Contract No:

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Disclaimer:

Title of Project
Deuterium Concentration Effects on Cell Cycle Progression

Project Start and End Dates
Project Start Date: October 1, 2020
Project End Date: July 30, 2021

Project Highlight
This project is conducting basic science to investigate the role of Deuterium (D) concentrations on cell cycle regulation over time. Evaluating the role of D in human cells should lead to a better understanding of cell cycle progression on normal and cancer human cells and if there is a link to radiation resistance through genomic investigation.

Project Team
Principal Investigator: Wendy Kuhne
Team Members: Candace Langan and Lucas Angelette
External Collaborators: None

Abstract
Deuterium (D) seems to play an important role in biology and is thought to be a missing piece in understanding cancer and radiation resistance. D is found in natural water at a concentration of ~150 parts per million (ppm), while D concentrations above 150 ppm are known to produce toxic effects in many organisms. There is evidence to suggest D levels significantly less than 150 ppm can cause delays in cell progression through the normal mitotic cell cycle. Some have theorized that the deuterium: hydrogen ratio (D:H) in cells may impact radiation resistance. Therefore, evaluating the role of D in human cells should lead to a better understanding of cell cycle progression and radiation resistance. To date, little has been revealed on the time-dependent effects of deuterium-depleted water (DDW – less than 150 ppm) on normal and cancer human cells *or* how the reduction of cell proliferation is associated with cell cycle regulation and consequence on gene expression profiles. Our studies will help further the mission of the Department of Energy to enhance the understanding of deuterium in fundamental biology by studying the cell cycle as a function of D concentration.

Objectives
- Perform kinetic studies of D levels with 5% CO_2 and temperature inside of the incubator over time.
- Perform phenotypic investigations of normal and cancer cells with varying concentrations of Deuterium Water (D_2O) treatment over time. Measure morphology changes and proliferation/growth rate changes using microscopy and BD FACSMelody^{TM} cell sorter.
- Perform molecular investigations of normal and cancer cells with varying concentrations of D_2O treatment over time- gene expression determination using RNA-seq, RT-qPCR and SDS-PAGE.

REVIEWS AND APPROVALS

1. Authors:

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Name and Signature Date

2. Technical Review:

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Name and Signature Date

3. PI's Manager Signature:

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Name and Signature Date

4. 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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Name and Signature

Introduction

Investigation into the effect of deuterium depleted water (DDW), concentrations below the normal 150 ppm, 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.[3,4] The lack of information in this regard creates an opportunity to investigate and understand the mechanisms associated with why the (or a) 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.

The purpose of the project is to unravel the novel mechanisms involved with the effect of DDW, in differing concentrations, on normal and cancerous human cell proliferation.

Approach

Investigate phenotypic changes on human cells upon treatment of varying concentrations of DDW/D_2O in a time-dependent manner. We will culture normal/cancer human cells in varying concentrations of DDW/D_2O in cell growth media/control media, and measure D concentrations using a PICARO Isotope and Gas Concentration Analyzer. Microscopy/Imaging will be used to monitor cell morphology changes with exposure to DDW. Proliferation/growth rate will be determined using cell counting techniques.

We will investigate molecular changes in the transcriptome upon treatment of varying concentrations of DDW/D_2O in a time-dependent manner. Cells will be collected at various time points for molecular analysis. RNA-sequencing (RNA-seq) 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.

Accomplishments

- Kinetics of Deuterium (D) exchange with ambient air and characterization of background deuterium levels in water and Dulbecco's Modified Eagle Medium (DMEM) cell media. No significant change in D concentration was measured over the course of three days in water (**Figure 1**) or in prepared cell culture media (**Figure 2**) at ambient conditions.
- Cell cultures were initiated with Biosafety Level 1 HEL 299 (human lung normal) and A549 (human lung cancer epithelial line).

Future Directions

- Complete kinetic studies of D levels with 5% CO_2 and temperature over time.
- Complete phenotypic investigations of normal and cancer cells with varying concentrations of DDW/D_2O treatment over time. Measure morphology changes and proliferation/growth rate

changes using microscopy and BD FACSMelody™ cell sorter. (Initial studies began in FY21 proposed continuation in FY22).

- Molecular investigations of normal and cancer cells with varying concentrations of DDW/D_2O treatment over time – gene expression determination using RNA-seq, RT-qPCR and SDS-PAGE verification (FY22).

FY 2021 Peer-reviewed/Non-peer reviewed Publications
None

Intellectual Property
None

Total Number of Post-Doctoral Researchers
Candace Langan, PhD

Total Number of Student Researchers
None

References
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3. Zlatska, O.V.; Zubrov, D.O.; Vasyliev, R.G.; Syroeshkin, A.V.; Zlatskiy, I.A. Deuterium Effect on Proliferation and Clonogenic Potential of Human Dermal Fibroblasts In Vitro. *Problems of Cryobiology and Cryomedicine.* **2018**.28(1):49-53. DOI:10.1155/2018/5454367
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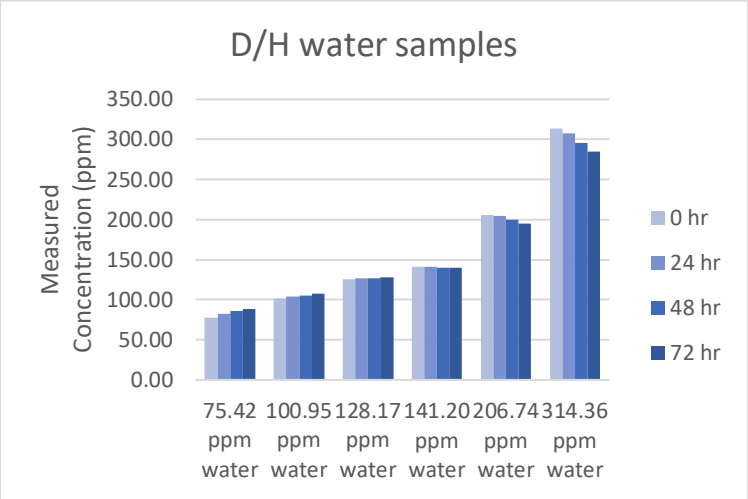

Figure 1: Deuterium concentration (ppm) measured in water only at ambient conditions for 72 hr.

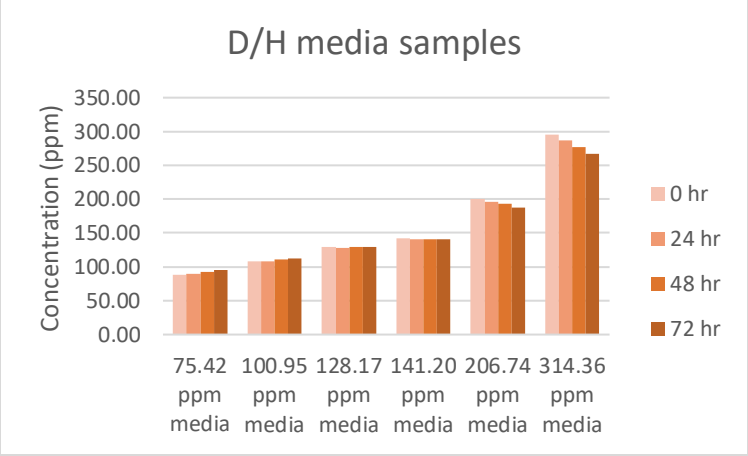

Figure 2: Deuterium concentration (ppm) measured in DMEM growth media at ambient conditions for 72 hr.