2566

CYT-0851 Suppresses RAD51 Focus Formation And Induces Replication Catastrophe Following AID Induced DNA Damage

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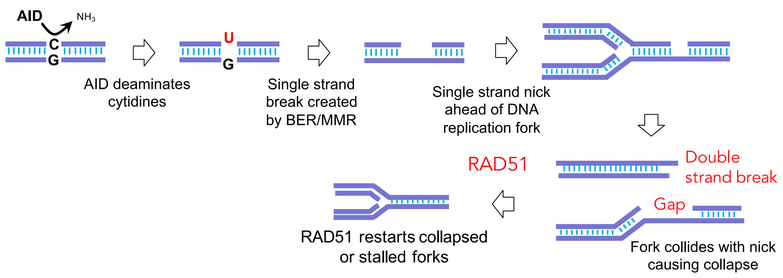
Abstract:

Activation Induced Cytidine Deaminase (AID) is a DNA-directed cytidine deaminase that plays a critical role in somatic hypermutation and immunoglobulin class switching in activated B-lymphocytes. AID is often ectopically expressed and promotes genomic hypermutation in a range of lymphoid malignancies. Deamination of genomic cytidines results in point mutations, single strand DNA breaks (SSB), and double strand DNA breaks (DSB). The resulting DNA damage stress leads to an obligate dependency on homologous recombination and RAD51 for cellular survival. We have previously shown that RAD51 depletion leads to accumulation of DNA breaks, and ultimately cell death, in AID expressing cells. We now describe the mechanism of action for a novel small molecule, CYT-0851, that targets RAD51 mediated recombination. Treatment of AID-expressing cells with CYT-0851 attenuates RAD51 focus formation and promotes accumulation of DNA damage. CYT-0851 treated cells undergo replication catastrophe associated with caspase 3/7 activation and persistent PCNA nuclear signal. We conclude that CYT-0851 is a novel homologous recombination inhibitor, and propose a new synthetic lethality mechanism, induced by CYT-0851, mediated by DNA replication stress and replication catastrophe.

Background:

AID Induced Damage And RAD51 Dependency



AID expressing cancers experience DNA replication stress resulting in double strand breaks. RAD51, a key component of the homologous recombination pathway, helps to stabilize DNA replication forks, repair the double strand break, and allow for the continuation of DNA synthesis.

The AID-RAD51 Axis In Cancer: A Selective Synthetic Lethality Target

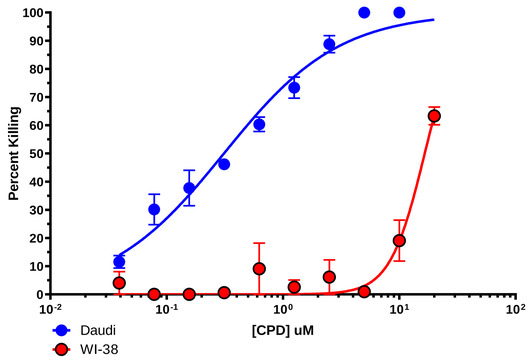


Figure 1. Daudi (AID+) and WI-38 (AID-) cells were treated with a range of concentrations of CYT-0851 for seven days and read with CellTiter-Glo. Viability was assessed relative to vehicle treated cells. Plotted values represent mean of 3 biological replicates and SEM.

Results:

CYT-0851 Suppresses RAD51 Foci Formation in Lymphoblastic Lymphoma

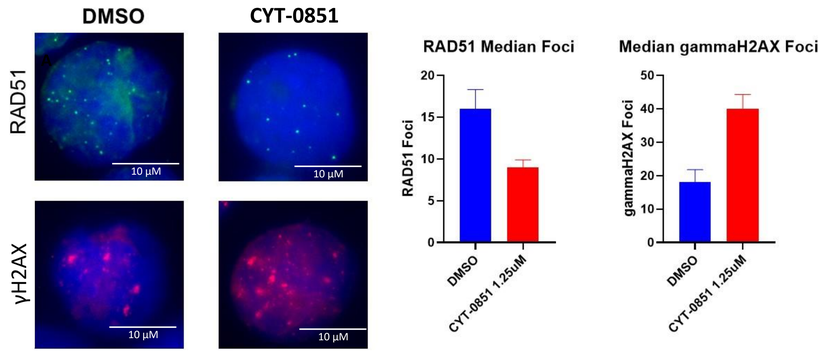


Figure 2. Upon DNA breakage the protein histone H2AX is phosphorylated (γH2AX). This acts as a DNA damage marker signaling the recruitment of DNA repair proteins, including RAD51, to the site**.** U698 cells, a B-cell lymphoma cell line, was treated with either DMSO or CYT-0851 for four days, before being fixed and stained for either phosphorylated H2AX or RAD51. Plotted values show mean and SEM.

CYT-0851 Suppresses RAD51 Foci Formation in Burkitt Lymphoma

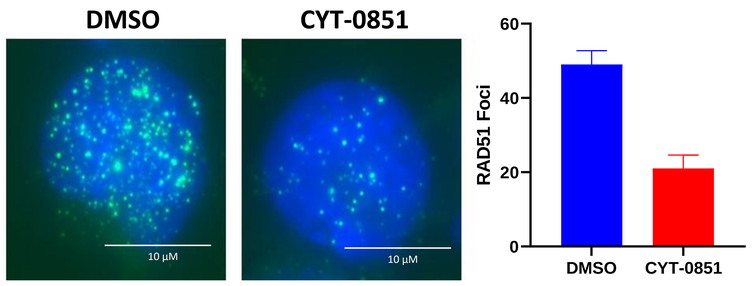


Figure 3. CYT-0851 reduces RAD51 foci after UV damage stimulation**.** Daudi cells, a Burkitt lymphoma cell line, was treated with either DMSO or CYT-0851 for 3 days, before being exposed to UV irradiation. The cells were allowed to recover for 3 hours and then fixed and stained for RAD51 (green). Plotted values show mean and SEM.

CYT-0851 Reduces Homologous Recombination

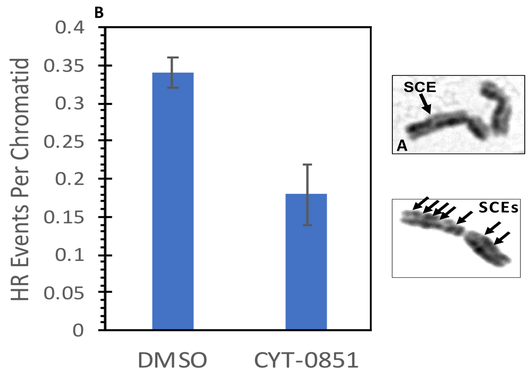
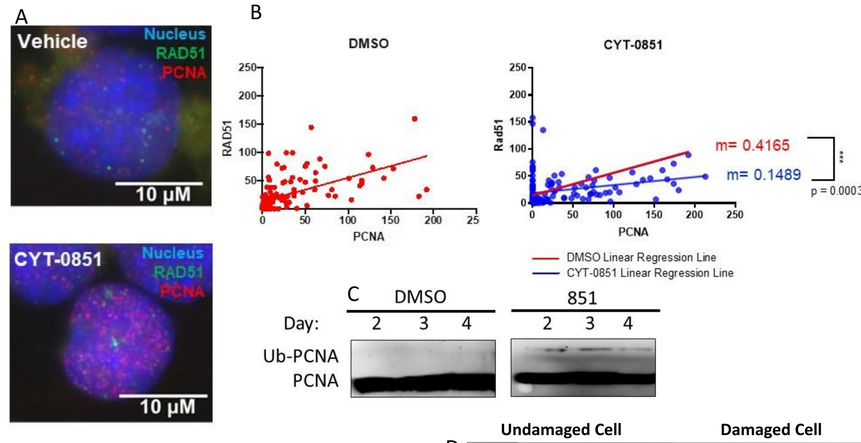


Figure 4. A sister chromatid exchange assay was performed to visualize and count homologous repair (HR) events (**A**)**.** This assay uses the incorporation of BrdU into chromosomes resulting in differential staining of parent and daughter chromatids to visualize the exchange of genetic material between the two. The exchanges occur via double strand breaks and HR. HR events were quantified for both CYT-0851 and DMSO treated conditions (**B**)**.**

CYT-0851 Promotes AID-Induced Replication Catastrophe



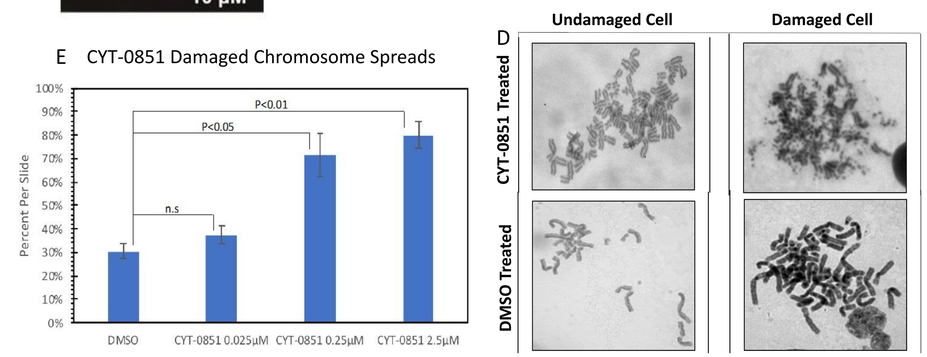


Figure 5. Daudi cells treated with 625nM CYT-0851 and stained for RAD51 and PCNA. **(A)** Blue is DAPI, green is RAD51, and red is PCNA. (**B**)Focus counts were plotted on a per-cell basis for both DMSO treated cells and CYT-0851 treated cells. A linear regression was performed on both plots. Western blot (**C**) was performed on nuclear protein extracts from Daudi cells treated for up to 4 days with either DMSO or CYT-0851 then probed for PCNA. (**D**) Metaphase spreads isolated from cells treated with CYT-0851 or vehicle. (**E)** Mean percentage of damaged chromosome spreads per slide. Error bars represent SEM.

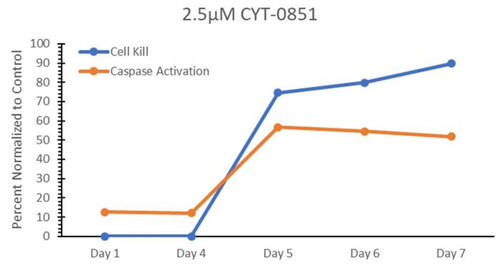


Figure 6. Simultaneous analysis of cell viability and caspase activation was measured in cells treated with CYT-0851 or vehicle using the Promega Apo-Tox-Glo Triplex assay**.** The graph represents the average of three repeats.

Conclusions:

- **CYT-0851 has a greater than 100-fold selectivity for AID positive cells *in vitro*.**
- **CYT-0851 reduces RAD51 foci formation and homologous recombination activity.**
- **CYT-0851 promotes the AID-dependent accumulation of DNA damage.**
- **Resulting unrepaired DNA damage leads to replication and mitotic catastrophe.**
- **Replication/mitotic catastrophe results in caspase activation and cell death.**

References:

- Feldhahnet al., JExp Med.2007May 14;204(5):1157-66
- Lamontet al.,J Exp Med.2013May 6;210(5):1021-33

*TM, MD, and KM are employees at Cyteir Therapeutics, JV, CM, and AC are paid consultants at Cyteir Therapeutics