Contract No:

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Disclaimer:

Deuterium Concentration Effects on Cell Cycle Progression

Deuterium (D), which is found in natural water at ~ 150 ppm, seems to play an important role in biology; however, research in this field has likely been stalled by the limited availability of D2O with varying D concentrations needed to accurately study the deuterium effects in biological systems. SRNL can currently manufacture D2O in varying concentrations, and we have assembled an interdisciplinary research group to study cell cycle in human normal and cancer cells as a function of D concentration over time in order to address several fundamental science questions.

Awards and Recognition

None

Intellectual Property Review

This report has been reviewed by SRNL Legal Counsel for intellectual property considerations and is approved to be publicly published in its current form.

SRNL Legal Signature

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Signature **Date**

Deuterium Concentration Effects on Cell Cycle Progression

Project Team: Wendy Kuhne (Primary), Candace J. Langan, Lucas Angelette,

Collaborators: Lesleyann Hawthorne (Augusta University)

Project Type: Discovery Science

Project Start Date: October 1, 2019
Project End Date: August 21, 2020

Deuterium (D), which is found in natural water at ~ 150 ppm, seems to play an important role in biology. For example, D concentrations above 150 ppm are known to produce toxic effects in many organisms. There is also evidence to suggest D levels significantly less than 150 ppm can cause delays in progression through the normal mitotic cell cycle. Some have even theorized that the D:H ratio in cells may impact an organism's radiation resistance. Therefore, evaluating the role of D and the D:H ratio in eukaryotic and prokaryotic cells should lead to a better understanding of cell cycle progression and radiation resistance in these organisms. Research in this field has likely been stalled by the limited availability of D_2O with varying D concentrations needed to accurately study the deuterium effects. However, SRNL can currently manufacture D_2O in varying concentrations, and we have assembled a unique team of radiation biologists, microbiologists, radiochemists, and health physics to form an interdisciplinary research group to study the cell cycle as a function of D concentration in order to address several fundamental science questions. Proposed work in FY20 was a collaborative effort with Augusta University to utilized BSL-2 mammalian cell lines. Lab work was halted due to the COVID-19 pandemic. An intensive literature review was performed and identified pertinent knowledge gaps that could be filled in future research efforts.

FY2020 Objectives

- Generation of deuterium depleted water (D_2O) for cell culture studies in concentrations of 50, 75, 100, 124 and 140 ppm). Additional D_2O above 150 ppm was also generated.
- Perform initial cell culture survival studies and measure effects to cell cycle progression.
- During cell culture survival studies, measure D_2O concentrations to ensure levels are maintained throughout the study using the PICARRO and measure changes to the cell culture media via ICP-MS

Introduction

Investigation into the effect of deuterium depleted water (DDW) on biological systems began less than 20 years ago.[1] Much of the published literature has focused on the effect of DDW on the phenotypic growth of normal/stem cells, tumor transplantation models, and tumor cell systems, with little emphasis on molecular mechanisms involved [2]. The few studies that attempt to investigate the effect of DDW on normal eukaryotic cells are either under hypoxic conditions, are poorly controlled and are ultimately largely uninformative [3]. The lack of information in this regard creates an opportunity to investigate and understand the mechanisms associated with why cell cycle is delayed under DDW conditions in a controlled manner.

The role of D in biology is thought to be a missing piece in understanding cancer and cancer epidemics in western populations. Little has been revealed on the time-dependent effects of DDW on normal/cancer human cells *or* how the reduction of cell growth/proliferation is associated with cell cycle

regulation/consequence on gene expression. Many different genes are involved in cell cycle regulation and activation of specific genes dictates response to cell proliferation (Figure 1).

The purpose of the project is to unravel the novel mechanisms involved with the effect of DDW, in differing amounts, on normal and cancerous human cell proliferation. Phenotypic and mechanistic changes will be monitored, focusing on cell cycle aberrations induced and genes dysregulated upon DDW treatment in a time-dependent manner. Genes relevant to cell cycle regulation and DNA repair mechanisms will be investigated further to determine the effects caused by depletion of deuterium.

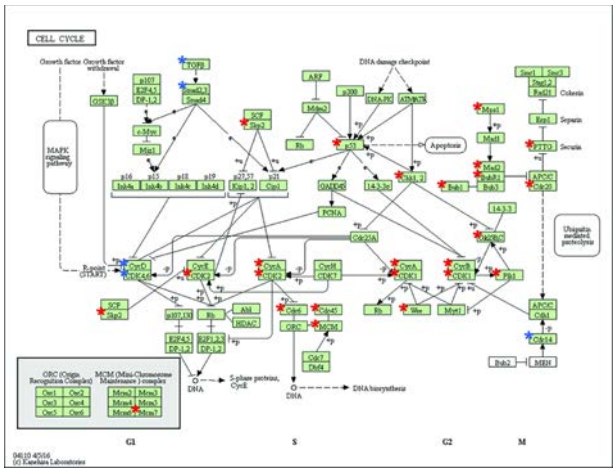

Figure 1. Genes and regulation of the human cell cycle pathway as described in KEGG (Kyoto Encyclopedia of Genes and Genomes) (http://www.genome.jp/kegg/).

These studies would provide basis for further investigation into the radioprotective effects of DDW onto normal cells. Research in mouse models indicate that DDW can reduce the effects of DNA damage caused by radiation [4], and with this study we hope to bridge our understanding between DDW use in normal cells, cell cycle arrest mechanisms, and eventually radioprotective effects in humans. By filling in this gap in the literature, we can potentially provide intervention opportunities to further explore, such as in situations when humans are exposed to short-term radiation and DNA damage-causing agents (focused-radiation chemotherapy, space-inflected radiation exposure).

Approach

FY20 proposed experiments were to investigate phenotypic changes on human cells upon treatment of varying concentrations of DDW and concentrations above 150 ppm in a time-dependent manner. The cell lines would represent normal and cancer cell lines from breast and colon tissues. Cell line assays typically last several days with nutrient media changes occurring at varying intervals during the assay. The concentration of D in the media during the assay would be measured using a PICCARO L2140-I Isotope and Gas Concentration Analyzer which has limits of detection to 50 ppb (Santa Clara, CA). SEM/TEM Microscopy/Imaging will be used to monitor cell morphology. Proliferation as measured by cell cycle progression and cellular growth rate will be determined using cell counting techniques using a FACSMelody™ (BD BioSciences, San Jose, CA).

Molecular changes in the transcriptome upon treatment of varying concentrations of DDW and concentrations above 150 ppm will be evaluated in a time-dependent manner. RNA-sequencing (RNA-seq) using the MinION sequencer (Oxford Nanopore Technologies, UK) will be performed to examine all transcripts/ gene patterns that may be dysregulated upon DDW treatment. We will focus on suspected pathways including cell cycle, metabolism, cell death and DNA repair. Genes/proteins of interest will be validated using RT-qPCR and SDS-PAGE protein detection.

Results/Discussion

All lab work was halted due to COVID-19 pandemic. All cell experiments will be conducted in FY21.

FY2020 Accomplishments

- Intensive literature review was completed and it was determined that significant knowledge gap exists and future work should focus on 1) the time-dependent effects of DDW on normal/cancer human cells and 2) investigate if a reduction of cell growth/proliferation is associated with cell cycle regulation/consequence on gene expression.
- Bio Safety Level 2 laboratory space has been identified and is under review by SRS Bio Safety Committee for FY21.

Future Directions

- Obtain cell cultures from approved vendors (example; ATCC)
- Experimental approaches described in FY20 will be completed in FY21 at SRNL.

FY 2020 Peer-reviewed/Non-peer reviewed Publications

None

References

1. Hohlefelder, L. S., Heavy water reduces GFP expression in prokaryotic cell-free assay at teh translation leel while stimulating its transcription *BioMed Research International* **2013**, 1-9.

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4. W Bild, I. S., I Haulica, C Lupuşoru, G Titescu, R Iliescu, V Nastasa, Research Concerning the Radioprotective and Immunostimulating Effects of Deuterium-Depleted Water. *Romanian journal of physiology : physiological sciences* **1999,** *36* (3-4), 205-218.

Acronyms

D	Deuterium
D_2O	Deuterium Oxide
DDW	Deuterium Depleted Water
L	Liter
µg	microgram (one millionth of a gram)
mg	milligram (one thousandth of a gram)
ppb	parts per billion (µg/L)
ppm	parts per million (mg/L)
RNA	Ribonucleic Acid
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy

Intellectual Property

There is no intellectual property to report for this effort.

Total Number of Post-Doctoral Researchers

One - (Dr. Candace Langan SRNL Postdoctoral Scientist)

Total Number of Student Researchers

None

External Collaborators (Universities, etc.)

Augusta University (Dr. Lesleyann Hawthorne)