Impact of Mgmt-Mediated DNA Repair on Mutation Susceptibility & Cancer in Mice

Lee J. Pribyl[1], Kay JE[1,2], Corrigan JJ[1], Owiti NA[1], Armijo AL[1], Fedeles BI[1], Carrasco SE[1], Croy RG[1], Dertinger S[3], Wadduwage DN[4], Essigmann JM[1], Engelward BP[1]

[1]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, [2]Silent Spring Institute, Newton, MA, [3]Litron Laboratories, Rochester, NY, [4]Center for Advanced Imaging, Harvard University, Cambridge, MA

SRP MIT CEHS MIT BE BIOLOGICAL ENGINEERING

NIH National Institute of Environmental Health Sciences Superfund Research Program

The Engelward Lab

Abstract

N-nitrosodimethylamine (NDMA) forms DNA lesions that are highly mutagenic and has been shown to be carcinogenic in animal models. Lesions produced by NDMA exposure include 3-methyladenine (3MeA), 7-methylguanine (7MeG), and O^6-methylguanine (O^6MeG). The direct reversal protein O^6-methylguanine-DNA methyltransferase (Mgmt) removes the methyl group from O^6MeG to restore the guanine. Failure to repair DNA damage leads to mutations, and since people are variable in their DNA repair capacity, Mgmt may be an important susceptibility factor for NDMA-induced cancer.

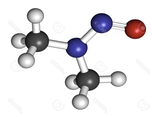
Using mouse models, we are studying Mgmt for variations in NDMA-induced DNA damage, mutagenesis and cancer. Utilizing the *Mgmt* null mouse we were able to query the impact of repair. Our experimental mice have been bred to harbor two additional transgenic markers: 1) the *Gpt* delta transgene, which enables high-resolution analysis of point mutations; and 2) the RaDR transgene, which allows for *in situ* fluorescence detection of sequence rearrangement mutations (homologous recombination events). In addition to detection of *de novo* recombination events, RaDR mice enable analyses of clonal expansion of mutant populations.

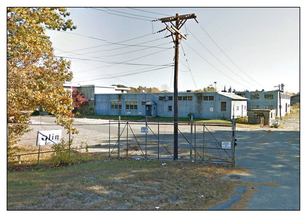
We are studying cellular responses within 1-10 days after NDMA exposure, including signaling pathways, inflammation, DNA damage, apoptosis, and associated regenerative proliferation. Mutations and sustained alterations in cell signaling/behavior are being analyzed at 10, 30, 50 and 70 days after exposure (prior to cancer development); and cell signaling, mutations, clonal expansion, and carcinogenesis at 10 months post exposure. Together, these analyses will provide an integrated view of the mechanistic progression from specific DNA lesions to cancer.

- Preliminary results from the liver 70 days (10 weeks) after NDMA exposure indicate that NDMA is a potent inducer of sequence rearrangement mutations and that *Mgmt*$^{-/-}$ animals accumulate substantially more mutant cells. NDMA-treated *Mgmt*$^{-/-}$ animals also display apparent liver fibrosis and enlarged spleens, indicative of accelerated disease progression relative to WT animals.

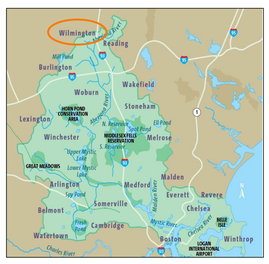
NDMA & DNA Repair

N-nitrosodimethylamine (NDMA) is a contaminant found at Superfund sites that causes cancer via DNA alkylation damage, mutations, and promotion of inflammation



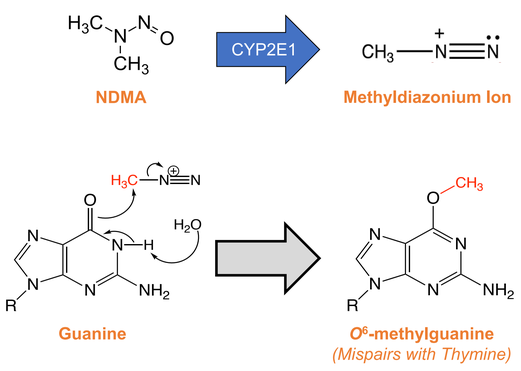


Olin Chemical Superfund Site
Wilmington, MA
Linked to 1990's childhood cancer cluster

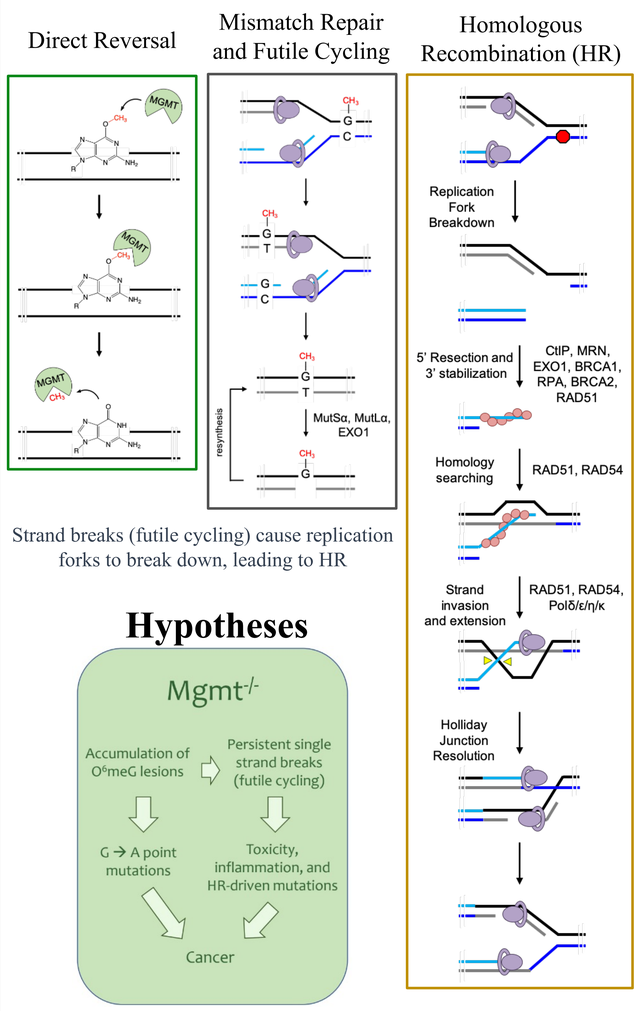


Mystic River Watershed

Metabolic activation of NDMA produces a reactive intermediate that methylates DNA, causing mutations

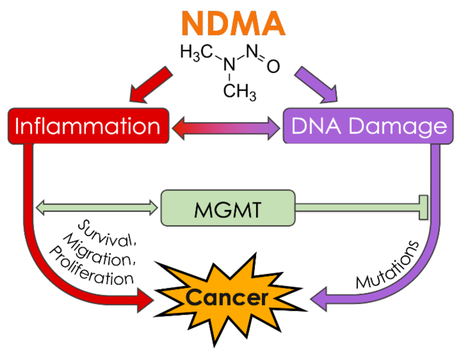


DNA Repair Pathways that Address NDMA-Induced DNA Lesions



Strand breaks (futile cycling) cause replication forks to break down, leading to HR

Hypotheses



DNA Damage, Mutations & Cancer

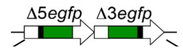
Detecting Mutations *In Situ*

RaDR mice contain a direct repeat of truncated EGFP sequences at the Rosa26 locus

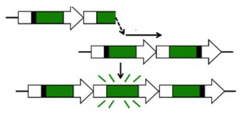
Homologous recombination allows exchange of genetic material between sister chromatids and formation of full length EGFP

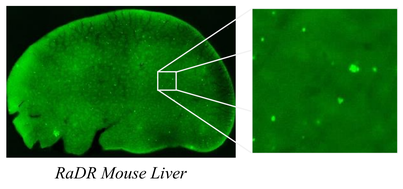
Mutations create fluorescence in the cell as well as its daughters, enabling detection of *de novo* mutation events and clonal expansion of mutant populations

RaDR Transgene



Example of RaDR Mutation Mechanism

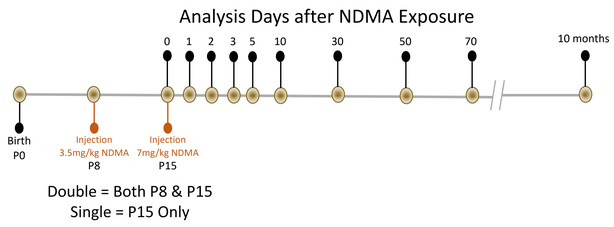




RaDR Mouse Liver

Detect mutant cells *in situ* with whole mount fluorescent microscopy

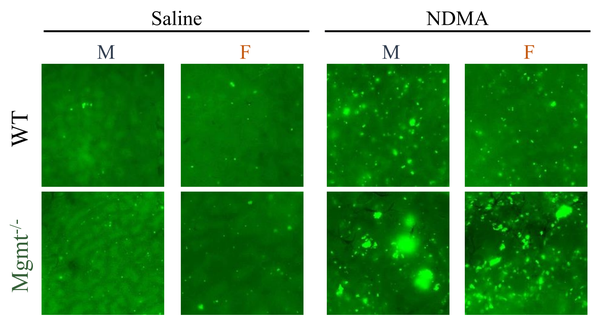
Experimental Design



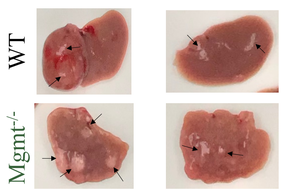
Initial Results

Liver Mutations

10 weeks after double exposure, NDMA has potently induced HR-driven mutations in the liver, most significantly in Mgmt$^{-/-}$ mice

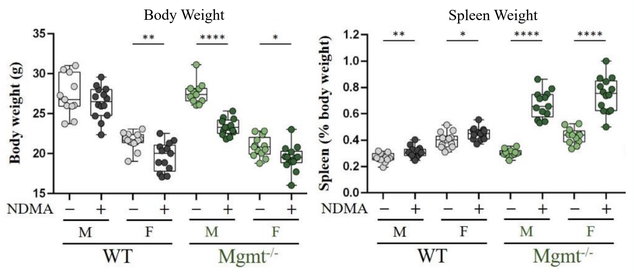


10 months after double exposure, NDMA induces liver tumors most significantly in Mgmt$^{-/-}$ male mice



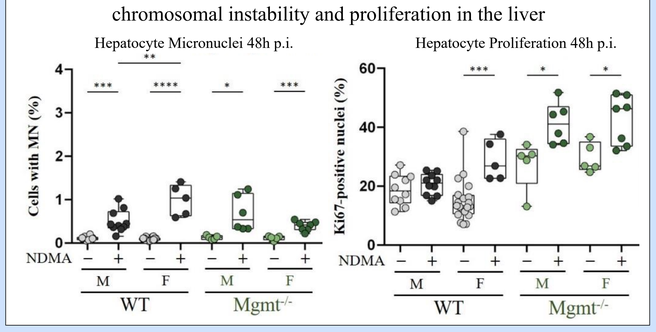
Overall Health

After 10 weeks, double NDMA exposure results in smaller body weight and larger spleens, primarily in the Mgmt$^{-/-}$ animals



Liver Damage

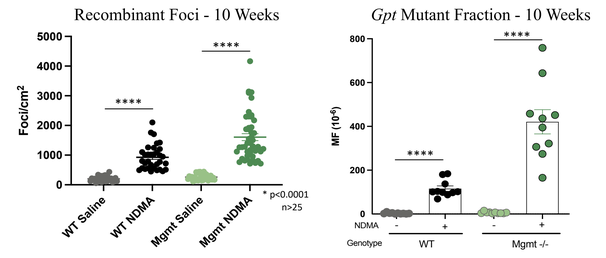
48 hours post double NDMA exposure induces rapid chromosomal instability and proliferation in the liver



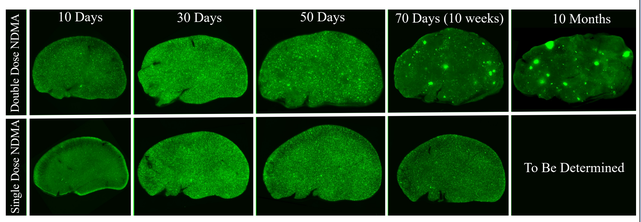
Results

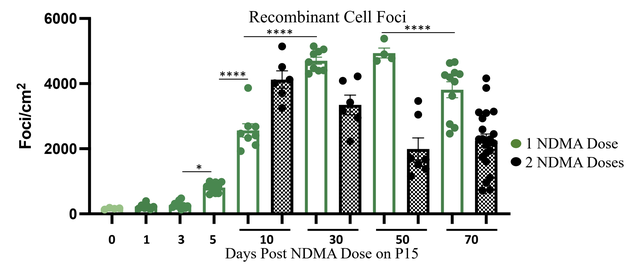
Mutation Accumulation

Recombinant foci (large-scale mutations) and *Gpt* (point mutations) are increased significantly post exposure to NDMA after 10 weeks

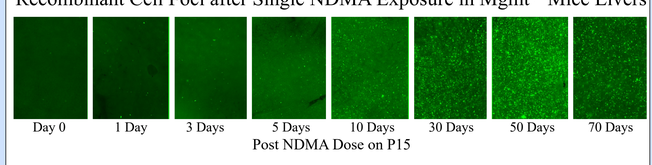


Single vs double exposure to NDMA creates distinct persistent recombination and mutational patterns within the liver of Mgmt$^{-/-}$ mice over time



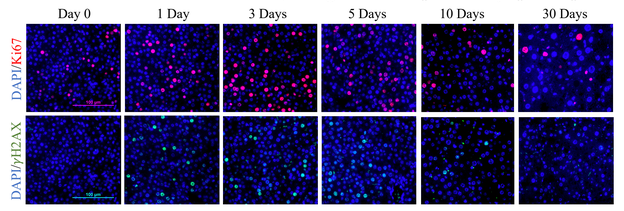


Recombinant Cell Foci after Single NDMA Exposure in Mgmt$^{-/-}$ Mice Livers

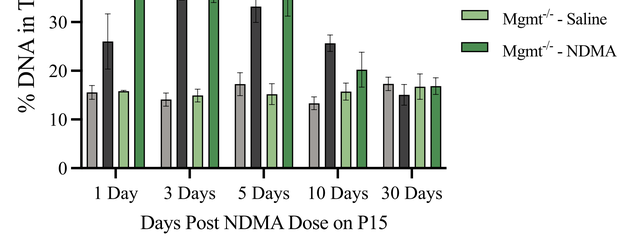


DNA Strand Breaks

Single exposure of NDMA induces early proliferation and DNA damage in the form of double strand breaks in the liver, in Mgmt$^{-/-}$ mice up to 10 days post exposure

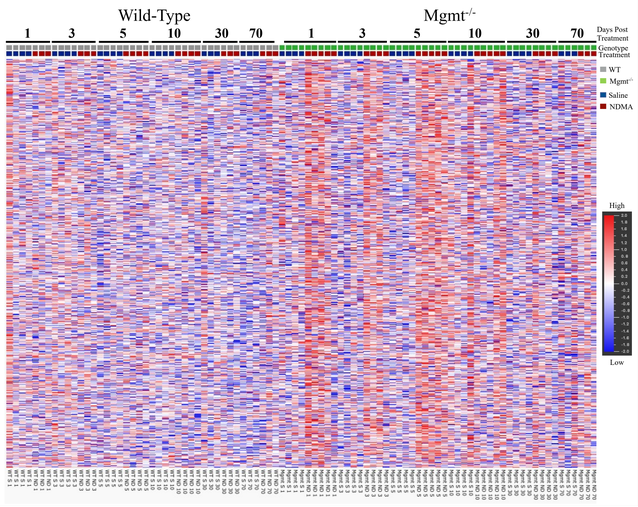


Single exposure of NDMA induces DNA damage in the form of single strand breaks in the liver, in WT and Mgmt$^{-/-}$ mice up to 10 days post exposure measured by the CometChip Assay



Gene Expression Analysis

Initial RNA-Sequencing analysis indicates increased gene expression in Mgmt$^{-/-}$ animals after 1-5 days post exposure of a single dose of NDMA



Acknowledgements

This work is supported by NIEHS Superfund Research Program Grant P42 ES027707, NIEHS Core Center Grant P30-ES002109, and NIH Training Grant T32-ES007020. We are grateful to the MIT Division of Comparative Medicine for assistance in creating animal strains and histological services.