Breaking Good

DNA Repair and Oncogenesis

Jeff Bednarski, MD, PhD
Pediatric Hematology-Oncology

03/21/17
DNA Damage Initiates Cellular Changes That Can Lead to Malignancy

- Environmental exposures
  - Radiation
  - Smoking
  - Alcohol
  - Drugs (chemotherapy)

- Defenses: limit exposure

- Outcomes: errors in DNA trigger malignant changes.
Daily Cellular Events Cause DNA Damage

- DNA replication
  - Synthesis of DNA
  - Unwrapping telomeres
- Cell Division – chromosome separation
- Transcription
- Reactive oxygen species
  - Metabolism
  - Inflammation
- Viruses
- Immune cell development
DNA Damage Response Networks

External Sources
- Radiation
- Chemotherapy
- Environmental toxins

Internal Sources
- Cell Division
- Immune Cell Development
- Reactive Oxygen Species

Signals → Sensors → Transducers → Effectors

- Cell cycle transitions
- Apoptosis
- Transcription
- DNA repair

Complex Signals Manage Broken DNA

- Activation of PI3K-like kinases (ATM, ATR, DNA-PK)
- Effector proteins
- Activation of HATs (e.g. Tip60)
- Chromatin relaxation
- DNA repair
- Histone deacetylation & chromatin condensation by HDACs
- Chromatin restoration

BRCA1
BRCA2
FANC

## DNA Damage and Disease

<table>
<thead>
<tr>
<th>Mutated Gene</th>
<th>Syndrome</th>
<th>Cancer Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH2, MSH5, MLH1</td>
<td>Hereditary nonpolyposis colorectal cancer</td>
<td>Colorectal carcinomas</td>
</tr>
<tr>
<td>BRCA1, BRCA2, RAD51</td>
<td>Familial breast cancer</td>
<td>Breast and ovarian cancer</td>
</tr>
<tr>
<td>FANC</td>
<td>Fanconi Anemia</td>
<td>AML, squamous carcinoma</td>
</tr>
<tr>
<td>RAG1, RAG2, XLF, LIG4</td>
<td>Immune deficiencies</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>DKC</td>
<td>Dyskeratosis congenital</td>
<td>Carcinomas</td>
</tr>
<tr>
<td>BLM</td>
<td>Bloom syndrome</td>
<td>Carcinomas, lymphomas</td>
</tr>
<tr>
<td>TREX1</td>
<td>Aicardi Goutieres syndrome</td>
<td></td>
</tr>
<tr>
<td>LIG1, APTX, SETX</td>
<td>Ataxia syndromes</td>
<td></td>
</tr>
<tr>
<td>XPA, XPB, XPC</td>
<td>Xeroderma pigmentosum</td>
<td>Melanoma</td>
</tr>
</tbody>
</table>

ATM Deficiency
(Ataxia Telangiectasia, A-T)

Increased sensitivity to ionizing radiation

Lymphoid tumors
(some with translocations but not most)

Lymphopenia

Cerebellar Ataxia
Multifaceted Responses to DNA damage

ATM phosphorylates hundreds of protein targets in response to DNA injury

ATM

Repair

Developmental Programs

Cell Death

?
B Cell Maturation Occurs in Distinct Stages

Lymphocyte Antigen Receptor Gene Assembly

http://www.biology.arizona.edu/immunology/tutorials/antibody/structure.html
DNA Breaks in Immune Development

- Heavy Chain (IgH)
- Light Chain (IgL)
- Class switch recombination

60,000,000 DNA Breaks each hour

1,600,000,000 DNA Breaks each day
B Cell Development Requires the Generation and Repair of Several DNA Breaks

Pro-B Cells

Pre-B Cells

Mature B Cell

Leukemia
Do DNA Breaks Signals Integrate with Developmental Programs?

DNA Break Activates Genes

DNA Damage Response
- Repairs of DNA break
- Controls balance of cell death and cell survival
- Slows cell growth

Cell Development
- Cell movement
- Cell maturation
- Cell signaling

Do DNA Breaks Signals Integrate with Developmental Programs?
Signals That Regulate B Cell Development

Pre-B Cells

Pre-B Cell Receptor
- Proliferation
- IgL transcription
- Differentiation
Pre-BCR Signaling

PU.1 (transcription factor)

VpreB

Pre-BCR (or BCR)

Blnk

Lyn

Fyn

Syk

Igα/Igβ

PI3K

Tec

Btk

Proliferation

Differentiation

Defects result in:
Immune Deficiency
Malignancy

Assessing the Effect of DNA Breaks in Pre-B Cells

+ IL-7

Pre-B Cells

- IL-7

Cell cycle arrest
Induction of RAG
Light Chain Rearrangement

<table>
<thead>
<tr>
<th>Rag&lt;sup&gt;−/-&lt;/sup&gt;</th>
<th>Artemis&lt;sup&gt;−/-&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-7</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

No DSBs

+ DSBs

ATM

DNA Damage Response
DNA Breaks Suppress SYK and BLNK Transcripts in Small Pre-B Cells

![Image showing bar graph and Western blots for Syk mRNA transcripts and protein levels with different genotypes and conditions.](image-url)
RAG DSBs Activate NF-κB2

<table>
<thead>
<tr>
<th></th>
<th>Rag⁻/⁻</th>
<th>Artemis⁻/⁻</th>
<th>Artemis⁻/⁻: Atm⁻/⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL7 wd (d)</td>
<td>1  2</td>
<td>3  4</td>
<td>1  2  3  4</td>
</tr>
<tr>
<td>p100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RelB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gapdh</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
RAG DNA Breaks Induce Spic Through the Activation of ATM and NF-κB2

RAG DNA breaks → ATM → NF-κB2

Artemis\(^{-/-}\) → Spic
Phlda3
IL4i1
Wnt10

Nfkb2\(^{-/-}\) →
Ltb
Tank
Cxcr5
DNA Breaks Regulate *Spic* Expression in Small Pre-B Cells

- SPIC is an ETS-family transcription factor
  - Binds to the same DNA sequence as PU.1
  - Function is unknown
  - Dysregulated expression blocks B cell development at the pre-B cell stage
SPIC suppresses transcription of Syk and Blnk
Pre-BCR Signaling Coordinates Multiple Functions in Pre-B Cell Differentiation

- Early proliferative burst
- Overactivation of SYK supports leukemic transformation

DNA Breaks (SPIC)

IgL transcription

IgL rearrangement
SPIC expression inhibits pre-BCR signaling

1. Inhibits proliferation

2. Reduces \( Igl \) transcription

3. Suppresses new RAG DSB generation

Activation of SPIC limits additional RAG DSB generation and prevents cells with DSBs from re-entering cell cycle
How does SPIC function?
SPIC Displaces PU.1 at Blnk and Igk Promoters

PU.1 Binding at Blnk promoter

SPIC Binding at Blnk promoter
SPIC binds to BCLAF1

Co-IP of FLAG-tagged SPIC expressed in Artemis−/− pre-B cells

BCLAF1
- functions in DNA damage responses as a transcriptional regulator
- enforces cell cycle arrest and binds to H2AX to support DSB repair in response to irradiation-induced DNA damage
- Loss of BCLAF1 results in abnormal T cell development but the role of BCLAF1 in B cells and in Igl gene recombination is unknown.

BCLAF1 binds SPIC but not PU.1
BCLAF1 suppresses Syk expression

Expression relative to b-actin

<table>
<thead>
<tr>
<th>Rag(^{-/-})</th>
<th>Artemis(^{-/-})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>250</strong></td>
<td><strong>120</strong></td>
</tr>
</tbody>
</table>

BCLAF1 and GAPDH Western Blot:
- BCLAF1: shBclaf1 (−/+) on Artemis\(^{-/-}\)
- GAPDH: shBclaf1 (−/+) on Artemis\(^{-/-}\)

Syk mRNA

Syk mRNA (relative to Actb)

<table>
<thead>
<tr>
<th>shBclaf1:</th>
<th>Artemis(^{-/-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>−/+</td>
<td><strong>50</strong></td>
</tr>
</tbody>
</table>
DNA Damage Response Networks

External Sources
- Radiation
- Chemotherapy
- Environmental toxins

Internal Sources
- Cell Division
- Immune Cell Development
- Reactive Oxygen Species

Cell cycle transitions
- Apoptosis
- Transcription
- DNA repair

IR-induced DSBs activate canonical NF-κB

- Well established that genotoxic DSBs trigger ATM-dependent activation of canonical NF-κB signaling

IR-induced DSBs do not activate NF-κB2
IR-induced DSBs do not induce *Spic*
IR-induced DSBs do not suppress SYK and BLNK

\[ \text{Rag}^{-/-}:\text{Lig4}^{-/-} \]

<table>
<thead>
<tr>
<th></th>
<th>IR (0.5 Gy)</th>
<th>-</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLNK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-p53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Reconstitution of RAG in $Rag^{-/-}$-$Lig4^{-/-}$ pre-B cells leads to NF-$\kappa$B2 activation

<table>
<thead>
<tr>
<th></th>
<th>Artemis$^{-/-}$</th>
<th>$Rag^{-/-}$: Lig4$^{-/-}$</th>
<th>$Rag^{-/-}$: Lig4$^{-/-}$ + Rag1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSB ind.</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Germline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J$\kappa$1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J$\kappa$2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Artemis$^{-/-}$</th>
<th>$Rag^{-/-}$: Lig4$^{-/-}$</th>
<th>$Rag^{-/-}$: Lig4$^{-/-}$ + Rag1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSB ind.</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>p100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
How do cells distinguish between RAG DSBs and genotoxic DSBs?

- **Locus-dependent**: site of DSB in the genome directs cellular response. *Igk* locus may be populated by signaling intermediates that can be modified by ATM once activated.

- **Rag-dependent**: The RAG complex itself may direct cellular response by modifying/recruiting signaling intermediates.

- Use CRISPR-based system to introduce DSBs at targeted sites in the genome and assess DNA damage response.
Inducible CAS9 expression in pre-B cells

Rag1^-/-:Lig4^-/- + Tet-Cas9

Doxycycline
- +

Cas9

GAPDH

Rag1^-/-:Lig4^-/- + Cas9-R2CT

Proliferating
+ -

Cas9

GAPDH

- Phosphorylated by cyclinA-CDK2 and degraded in G2-S
- Stable expression in G1

Doxy
Tet-on
Cas9
RAG2 c-term

Phosphorylated by cyclinA-CDK2 and degraded in G2-S
Stable expression in G1
Generation of targeted DSBs using inducible CAS9 system

- Pre-B cells are arrested in G1 then transfected with gRNAs that target specific loci.
CAS9-mediated DSBs at *Tcrb* do not activate NF-κB2
Mechanism of DNA Injury Dictates Cell Response

**Physiologic DNA breaks**
- NF-κB1
- NF-κB2
- SpiC

**Genotoxic DNA breaks**
- p53
- Cell death

Why?
- Magnitude of DNA injury
- Location of DNA break
- Local context of the DNA break

Differentiation
RAG DSBs Activate Canonical Checkpoints

ATM

SPIC
- Cell cycle arrest
- Block new RAG DSBs

p53
- Cell cycle arrest
- Cell death
p53-Deficient Cells with RAG DSBs Arrest in G1

<table>
<thead>
<tr>
<th>CD40:</th>
<th>neg</th>
<th>pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p53^{+/+}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p53^{-/-}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- p53$^{-/-}$
  - No DSBs: 41% BrdU$^+$
  - + DSBs: 9%

- p53$^{+/+}$
  - No DSBs: 41% BrdU$^+$
  - + DSBs: 4%
Expression of SPIC and Activation of p53 Cooperate to Enforce G1 Arrest

DNA Breaks (SPIC) → Pre-BCR → SYK → proliferation → % S-phase

p53−/−

SYK: - - +
DSBs: - +
Canonical and Cell-Type Specific Checkpoint Pathways Cooperate to Enforce G1-arrest

ATM

SPIC

p53

SYK

Cell cycle arrest

Does loss of both Spic and p53 results in defective B cell development or generation of pre-B leukemia?
SYK in Leukemia

• Pediatric B cell leukemia
  – Often hijack components of normal signaling pathways to support survival and proliferation of leukemic cells

• Many pediatric pre-B ALL have increased SYK activity
  – There have not been any SYK coding mutations identified in pediatric leukemia
  – Constitutive SYK activity is required for survival of B cell chronic lymphocytic leukemia (CLL)
Inhibiting Syk as Potential Therapy for ALL

Bone Marrow

Spleen

CNS

Perova T et al. Sci Transl Med 6:236
Can We Capitalize on Unique Properties of DNA Damage Responses to Devise New Treatments?

- Combine pathway inhibitors or stimulators to force a desired response to DNA damage
  - Use genotoxic therapies to promote differentiation or achieve target cell effects

Genotoxic DNA breaks
(chemotherapy, radiation) → Cell-type specific response
Acknowledgements

Lynn White
Deepti Soodgupta
Katharina Wiendahl
Rachel Johnston
Brendan Mathias

Gene Oltz
Jackie Payton
Ken Murphy
Malay Haldar
Masako Kohyama
Peter McPherson
Razq Hakem

Funding
NIH K08
Alex’s Lemonade Stand Foundation
Siteman Investment Program