Targeting the Ubiquitin Proteasome System

Financial Disclosures: C4 Therapeutics, Pfizer, Celgene, Novartis, Astellas, Deerfield, AbbVie

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Boston 11-14-2018
Cellular Waste Disposal: The Ubiquitin Proteasome System
The UPS is Vast and Causally Linked to Human Disease

The Ubiquitin-Proteasome System (UPS) is a cellular system that involves three main enzymes: E1 (Ub-activating enzyme), E2 (Ub-conjugating enzyme), and E3 (Ub-ligase). ATP is used to activate Ub, and ADP + Pi is used to regenerate ATP. The process involves the conjugation of Ub to substrates by E1 and E2, followed by the recognition and degradation of the Ub-protein conjugates by the proteasome. DUBs (deubiquitinating enzymes) can remove Ub from substrates, reversing the conjugation process.
The UPS is vast and causally linked to human disease.

- ~ dozen UBLs / E1
- ~ 50 E2s
- ~ 600 E3s
- ~ 100 DUBs
- ~ 300 UBPs
- ~ 50+ regulators

ATP → ADP + Pi
Catalysis by Proximity

DDB1
Adaptor

DDB2
Substrate
Receptor

Cul4 (E3 ligase)

Nedd8

RBX1/E2

Fischer et al., 2011 CELL
HIV-1 hijacks Cullin-RING ligase to degrade UNG2

Fischer et al., 2011 CELL
Wu et al., 2016 NSMB
Small Molecule Targeting of Ubiquitin Ligases

Thalidomide / IMiDs / PROTACs

Indisulam / E7820

Auxin / Jasmonate
Thalidomide and Derivatives (IMiDs)

Thalidomide

1956
Approved: sedative & antiemetic agent

1961
Withdrawn: classed as teratogen

1994
Anti-angiogenic properties observed

1998
Approved: to treat leprosy

2000
Effective in multiple myeloma (MM) trial

2005
Approved: multiple myeloma

2006 lenalidomide
2013 pomalidomide

Thalidomide
Lenalidomide
Pomalidomide
CRBN (Cereblon): The direct cellular target mediating Thalidomide teratogenicity and anti-proliferative activity.

Ito et al., 2010 Science; Lopez-Girona et al., 2012 Leukemia
CRBN resembles a DCAF on structural grounds

DDB1-BPA

DDB1-BPB

CRBN

DDB1-BPC

RBX1

Thalidomide

CUL4A

Fischer et al., 2014 Nature
Thalidomide and analogs are bi-functional molecules

Ebert lab, Handa lab, Kaelin lab, Thomä lab, Basserman lab, Celgene, Fischer lab
(published/unpublished)
Structure of DDB1-CRBN-Lenalidomide-Ck1

Petzold, Fischer, Thomä 2016
Hundreds of $C_2H_2$ Zinc-Finger Proteins Resemble CRBN Binding Motif

IKZF1
IKZF3
ZFP91
CK1α
GSPT1

An et al., 2017
Petzold, Fischer, Thomä 2016
Matyskiela et al., 2016
IMiD Induced Degradation in H9 Human Embryonic Stem Cells

Thalidomide

Lenalidomide

Pomalidomide

SALL4

RAB28

FAM83F

CSNK1A1

Donovan et al., (Elife 2018);
Fischer et al., US Patent 62/5755059
IMiD’s Disrupt a Network of ZnF Transcription Factors

Donovan et al., (Elife 2018); Fischer et al., US Patent 62/5755059
Phenotypic Overlap Between SALL4 and Thalidomide Syndromes

Thalidomide Syndrome

- Eyes
- Ears
- Upper limbs
- Heart & inner organs
- Lower limbs

SALL4 mutations

Donovan et al., (Elife 2018)
IMiDs Exhibit Characteristic and Species Specific Birth Defects

Teratogenic Effects of Thalidomide in Rabbits, Rats, Hamsters, and Mice

I. D. Fratta, E. B. Sigg, and K. Maiorana

Department of Pharmacology, Geigy Research Laboratories, Ardsley, New York

Received March 12, 1964
CRBN$^{V388I}$ Segregates With Sensitivity to Thalidomide

**Kronke et al., Nature 2015**

**Donovan et al., Elife 2018**
Crbn^{I391V/I391V} Knock-in Mouse Model Restores ZnF Degradation

Crbn^{wt/wt}

Crbn^{I391V/I381V}

1 µM Pomalidomide

Fink et al., (Blood 2018)
Crbn\textsuperscript{1391V/1391V} Knock-in Mouse Model Remains Insensitive to Thalidomide Teratogenicity.

Crbn\textsuperscript{wt/wt}

Crbn\textsuperscript{1391V/1381V}

Fink et al., (Blood 2018)
# Mutations in SALL4 Prevent IMiD Dependent Binding to CRBN

![Mutation Alignment]

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
<th>Length</th>
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<tr>
<td>Human</td>
<td>FVCSCVCGHRFTTTKGNLKVHFHRH</td>
<td>432</td>
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<tr>
<td>Macaque</td>
<td>FVCSCVCGHRFTTTKGNLKVHFHRH</td>
<td>330</td>
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<tr>
<td>Marmoset</td>
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<td>432</td>
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<tr>
<td>Bushbaby</td>
<td>FVCSCVCGHRFTTTKGNLKVHFHRH</td>
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<tr>
<td>Rabbit</td>
<td>FVCSCVCGHRFTTTKGNLKVHFHRH</td>
<td>384</td>
</tr>
<tr>
<td>Mouse</td>
<td>YVCPIICGHRFTTTKGNLVHQLQRH</td>
<td>437</td>
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<tr>
<td>Rat</td>
<td>YVCPCICGHRFTTTKGNLKVHFHRH</td>
<td>435</td>
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<tr>
<td>Zebrafish</td>
<td>FKCNICCGNRFTTTKGNLKVHFQRH</td>
<td>411</td>
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<tr>
<td>Chicken</td>
<td>YKCNICCGNRFTTTKGNLKVHFQRH</td>
<td>420</td>
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**Graph:**

- hsSALL4$_{ZnF2}$
- mmSALL4$_{ZnF2}$
- drSALL4$_{ZnF2}$

**Table:**

<table>
<thead>
<tr>
<th>log[Thal] in M</th>
<th>520/490</th>
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<td>-4</td>
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</tbody>
</table>

Donovan et al., (Elife 2018)
Amino Acid Differences in CRBN & SALL4 Prevent Degradation

Donovan et al., (Elife 2018)
Targeted Protein Degradation
Hijacking the Ubiquitin Proteasome System for a Good Cause

IKZF1/3
CSNK1A1
ZFP91
RNF166

poly-Ubiquitin

DEGRADER MOLECULE

TARGET PROTEIN

DEGRADATION

Ebert lab
Fischer lab
Handa lab
Kaelin lab
Thomä lab
Celgene
Small molecule degraders / PROTACs

Winter et al., 2015; Raina et al., 2016
Multi-targeted kinase scaffold results in selective degradation

Huang et. al, (2017)
Structure of DDB1-CRBN-dBET23-BRD4_{BD1} complex

Nowak et al., (2018) NCB
Contacts between CRBN and BRD4\textsubscript{BD1} contribute to binding

Nowak et al., (2018) NCB
Contacts between CRBN and BRD4_{BD1} contribute to binding.
dBET1/57 incompatible with dBET6/23 binding mode
How can a single ligase accommodate multiple substrates?

Nowak et al., (2018) NCB
Structure of DDB1-CRBN-dBET57-BRD4$_{BD1}$ complex

Nowak et al., (2018) NCB
dBET57 and dBET23 bound CRBN-BRD4_{BD1} complex structures exhibit distinct binding mode
dBET57 incompatible with dBET23 binding mode

Nowak et al., (2018) NCB