Histone ubiquitination in DNA damage response

Xiaochun Yu
University of Michigan
Protein ubiquitination

**E1** → **Ub** → **E2** → **Ub** → **E3** → **substrate**

**degradation by proteasome**

**Signaling??**
Protein ubiquitination participates in the DNA damage response

Ubiquitin  γH2AX  DAPI

-  -  -

IR  -  -
Histone H2A is ubiquitinated at the DNA damage sites

DNA damage sites
RNF8 participates in the DNA damage response.

The diagram shows a schematic representation of RNF8, highlighting the FHA and Ring domains. The pThr binding domain interacts with the E3 ligase and subsequently binds to a substrate. The process involves the attachment of Ub (ubiquitin) molecules, leading to a marked response under 2 Gy irradiation compared to 0 Gy.
FHA domain targets RNF8 to the DNA damage sites
RNF8 acts downstream of H2AX and MDC1

Many other factors including RNF8

FHA
RNF8 controls Ub-H2A foci

Ub-H2A  γH2AX  DAPI

RNF8  +/+  

RNF8  -/-
RNF8-dependent DNA damage response

ATM → H2AX

H2AX → MDC1

MDC1 → H2A, H2B

H2A, H2B → Ub

Ub → RNF8 complex

RNF8 complex → BRCA1

BRCA1 → 53BP1

RNF168 → RNF8

Ub → Ubc13

?
Phenotypes of RNF8 deficient mice

In vivo:

1. Few RNF8-null mice develop tumors.
2. The male RNF8-null mice are sterile.

Why??
Chfr is a potential paralog of RNF8, and is often downregulated in cancers.

RT-PCR

Primary colon cancers

N = Normal tissue  T = Tumor tissue

N= Normal tissue  T= Tumor tissue
RNF8 and Chfr DKO mice develop T-cell lymphoma
SKY analysis of T-cell lymphoma in DKO mice

Similar to ATM -/- tumors

Chromosome 6, 14 rearrangement

Similar to ATM -/- tumors
Loss of RNF8 and Chfr abolishes ATM-dependent DNA damage response
RNF8 controls histone removal during spermiogenesis

WT

KO

Spermiogenesis:

Round spermtid

Elongating spermtid

Condensing spermtid

Sperm

Transition Protein 1 and 2

Protamin 1 and 2 deposition

Elongating spermtid

Histone removal

Condensing spermtid
H4 acetylation controls chromatin relaxation

H4 ac (H4 K16 ac)
Histone ac and Ub are abolished in RNF8-deficient spermtids

Histone H4 acetylation is the marker of elongating spermtids.
RNF8 and Chfr regulate chromatin relaxation.
RNF8 and Chfr regulate chromatin-association of MOF and Tip60
MRG15 associates with Ub-H2B

MRG15: Common component of both MOF and Tip60 complexes
ATM-dependent DNA damage response is impaired in MRG15-depleted cells
TSA rescues ATM-dependent DNA damage response in DKO MEFs

Trichostatin A (TSA):
Class I and II histone deacetylase (HDAC) inhibitor

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<th>Wild type</th>
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<th>DKO</th>
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<tr>
<td>IR</td>
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<td>TSA</td>
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TSA

↑ H4 ac

ATM activation
TSA restores 53BP foci in DKO cells
MRG15 → MOF → Tip60 → DNA damage → ATM activation → Checkpoint activation → DNA damage repair

Recruit more RNF8
Global histone eviction

spermiogenesis

RNF8, Chfr

Ub-H2A/H2B

H4 ac

Histone like proteins

local histone eviction

DNA damage response

DNA damage repair proteins

Global histone eviction

spermiogenesis

RNF8, Chfr

Ub-H2A/H2B

H4 ac

Histone like proteins

DNA damage repair proteins
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