dominant mutations in human cancer.

Point mutations that activate RAS genes are clustered in the regions encoding amino acids 12, 13, and 59 to 61.

These mutations act by interfering with the guanine triphosphate (GTP) hydrolysis step of the RAS-GNP cycle.

Quantitative changes (amplification or over-expression) of c-ras gene can also transform normal cells.

ras protein is a monomeric guanine nucleotide-binding protein with intrinsic GTPase activity.

Mutations that create oncogenic ras inhibition of GTPase activity.

Canonically activated ras may be oncogenic.

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Translocation can generate hybrid oncogenes & human cancers.

CML & Philadelphia chromosome: c-abl gene on chromosome 9 and bcr gene on chromosome 22.

Why is the hybrid bcr-abl protein oncogenic? Activation of ras pathway for transformation.
Four basic types of effects:

- Progression from G1 to S phase in the cell cycle
- Apoptosis
- Genome stability
- Cellular maturation

ONCOGENES AS TRANSCRIPTION FACTORS

Amplification, Insertion, or Translocation

Genomic changes (amplification, insertion & translocation) that cause proto-oncogene activation:

- Amplification: c-myc, c-abl, c-myb, c-erbB, c-K-ras, mdm-2
- Presence of known oncogenes in amplified region
- Amplification of same oncogenes in many cancers

- Insertion: Insertion of retrovirus LTR over-expresses c-myc
- Insertion of ALV activates c-myc gene; [Fig 28-11]

- Translocation:
  - [Reciprocal translocation by illegitimate recombination; Fig 28-12]
  - Immunoglobulin or TCR gene and c-myc oncogene
  - Increased c-myc expression after translocation
  - c-myc coding sequences are unaltered in all cases

ONCOGENES AS TRANSCRIPTION FACTORS

Growth factor-stimulated cells: rapid rise in "immediate early" mRNAs for nuclear proteins: Myc, Fos, Jun

- Regulation of gene expression: cell proliferation and differentiation
- Bind DNA in a site-specific manner
- Activate or repress gene transcription

ONCOGENES AS TRANSCRIPTION FACTORS

Cell culture and animal models: Oncogene cooperation between c-MYC and activated Ha-RAS: neither oncogene is fully oncogenic

- Unlike other oncogenes: inappropriately regulated expression of c-MYC, rather than mutations in the protein, contributes to tumorigenesis

ONCOGENES AS TRANSCRIPTION FACTORS

- c-MYC
  - Initial discovery as a viral oncogene in the avian myelocytomatosis virus
  - c-MYC activation by chromosomal translocation in Burkitt's lymphoma
  - Amplification of c-MYC or of MYC family members (N-MYC) in numerous human tumors, including neuroblastomas, and retinoblastomas

Figure 30.19 A chromosomal translocation is a mechanism by which two chromosomes exchange parts of the genes. The translocations that activate the human c-myc protooncogene involve either in thrombopoiesis and TCR loci in T cells.

ONCOGENES AS TRANSCRIPTION FACTORS
**ONCOGENES AS TRANSCRIPTION FACTORS**

- JUN, FOS, and AP1

  FOS and JUN form dimers that initiate gene transcription of targeted genes

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**ONCOGENES AS REGULATORS OF THE CELL CYCLE**

- Cyclins and CDKs are the core apparatus of cell cycle progression

  - In mantle cell lymphoma, a reciprocal translocation between 11q13 and 14q32 places cyclin D under regulatory control of Ig heavy chain sequences

  - Cyclin D expression is also frequently upregulated through demethylation of the gene, permitting transcriptional activation.

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**ONCOGENES AS ANTI-APOPTOTIC GENES (BCL-2)**

- **BCL-2 overexpression can block apoptosis** that is induced by any of a number of signals, including radiation, chemotherapeutic agents, growth factor withdrawal, steroids, and heat shock

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**ONCOGENES AS ANTI-APOPTOTIC GENES (BCL-2)**

- Follicular lymphomas: t(14;18) that puts the BCL-2 gene (on chr 18) under transcriptional control of the Ig heavy chain gene (on chr 14), resulting in BCL-2 overexpression

  - In transgenic animals, BCL-2 overexpression in lymphoid cells results in increased survival of these cells and immune dysfunction, rather than increased cellular proliferation

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**ONCOGENES AS INHIBITORS OF CELLULAR DIFFERENTIATION**

- Human cancers typically arise after long latency and accrue multiple abnormalities in their control over cellular growth and phenotype

- One distinguishing feature of malignant cells is their inability to attain a normal, functional, **terminally differentiated state**

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**ONCOGENES AS INHIBITORS OF CELLULAR DIFFERENTIATION**

- The nuclear oncogene v-ERBA was isolated, along with v-ERBB, as one of two viral oncogenes in the chicken erythroblastosis virus

  - The cellular homologue, c-ERBA, is a **nuclear receptor for the thyroid hormones**

  - v-ERBA by itself does not appear to express transforming potential, but its presence potentiates the transforming potential of v-ERBB (which encodes a truncated and constitutively active EGF receptor) by specifically interrupting the differentiation of erythroblasts
The v-ERBA product is thought to alter the expression of genes that play an important role in erythroid differentiation.

The v-ERBA gene does not bind triiodothyronine or thyroxine and is therefore ligand independent, but it can either suppress or transactivate certain promoters.

Acute promyelocytic leukemia, the M3 subtype of AML, is associated with a block at the promyelocyte stage of differentiation.

- High doses of retinoic acid can induce a hematologic remission, which is accompanied by a release of the block to cellular maturation.
- The tumor typically occurs with a t(15;17) that involves two genes - PML (for promyelocyte) on chromosome 15 and the retinoic acid receptor alpha (RARalpha) gene on chromosome 17.

PML is a gene of unknown function that localizes to the nucleus and may act as a factor regulating apoptosis.

RARalpha is one of three nuclear receptors for retinoic acid and is a ligand-dependent transcription factor.

In a cell culture assay, the PML-RARalpha chimeric protein can block the differentiation of human promyelocytic cells, and overexpression of the chimeric protein in the myeloid compartment of mice results in massive overproduction of immature myeloid cells and, at lower frequency, myeloid leukemias.

It is likely that the PML-RARalpha chimera inappropriately represses or activates key targets that play a role in myeloid maturation and that this results in a block to the differentiation process.

With pharmacologic dosing of retinoic acid, it is possible to overcome this block, most likely by alleviating the effect of this oncogenic chimera.

In general, oncosuppressor genes act as recessive genes. It follows that if only one allele is mutated or deleted, the wild-type allele compensates and no altered phenotype is apparent. Thus, in heterozygous oncosuppression genes are not effective as long as the other allele is functional. Loss or heterozygous (LOH) is a condition in which the normal allele is deleted or not expressed (silenced l); in this case the combination with the mutated allele determines a loss-of-function leading to the transformed phenotype. Haploinsufficient oncosuppressors manifest their effect also when only one allele is mutated.
MOST IMPORTANT ONCO-SUPPRESSORS

NEGATIVE REGULATORS OF CELL CYCLE

- p-53 (p105Rb)
- WT-1 (p45-49/T)
- MTS-2 (p205), inhibitor of cdk-4/cyclin D
- MTS-1 (p6), inhibitor of cdk-4/cyclin D
- RB-1 (p110RB), inhibitor of cdk-4/cyclin D

NOTCH

INHIBITORS OF SIGNAL TRANSDUCTION

- NF-1 (neurofibromin)
- PTEN (protein phosphatase)
- NF-2 (Shwannina)
- ARK-1 (ovomorulin)
- APC (APC family)

REGULATORS OF AUTOPHAGY

- Becn1 (Beclin1, it binds and activates PI3k III)

TUMOR SUPPRESSOR GENE RB PRODUCT p105-RB:
A Nuclear Phosphoprotein Involved in Cell Cycle Regulation

- RB is a gene that was first identified as a tumor suppressor gene mutated in the familial cancer, retinoblastoma
- RB mutations are not restricted to familial retinoblastomas; they occur in many human cancers
- Loss-of-function mutations in RB, or production of DNA tumor virus RB binding proteins, abrogate the need for a major cyclin D function, which is the cell cycle-dependent phosphorylation of RB

INVOLVEMENT of p53 in the CONTROL OF CELL PROLIFERATION

- p53 suppresses growth or triggers apoptosis
- >50% of cancers lost p53 or have mutations in p53 gene
- p53 protein level ↑ in many tumor cells. Oncogene?
- Mutant proteins act as dominant negative mutants.

Loss of p53:
- Cell growth advantage, not tissue-specific (many cancers)
- Wild type p53 restrains cell growth

Implication: p53 inhibits normal cells’ capacity of unrestrained growth?

Evidence that p53 is indeed a tumor suppressor gene:
- p53 mice develop a variety of tumors early in life
- p53 DNA inhibits transformation by oncogenes in cultured cells
- Human Li-Fraumeni Syndrome (rare inherited cancer; heterozygous p53 mutation acted as dominant negative or autosomal dominant)

P53 Suppresses Growth or Triggers Apoptosis

- p53 protects cells from consequences of DNA damages (repair it or destroy if it is unable to repair)
- Activation of p53 → growth arrest or apoptosis
- Depends on cell cycle
- Deregulated activities of p53
- p53 can also activate various pathways as an inflammation factor;
- Possible involvement of p53 activity forms a negative feedback circuitry
- How p53 triggers apoptosis? Separable from growth arrest
- Is p53 function essential for survival?
P53 Suppresses Growth or Triggers Apoptosis

How p53 trigger apoptosis?
- separable from growth arrest at the G1 checkpoint

Connection between tumorigenesis & loss of apoptosis
- apoptosis inhibits tumorigenesis by eliminating tumorigenic cells

p53 function is probably not essential for survival
- p53 knockout animals
- Definitive proof the p53 & Rb suppress tumorigenesis still lacking

P53 acts as a sensor that integrates information from many pathways that affect the cell’s ability to divide

p53 and Drug Resistance
- Loss of normal p53 function reduces drug-induced apoptosis and tumor regression.
  - p53 mutations
  - Defects in the p53 pathway
    - Functional mutations or altered expression of its downstream effectors (PTEN, Bax, Bok, and Apaf-1) or upstream regulators (ATM, Cdk2, Mdm2 and E2F-6/ABF)
    - In many tumor cells, pro-apoptotic signaling via BH3-only proteins is impaired, typically due to mutations in p53 (e.g., Bax, Bok, Puma, and Noxa)
  - Effects of p53 on drug-induced apoptosis is determined by a variety of factors
  - Functional p53 does not appear to be a general determinant of anticancer drug activity in solid tumors

TUMOR SUPPRESSOR GENE p53
- In many tumors, both p53 alleles are deleted and there is no p53
- However, malignant transformation can also occur when certain mutants of p53 are expressed in a cell containing at least one normal p53 allele. These mutations, referred to as dominant negative, probably act by binding to and inhibiting the function of the normal p53 protein.
  - (most frequent mutation R273H)

PTEN
- PTEN phosphatase and tensin homolog deleted on chromosome 10
- MMAC1 mutated in multiple advanced cancers
- TEP-1 TGFβ-regulated and epithelial cell enriched phosphatase

Models of p53 action:
- Transcriptional upregulation of pro-apoptotic genes
  - Pro-apoptotic Bcl-2 members
  - Death receptors (e.g., CD95 & Fas)
- Transcription-independent activation of Bax (BH1-like activity), initiating cytochrome release.

In many tumors, both p53 alleles are deleted and there is no p53. However, malignant transformation can also occur when certain mutants of p53 are expressed in a cell containing at least one normal p53 allele. These mutations, referred to as dominant negative, probably act by binding to and inhibiting the function of the normal p53 protein. (most frequent mutation R273H)
PTEN antagonizes PI 3-kinase signalling by dephosphorylating the 3-position of the lipid phosphatase is a ubiquitous regulator of the cellular PI (phosphoinositide) 3-kinase signaling pathway

PTEN and P53 oncosuppressors regulate autophagy

Lipid phosphatase activity

- lipid phosphatase is a ubiquitous regulator of the cellular PI (phosphoinositide) 3-kinase signalling pathway
- PTEN antagonizes PI 3-kinase signalling by dephosphorylating the 3-position of the inositol ring of PtdIns(3,4,5)P3, and thus inactivating downstream signalling

PTEN AND P53 ONCOSUPRESSORS REGULATE AUTOPHAGY

De-regulation of PTEN function in disease

alternations in the expression and activity of the phosphatase have been proposed to play a causal role in the development of several conditions other than cancer, including rheumatoid arthritis, chronic obstructive pulmonary disease and pulmonary fibrosis
Evidence for mutation of the PTEN coding sequence in many diverse tumour types strongly support the status of PTEN as an important tumour suppressor for many types of cancer. In addition, soon after the identification of PTEN, it was discovered that inherited mutations in PTEN can cause several human disorders, including Cowden disease, Bannayan-Riley-Ruvalcaba syndrome and Proteus syndrome. Cowden disease in particular is accompanied by an increased cancer risk, specifically of breast and thyroid tumours.

Of the 150 or so mutations in PTEN that have been identified in patients suffering from these inherited disorders, none have been described in the C-terminal tail

loss of PTEN drives tumour development through deregulation of PI 3-kinase signalling and, in turn, processes including cell growth, proliferation and survival

PTEN AND P53 ONCOSUPPRESSORS REGULATE BOTH AUTOPHAGY AND CELL SURVIVAL

BOTH AUTOPHAGY AND CELL SURVIVAL

aa 88 to aa150 in BECLIN 1 mediates the interaction with anti-apoptotic BCL2

BECLIN1 interacts with PI3-K class III to trigger AUTOPHAGY

APC oncosuppressor: its Loss promotes intestinal tumours

Wnt Pathway

- APC regulates Wnt signalling by promoting degradation of β-catenin
- β-catenin accumulation leads to increased Wnt target gene expression
- Loss of APC function results in increased β-catenin and Wnt target gene expression, promoting cell proliferation and tumour growth
BRCA-1 and BRCA-2

- Deletion and Loss-Of-Function mutations of both alleles increases the risk of cancers (breast, ovary)
- 5-10% of breast cancers are familial: 80% of these cancers present with mutations in BRCA1 or BRCA2 (BRCA mutations are rare in sporadic breast cancers)
- BRCA proteins are involved in Double-Strand break DNA repair.

ATM

- Analysis of families carrying the trait suggests that ATM heterozygotes are at a somewhat greater risk for cancer development, notably breast cancer
- Ataxia-telangiectasia mutant cells show defects in the G1/S, S, and G2/M checkpoints, indicating that ATM is a common element in all three of these responses

INHIBITORS OF CYCLIN-DEPENDENT KINASES IN CANCER

- Inhibitors of cell cycle progression are candidate tumor suppressor genes since they function normally to restrain cell division
- There are several inhibitors of cyclin-dependent protein kinases
- The genes encoding two related CDKIs, p15 and p16, are located in the neighborhood of a tumor suppressor locus involved in familial melanoma and other cancers, and several alleles of p16 derived from tumors are deficient in p16-mediated cell cycle arrest.

COOPERATION BETWEEN ONCOGENES & TUMOR SUPPRESSOR GENES

- Inappropriate advancement of the cell cycle (e.g. following viral infection, oncogene activation, or loss of tumor suppressors that regulate the cell cycle) can trigger apoptosis:
  - Following adenovirus infection, the adenovirus protein E1A can cause G1 to S transition, in part by binding to and inactivating RB
  - A normal cell responds to this by initiating apoptosis
  - Thus, by itself, E1A is not a potent oncogene in normal cells because its expression can cause cell death
  - However, in the case of oncosuppressor loss, E1A can induce a tumoral phenotype (E1A is transforming when p53 or RB is inactivated)
- A similar effect is seen with loss of RB or overexpression of E2F.

COOPERATION BETWEEN ONCOGENES & TUMOR SUPPRESSOR GENES

- Also other oncogenes, such as MYC and FOS, encode proteins that can cause advancement of the cell cycle. In normal cells, the cellular response to these alterations is apoptosis, while in cells lacking certain oncosuppressor the hyper-expression of MYC or FOS can lead to transformation.
- Note: MYC also cooperate with RAS !!!

COOPERATION BETWEEN ONCOGENES & TUMOR SUPPRESSOR GENES

- The combination of a stimulus to the cell cycle and an anti-apoptotic factor or gene results in cell growth, usually at a more rapid rate than their normal counterparts, and often with an altered, or transformed morphology
- This is a possible explanation for many instances of oncogene cooperativity that occurs when two different genes can fully transform primary cells, whereas each gene on its own cannot