Artemis Function in DNA repair and Immunogenesis

DNA REPAIR VIDEOCONFERENCE

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A founder mutation in Artemis, an SNM1-like protein, causes SCID in Athabascan-speaking Native Americans.

Intersect of DNA Repair and Immunity

V(D)J Recombination

RAG 1/RAG2

Hairpin Opening

Artemis
Ku70/80
DNA-PKcs

Ligase IV/XRCC4

Essential for B and T Cell Maturation

Non-Homologous End Joining

Double-Strand Break

Unknown? Processing?

Critical for Cellular Survival
Treating Immunodeficiency in SCDA Children

The Problem

- Bone marrow transplantation is the only effective therapy for SCID.
- Both RAG and Artemis mutations present clinically as B-T- NK+ SCID.
- Effective bone marrow transplant requires immunoablative therapy (X-Rays, Chemotherapies)

**RAG SCID:** Immunoablation - better BMT engraftment.

**Artemis SCID:** Immunoablation - poor prognosis.

Can defining Artemis substrates allow us to identify a less toxic immunoablative therapy for Artemis-deficient SCID children?
Artemis Defects are Unlike other NHEJ Defects

Like all NHEJ defects: SCIDA Cells are Radiation Sensitive

Unlike Other NHEJ defects: Double-Strand Break Repair is NOT Grossly Defective in SCIDA cells


Hypothesis: Artemis function is essential for repair of a subset of DSBs.
SCIDA Cells are Not Equally Sensitive to IR and Etoposide Induced DSBs

**X-Rays**

- Heterogeneous DSBs (~50% 3-PG termini)

- Damage dose monitored by phosphorylation events

**Etoposide**

- Homogeneous DSBs 5’ blocked termini

![Graphs showing survival fraction vs. dose for X-Rays and Etoposide treatments]
So What Are Artemis Substrates In Vivo?

1) Only a small subset of IR induced DSBs require Artemis.

2) Are more abundant after IR than etoposide treatments.

3) Are a significant determinant of cellular survival.

**Characteristics of IR DSBs**

- Heterogeneous Breaks and Termini
- Clustered Damage on DNA
- Randomly Distributed in Genome
- 3’ Phosphoglycolates at Termini (~50%)
Artemis Efficiently Removes 3’ Blocking Groups

Artemis Registers nuclease on ds/ss DNA transition

36/23

42/23

48/23

Cleavage at ss/ds +5

APE1 and TDP1
Very Inefficient

Artemis can remove blocking moieties by ‘bypass’

36/27

36/23

36/21

X = -PG or -OH, or P-Tyr
Artemis Biochemical activities

- Artemis activity is strictly dependent on DNA-PK and ATP
- Artemis/DNA-PK can efficiently remove 3’ blocking moieties on a 3’ overhang via bypass.
- Artemis/DNA-PK is processive and can make multiple cuts


**Hypothesis:** Artemis functions to remove 3’ blocking groups at DSBs.
3’ Phosphoglycololate-Inducing Drugs

Are Treatments causing more 3’ blocking groups more toxic?

~ 50% PG  ~ 75% PG  ~ 100% PG

Toxicity does NOT obviously trend with PG induction.
Inverted Repeat

1) Would certainly be a small fraction of total DSBs.

2) Is consistent with Artemis substrate specificity known from V(D)J recombination (non-redundant).

3) Hairpin formation has long been suspected to be the cause of genomic instability arising from inverted repeats.

4) Consistent sequence independence of X-rays as opposed to drugs.

5) Slow 3’-PG removal may encourage hairpin formation by promoting 5’-3’ resections.
Take-Home Messages

- Artemis is only active in the context of DNA-PK.
- SCIDA cells are marginally defective in gross DSB repair.
- Artemis functions on a subset of DSBs in the cell.
- Not all DSBs are equally toxic to SCIDA cells.
- Derivative hairpin structures may be an Artemis substrate.
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