Insights into DNA damage response signaling from an oncovirus

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Oncogene induced senescence
Circumventing DDR during oncogene-driven cell proliferation

• For oncogenes to successfully drive cell proliferation and cancer, DNA damage checkpoint barriers need to be overridden

• Mechanisms of DDR attenuation: inherited versus sporadic cancers

• STAT3 – transcription factor
  – frequently activated by growth factors and cytokines
  – prosurvival, angiogenesis, metastasis
  – constitutively active in many human cancers
  – precise contribution to tumorigenesis unknown
Does/Can STAT3 mediate DDR suppression during the initial rounds of oncogene-driven cell proliferation?
Epstein-Barr virus (EBV)

- Post-transplant lymphomas, AIDS-lymphomas, Burkitt lymphoma, NPC, Hodgkin lymphomas, T/NK cell lymphomas, gastric carcinomas

- Oncogenic human gammaherpesvirus
  - co-evolved with humans, infects nearly everyone
  - excellent means to uncover fundamental cellular processes
  - encodes several oncoproteins, drives cellular DNA replication, causes cellular DNA damage
  - post-transplant lymphomas develop within weeks of infection
  - primary human B cells + EBV………cell lines in 2 weeks
  - STAT3 constitutively active in EBV-related cancers
Does STAT3 mediate DDR suppression during the initial rounds of EBV oncogene-driven cell proliferation?
EBV infection results in early STAT3 activation and increased expression.

Koganti et al., J. Virology, 2014
Inhibition of STAT3 results in fewer EBV+ cells

Day 4 Culture

Koganti et al., J. Virology, 2014
STAT3 is necessary for outgrowth of cell lines

Koganti et al., J. Virology, 2014
**STAT3 is necessary for survival of LMP1+ cells**

- **Graph 1:**
  - **X-axis:** LMP1-pos, LMP1-neg
  - **Y-axis:** % Annexin V-pos cells
  - **Legend:**
    - EBV
    - EBV + AG490
    - AD-HIES B cells + EBV
  - **Day 2:**
    - LMP1-pos: EBV, EBV + AG490, AD-HIES B cells + EBV
    - LMP1-neg: EBV, EBV + AG490, AD-HIES B cells + EBV
  - **Day 3:**
    - LMP1-pos: EBV, EBV + AG490, AD-HIES B cells + EBV
    - LMP1-neg: EBV, EBV + AG490, AD-HIES B cells + EBV
  - Stars for significant differences

- **Graph 2:**
  - **X-axis:** EBV, EBV + AG490
  - **Y-axis:** Relative quantity of mRNA
  - **Legend:**
    - N.S.
  - **Results:**
    - EBV: 1.4
    - EBV + AG490: 1.2

*Koganti et al., J. Virology, 2014*
AG490 inhibits STAT3 and its targets including pro-survival genes

Koganti et al., J. Virology, 2014
STAT3 is necessary for proliferation past the S phase
.........or impairment of STAT3 causes S phase delay/arrest

Koganti et al., J. Virology, 2014
STAT3 is necessary for proliferation past the S phase
or impairment of STAT3 causes S phase delay/arrest

Koganti et al., PNAS, 2014
STAT3 blocks signaling downstream of ATR

Koganti et al., PNAS, 2014
STAT3 suppresses pChk1 levels

Healthy B + EBV, LMP1 pos
AD
- HIES
B + EBV, LMP1 pos
Un-infected
AD
- HIES B
cells
Un-infected
healthy B
cells

Day 4 Culture

pChk1

Healthy LCL

AD-HIES LCL

pChk1

β-Actin

LCL 1
LCL 2

pChk1

β-Actin

siRNA
Sc
STAT3
Sc
STAT3

Koganti et al., PNAS, 2014
**Proliferating EBV-infected cells in vivo show high STAT3 but low pChk1**

Koganti et al., PNAS, 2014
STAT3 functions via Chk1 to promote progression of EBV-infected cells past the S phase.

Koganti et al., PNAS, 2014
Cells with functional STAT3 demonstrate loss of nuclear Claspin

Day 4

EBNA2  Claspin  DAPI
EBV

EBV  E+A

Mean nuclear fluorescence (relative unit)

Claspin mRNA (relative level)

Claspin

EBV  E+A

EBV  E+A

EBV

Time (hr)

0  4  8  12  24  48  72  96

pATR

Claspin

β-actin

7.8  16.1

Koganti et al., PNAS, 2014
EBV infection in the presence of STAT3 results in caspase 7 activation.

Koganti et al., PNAS, 2014
Caspase inhibition causes Claspin recovery and failure of EBV-mediated growth transformation

Day 4

<table>
<thead>
<tr>
<th>ZVAD-FMK (µM)</th>
<th>Claspin</th>
<th>pChk1</th>
<th>β-Actin</th>
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<td><img src="image3" alt="β-Actin" /></td>
</tr>
</tbody>
</table>

Number of live cells

- Donor 1+EBV
- Donor 1+EBV+ZVAD
- Donor 2+EBV
- Donor 2+EBV+ZVAD

Days post-infection

Koganti et al., PNAS, 2014
Inhibition of Caspase 7 (but not Caspase 6) causes recovery of Claspin and pChk1

Koganti et al., PNAS, 2014
Model for DDR suppression during the initial rounds of EBV-oncogene-driven cell proliferation
Summary

- Link STAT3 to DDR suppression during oncogene-driven cell proliferation

- A newly discovered function for STAT3 (constitutively active in most cancers)

- STAT3 mediated relaxation of the intra-S phase checkpoint is a previously unknown mechanism for genomic instability

- Caspase 7 in a non-apoptotic role

- STAT3, a major transcription factor, is central to cell proliferation – implications beyond EBV infection and tumorigenesis
Contributions

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