Searching for Substrates of the ICP0 Ubiquitin E3 Ligase of HSV-1

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Ubiquitination

Ubiquitin activating enzymes (2)

Ubiquitin conjugating enzymes (~40)

Ubiquitin ligases (~500)

DUBs (~100)

Thioester transfer

Ubiquitin

K48-linked: Proteasomal degradation
K63-linked: Cell signaling, DNA damage response
K11-linked: Cell cycle regulation

Many viruses encode E3s and DUBs → viral exploitation of the host ubiquitin system

HSV-1 proteins

• ICP0 - E3 ligase
• UL36 - DUB

Mono 

Multi-mono

Poly

Substrate

Substrate

Substrate

Ub

Ub

Ub

Ub

Ub

Ub

Ub
ICP0 (Infected Cell Protein 0)

- An immediate-early protein of HSV-1
- A viral transactivator: a key regulator of the lytic phase and reactivation from latency
- An ubiquitin E3 ligase (RING finger type) for proteasomal degradation
- Counteracts host intrinsic and innate immunity
- ICP0 functions are dependent on its E3 ligase activity

Degradable targets of ICP0
- PML
- IFI16
- RNF8
- RNF168
- USP7
- IκBα
- E2FBP1
- CENPs
- SUMO
- TRIM27

C3HC4 zinc (Zn2+)-binding RING-finger domain
ICP0 increases ubiquitin chains with multiple linkages

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ub</th>
<th>K48-Ub</th>
<th>K63-Ub</th>
<th>K11-Ub</th>
<th>ICP0</th>
<th>ACTB</th>
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<tbody>
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<td>MG132</td>
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<tr>
<td>HSV-1 ΔRING</td>
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<td>-</td>
<td></td>
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<td>+</td>
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<tr>
<td>HSV-1 WT</td>
<td>-</td>
<td>+</td>
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<td>-</td>
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<tbody>
<tr>
<td>Dox</td>
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<td>rAd.C-rtTA</td>
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Transduced cells

Ad-ICP0

M_r(k) [kDa]

Actin (ACTB)

GAPDH
I. Searching for **Non-Proteolytic** Substrates of ICP0

II. Searching for **Proteolytic** Substrates of ICP0
Identification of ICP0-mediated Ubiquitin targets

Cotransfection
GFP-ICP0 & HA-Ub

→ IP: HA-Ub

\[\begin{array}{ccc}
\text{eGFP-ICP0} & - & - \\
\text{HA-Ub} & - & + \\
\text{WTΔRING} & + & + \\
\text{HA-UB}\_n & * & * \\
\end{array}\]

Silver stain

 Ub linkages enriched by ICP0

<table>
<thead>
<tr>
<th>Ub linkage</th>
<th>Log2 fold change (WT vs ΔRING)</th>
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<tbody>
<tr>
<td>K11</td>
<td>5.76</td>
</tr>
<tr>
<td>K63</td>
<td>4.62</td>
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<tr>
<td>K6</td>
<td>3.98</td>
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<tr>
<td>M1</td>
<td>2.87</td>
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<tr>
<td>K48</td>
<td>2.54</td>
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Previously known ICP0 substrates

\[\begin{array}{c}
P53, USP7, SUMO-1, DNA-PKcs, SUMO-2, ΔRING, ICP0\end{array}\]
RNA binding proteins within the ICP0-mediated ubiquitome
I. Searching for Non-Proteolytic Substrates of ICP0

II. Searching for Proteolytic Substrates of ICP0
Identification of proteins associated with replicating viral DNA using iPOND (isolation of proteins on nascent DNA)

Pull-down of EdU-labeled viral DNA and protein complex

- Mock
- HSV-1
- ΔICP0

Recovered Proteins

LC-MS/MS

Data Analysis
- Mock
- HSV-1
- ΔICP0

ICP0

Degradation
Known ICP0 substrates are decreased on WT HSV genomes compared to HSV ΔICP0 mutant virus genomes.

Clustering of proteins identified in iPOND proteome predicts additional ICP0 substrates.
SLFN5 (Schlafen 5)

- May have roles in hematopoietic cell differentiation and in controlling motility and invasiveness of carcinoma cells
- Contains a divergent AAA domain that may function in GTP/ATP binding
- Predicted putative DNA/RNA helicase domain
- Member of a family of related SLFN proteins
- SLFN11 has anti-HIV activity
- SLFN5 has not been investigated during virus infection

SLFN5 detected in Nucleoplasm and Vesicles
(adapted from the human proteins ATLAS)
HSV-1 infection reduces SLFN5 through proteasomal degradation

ICP0 RING domain dependent degradation

Proteasome dependent degradation

CYH chase assay
ICP0 ubiquitinates and degrades SLFN5

**ICP0 cotransfection**

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<tr>
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<th>GFP-ICP0</th>
<th>SLFN5-V5</th>
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<tr>
<td>WT △RING</td>
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<tr>
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<th>V5</th>
<th>GFP</th>
<th>GAPDH</th>
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**In vivo ubiquitination assay**

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<tr>
<th>Condition</th>
<th>Dox</th>
<th>Ad.C-rtTA</th>
<th>Ad.T-ICP0</th>
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<tbody>
<tr>
<td>Mock</td>
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**Mock**

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<tr>
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<th>2hpi</th>
<th>4hpi</th>
<th>6hpi</th>
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<td>ICP0</td>
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<tr>
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**WT**

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**△RING**

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SLFN5 localizes to viral replication centers when not degraded during HSV-1 infection

MOI 3 at 6 hpi

Virus Replication Center (VRC)
Depletion of SLFN5 increases HSV-1 DNA replication and virus progeny production

- SLFN5 is inhibitory to HSV-1 replication
- Overcome by the degradation of SLFN5 during WT virus infection
Ectopic expression of SLFN5 inhibits HSV-1 replication

SLFN5 depletion by CRISPR/Cas9 → Reconstituted with Tet-SLFN5-HA by lentiviral vector transduction

** p < 0.05
*** p < 0.005
SLFN5 suppresses transcription of viral genes

HSV-1 ΔRING
MOI of 0.5 for 8 h

ICP27
Immediate-early

TK
Early

UL36
Late

** p < 0.05
*** p < 0.005

* PAA (Phosphonoacetic acid): a viral DNA polymerase inhibitor
FINDINGS

I. HSV-1 E3 ligase ICP0 induces various non-proteolytic ubiquitination as well as proteolytic ubiquitination

II. ICP0 modifies RNA binding proteins with non-proteolytic ubiquitination

III. Using PCA-based clustering of iPOND data, we identified SLFN5 as a potential ICP0 target

IV. ICP0 ubiquitinates and degrades SLFN5 via the proteasome

V. SLFN5 represses HSV-1 replication

VI. SLFN5 down-regulates viral genes transcription during HSV-1 infection
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