NEOPLASIA 2: MECHANISMS

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CANCER IS A GENETIC DISEASE

- Certain genes are CANCER-CRITICAL
- Two major categories:
  - GAIN OF FUNCTION
    - Overexpression of gene product
    - DOMINANT
  - LOSS OF FUNCTION
    - Creates lack of something needed to control cell
    - RECESSIVE
ONCOGENES

• From Greek *onkos* = “mass”
  – *Dominant* mutations conferring new function(s)
    • Wild-type (normal) is a “Proto-oncogene”
    • Mutated genotype adds function
    • Usually only one allele needed for change
    • May be large enough to cause visible chromosomal aberration
    • At least 100 known oncogenes

![Diagram illustrating the concept of oncogenes and their function](image-url)
ONCOGENE CONVERSION

• 3 possible routes to hyperactivity:
  – Mutation: ↑ activity of oncogene product
  – Amplification: ↑ expression of normal product
  – Rearrangement: Creates fusion product
TUMOR SUPPRESSOR GENES

- LOSS OF FUNCTION is key feature
  - Wild-type confers control
  - Loss of control occurs in mutated forms
  - RECESSIVE mutation
    - Two “hits” needed for expression: *i.e.*, *loss of heterozygosity*
LOSS OF HETEROZYGOSITY

• *Rb* gene (retinoblastoma) classic example of a TUMOR SUPPRESSOR gene
  – *Rb* product controls entry into S phase
  – Loss of *Rb* product bypasses control point, triggers S phase inappropriately
THE CELL CYCLE

• Most normal cells in INTERPHASE (= G1 + G2 + S)

• G1 critical to monitoring internal & external environment
  – Signals to grow & divide must be present & received to progress to S

• G2 time to check accuracy of DNA replication
THE CONTROLLER

• COMPONENTS
  – Clock
    • Fixed time for each stage to complete
  – Anti-backlash control
    • Can’t run in reverse!
  – On/Off switch for each stage
  – Redundancy of controls
  – Flexibility to adapt to changing conditions
CHECKPOINTS

• Provide for verification that everything is “GO” for launch of next round

• Controller responds to intra- & extra-cellular signals

• Regulates entry into next stage if all is not “nominal”
  – Allows time for repair
  – Can signal total halt
  – G1 checkpoint especially important
NEGATIVE SIGNALS CONTROL
CHECKPOINTS

• Intracellular stop signals generated if all is *not* “GO”
  – Cycle halts at that point
    • Diminution/end of stop signal re-starts cycle

• Defective or unreplicated DNA sends a stop signal
  – Mutations can prevent generation of this signal
    • Cell proceeds to divide with defective DNA
CYCLINS & KINASES

• CYCLINS regulate cyclin-dependent kinases (CDKs)
  – One pairing for each checkpoint
    • G1/S cyclins commit cell to DNA replication
    • S cyclins initiate replication
    • M cyclins trigger mitosis
    • G1 cyclins pass cell through the G1 checkpoint

• Kinases must be bound to cyclin to activate substrate proteins & continue cycle
  – Intracellular CDK levels constant
  – Cyclin levels rise & fall
  – Complex has to be formed for functionality
REGULATION OF THE COMPLEXES

• **Cyclin levels** controlled by synthesis vs proteolysis
  
  – Main source of control of CDK activity
    • Regulated by signal transduction pathways
      – Intracellular proteases ↓ cyclin levels
      – Balance of synthesis/proteolysis regulates cyclin availability
    • No cyclin, no complex formation
  
  – CDK/Cyclin complex *activated* by Cyclin Activating Kinase (CAK)
  
  – CDK/Cyclin complex *inactivated* by enzymatic phosphorylation & inhibitory proteins (CKIs)
    • Both under control of signal transduction pathways
EXEMPLAR OF CDK ACTIVATION: M-CDK & ENTRY INTO MITOSIS

- Interference with any component = TROUBLE
DNA REPLICATION CHECKPOINT

- Unknown sensors detect DNA damage
  - Signal is sent to block activation of M-CDK
    - Target protein is Cdc25 Phosphatase
      - CdC25 remains phosphorylated, remains inactive
    - Progress into mitosis is stopped
RETINOBLASTOMA

- **Rb** was the first identified tumor suppressor gene
- **Rare childhood cancer**
  - Hereditary & sporadic forms
  - Hereditary form often has visibly aberrant karyotype on Ch 13
    - Deletion mutation
    - Recessive
    - 2nd "hit" causes disease
    - Paradigm of Knudson's model
- **Rb** later found missing in many other cancers
- **Rb** protein a major regulator
  - Universal “brake” on cell cycle at G1/S
  - Loss triggers abnormal cell cycling

![Diagram showing the stages of Rb gene inactivation in normal, hereditary, and non-hereditary cases.](image)
G1/S CHECKPOINT: Rb PROTEIN

- Primary regulation via protein E2F
  - E2F regulates DNA sequences needed for entry into S phase, including cyclins
  - E2F is regulated by Rb Protein
    - Rb blocks E2F regulated transcription
    - Rb blocks expression
    - Rb halts cell cycle in G1, stops entry into S
Promote expression of CDK Inhibitors

GROWTH INHIBITORS (TGF-β, p53, others)

Promote formation of Cyclin/CDK complex

GROWTH FACTORS (EGF, TGF-α, HGF, PDGF)

ONCOGENIC VIRUSES (HPV-E7 protein; SV40-T antigen)

G2
M
S
G1

Checkpoint

Stimulate

CDK Inhibitors (Cip/Kip and INK4a* cell cycle inhibitors)

Inactivate

Hyperphosphorylated RB

Cyclin D/CDK4

Cyclin E/CDK2

Hypophosphorylated RB

Bind

Rb inactivated: Cell can proceed past G1/S checkpoint

Rb Active: Cycle halted at G1/S checkpoint
More than 50% of tumors have mutations involving \textit{p53}
The p53 tumour suppressor protein

Diagram showing the domain structure of p53:
- Activation domain
- Sequence specific DNA binding domain
- Non-specific DNA interaction domain

Domains and sites mentioned:
- dsDNA=PK and CKI sites
- TFIIID, TBP, TAF40, TAF60
- TFIH, p62
- mdm-2, RP-A, AdE1B 55kD
- SY40 T Ag, p53BP1, p53BP2
- CDK site, CKII site
- PKC site, TBP, TFIH, XBP, XPD, CSB
FUNCTIONS OF p53

- Cell cycle arrest
- Initiation of apoptosis
  - In response to DNA damage
- Controls transcription of p21, the actual inhibitor of G1/S cyclin/CDK complex
  - Mutations usually in DNA binding portion of p53
  - Mutated protein unable to bind DNA to trigger p21 transcription
- Up-regulates transcription of GADD45 to make repair protein & BAX
  - Facilitates DNA repair mechanism
  - Initiates apoptosis through BAX of unrepairable cells
- p53 Half life is short
  - Proteolytic degradation
- Homozygous loss leaves cell unprotected from DNA injury
- Add in expression of telomerase...
Products of genes transcriptionally activated by p53

DNA damage
Stress

Increased levels
Modification

↑ transcriptional activation
↓ transcriptional inhibition

G1 arrest

MDM2
Cyclin
CDK

p21/WAF1
Rb E2F

GADD45

G1 phase → S phase

PCNA
DNA polymerase

BAX

FAS

IGF-BP3

Apoptosis
SELF-SUFFICIENCY IN GROWTH SIGNALS

• A major feature of cancer cells conferred by oncogenes
  – Mutated cells can make their own growth factors
    • Autocrine signalling
  – Mutations can genes cause overexpression of growth factors and receptors for growth factors (*HER 2/neu*)
    • ↑ GF or GFR expression alone not sufficient for neoplasia
      – But ↑ likelihood of deleterious mutations in a sub-clone
  – Mutations can cause mimicry of normal signal transduction (*Ras*)
HER 2/neu

- A receptor for epidermal growth factor
- Mutation causes overexpression of receptor protein & insertion into PM
- Cell more sensitive to growth factors
- In presence of other appropriate mutations, leads to neoplasia
• Normal component of inner PM
• Part of many signal cascade sequences
• A monomeric G-protein
• Transduces growth factor signals
  – Initiates a kinase cascade that switches on regulatory proteins in nucleus
• Can indirectly regulate levels of cyclins
  – Involvement in cell cycle
• *Ras* point mutations in $\geq 20\%$ of human tumors
  – Dominant mutation of this oncogene
  – $\downarrow$ GTPase activity of Ras
  – Ras GTPase activity normally self-inactivates & terminates action
  – Hyperactive Ras is constantly active
GTP bound = ON
GDP bound = OFF
- GNRP (Guanine Nucleotide Releasing Protein) activates Ras by ↑ rate of exchange of GTP for GDP
- GAP (GTPase Activating Protein) inactivates Ras by ↑ hydrolysis of GTP
Ras Control

- GTP bound = **ON**
- GDP bound = **OFF**
  - GNRP (Guanine Nucleotide Releasing Protein) activates Ras by ↑ rate of exchange of GTP for GDP
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GENERAL SCHEMATIC OF RTK/Ras LINKING

- Receptor Tyrosine Kinase
- Ligand for receptor tyrosine kinase
- plasma membrane
- Linking protein with SH2 domain(s)
- GNRNP protein
- CYTOSOL
- GTP
- GDP
- GAP
- inhibition of GAP
- Ras
- Ras protein bound to PM
- Downstream Phosphorylation Cascade
Phosphorylation cascade resulting from Ras activation prolongs effect of ligand-binding after ligand is released.

Activation of MAP-kinase-kinase-kinase (a SER/TRH kinase)

MAP-kinase-kinase-kinase phosphorylates SER & THR on MAP-kinase-kinase to activate it

MAP-kinase kinase then phosphorylates MAP-kinase in the same way

And finally MAP-kinase phosphorylates miscellaneous regulatory proteins of the nucleus

Activated MAP-kinase migrates into nucleus via nuclear pores to activate regulatory proteins

- P Jun
- P Elk-1
- SRF
- protein kinases
- other proteins
THE MYC ONCOGENE

- MYC protein promotes cell cycle entry
  - ↑ transcription of genes for cyclins
  - ↑ transcription of genes for degradation of cyclin inhibitors
  - ↑ transcription of gene for E2F protein
- MYC linked to Ras protein by the MAP kinase cascade
- Mutagens initiate the cascade
MYC ACTIONS

- Mutations of *MYC* are common in tumors
  - Persistent expression & overexpression
    - Leads to sustained transcription of other genes for proliferation
  - Known mutations of *MYC* occur in some lymphoma & leukemia states

Translocation of *MYC* from 8 to 14 causes overproduction of MYC protein in Burkitt’s lymphoma
ANGIOGENESIS

• Everyone needs a blood supply!

• Tumors initiate formation of new blood vessels
  – Diffusion inadequate > 2.0 mm diameter

• Adaptive mutation includes making angiogenesis stimulators
Tumors produce and release angiogenic growth factors (proteins)

Growth factors bind to specific receptors located on endothelial cells (EC) of preexisting blood vessels

Endothelial cells become active, produce enzymes to dissolve the basement membrane

Endothelial cells proliferate, migrate towards the tumor

Adhesion molecules (integrins) pull the blood vessel sprout forward

Matrix metalloproteinases dissolve the tissue in front of the sprouting vessel; the vessel extends, the tissue is remolded

Sprouting endothelial cells roll up to form a blood vessel tube

Individual blood vessel tubes connect to form blood vessel loops

Newly formed blood vessels pericytes for structural support
ANTI-ANGIOGENESIS

• Angiogenesis inhibitors are potential therapies

• **SWAINSONINE**
  – Plant alkaloid from “locoweed”
  – Suppresses angiogenesis in caprine placenta

• Inhibits expression of VEGF
STAY TUNED FOR MORE!