Novel tools for DUB inhibitor specificity profiling in cancer

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**Group and Interests**

Ubiquitin-Proteasome System

Chemical Biology & Mass Spectrometry

- MHC presentation
- Immune recognition (T-cells)
Ubiquitin System in Human Diseases

Neurodegenerative disease

Inflammation

Cancer

Bacterial and Viral Infections

Ub linkage type adds to biological complexity
# DUBs in Disease (1)

Cancer Target discovery & "Learn" how to use them for disease intervention

## Neurodegeneration Identification for Pathogens

<table>
<thead>
<tr>
<th>DUB enzyme</th>
<th>Biology</th>
<th>Human Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCH-L1</td>
<td>AKT – β-Catenin – NF-κB</td>
<td>Parkinson’s disease (mutation/OE)</td>
</tr>
<tr>
<td>USP6 (Tre2)</td>
<td>RTK, Wnt and HH pathways</td>
<td>B-cell malignancies, pancreas, colorectal, breast (OE)</td>
</tr>
<tr>
<td>USP8</td>
<td>26S editing</td>
<td>Oncogene</td>
</tr>
<tr>
<td>USP14</td>
<td>Wnt, NF-κB</td>
<td>Oncogene</td>
</tr>
<tr>
<td>CYLD</td>
<td>bind VHL</td>
<td>Ataxia (mouse)</td>
</tr>
<tr>
<td>VDU1, 2</td>
<td></td>
<td>Cylindromatosis (mutations)</td>
</tr>
<tr>
<td>USP2</td>
<td>Mdm2/4, FASN, NF-κB, c-Myc</td>
<td>Prostate / breast cancer (OE)</td>
</tr>
<tr>
<td>USP7 / 7S*</td>
<td>Mdm2/4, PTEN, FOXO4 (p53)</td>
<td>Diverse cancers (OE)</td>
</tr>
<tr>
<td>USP15</td>
<td>TGF-β-R1, β-Catenin, SMADS</td>
<td>Glioblastoma, breast &amp; ovarian (OE)</td>
</tr>
<tr>
<td>Cezanne 1</td>
<td>EGRF turnover</td>
<td>Breast cancer (amplification, OE)</td>
</tr>
<tr>
<td>OTUB1</td>
<td>UBC13/RNF 168, p53, RhoA</td>
<td>DNA damage, prostate cancer</td>
</tr>
<tr>
<td>USP1/UAF1</td>
<td>Chk1 &amp; ID1-3 (CSC)</td>
<td>Melanoma, colon, lung, osteosarcome (OE, activation)</td>
</tr>
<tr>
<td>USP9X</td>
<td>Mcl-1, β-catenin, TGF-β</td>
<td>Colorectal, breast, lung, lymphoma (OE)</td>
</tr>
<tr>
<td>USP10</td>
<td>p53, AR, autophagy</td>
<td>Melanoma (OE)</td>
</tr>
<tr>
<td>USP13</td>
<td>MITF oncogene</td>
<td>10-20% of melanomas</td>
</tr>
<tr>
<td>USP22</td>
<td>p53, MYC</td>
<td>Aggressive cancers (OE)</td>
</tr>
<tr>
<td>USP4</td>
<td>TGF-β-R1, β-Catenin</td>
<td>Breast, lung, colon hematopoietic cancers (OE)</td>
</tr>
<tr>
<td>USP17 (Dub3)</td>
<td>Cdc25A turnover – GTPases</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>USP33</td>
<td>Met signaling - apoptosis</td>
<td></td>
</tr>
</tbody>
</table>

*Khoronenkova Mol.Cell. 2012*
### Problems, Challenges & Opportunities:

- How to discover & choose the right target(s) relevant for disease?
- Best way to manipulate these targets for effective intervention?

- Good knowledge about molecular target and pathway
- Substrate identity and function are unknown for most DUBs

- **Understand how your target functions**
  - Inhibitor development and substrate ID *in vitro*
  - Need to explore them in a cellular environment

### DUBs in infection:

<table>
<thead>
<tr>
<th>DUB-like enzyme</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>UL36&lt;sup&gt;USP&lt;/sup&gt;</td>
<td>HSV</td>
</tr>
<tr>
<td>CoV PLpro</td>
<td>SARS</td>
</tr>
<tr>
<td>L protein</td>
<td>Hemorrhagic fever virus</td>
</tr>
<tr>
<td>Avp</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>YopJ</td>
<td><em>Yersinia</em></td>
</tr>
<tr>
<td><em>ChlaDub1, ChlaDub2</em></td>
<td><em>Chlamydia</em></td>
</tr>
<tr>
<td>PFDub1</td>
<td><em>Plasmodium Falciparum</em></td>
</tr>
</tbody>
</table>
Know How Your DUB Works: Structural Information for Inhibitor targeting & design

Altun M, 2013

Otubain-2 catalytic centre
- Specific features of DUB cysteine proteases:
- Unusual triade
- Often in an “unproductive conformation” in apo form

OTUB2-UbBr2
yOTU1-UbBr3

OTUB2-UbBr2
vOTU-Ub

OTUB2-UbBr2
OTUB1-Ubal-UBC13-Ub

OTUB2-UbBr2
DEN1-NEDD8
<table>
<thead>
<tr>
<th>DUB Inhibitor</th>
<th>Target/Disease association/Therapeutic potential</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>USP Inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td>USP7</td>
<td>USP7: prostate cancer, non-small cell lung adenocarcinoma,</td>
</tr>
<tr>
<td>USP8</td>
<td>USP8: Sensitivity to glioblastoma</td>
</tr>
<tr>
<td>USP14</td>
<td>USP14: Neurodegeneration, ataxia</td>
</tr>
<tr>
<td><strong>UCH Inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td>UCH-L1</td>
<td>UCH-L1: Parkinson’s disease</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
</tr>
<tr>
<td>PR-619</td>
<td>Broad specificity DUB inhibitor</td>
</tr>
<tr>
<td>GRL0617</td>
<td>Plpro: SARS corona virus</td>
</tr>
</tbody>
</table>

Structures of DUB inhibitors PR-619 and P22077 and in vitro DUB inhibition profiles

Altun M et al. Chem&Biol 2011


In vitro enzyme activity EC50 [M]

PR-619

P22077

Cell death

Cell survival
Chemoproteomics
Looking at DUBs in Cells: Activity-Based Probes

Visualization
Retrieval
Specificity
Trap

$^{125}$I
Biotin
Fluorescent
Group
Peptide
Non-peptidyl
Moieties
Epoxide
Vinyl Sulfone
Fluorophosphonate
Acyloxyketone

-SH + \[\text{active enzyme}\] \rightarrow \text{detected}

-SH + \[\text{inactive enzyme}\] \rightarrow \text{not detected}
HAUb-derived probes

Michael acceptors

HAUb

HAUb

HAUb

HAUb

HAUb

Alkyl halides

HAUb

HAUb

HAUb

HAUb

HAUb

HAUb

HAUb

HAUb

Biochemical Validation of DUB Inhibition in Cells

DUB inhibitors
PR-619
P22077

HA Tag
Ubiquitin

VME

Enzyme
Active site

Cells treated with DUB inhibitors for 6h

Extracts incubated with HA-Ub probes

HA-epitope
Electrophile

HA-Ub2

HA-UbVME

Altun M et al. Chem&Biol 2011
DUB Inhibitor Profiling in Cells using a Mass Spectrometry Approach

1. Cells treated with DUB inhibitors for 6h
2. Extracts incubated with HA-Ub probes
3. HA immunoprecipitation
4. Elution, precipitation and trypsin digestion
5. Relative quantitation by mass-spec
6. Tryptic Digestion
7. Beads with Antibody
8. HA-epitope
9. Electrophile
10. Small-molecule

Altun et al., BBA 2012
1. Label-Free Quantitation - UPLC-MS<sup>E</sup>
2. Label-Free Quantitation / SILAC – UPLC-Orbitrap Velos – LC-Progenesis / MaxQuant
1. Normalisation based on Ubiquitin derived peptides
Activity-Based Proteomics for DUB Inhibitor Profiling in Cells

49 DUBs covered
Expanding to NEDD8, SUMO, etc...

(out of 71 known human Cysteine protease DUBs = 69%)

Altun M. et al.
Chem&Biol 2011
DUB Activity Versus Abundance

DUBs in HEKs

Correlation between Abundance and Labelling (activity)

+   -

Adapted from Geiger T. Mol Cell Proteomics 2012
Kessler BM. Curr. Opin. Chem. Biol. 2013

Altun. Chem & Biol. 2011
P5091 is a USP7 Selective Inhibitor

Chauhan D. et al.
Mol Cell 2012
USP7: A Therapeutic Target in Multiple Myeloma (MM)

USP7 Expression And Prognostic Relevance in MM Cells

P5091 Overcomes Bortezomib-Resistance Combination of P5091 and Lenalidomide, SAHA, or Dex Trigger Synergistic Anti-MM Activity

Chauhan D. et.al. Mol Cell 2012
Development of Fluorescent Ub probes

McGouran et al., OBC 2012

Active DUB profiles in cells

Blue: DAPI; green: lyso-tracker; red/yellow: DUBs
“Chariot” protein transfection reagent
HEK293T cells

CTRL (dye alone)  HA-Ub-VA-Cy3  HA-Ub-VA-Cy5

Patent P38458GB

⇒Information about localized DUB activity & inhibition in cells

McGouran, 2012
“Branched” Ub Probes to Profile DUB Linkage Specificity

Information about DUB inhibition & Ub linkage specificity in a cellular environment
Novel di-Ubiquitin Probes Coupled by "Click" Chemistry

Joanna McGouran et al., 2013. Submitted
Purification and Characterisation of di-Ubiquitin active site probes

Joanna McGouran et al., 2013. Submitted
Challenges for future UPS/DUB drug development

- Obtaining specificity in targeting DUBs – demonstrated with selective USP7 inhibitors
- Chemoproteomics for DUB (inhibition) mechanism of action in cells – DUBs as drug targets
- USP7 inhibition has anti-tumour activity in vivo – synergistic with other drugs
- Novel di-Ub probes begin to address DUB Ub-linkage specificity in cells

Opportunities for

- Defining DUB subsets for different Ub-linkages
- DUB Ub-linkage inhibitor screening
- Deconvoluting DUB function:
  - DUB – substrate probes to capture DUB(s) for a given substrate in cells
Thank You!

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