Genotoxicity Disrupts Intestinal Proliferating Cells

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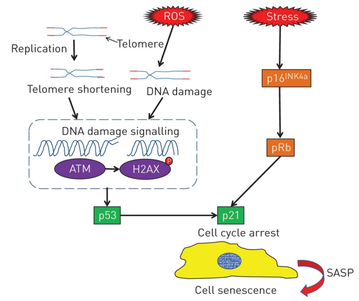
ABSTRACT

Many bacterial infections have been shown to cause DNA damage in host cells, and a number of pathogens can directly damage host DNA by producing genotoxins. Due to the importance of maintaining genomic integrity for cellular function, cells possess a coordinated DNA damage response (DDR) mechanism to sense damage in DNA and generate a signal amplification cascade to activate DNA repair mediators. Our study has shown that cytolethal distending toxins (CDTs), a genotoxin secreted by mucosal pathogenic bacteria, is responsible for DNA damage and thus prolonged cell cycle arrest in intoxicated intestinal proliferating crypt cells. Our data support that CDT intoxication results in a large increase in activated H2AX level, an early DNA damage signal, and in cellular p53 levels, a major cellular protein responsible for cell cycle arrest in response to DNA damage. Also, we found that CDT-mediated DNA damage results in reduction of a transcription factor involved in the differentiation potential of intestinal proliferating cells, Snai1, which is responsible for lineage allocation in differentiating cells. In order to evaluate whether pathogen-independent DNA damaging agents can lead to reduction in cellular levels of Snai1 in normal human intestinal proliferating cells, used three DNA damage agents, including 5-fluorouracil and etoposide, each of which causes DNA damage in different ways. Our data support that CDT-independent DNA damage lead to dose-dependent reduction in Snai1 level. These experiments enhance our understanding of potential side effects of widely used chemotherapeutics on epithelial barrier homeostasis and overall innate immunity.

DNA Damaging Agents and DNA Damage Response (DDR)

DNA Damage Response Pathway

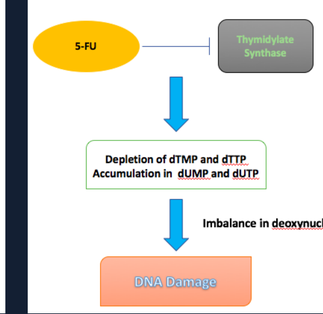


Barnes 2015

- pH2AX adjacent to DNA damage site serves as early DNA damage signal, which then triggers ATM/ATR pathway in DDR.
- Amplification of DNA damage signal results in stabilization of cellular p53 level, known as "Guardian of the Genome".
- p53, a major cellular protein, is responsible for cell cycle arrest and cell fate determination in response to DNA damage.

How does Anti-Cancer Drugs Cause DNA Damage?

5-Fluorouracil (5-FU)



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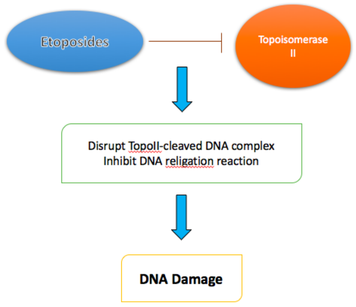
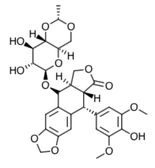
Etoposides



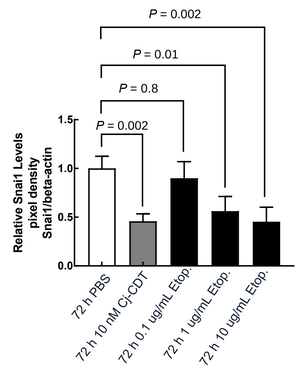


New Global Enterprises

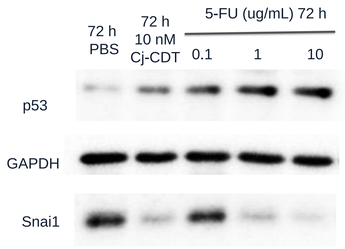
RESULTS

Etoposides (Etop.) mediated DNA Damage causes Snai1 reduction

Yingxing Li, Blanke SR. Unpublished 2018



5-Fluorouracil (5-FU) mediated DNA Damage causes Snai1 reduction



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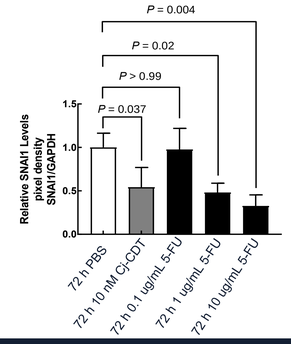
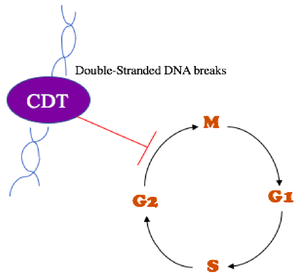
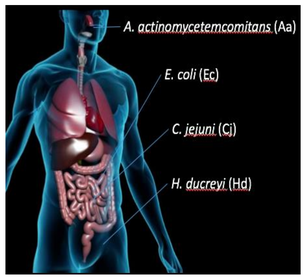


Cytolethal Distending Toxin (CDT)

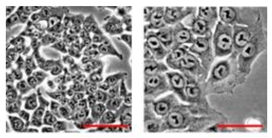


Cytolethal Distending Toxin (CDT):

- Secreted toxin
- Produced by gram-negative pathogenic bacteria
- Changes cell morphology
- DNase I activity
- Generates single-stranded nicks and double-stranded breaks in DNA
- Causes G2/M cell cycle arrest



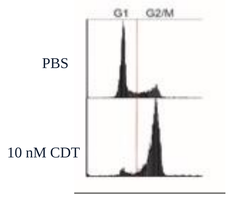
https://happydeviant.files.wordpress.com/2011/01/gi.jpg



PBS 10 nM CDT

CDT intoxication induces cellular distension (24h treatment)

Lieu DJ, Blanke SR. Unpublished



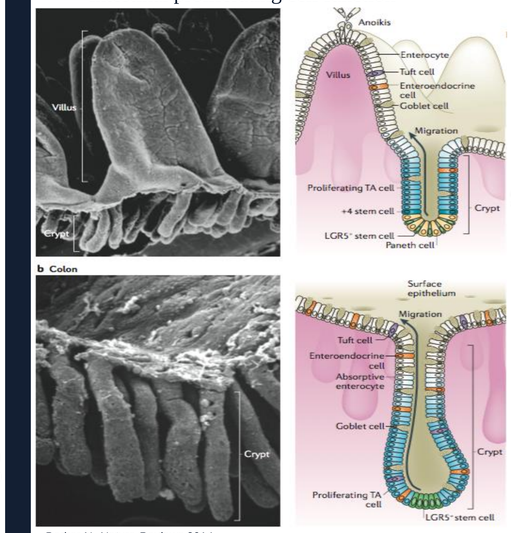
CDT intoxication induces G2/M cell cycle arrest (24h treatment)

Lieu DJ, Blanke SR. Unpublished

Pathogen-Mediated DNA Damage Effects during Infection

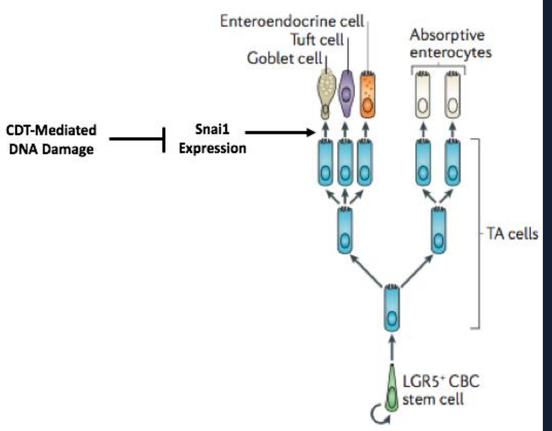
Gap in Knowledge:

- How DDR and cell cycle arrest affect intestinal proliferating cell function



Barker N. *Nature Reviews* 2014

We believe that pathogen-dependent and independent DNA damage leads to reduction in cellular Snai1 level in normal intestinal proliferating cells



Barker N. *Nature Reviews* 2014

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- Paul Kaminski
- Michidmaa Enkhbaatar

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