Chapt 15: Molecular Genetics of Cell Cycle and Cancer
Student Learning Outcomes:
• Describe the cell cycle: steps taken by a cell to duplicate itself = cell division; Interphase (G1, S and G2), Mitosis.

• Describe how progression through cell cycle is controlled by cyclin-dependent protein kinases (cyclins + cdks) and by protein degradation.

• Explain how checkpoints monitor cell for DNA damage; Abnormalities signal cell cycle arrest to permit repair

• Explain how cancer cells show uncontrolled proliferation: requires several genetic changes.

• Inherited cancer tendencies involve germline mutations -> easier to get a second mutation of another gene. ex: Rb, p53, Ras, cyclin D

• Explain how cancer cells have defects or over-expression of genes involved in cell cycle regulation or checkpoint control (tumor suppressors, oncogenes).

• Important Figures: 1*, 3*, 8, 10*,11*,12*,14*,15*, 16,17, 23, 25*, 29*, 30*; Table 1*, 3*, 4*;

• Important problems: 1-8, 11-19, 21-27

Mammalian cell cycle

mitotic

Fig 15.1: Typical mammalian cell cycle is 24 hrs
Main events in cell cycle

<table>
<thead>
<tr>
<th>G1</th>
<th>S</th>
<th>G2</th>
<th>M</th>
</tr>
</thead>
</table>
| Cell cycle of budding yeast *Saccharomyces cerevisiae*

Yeast model system:
- haploid or diploid
- easy to get mutants (ts for essential genes)
- grows fast (90 min)
- size of bud indicates stage of cell cycle

Replication of DNA in ts *cdc* mutant cells

At restrictive temperature in ts mutant, cells with diploid DNA content accumulate: division is blocked.
Cyclin-CDK (cyclin-dependent kinases) complexes regulate cell cycle progression

- CDK only active as kinase bound to specific cyclin(s)
- phosphorylates target proteins
- Yeast have many cyclins, only one CDK (cdc2 fission yeast)
- Higher eukaryotes have 4 CDKs, 7 cyclins
  cyclin D critical

Cyclin-CDKs are protein kinases

- **Protein kinases** add phosphates to –OH of ser, thr, tyr
- CDK protein kinases are active when bind cyclin
- **Cyclin** binds specific target and CDK; complex dissociates after phosphorylates target (PO₄⁻)

Phosphorylation activate or inhibit enzymes
- Phosphatases remove phosphates; reset system.

- **Activity** of cyclin-CDK also controlled by phosphorylation:
  ex. Cyclin D-CDK complexes controlled by inhibitor p16 protein and by dephosphorylation

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### Table 15.1 Cyclin-CDK interactions in eukaryotic cells

<table>
<thead>
<tr>
<th>Organism</th>
<th>S phase</th>
<th>G₁ phase</th>
<th>S phase</th>
<th>G₁, M phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher eukaryotes</td>
<td>Cyclin B, C, D</td>
<td>Cyclin A, B</td>
<td>Cyclin A, B</td>
<td>Cyclin B, C, D</td>
</tr>
</tbody>
</table>

These regulate growth and DNA replication
These regulate mitosis

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Cyclin levels rise and fall of during cell cycle

If proper cyclin-CDK complex is not present, cell cycle does not progress

**Fig 15.10: cdc2 = cdk1**

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Ref: Figure 15.10: v15.1001
Temporal expression of cyclin-CDKs controls mammalian cell cycle

Big decision points:
START (G1/S)  
G2/M

- New cell cycle begins
- Mitosis begins

CELL CYCLE

- G1
- G2
- S
- M

Fig 15.11 mammalian

Phosphorylation controls transition G1 to S:

**cycD-Cdk** inactivates Rb  -> E2F activates transcription

**Fig 15.12 A**

Phosphorylation controls transition G1 to S:

after E2F activated transcription:
**cyclins A, E, Cdk2** activate prereplication complexes

**Fig 15.12 B**
Phosphorylation controls transition G2 to M:

**MPF** (Maturation-promoting factor) = cycB-Cdc2
Phosphorylates key substrates:
- Duplicate spindle poles
- Break down nuclear membrane

Protein degradation regulates cell cycle:
Ex. Cyclins must be destroyed to reset
Ex. Activated APC/C controls metaphase to anaphase:
Marks unneeded proteins for degradation (proteasome)

Cell-cycle checkpoints control cell division
- **Checkpoints** permit pause: (Fig. 14)
  - check if ready for next step
  - repair damage
- Failure to stop at checkpoints causes
  - aneuploidy, polyploidy or mutations
- Unregulated cell division is hallmark of cancer

1. DNA damage checkpoint (G1/S or G2/M)
2. Centrosome duplication checkpoint (G2/M)
3. Spindle checkpoint (metaphase/anaphase)
Some Cell-cycle checkpoints and events that trigger arrests

<table>
<thead>
<tr>
<th>Checkpoint</th>
<th>G1/S</th>
<th>G2/M</th>
<th>S</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry to S</td>
<td>DNA replication and cytokinesis in some cells at the G1/S transition.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exit from G2</td>
<td></td>
<td></td>
<td>DNA replication and cytokinesis in some cells at the G1/S transition.</td>
<td></td>
</tr>
<tr>
<td>Blocks</td>
<td></td>
<td></td>
<td></td>
<td>DNA replication and cytokinesis in some cells at the G1/S transition.</td>
</tr>
<tr>
<td>Induced by</td>
<td></td>
<td></td>
<td></td>
<td>DNA replication and cytokinesis in some cells at the G1/S transition.</td>
</tr>
<tr>
<td>Fig 15.14:</td>
<td></td>
<td></td>
<td></td>
<td>DNA replication and cytokinesis in some cells at the G1/S transition.</td>
</tr>
</tbody>
</table>

Activation of transcription factor p53 is critical for DNA damage checkpoints

- In normal cells, p53 levels are low. Mdm2 removes p53 from the nucleus and leads to its degradation by the proteasome.
- Damage to DNA results in p53 phosphorylation (P) and acetylation (Ac) and activation of p53 as a transcription factor.
- Mdm2 cannot bind to modified p53.

Different downstream events can be triggered by activated p53

- p53 activates or represses different genes to help cell cope (or die by apoptosis)
- Route depends on nature of DNA damage, presence of other growth factors
- p53 tumor suppressor:
- Mutated p53 increases cancer risk

Fig 15.16; see Table 2 for p53 targets:
Central role of p53 in DNA damage checkpoint

Loss of p53 and cell does not arrest; loss of p21 -> polyploid

Activation of the spindle checkpoint

Checks if chromosomes are attached

Mutations in sensor proteins Mad, Bub lead to aneuploidy

How are checkpoints controlled?
- Regulated by cyclin/CDK complexes;
- Correct cyclin-CDK needed at right time.
- If proper cyclin-CDK is not active, cell cycle stalls.

So what controls cyclin-CDKs?
- Cell asks questions at checkpoints:
  - Presence of growth hormones, growth factors, cell size, DNA damage, DNA replication, spindle assembly
  - Senses environment through proteins in cell membrane (signal transduction pathway) and intracellular transduction pathway; affects cyclin synthesis, and CDKs' activities to phosphorylate, dephosphorylate or inhibit other proteins.
Checkpoint failures contribute to genetic instability

cancer cells have mutated checkpoint controls

Cancer cells are out of control

- Not contact inhibited
- Immortal
- Evade apoptosis
- Lower requirement for growth factors
  - Insensitive to anti-growth signals
- Metastasize – invade tissues
- Angiogenesis (new blood vessels)
- Clonal origin from ancestral cell

Genetic instability (aneuploid)

See also (Fig. 23)

**Oncogenes and tumor suppressor genes**

**Oncogenes – ‘stimulators’** Cyclin D, Ras, EGFR
- gain-of-function mutations
- contribute to cancer progression
- from proto-oncogenes (overactive, over-expressed)
- cell responds as if signal for growth

**Tumor suppressor genes – ‘brakes’** p53, Rb
- normally negatively regulate cell proliferation (or activate apoptosis)
- loss-of-function mutations
- contribute to cancer progression
Oncogenes and tumor suppressors: Cell cycle regulatory genes affected in tumors

<table>
<thead>
<tr>
<th>Protein</th>
<th>Allelic</th>
<th>Germline</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Myc</td>
<td>Amplification</td>
<td>Promotes entry into G1 phase</td>
</tr>
<tr>
<td>RAS</td>
<td>Mutations</td>
<td>Activates Ras, growth factor receptors</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Mutations</td>
<td>Causes breast cancer</td>
</tr>
</tbody>
</table>

**Gain-of-function mutations of Ras, growth factor receptors -> uncontrolled growth, can cause cancer**

Ras protein normally transduces environmental signals from surface receptors to affect gene expression in nucleus.

*Fig15.25: Second messenger signalling: EGFR tyrosine kinase receptors in pathway involving GTP-binding Ras protein*

15.6 Hereditary cancer syndromes, tumors

- Only ~ 1% of all tumors are familial (Table 4):
- Inherit 1 mutated gene -> more likely to get mutation in other copy, or mutation in second gene
- Often autosomal dominant cancer susceptibility, even though mutant protein recessive.

- Defects in DNA repair:
  - Xeroderma pigmentosum (Excision repair genes)
  - Breast cancer (BRCA1 gene)
  - HNPCC (mismatch repair genes)

- Defects in cell cycle regulation:
  - Rb (retinoblastoma, many cancers)
  - p53 (Li-Fraumeni syndrome – many cancers)
Mutated p53 in Li-Fraumeni syndrome: increased incidence of cancer
Autosomal dominant inheritance.

Fig 15.27:

Retinoblastoma: mutated tumor suppressor gene

Disease often seen in young children – cause blindness, treatment is removal of eye

Inheritance of one mutated Rb gene carries high risk of retinoblastoma or other cancers:
• (loss of heterozygosity uncovers bad allele)

Genetic mechanisms for loss of heterozygosity of wildtype RB1

Fig 15.29:
Heterozygosity should protect from mutant allele; often, loss of normal Rb allele uncovers mutant allele
Chromosomal translocations cause cancer

**Acute leukemias** often involve *translocations*
- Cells of hematopoietic system divide rapidly:
  - bone marrow stem cells -> red blood cells, white blood cells

**Promoter fusions** ex. Immunoglobulin promoter to Bcl2 to proto-oncogene -> overexpress normal protein in B lymphocytes -> promote excessive cell division

**Gene fusions** create novel or overactive proteins
- **CML** = chronic myeloid leukemia: fusion of Bcr and abl genes - overactive tyrosine kinase
- **APL** = acute promyelocytic leukemia: PMLRAR from fusion of PML and RAR (retinoic acid receptor); aberrant protein

**Conclusions**
- Cell cycle control is highly regulated:
  - Cyclins are synthesized, bind CDKs, phosphorylate targets
  - Checkpoints for assessing damage
    - Key players: p53, Rb, cyclinD, cdk2, E2F
- Cancer cells are out of control:
  - Mutation of at least 2 different genes:
    - Tumor suppressor ruined, proto-oncogene -> oncogene
  - New terms: translocation, loss of heterozygosity
    - Key players: p53, Rb, Ras, cyclin D, EGFR
  - Molecular diagnostics, prognostics