Oncogenic herpesvirus hijacks components of the DNA repair machinery to promote its own replication

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Bhaduri-McIntosh laboratory
The DNA Damage Response cascade and ATM

Yosef Shiloh et al., Nature Reviews, 2013
Phosphorylation of Krüppel-associated Box (KRAB)-associated protein (KAP)-1 by ATM is a key event during DDR

Yael Ziv et al., Nature Cell Biology, 2006
Krüppel-associated Box (KRAB)-associated protein (KAP)-1

KAP1/TRIM28 (triptic motif-containing protein 28) structure

KAP1 recruits HDACs and HMT to modify the epigenetic status around its binding locus.

KAP1 facilitates repair of DNA breaks in heterochromatin by allowing access to repair factors.

- H3-K9 trimethylation
- H3-acetylation
Epstein-Barr virus (EBV)

- Member of human gammaherpesvirus family
  - Enveloped, encapsidated large dsDNA genome
  - Mainly lymphoid, epithelial tropism

- Associated with numerous human malignancies
  - Burkitt lymphoma
  - Nasopharyngeal cell carcinoma
  - Hodgkin’s disease
  - Post-transplant Lymphoproliferative diseases (PTLD)
  - NK/T-Cell lymphomas
  - Gastric carcinoma

In situ hybridization for Epstein-Barr virus–encoded small RNAs (EBERs) in
A. Burkitt lymphoma, B. Nasopharyngeal cell carcinoma, C. Hodgkin’s disease; D. PTLD
Latency to lytic switch of EBV

Latent infection
- Limited viral genes expressed
- Viral genome duplicates in S-phase
- No progeny production

Lytic (re)activation
- Immediate
- Early
- Late

HDACi, TGF-β, hypoxia, etc
Mechanisms underlying EBV latency to lytic switch

Cellular components that regulate latency-to-lytic switch

- PCBP2  Siva Koganti, et al., *JVI*, 2015;
- KRAB-ZFPs: large family of transcriptional repressors

thought to function via the **KAP1** corepressor
Manipulation of KAP1 levels modulates EBV lytic activation

A

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<th>scrambled</th>
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<tr>
<td>β-actin</td>
<td>50kD</td>
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B

- BZLF1 mRNA (relative amount)
- BMRF1 mRNA (relative amount)
- BFRF3 mRNA (relative amount)

C

EBV DNA copies (relative amount)

D

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<tr>
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<td>β-actin</td>
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E

- BZLF1 mRNA (relative amount)
- BMRF1 mRNA (relative amount)
- BFRF3 mRNA (relative amount)

F

EBV DNA copies (relative amount)
KAP1 is phosphorylated at Serine 824 upon viral lytic activation in EBV+ BL cells

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B

p-S824 KAP1  ZEBRA  DAPI

C

p-S824 KAP1  EA-D  DAPI

D

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<th>Dox (μg/ml)</th>
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E

p-S824 KAP1  ZEBRA  DAPI

F

p-S824 KAP1  EA-D  DAPI

Xiaofan Li, et al., PLOS Pathogens, 2017
**Phosphorylated KAP1 at S824 is impaired in its ability to restrain EBV lytic activation**

Phospho-dead  
phosphomimetic  
phosphorylated

Diagram A:
- **ZEBRA**
- β-actin
- KAP1
- FLAG

Diagram B:
- **BZLF1 mRNA** (relative amount)
- **BMRF1 mRNA** (relative amount)
- **BFRF3 mRNA** (relative amount)

Diagram C:
- **EBV DNA copies** (relative amount)

Xiaofan Li, et al., *PLOS Pathogens, 2017*;
How is KAP1 phosphorylated at S824?

DNA damage response

ATM → KAP1

HCMV lytic activation

KAP1 → mTOR

David White et.al, *Cancer Res*, 2006;

Benjamin Rauwel et al., *Elife*, 2015
ATM is responsible for phosphorylation of KAP1 at S824 during EBV lytic activation

KU-55933: ATM inhibitor
Torin1: mTOR inhibitor

KU-55933 (μM) | NaB- | NaB+ 
---|---|---|
0 | p-S824 KAP1 | 98kD | 98kD |
0.1 | 98kD | 98kD |
0.5 | 36kD | 36kD |
1.0 | 50kD | 50kD |

Torin1 (μM) | NaB- | NaB+ 
---|---|---|
0 | p-S824 KAP1 | 98kD | 98kD |
0.1 | 98kD | 98kD |
0.2 | 36kD | 36kD |
0.5 | 50kD | 50kD |

NaB | scrambled | si-ATM 
---|---|---|
1.0 | p-S824 KAP1 | 98kD | KAP1 |
0.63 | 98kD | 250kD | ATM |

Xiaofan Li, et al., PLOS Pathogens, 2017
ATM binds to KAP1 during EBV lytic activation

Xiaofan Li, et al., PLOS Pathogens, 2017
**Working model**

1. **Viral lytic product (IE, E, L)**
   - EBV genome
   - KAP1
   - Chloroquine

2. **ATM**
   - ZEBRA
   - Lytic signal
   - EBV genome
   - KAP1
   - Viral lytic product (IE, E, L)
ATM activator chloroquine induces phosphorylation of KAP1 at S824 and EBV lytic activation.

A. p-S824 KAP1, ZEBRA, DAPI

B. CQ 0h 4h 8h 16h 24h 48h 72h

C. KAP1 wt KAP1 S824A

D. CQ +KU

E. p<0.01

F. Mock 10μM CQ 200μM CQ NaB

Xiaofan Li, et al., PLOS Pathogens, 2017;
Chloroquine and NaB activate ATM in the absence of observable DNA damage

Xiaofan Li, et al., *PLOS Pathogens*, 2017
Conclusion

The cancer-causing virus EBV exploits a cellular mechanism (involving ATM and KAP1) that repairs breaks in heterochromatin to promote its own replication.
Acknowledgements

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