Targeting Cell Cycle Proteins for Cancer Therapeutics

Group Meeting
November 3, 2021
Cancer

**Family of diseases** arising from uncontrolled division of abnormal cells

- Second leading cause of death globally (1 in 6)
- 90 million newly reported cases annually
- Global economic burden exceeds $2 trillion USD

**targeted therapies** selectively kill certain types of cancer cells in patients with relevant **biomarkers**

Surgery, radiotherapy, chemotherapy

Outline

- Part I: Introduction to the Cell Cycle and Basic Concepts
- Part II: Biochemical Regulation of Cell Cycle Progression
- Part III: Strategies for Therapeutic Intervention
DNA is Packaged into Chromosomes and Chromatin

Kinases and Phosphatases Control Signaling via Phosphorylation State

~520 human protein kinases

~200 human phosphatases

inhibitory S113 phosphorylation of isocitrate dehydrogenase

effects of phosphorylation

- direct interference
- conformational change
- creation of binding sites

kinases can be inhibited or activated themselves by phosphorylation

Cyclin-Dependent Kinases (CDKs) Drive Cell-Cycle Transitions

- **T-loop blocks catalytic site between N- and C-lobes**
  - cyclin binds PSTAIR helix frees T-loop & permits ATP entry
  - Cdk-activating kinase (CAK) phosphorylates T-loop

**Ubiquitylation Targets Proteins to the 26S Proteasome for Destruction**

**Activation of ubiquitin by E1**
E1-enzyme conjugates C-terminal carboxyl of ubiquitin onto itself

**Transfer to E2**
Activated ubiquitin is transferred to E2 carrier enzyme at cysteine

**Ubiquitylation of Target**
E3 ligases facilitate transfer either directly or via E3 intermediate

**Poly-ubiquitylation**
Poly-ubiquitin chain recognized by proteasome receptor machinery

**Other outcomes:** directs protein sorting, protein-protein interactions, removal by deubiquitinases

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E3 Ligases APC/C and SCF Control Protein Degradation in the Cell Cycle

Anaphase-promoting complex/cyclosome
Co-activators target complex to specific **degrons**

- **Cdc20** coactivator
  - active during mitosis

- **Cdh** coactivator
  - active during **G1**

Skp/cullin/F-box complex is active during **S/G2**
Specificity controlled by **78 F-box proteins**

- **Skp2**
  - connects to the F-box protein

- **Cullin 1**
  - forms a scaffold

- **Rbx1**
  - functions in ubiquitin ligase activity

- **E2**
  - ubiquitin-conjugating enzyme

- **p27Kip1**
  - Cdk2 inhibitor

- **Cdc25A**
  - Cdk1 activator

- **Wee1**
  - Cdk1 inhibitor

- **Emi1**
  - APC/C<sup>Cdh1</sup> inhibitor

- **β-Catenin**
  - Cell-proliferation regulator

DNA Surveillance Mechanisms Operate Throughout Interphase

**DNA damage** and **DNA replication stress** checkpoints control cell cycle progression and DNA repair

- **DNA breaks** and **problems at replication forks**
- **ATM dimer**, **ATM monomer**, and **ATR + cofactors** bind ssDNA
- **Chk2 kinase**, **p53 transcription factor**, and **Chk1 kinase**
- **Cell cycle proteins**, **DNA repair proteins**
- **Apoptosis**, **cell-cycle arrest**, **DNA repair**

**Genotoxic stress**

- **Sensors**
  - recognize & bind sites of damaged DNA
- **Transducers**
  - produce and amplify biochemical signals in response to damage
- **Effectors**
  - stall cell cycle and repair lesions
- **Responses**
  - restrict propagation of damage

Caused by chemical agents, UV light, normal metabolism, viral infection, etc.
Cells in $G_0$ states

*quiescent* (reversible)

vs.

*senescent* and *differentiated* (irreversible)

**Restriction Point**

biochemical “gate” controlling progression

activates cell-cycle genes for transcription
1. **Mitogens** stimulate expression of cyclin D via surface receptor transduction pathways.

2. **Cyclin D** binds Cdk4/6 and activates its kinase activity.

3. **Cyclin D–Cdk4/6** phosphorylates Rb, releasing the E2F transcription factor.

4. Rb dissociation permits histone acetylation and polymerase access.

5. **E2F** activates transcription and expression of cell-cycle genes.
During progression from G1 to S phase, proteolysis plays a key role in the degradation of many cell-cycle regulatory proteins. This process allows the cell to progress through the G1 phase and enter S phase, where DNA replication occurs.

In normal G1 progression, the cell cycle is regulated by the balance between transcription of cyclins A, E, and Cdk1 DNA replication genes and the activity of Cdk4/6–cyclin D. The Ink4a protein acts as a tumor suppressor, preventing the cell from entering S phase. Cdk4/6–cyclin D activation is inhibited by the Rb protein, which is phosphorylated by Cdk4/6 in response to external signals. Without external signals, the cell remains in G1 and is arrested at the restriction point.

In oncogenic G1 progression, mutations in the cyclin D–Cdk4/6–Rb–E2F pathway or active signaling by an oncogene (e.g., Ras) can trick the cell into passing the restriction point without completing the G1 phase. This allows the cell to proceed into S phase, where DNA replication occurs.

External signals can activate Cdk4/6–cyclin D, promoting the passage of the restriction point. Conversely, the absence of external signals prevents the cell from entering S phase and progressing through the cell cycle.

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$G_1/S$ Checkpoint for DNA Damage: Two Pathways for Cell Cycle Arrest

**Undamaged DNA**

- $(ATM)_2$ inactive
- ATR not signaling
- E2F drives expression of cell cycle genes
- p53 present in low amounts in cytoplasm
- Normal cell-cycle progression

**dsDNA breaks**

- $(ATM)_2$ active
- ATM active
- E2F active
- Mdm2 inactive
- p53 active in nucleus
- p21
- Apoptosis
- Stable cell-cycle arrest
- Rapid cell-cycle arrest

**Exposed ssDNA**

- ATR active localized to damage site
- Chk1 kinase
- Cdc25A
- Degraded

**p53 “guardian of the genome”**

**ATM/p53 transcription factor activation:** removing the wheels

**ATR/Chk1 kinase activation:** applying the brakes

**p53 Regulation of the G₁/S Checkpoint for DNA Damage**

**healthy cell**

- ATM phosphorylates and activates p53
- Mdm2 binding is blocked
- p53 levels low throughout cell

**irradiation**

- ATM phosphorylates and activates p53
- Mdm2 binding is blocked

**oncogenic stress**

- E2F (from activated oncogene, e.g. K-Ras) transcribes p19
- p19 sequesters Mdm2 in nucleolus

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**p53** tumor suppressor
- mutated/deleted in ~50% of human cancers

**Mdm2** oncogene
- amplified/mutated in 57% of sarcomas

**p19** tumor suppressor
- mutated/deleted in up to 70% of human cancers

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S Phase and DNA Replication

Replicating DNA
Mechanism of chain elongation

Growing DNA chain
Free 3'-hydroxyl end

Nucleophilic attack

Diacidic acid

Base 1
Base 2
Base 3
Base 4

S-phase cell with thousands of replicons

**S Phase and DNA Replication**

**Origin “Licensing” & “Firing”**

origins are “licensed” in late G₁ (low Cdk-activity); subset (10k) is “fired” during S-phase (high Cdk-activity)

**Portion of eukaryotic chromosome**

- Active origin
- Dormant origins
- Active origin

130,000 bp

**licensing**

**firing**

human cervical epithelium (anti-Mcm5)

**DNA Replication Stress Checkpoint**

**What happens if the replication fork hits an obstacle and stalls?**

e.g., bulky lesions, ribonucleotide, intra-strand H-bonding, etc.

- Replication stress
- Accumulation of ssDNA

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**ATR activation** protects stalled forks from replication fork collapse

**Firing of nearby dormant origin** leads to convergence at site of damage

**Small “gap” is traversed by a special translesion DNA polymerase**

G₂/M transition promoted by Cdk1 feedback

molecular “switch” flipped by Polo kinase

positive feedback
active Cdk1 phosphorylates and activates its own activator phosphatase Cdc25

inhibition of inhibition
active Cdk1 phosphorylates and inactivates its own inhibitory kinases Wee1 and Myt1

G₂/M transition events
- microtubule stability drops
- centrosomes migrate apart
- chromosomes condense
- kinetochore assembly starts

anti-cyclin B1

common DNA repair pathways in vertebrates

- base excision repair
- nucleotide excision repair
- mismatch repair
- double-stranded break repair (NHEJ or HR)
**Morphological Changes During Mitosis**

### Interphase
- Chromosomes duplicate
- Cell grows in size

### Prophase
- Chromosomes condense
- Asters form around centrosomes
- Cell rounds up

Morphological Changes During Mitosis

**Prometaphase**
- Nuclear envelope breaks down
- Kinetochores capture microtubules

**Metaphase**
- Chromosomes align at spindle equator

Morphological Changes During Mitosis

Anaphase A

- securin is degraded
- sister chromatids move toward poles

Anaphase B

- central spindle (CS) assembles
- poles separate
- cleavage furrow (CF) assembles

Morphological Changes During Mitosis

**Telophase**
- cleavage furrow (CF) constricts
- nuclear envelope (NE) reassembles

**Cytokinesis**
- chromosomes decondense
- microtubule cytoskeleton reassembles
- daughter cells separate

Chromosomal Passenger Complex (CPC) Corrects Kinetochore Attachment Errors

What happens if spindle microtubules capture kinetochores incorrectly?

Aurora B phosphorylates kinetochore components, promoting dissociation.
1. **Aurora B kinase** signaling (not shown) recruits Mad1/2 to the unattached kinetochore

2. The **mitotic checkpoint complex** (MCC) assembles, inhibiting the **APC/C** (E3 ubiquitin ligase)

3. Kinetochore attachment frees APC/C to target substrates for onset of anaphase (cyclin B, securin)

Targeting Cell Cycle Proteins for Cancer Therapeutics

- **Induce genome instability**
  - e.g., barasertib
  - (Aurora B inhibitor)

- **Exploit replication stress**
  - e.g., prexasertib
  - (Chk1 inhibitor)

- **Force cell cycle exit**
  - e.g., palbociclib
  - (Cdk4/6 inhibitor)

- **Force cycle progression**
  - e.g., adavosertib
  - (Wee1 inhibitor)

**Cdk4/6 Inhibitors Force Cell Cycle Exit**

- Cdk4/6 very commonly upregulated in cancers

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**Diagram Description**

- **Cdk4/6** very commonly upregulated in cancers.

**Figure 41.9**

- **A. Absence of mitogens**
  - Cdk2–cyclin E participates in a second wave of Rb phosphorylation.
  - Cyclin D, etc.

- **B. Mitogens present**
  - Acetylated "open" chromatin favors transcription.
  - Histone deacetylation results in chromatin compaction and repression of transcription.

**Figure 41.10**

- **Restriction Point and Cancer**
  - The transcriptional regulator Myc acts as a selective inhibitor of Cdk4/6 activity.
  - Activity should induce cytostatic G0/G1 arrest.

**Graph**

- Alteration frequency (%)
  - Mutation, Deletion, Amplification, Multiple alterations

**Table**

- AML, ACYC, ACC, Bladder, Glioma, Glioblastoma, Head and neck, Renal, Liver, MBL, Breast, CCLE, Cervical, Colon, Oesophagus, MM, Ovarian, Melanoma, Lung, Stomach, Thyroid, NCI60, Pancreatic, Uterine CS, Uterine

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**Text References**

Pyrido[2,3-\textit{d}]pyrimidin-7-one scaffold provided highly selective inhibition for Cdk4/6 over other Cdks.

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<th></th>
<th>Cdk4–cyclin D</th>
<th>Cdk2–cyclin A</th>
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<tr>
<td>IC\textsubscript{50} (µM)</td>
<td>0.145</td>
<td>5.010</td>
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<tr>
<td>PD-0332991 (palbociclib)</td>
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</table>

Cdk4/6 Inhibitors Force Cell Cycle Exit

- In vivo studies with human tumor xenografts showed promising tumor regression and confirmed MoA

Inhibition of Rb phosphorylation at Cdk4-specific sites

Antiproliferative activity arrests human breast carcinoma cells in G1

In vivo studies with human tumor xenografts showed promising tumor regression and confirmed MoA.

approved in February 2015 for ER+ Her2- advanced breast cancer in combination with letrozole (aromatase inhibitor)

Wee1 Inhibitors Force Cell Cycle Progression

- Strategy targets regulation of the G2/M transition, forcing premature cell cycle progression

Wee1 restricts G2/M transition by phosphorylating Cdk1 (Cdc2) at Y15

Wee1•adavosertib

Wee1 inhibition permits sensitization to co-dosed genotoxics by suppressing DNA damage response

Wee1 Inhibitors Force Cell Cycle Progression

Wee1 inhibition permits sensitization to co-dosed genotoxics by suppressing DNA damage response.

MK-1775 was licensed to AstraZeneca (AZD-1775, adavosertib) in 2013, with ~60 phase I/II clinical trials currently ongoing for various cancer types.
**Chk1 Inhibitors Impair Oncogenic Replication Stress Tolerance**

- ATR and Chk1 are two promising targets associated with oncogene-induced DNA replication stress.

![Diagram](image)

Actively dividing cancer cells experience higher levels of **replication stress** (ssDNA) and are more susceptible to **replication catastrophe**.

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**Chk1 Inhibitors Impair Oncogenic Replication Stress Tolerance**

- **Chk1** inhibition by LY2606368 causes accumulation of DNA damage and replication catastrophe.

![Graph showing pH2AX and RPA levels over time with LY2606368 concentrations.](image)

**Chk1 Inhibitors Impair Oncogenic Replication Stress Tolerance**

- Prexasertib induces DNA damage in tumors and inhibits lung carcinoma in xenograft model

- Activity in PARP-inhibitor resistant ovarian cancer

- **Olaparib (Lynparza)** – PARP inhibitor
  poly(ADP-ribose) polymerase critical for genome stability

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Inhibitors of the mitotic spindle and spindle assembly checkpoint induce chromosome mis-segregation.

**Aurora B** phosphorylates kinetochore components, promoting dissociation.

**in vitro selectivity**

<table>
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<tr>
<th>kinase</th>
<th>IC$_{50}$, $\mu$M$^a$</th>
<th>kinase</th>
<th>IC$_{50}$, $\mu$M$^a$</th>
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<tr>
<td>Aurora A</td>
<td>1.4</td>
<td>KDR</td>
<td>1.8</td>
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<tr>
<td>Aurora B-INCENP</td>
<td>&lt;0.001</td>
<td>PHK</td>
<td>1.8</td>
</tr>
<tr>
<td>Aurora C-INCENP</td>
<td>0.017</td>
<td>ZAP70</td>
<td>8.2</td>
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<tr>
<td>LCK</td>
<td>0.17</td>
<td>others$^b$</td>
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Aurora B Inhibitors Interfere with Chromosomal Segregation

Aurora B inhibitor barasertib induces polyploidy and inhibits cancer growth in xenograft models.

DNA content in treated cells

DNA replicates but cells do not divide

Inhibition of colorectal cancer xenografts

Histology consistent with apoptotic induction in cancer tissues


Aurora B Inhibitors Interfere with Chromosomal Segregation

Ongoing trials in phase I/II for hematological cancers, small-cell lung cancer, and prostate cancer