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TITLE: Effects of Ionizing Radiation (IR) on Myocardial DNA Methylation Profiles in Relation to Cardiomyopathy in a Nonhuman Primate Model

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Effects of Ionizing Radiation (IR) on Myocardial DNA Methylation Profiles in Relation to Cardiomyopathy in a Nonhuman Primate Model

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Specific Aims were to determine 1: The effects of radiation exposure on the myocardial DNA methylome; 2: Relationships between myocardial methylation profiles and cardiomyopathy (myocardial fibrosis, heart structural and functional characteristics; and 3: Relationships between myocardial DNA methylation profiles and myocardial transcriptional profiles, the latter supported by another mechanism. **Progress**: Aim 1: We have completed the tissue distribution and processing, DNA isolation and characterization, and are finalizing plans for the DNA methylation and analysis work. Aim 2: Cardiac phenotyping completed. Aim 3: We are conducting analysis of the transcriptomes of these same tissues using RNAseq as well as Nanostring technologies, providing significant support for the planned methylation profiling. Our work on the myocardial gene expression profiles and other cardiac phenotyping required for final completion of a manuscript has been substantial and sets the stage for a significant body of work when combined with the proposed work.

15. SUBJECT TERMS
Epigenetics, genomics, myocardium, heart disease, fibrosis, DNA methylation, radiation, countermeasures, inflammation

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REPORT OUTLINE

The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

1. **INTRODUCTION:**

Cardiomyopathy is a condition in which heart muscle becomes damaged and dysfunctional due to a variety of causes, including exposure to ionizing radiation such as one might experience in a nuclear event, and radiation Induced Heart Disease (RIHD) is common delayed effect of acute radiation exposure in survivors of cancer related radiotherapy and military and accidental radiation exposures. Japanese survivors of the Hiroshima and Nagasaki atomic bombings have a markedly increased predisposition to heart and cardiovascular disease. Unfortunately, there are no accepted recommendations for how to prevent or treat RIHD. Expanding global threats which could lead to malicious military or terrorism related radiation injury are considerable and relevant in today's world. Biological pathways involved in radiation induced cardiomyopathies are poorly understood. DNA can be modified chemically by methylation leading to an alteration in the way in which cells and tissues function. Changes in DNA methylation has been recently identified as a potential factor in the development of cardiomyopathies induced by other means, and there are treatments which can alter DNA methylation systemically using specific inhibitors which could provide a strategy for intervention. The importance of myocardial DNA methylation in radiation responses is not known. The proposed project seeks to identify the effects of radiation exposure of the heart on myocardial DNA methylation profiles in non-irradiated and irradiated non-human primate (NHP) survivors, which are excellent models of human health and known to develop cardiomyopathy similar to that seen in the human population. The central **hypothesis** of the proposed work is that radiation exposure to the heart will induce specific and long lived alterations in DNA which may play important roles in myocardial fibrosis and the future development of cardiomyopathy. We will determine the effects of radiation exposure on myocardial DNA methylation, and relationships between these changes and adverse effects of radiation on the heart. The proposed studies are **innovative,** will provide new insights into potential mechanisms by which radiation produces long term delayed effects on the heart, and should help to identify pathways for interventions to delay or prevent radiation induced cardiomyopathies in individuals exposed to malicious, accidental, or medical ionizing radiation. The proposed work is economical, making use of already available nonhuman primate myocardial tissue and data which will complement the proposed outcomes. The findings will help inform future grant applications to understand **cardiomyopathy** and develop and test new interventions for prevention and treatment.

2. **KEYWORDS:**

Epigenetics, genomics, myocardium, heart disease, fibrosis, DNA methylation, radiation, countermeasures, inflammation

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Specific Aim 1: Determine the effects of radiation exposure on the myocardial DNA methylome.

Milestones for Aim 1 were to complete 1) Distribution of tissues; 2) DNA isolation from Rhesus Myocardium and QC, and 3) DNA methylation profiling in isolated DNA using DNA Illumina Infinium Human 450k Methylation Arrays (Target was 6 months). We have completed the tissue distributions, DNA isolation and characterization, and are in the process of finalizing plans for the DNA methylation study.

Percent completion: 65%

Specific Aim 2: Determine relationships between myocardial methylation profiles and cardiomyopathy including myocardial fibrosis and heart structural and functional characteristics.

Milestones for Aim 2 were to complete Bioinformatics and Statistical Analysis of DNA methylation profiles in relation to cardiac phenotypes (Target was 12 months). We have made progress in this area through analysis of the transcriptomes of these same tissues using RNAseq as well as Nanostring technologies. This provides significant support for the planned analysis of the methylation profiles.

Percent completion: 10%

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Specific Aim 3: Determine relationships between myocardial DNA methylation profiles and myocardial transcriptional profiles, the latter of which are being supported by another mechanism.

Milestones for Aim 3 were to complete 1) Statistical Analysis of DNA methylation profiles in relation to myocardial gene expression profiles; 2) produce Abstracts and Manuscript(s) reporting the results of the study and 3) Initiate a Grant Application based on the findings. (Target was 18 months). Our work on the myocardial gene expression profiles and other cardiac phenotyping required for final completion of a manuscript has been substantial and sets the stage for a significant body of work when combined with the proposed work.

Percent completion: 20%

- **What was accomplished under these goals?**

  Major activities were distributions of myocardial tissues, isolation and quality control characterizations of DNA, and preparation for analysis of DNA methylation bionformatic and are in the process of finalizing plans for the DNA methylation study.

  Percent completion: 65%

  Specific Aim 2: Determine relationships between myocardial methylation profiles and cardiomyopathy including myocardial fibrosis and heart structural and functional characteristics.

  Milestones for Aim 2 were to complete Bioinformatics and Statistical Analysis of DNA methylation profiles in relation to cardiac phenotypes (Target was 12 months). We have made progress in this area through analysis of the transcriptomes of these same tissues using RNAseq as well as Nanostring technologies. This provides significant support for the planned analysis of the methylation profiles.

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- **What opportunities for training and professional development has the project provided?**

  Nothing to report

- **How were the results disseminated to communities of interest?**

  Nothing to report

- **What do you plan to do during the next reporting period to accomplish the goals?**

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

  Nothing to report

- **What was the impact on other disciplines?**

  Nothing to report

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What was the impact on technology transfer?

Nothing to report

- **What was the impact on society beyond science and technology?**

  Nothing to report

5. **CHANGES/PROBLEMS:**

   - **Changes in approach and reasons for change**

     The project has been delayed slightly due to 1) putting in place the appropriate sourcing of the DNA methylation array data acquisition and 2) the COVID-19 pandemic.

   - **Actual or anticipated problems or delays and actions or plans to resolve them**

     The COVID-19 pandemic has created challenges across the scientific community by altering some sourcing of reagents as well as Professional effort, and technical administrative staff availabilities. Academic institutions restricted basic research to essential activities. Our institution is starting to open back up on July 1, although some administrative and technical staff will undergo some staggered furloughs. We expect some delays but full completion of the project.

   - **Changes that had a significant impact on expenditures**

     Delay in hiring staff and performing DNA methylation analyses have resulted in fund preservation.

   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

     Nothing to report.

   - **Significant changes in use or care of human subjects**

     Nothing to report.

   - **Significant changes in use or care of vertebrate animals**

     Nothing to report.

   - **Significant changes in use of biohazards and/or select agents**

     Nothing to report.

6. **PRODUCTS:**

   Nothing to report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

   - **What individuals have worked on the project?**

Name:	Thomas Register
Project Role:	Principal Investigator
Researcher Identifier:	N/A
Nearest person month worked:	1.8
Contribution to Project:	Dr. Register will oversee all aspects of the project, orchestrating interactions between collaborating parties, data collections, archiving, and analysis.
Name:	J. Mark Cline

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Project Role:	Co-Investigator
Researcher Identifier:	N/A
Nearest person month worked:	0.6
Contribution to Project:	Dr. Cline will work with Dr. Register on approaches to analysis and interpretation of the data

Name:	Timothy Howard
Project Role:	Co-Investigator
Researcher Identifier:	N/A
Nearest person month worked:	0.6
Contribution to Project:	Dr. Howard will work with Dr. Resister in the conduct of the DNA methylation profiling studies

Name:	Carl Langefeld
Project Role:	Principal Investigator
Researcher Identifier:	N/A
Nearest person month worked:	0.6
Contribution to Project:	Dr. Langefeld will provide biostatistical and bioinformatics support.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Thomas Register (PI)

Changes in *Current* Support as reported since the last reporting period (*original application*):

Project Title:	Dietary Mitigation of Psychosocial Stress Effects on CVD Risk in Female Primates R01 HL087103 (Shively-PI)
Change:	Project Complete

Project Title: (Register PI)	Monocyte Polarization in Acute and Delayed Responses to Total Body Irradiation
Change:	Project Complete

Project Title:	Effects of Western and Mediterranean Diets on Metabolic and Neuropathological Risk Factors for Alzheimer's disease in NHPs
Change:	New Award

J Mark Cline (Co-Inv.)

Project Title:	Assessment Study to Evaluate Breast Cancer Tumor-bearing Non-human Primates
Change:	New Award

Project Title:	Wake Forest Primate Cancer Initiative – Model Development
Change:	New Award

Project Title:	Modulating the Breast Microbiome To Prevent ER+ Breast Cancer.
Change:	New Award

Timothy Howard (Co-Inv.)

Project Title:	Human Pesticide Exposure and Epigenetic Changes in Sperm DNA R01ES025066 (Howard)

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Change: Project Complete

Project Title: Integrated Omics Analysis of Pain: Omics Data Generation Center (MPI: Olivier, Howard, Langefeld)
Change: New Award

Project Title: Predicting uveitis onset in children with juvenile idiopathic arthritis (Angeles-Han)
Change: New Award

Project Title: Molecular and epigenetic predictors of treatment response to topical steroids in eosinophilic esophagitis (Dellon)
Change: New Award

Project Title: North Carolina Diabetes Research Center (McClain)
Change: New Award

Carl Langefield (Co-Inv.)

Project Title: Exome Sequencing to Identify CVD Risk Variants in Hispanic and African Americans
Change: Project Complete

Project Title: Biomarkers for Molecular-Based-Decision-Making in Diagnosis and Treatment of Interstitial Cystitis
Change: Project Complete

Project Title: Evaluating of Hydrodistention Response as a Clinical Sub-Phenotype in IC/BPS
Change: Project Complete

Project Title: Nephrology Pilot Project
Change: Project Complete

Project Title: Genetic Risk for Granulomatous Interstitial Lung Disease
Change: Project Complete

Project Title: The Role of Hepatocyte ABCA1 in Lipid Metabolism and Transport
Change: Project Complete

Project Title: Genetics and the Microbiome in Anti-Ro Preclinical and Established Autoimmunity
Change: Project Complete

Project Title: Altered DNA methylation modulates adipose tissue transcript expression and insulin resistance in African Americans
Change: New Award

Project Title: Identifying Genetic Drivers of Expression Networks Causing Insulin Resistance
Change: New Award

Project Title: Early Life Factors, Gene-Environment Interaction and Eosinophilic Esophagitis
Change: New Award

Project Title: Genetic and Epidemiological Predictors of Glucose Homeostasis Measures
Change: New Award

Project Title: Integrated Omics Analysis of Pain: Omics Data Generation Center
Change: New Award

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Project Title:	Predicting Uveitis Onset in Children with Juvenile Idiopathic Arthritis
Change:	New Award
Project Title:	Lung Organ Tissue Equivalent Platform For Modeling Chlorine Gas Toxicology And Medical Countermeasure Efficacy
Change:	New Award

- **What other organizations were involved as partners?**

  Nothing to report

8. **SPECIAL REPORTING REQUIREMENTS**

   - **COLLABORATIVE AWARDS:**

     N/A

   - **QUAD CHARTS:**

9. **APPENDICES:**

   N/A