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TITLE: A Systems Biology Approach to Radiation Biodosimetry and the Host-Environment Interaction: Applications to Mass Casualty Triage in the Polytrauma Patient

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6. AUTHOR(S)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
We have successfully utilized rodent models of radiation and thermal injury to examine existing and novel biomarkers for radiation exposure alone, thermal injury alone, or their combination. We quantified markers at varying levels in skin, blood, and major organs. The emphasis was on pan–omic work, but we related findings to ongoing and previous histopathology and immunohistochemistry (IHC) to elucidate relationships between biomarker change and potential clinical impacts in samples collected. A focus was on identification of candidate markers that are obtained least invasively (i.e., blood or skin biopsy) and therefore can be applied readily to the field for rapid assessment of military and civilian populations that have had potential radiation exposure and/or thermal injury.

15. SUBJECT TERMS
radiation, burn injury, mass casualty, biomarkers, systems biology

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1. INTRODUCTION

We have successfully utilized rodent models of radiation and thermal injury to examine existing and novel biomarkers for radiation exposure alone, thermal injury alone, or their combination. We quantified markers at varying levels in skin, blood, and major organs. The emphasis was on pan–omic work, but we related findings to ongoing and previous histopathology and immunohistochemistry (IHC) to elucidate relationships between biomarker change and potential clinical impacts in samples collected. A focus was on identification of candidate markers that are obtained least invasively (i.e., blood or skin biopsy) and therefore can be applied readily to the field for rapid assessment of military and civilian populations that have had potential radiation exposure and/or thermal injury.

2. KEYWORDS

radiation, burn injury, mass casualty, biomarker, systems biology

3. ACCOMPLISHMENTS

What were the major goals of the project? (Goals to be accomplished and status.)

Aim 1: Biomarker Identification - 100% complete

Major Task 1: Secure IACUC and ACURO Approval and Plan for Exposures with Consultation from AFRRI - 100% complete

Major Task 2: Perform Single Injury Animal Exposures (n = 432 animals, C57BL6 mouse) - 100% complete

Major Task 3: Perform Combined Injury/Exposure groups (n= 486 animals, C57BL6 mouse) – 100% complete

Major Task 4: -omics Assays for molecular biomarker identification - 100% complete

Major Task 5: H2AX/Histologic Assays for histologic biomarker identification and confirmation. - 100% complete

Aim 2: Biomarker Detection and Timeline Assessment - 100% complete

Major Task 1: Temporal/Chronologic Analyses of Data to assess for biomarker dynamics over time and optimal window for detectable signals. - 100% complete

Determine whether there is a dose-dependent and time-dependent response that can be seen with this molecule(s) or other biomarker, such as RIN value. - 100% complete

Aim 3: Biomarker Sample Source Optimization - 100% complete

Major Task 1: Comparison of sample types for identification of best sample source - 100% complete

Determine whether this biomarker signal can be detected strongly enough in the least-invasively obtained sample (saliva, then feces/urine, then blood, then skin biopsy) to make it translatable to mass casualty/field triage. - 100% complete

Aim 4: Analysis, Reporting, and Communications of Findings - 100% complete

Major Task 1: Final Data Analysis - 100% complete

What was accomplished under these goals? (Detailed progress and results.)

Aim 1: Biomarker Identification

Major Task 1: Secure IACUC and ACURO Approval and Plan for Exposures with Consultation from AFRRI

All appropriate regulatory approvals have been maintained for animal use in this study. Protocols have been renewed at the MedStar Health Research Institute IACUC, and subsequently were maintained with the ACURO office.

Major Task 2: Perform Single Injury Animal Exposures, n = 432 animals

All of the animal exposures have been completed for both burn and radiation only exposures, with the majority of animals exposed completing a 14 day time course. The highest radiation only exposure group however only survives until day 6, therefore this group did not complete the full proposed time course.

Major Task 3: Perform Combined Injury/Exposure groups, n= 486 animals

All of the combination injury animal exposures have also been completed. We have observed a clear combinatorial effect on mortality however, and have had to adjust our experimental groups accordingly to end the time courses following the combination 20% burn injury and radiation exposure to 2 days, in order to ensure viable sample collection. We also modified the lowest dose of exposure down to 0.5 gray in order to see if we could minimize mortality and gain additional data.

Major Task 4: -omics Assays for molecular biomarker identification

All samples generated to date and preserved for subsequent molecular analysis have been transferred to USACEHR for processing and assay. Additionally, samples have been included as "extra" in order to optimize processing from skin samples. Optimization is complete and all blood, skin, and liver RNA samples have been purified. Blood and skin samples sets have been applied to DNA microarray hybridization and scanning. Initial quality control (QC) analysis of the blood and samples is complete and is underway for the liver samples. Downstream analysis on blood and skin microarray results is complete.

Major Task 5: H2AX/Histologic Assays for histologic biomarker identification and confirmation.

H&E stains have been completed on skin samples representing every exposure group and other assays will need to remain underway.

Aim 2: Biomarker Detection and Timeline Assessment

Major Task 1: Temporal/Chronologic Analyses of Data to assess for biomarker dynamics over time and optimal window for detectable signals.

Determine whether there a dose-dependent and time-dependent response that can be seen with this molecule(s) or other biomarker, such as RIN value.

We assessed the RNA Integrity Number (RIN) for the blood RNA samples, as a potential biomarker for dose and time dependent effects of ionizing radiation. Manuscript has been published which describes this, and a presentation given at the RAND sponsored DoD State of the Science meeting on Blast-related burn injuries.

Aim 3: Biomarker Sample Source Optimization

Major Task 1: Comparison of sample types for identification of best sample source

Determine whether this biomarker signal can be detected strongly enough in the least-invasively obtained sample (saliva, then feces/urine, then blood, then skin biopsy) to make it translatable to mass casualty/field triage.

We have compared skin and blood as sample sources for exposure markers for each experimental group.

Aim 4: Analysis, Reporting, and Communications of Findings

Comparisons of skin and blood microarray results have taken place. Comparison between current and previously obtained skin microarray results is near completion. Two additional manuscripts are in preparation

describing findings, and multiple abstracts are being submitted to the American Burn Association annual meeting (2021 meeting).

Major Task 1: Final Data Analysis

Data analysis is near completion. This was somewhat delayed as a change of primary analyst from Dr. Clifford to Dr. Alkhalil occurred in approximately the end of Y02.

Key Findings or Accomplishments:

Preliminary data indicate differential gene expression that is time and dose dependent. The highest dose previously tested (20Gy) is lethal by >7days and exerts effects related to inflammatory disease states in skin. We have verified this in the present animal work and have completed animal model work according to modifications related to observations of mortality.

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

A surgical research fellow was an active participant in the animal modeling and data analysis during the course of this project. He presented some of the data at a conference and worked in collaboration with Dr. Alkhalil. Three medical students have had the opportunity to learn skill sets in sample preservation and processing for histology with samples generated in this project. This learning occurred with the students under the mentorship of more senior lab staff. In addition, one summer student that was part of the Army Educational Outreach Program (AEOP) has gained hands on experience purifying RNA from collected skin and blood samples from the experiment.

How were the results disseminated to communities of interest?

Results were presented at the 2018 and 2019 MHSRS conferences, and at the 2019 Wound Healing Society conference. Additionally the first of at least 3 manuscripts has been published in the peer reviewed literature on data resulting from the present work (PMID: 32282289).

Plans for the next reporting period to accomplish the goals

Although this is the final report and POP is over, we plan to continue to complete manuscripts, tie up data analyses, and potentially seek additional funding to be able to process and analyze additional samples that have been preserved from the animal models.

4. IMPACT

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

We believe that our findings on RIN values and their association with exposure and potential prediction of outcome are significant and may be highly translatable to human subjects in mass casualty incidents. Additionally, this may even be applicable to other radiation exposure scenarios.

What was the impact on other disciplines?

As stated above, there could be potential for other fields to apply the biomarkers we are identifying, including RIN value change, to areas such as radiation oncology and other occupational sources of radiation.

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

Nothing to report.

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

Nothing to report.

Changes that had a significant impact on expenditures

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

Journal publications

Alkhalil A, Clifford JL, Ball R, Day A, Chan R, Carney BC, Miller SA, Campbell R, Kumar R, Gautam A, Hammamieh R, Moffatt LT, Shupp JW. Blood RNA Integrity is a Direct and Simple Reporter of Radiation Exposure and Prognosis: A Pilot Study. Radiat Res. 2020 Jun 1;193(6):543-551. doi: 10.1667/RR15527.1. PMID: 32282289

Books or other non-periodical, one-time publications

Nothing to Report.

Other publications, conference papers, and presentations

Alkhalil A, Clifford J, Ball R, Day A, Miller S, Campbell R, Kumar R, Hammamieh R, Moffatt L, Shupp J. May 2019. Blood mRNA Integrity is a Direct and Simple Reporter of Radiation Exposure. Wound Healing Society 31st Annual Meeting, San Antonio, TX

Garg G, Alkhalil A, Clifford J, Ball R, Day A, Miller S, Campbell R, Kumar R, Hammamieh R, Moffatt L, Shupp J. Jun 2019. Assessment of Radiation Exposure by MRNA Integrity Bioanalysis. Shock Society, 42nd Annual Conference on Shock, Coronado, CA

Alkhalil A, Clifford J, Ball R, Day A, Miller S, Campbell R, Kumar R, Hammamieh R, Moffatt L, Shupp J. Aug 2019. Blood mRNA Integrity is a Direct and Simple Reporter of Radiation Exposure. Military Health Services Research Symposium (MHSRS), Kissimmee, FL

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Biospecimen collections; we have collected all organs from experimental animals and therefore will have accumulated samples that will be kept in a repository if full samples are not used in presently described assays.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: John Clifford, PhD
Project Role: Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.0
Contribution to Project: Planning, coordination, re-analysis of preliminary data, experimental design development and initial execution

Name: Lauren Moffatt, PhD
Project Role: Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.0
Contribution to Project: Regulatory approvals, planning, coordination, experimental design development and initial execution

Name: Abdulnaser Alkhalil, PhD
Project Role: Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.0
Contribution to Project: Planning, coordination, re-analysis of preliminary data, experimental design development and execution and coordination of animal exposures

Name: Campbell, Ross, PhD
Project Role: Statistician, Data Manager
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.5
Contribution to Project: Re-analysis of preliminary data, experimental design development

Name: Duncan Donohue, PhD
Project Role: Statistician, Data Manager
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.0
Contribution to Project: Re-analysis of preliminary data, experimental design development

Name: Robert Christy, PhD
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.5
Contribution to Project: Oversight of planning, coordination, experimental design development

Name: Sanchita Ghosh, PhD
Project Role: Consultant

Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.0
Contribution to Project: Radiation-related regulatory approvals, and associated planning, coordination, experimental design development

Name: Anna Day, BS
Project Role: Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3.0
Contribution to Project: Execution of animal exposures and materials acquisition, sample tracking
to Project: Execution of animal exposures, sample tracking

Name: Karina Charipova, BS
Project Role: Technician/Student
Researcher Identifier: N/A
Nearest person month worked: 1.0
Contribution to project: Sample processing, initial histology

Name: Kyle Monger, BS
Project Role: Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3.0
Contribution

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

Nothing to Report

What other organizations were involved as partners?

No additional organizations were involved, other than those originally listed in the SOW and funded as subawardees.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHART
Convert this report to a PDF file and append updated quarterly Quad Chart in PDF as an appendix.

9. APPENDICES

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

A Systems Biology Approach to Radiation Biodosimetry and the Host-Environment Interaction: Applications to Mass Casualty Triage in the Polytrauma Patient

DM160525

Clinical Research Intramural Initiative Program, Precision Medicine Research Award FOA# DHA-16-CRII-PMRA

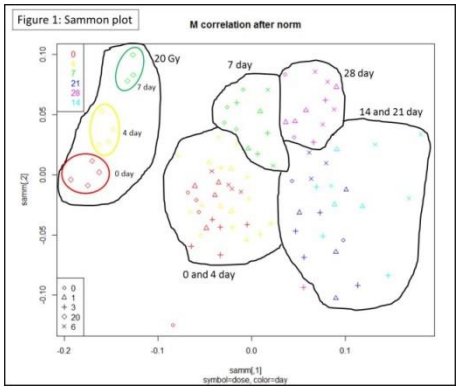
PI: Robert J. Christy, PhD **Org:** Institute Of Surgical Research **Award Amount:** 750,000

Study/Product Aim(s)

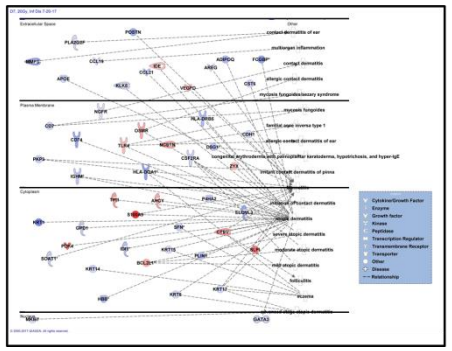
1)Identify the signature molecular biomarker modulation that occurs in animals exposed to variable doses of X-ray radiation, or variable severity burn injury.
2) Identify the signature molecular biomarker modulation that occurs in animals with a combination of both of the above injuries.
3) Determine whether there a dose-dependent and time-dependent response that can be seen with this molecule(s).
4)Determine whether this biomarker signal can be detected strongly enough in the least-invasively obtained sample (saliva, then feces/urine, then blood, then skin biopsy) to make it translatable to mass casualty/field triage.

Approach

We will use a rodent model to examine existing and novel biomarkers of combination exposure at varying levels in hair, skin, blood, and major organs. Though the emphasis will be on pan–omic work, we will also implement histopathology and IHC to elucidate relationships between biomarker change and potential clinical impacts. A focus will be on identification of candidate markers that are obtained least invasively (i.e., blood or hair) and therefore can be applied readily to the field for rapid assessment of military and civilian populations that have potential exposure.



B. Processes related to inflammatory disease (Day 7, 20Gy)



Accomplishment: Preliminary data indicate differential gene expression that is time and dose dependent. The highest dose previously tested (20Gy) is lethal by >7days and exerts effects related to inflammatory disease states in skin. We have verified this in the present animal work and have completed animal model work according to modifications related to observations of mortality.

Timeline and Cost

Activities / CY	17	18	19
Animal Work			
-Omics and Histologic Assays			
Data analysis and harmonization			
Translatability Assessment			
Estimated Budget ($K)	**$150**	**$350**	**$250**

Updated: SEP2020

Goals/Milestones

CY17 Goal – Animal Model
☑Secure ACURO approval and begin animal model
☑Sample collection and processing
☑Assay initiation
CY18 Goals – Continue Animal Model and Assays
✓ Animal model completion
✓Continue assays
CY19 Goal – Analysis and biomarker suitability assessment
✓ Validate markers