Data in Brief

Transcriptional profile of immediate response to ionizing radiation exposure

Eric C. Rouchka a,b,* , Robert M. Flight c , Brigitte H. Fasciotto d , Rosendo Estrada e , John W. Eaton f,g,h , Phani K. Patibandla i,j, Sabine J. Waigel h,a, Dazhao Liu a, John K. Kirtley a, Palaniappan Sethu i,j, Robert S. Keynton f

a Department of Computer Engineering and Computer Science, University of Louisville, Louisville, KY 40292, United States
b Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY 40356, United States
c The ElectroOptics Research Institute and Nanotechnology Center, University of Louisville, Louisville, KY 40292, United States
d Department of Bioengineering, University of Louisville, Louisville, KY 40292, United States
e Department of Medicine, University of Louisville, Louisville, KY 40292, United States
f Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY 40292, United States
g James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, United States
h Division of Cardiovascular Disease, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, United States
i Department of Biomedical Engineering, University of Alabama at Birmingham, Birmingham, AL 35294, United States

A R T I C L E  I N F O

Article history:
Received 16 November 2015
Accepted 30 November 2015
Available online 1 December 2015

Keywords:
Ionizing radiation
Radiation exposure
Astronaut
Long duration space travel

A B S T R A C T

Astronauts participating in long duration space missions are likely to be exposed to ionizing radiation associated with highly energetic and charged heavy particles. Previously proposed gene biomarkers for radiation exposure include phosphorylated H2A Histone Family, Member X (γH2AX), Tumor Protein 53 (TP53), and Cyclin-Dependent Kinase Inhibitor 1A (CDKN1A). However, transcripts of these genes may not be the most suitable biomarkers for radiation exposure due to a lack of sensitivity or specificity. As part of a larger effort to develop laboratory methods for detecting radiation exposure events using blood samples, we designed a dose–course microarray study in order to determine coding and non-coding RNA transcripts undergoing differential expression immediately following radiation exposure. The main goal was to elicit a small set of sensitive and specific radiation exposure biomarkers at low, medium, and high levels of the biomarker radiation exposure. Four separate levels of radiation were considered: 0 Gray (Gy) control; 0.3 Gy; 1.5 Gy; and 3.0 Gy with four replicates at each radiation level. This report includes raw gene expression data files from the resulting microarray experiments from all three radiation levels ranging from a lower, typical exposure than an astronaut might see (0.3 Gy) to high, potentially lethal, levels of radiation (3.0 Gy). The data described here is available in NCBI's Gene Expression Omnibus (GEO), accession GSE64375.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

http://dx.doi.org/10.1016/j.gdata.2015.11.027
2213-5960/© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
three distinct levels of radiation: 0.3 Gy (low), 1.5 Gy (medium), and 3.0 Gy (high).

- Immediate transcriptional response biomarkers to radiation exposure can be elucidated by combining the radiation exposure data and determining common transcriptional responses.
- Dose-specific transcriptional responses immediately following radiation exposure can be determined using the available dataset which can be used to extract sensitive and specific biomarkers.
- Identification of appropriate biomarkers for general radiation exposure as well as dose-dependent markers found within blood plasma samples makes it possible to design appropriate diagnostic tests for measuring radiation exposure. Such a test could be employed on long-term space flights to diagnose whether or not an astronaut has been exposed to radiation and at what level so appropriate treatment options can be explored.

3. Experimental design, materials and methods

3.1. Experimental design

All procedures were performed in accordance with published NASA and NIH Guidelines, the University of Louisville Institutional Review Board (IRB), and the University of Louisville Institutional Biosafety Committee (IBC). In this study, we sought to understand transcriptional changes in human blood samples resulting from exposure to different levels of radiation. The experimental design consisted of blood draws from four volunteers which was separated into four samples. Blood from each volunteer was then exposed to 0.0 Gy, 0.3 Gy, 1.5 Gy, and 3.0 Gy of radiation independently as described in Section 3.2.

### 3.2. Sample preparation

Whole blood was drawn from four (4) volunteers using a Safety Winged IV blood draw set (Exel International, St. Petersburg, FL) in 7-ml lavender topped Ethylenediaminetetraacetic acid (EDTA) anticoagulant-containing vacutainers. Blood samples were aliquoted and kept at room temperature throughout the radiation and white blood cell (WBC) isolation process.

Whole blood samples were radiated at the Kentucky Lion Eye Center using a Gammacell 1000 Elite (Cs-137) (Best Theratronics Ltd., Ottawa, Canada) for 0 s (control – 0.0 Gy exposure), 3 s (0.30 Gy exposure), 16 s (1.5 Gy exposure), or 32 s (3.0 Gy exposure).

Approximately 30 min after completion of the radiation cycle, red blood cells (RBC) were lysed by adding 15 ml of NH₄Cl RBC lysis buffer for each ml of whole blood (1:15 v/v dilution) in order to isolate leukocytes. The tubes were agitated for 5 min on a rocker platform and centrifuged for 5 min at 1500 RPM at room temperature. Cells were suspended in 10 ml of phosphate-buffered saline (PBS) and centrifuged again twice for 5 min at 1500 RPM. WBCs were suspended in 2 ml PBS, equivalent to the initial volume of the whole blood. WBCs were centrifuged 5 min at 1500 rpm. Supernatant was discarded and cell pellets were suspended in 600 ul RTL lysis buffer (Qiagen, Venlo, The Netherlands) and tubes were vortexed vigorously and stored at −70 °C until RNA purification. Purification of total RNA was performed using the RNeasy Mini Kit (Qiagen). Optional on-column DNase digestion was performed to eliminate genomic DNA contamination. Total RNA was eluted in 60 ul of RNase-free water. The quantity analysis of the total RNA was performed with a Nanodrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The quality of the total RNA was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA) (Table 1).

Biotinylated cDNA were prepared according to the standard protocol for Affymetrix® GeneChip® WT Expression protocol (Affymetrix® Inc., Santa Clara, CA) from 100 ng total RNA, which includes an Ambion WT Expression kit followed by a Genechip® WT Terminal Labeling and Hybridization kit. Following fragmentation, microarrays were hybridized at the University of Louisville Genomics Core Facility in a single batch. cDNA were hybridized for 16 h at 45 °C to Affymetrix® GeneChip® Human Gene 1.0 ST v1 Arrays (GEO platform GPL6244) according to the GeneChip® WT Terminal Labeling and Hybridization User Manual from Affymetrix®.

3.3. Data acquisition

GeneChips® were scanned using an Affymetrix® GeneChip® Scanner 3000 7G (Affymetrix®) and the GeneChip® Command Console® software version 3.1 (Affymetrix®), resulting in 16 raw CEL files.

### Table 1

| Sample Number | Voluneteer Number | Radiation Level | Concentration ng/ul | 250/280 | 260/230 | RIN |
|---------------|-------------------|-----------------|---------------------|---------|---------|-----|
| 1             | 1                 | 0.0 Gy          | 81                  | 2.08    | 2.00    | 8.20|
| 2             | 2                 | 0.0 Gy          | 99                  | 2.09    | 2.02    | 5.90|
| 3             | 3                 | 0.0 Gy          | 93                  | 2.08    | 2.13    | 6.60|
| 4             | 4                 | 0.0 Gy          | 62                  | 2.08    | 2.05    | 7.90|
| 5             | 1                 | 0.3 Gy          | 67                  | 2.06    | 2.14    | 8.30|
| 6             | 2                 | 0.3 Gy          | 130                 | 2.08    | 2.23    | 5.40|
| 7             | 3                 | 0.3 Gy          | 97                  | 2.03    | 2.04    | 6.70|
| 8             | 4                 | 0.3 Gy          | 68                  | 2.08    | 2.14    | 7.30|
| 9             | 1                 | 1.5 Gy          | 68                  | 2.06    | 2.19    | 7.90|
| 10            | 2                 | 1.5 Gy          | 167                 | 2.08    | 2.13    | 6.50|
| 11            | 3                 | 1.5 Gy          | 99                  | 2.09    | 2.13    | 6.20|
| 12            | 4                 | 1.5 Gy          | 77                  | 2.07    | 2.18    | 7.50|
| 13            | 1                 | 3.0 Gy          | 62                  | 1.99    | 2.06    | 7.30|
| 14            | 2                 | 3.0 Gy          | 88                  | 2.11    | 2.06    | 6.90|
| 15            | 3                 | 3.0 Gy          | 94                  | 2.09    | 2.02    | 6.70|
| 16            | 4                 | 3.0 Gy          | 68                  | 2.10    | 2.04    | 6.80|

### Table 2

Sample information.

| Sample number | Sample name         | CEL file                  | Volunteer number | Dose | GEO sample ID |
|---------------|---------------------|---------------------------|------------------|------|---------------|
| 1             | SAMPLE_0.0Gy_1h-1   | PS_Vol1_0.0GY.CEL         | 1                | 0.0 Gy | GSM1569806    |
| 2             | SAMPLE_0.0Gy_1h-2   | PS_Vol2_0.0GY.CEL         | 2                | 0.0 Gy | GSM1569807    |
| 3             | SAMPLE_0.0Gy_1h-3   | PS_Vol3_0.0GY.CEL         | 3                | 0.0 Gy | GSM1569808    |
| 4             | SAMPLE_0.0Gy_1h-4   | PS_Vol4_0.0GY.CEL         | 4                | 0.0 Gy | GSM1569809    |
| 5             | SAMPLE_0.3Gy_1h-1   | PS_Vol1_0.3GY.CEL         | 1                | 0.3 Gy | GSM1569810    |
| 6             | SAMPLE_0.3Gy_1h-2   | PS_Vol2_0.3GY.CEL         | 2                | 0.3 Gy | GSM1569811    |
| 7             | SAMPLE_0.3Gy_1h-3   | PS_Vol3_0.3GY.CEL         | 3                | 0.3 Gy | GSM1569812    |
| 8             | SAMPLE_0.3Gy_1h-4   | PS_Vol4_0.3GY.CEL         | 4                | 0.3 Gy | GSM1569813    |
| 9             | SAMPLE_1.5Gy_1h-1   | PS_Vol1_1.5GY.CEL         | 1                | 1.5 Gy | GSM1569814    |
| 10            | SAMPLE_1.5Gy_1h-2   | PS_Vol2_1.5GY.CEL         | 2                | 1.5 Gy | GSM1569815    |
| 11            | SAMPLE_1.5Gy_1h-3   | PS_Vol3_1.5GY.CEL         | 3                | 1.5 Gy | GSM1569816    |
| 12            | SAMPLE_1.5Gy_2h-1   | PS_Vol1_1.5GY.CEL         | 1                | 1.5 Gy | GSM1569817    |
| 13            | SAMPLE_1.5Gy_2h-2   | PS_Vol2_1.5GY.CEL         | 2                | 1.5 Gy | GSM1569818    |
| 14            | SAMPLE_1.5Gy_2h-3   | PS_Vol3_1.5GY.CEL         | 3                | 1.5 Gy | GSM1569819    |
| 15            | SAMPLE_3.0Gy_2h-2   | PS_Vol1_3.0GY.CEL         | 1                | 3.0 Gy | GSM1569820    |
| 16            | SAMPLE_3.0Gy_2h-3   | PS_Vol2_3.0GY.CEL         | 2                | 3.0 Gy | GSM1569821    |
which were subsequently submitted to GEO (Table 2). These CEL files were analyzed in RStudio version 0.98.501 [3] using R (version 3.0.1 2013-05-16 “Good Sport”) [4] and Bioconductor packages [5]. CEL files were pre-processed and normalized in R using the oligo package [6] and robust multichip averaging (RMA) [7]. CEL files were organized into a single category for comparison, based on dose-dependent responses at an early time point averaging roughly 1 h post-exposure.

Low radiation is defined as 0.3 Gy; mid radiation as 1.5 Gy; and high radiation as 3.0 Gy.

Differentially expressed genes (DEGs), defined as Affymetrix® transcript sets, were determined using Limma [8] and a p-value cutoff of 0.05. Using these levels, approximately 400 differentially expressed genes (DEGs), detected by Limma at p ≤ 0.05 for low (0.3 Gy vs. 0.0 Gy), mid (1.5 Gy vs. 0.0 Gy) and high (3.0 vs. 0.0 Gy) radiation levels.

Many of these DEGs appear to be specific to a particular level of radiation exposure, while a small number are shared as general radiation response biomarkers. Further analysis of these genes based on categorical enrichments was performed using categoryCompare [9] (results not shown).

3.4. Expression of radiation-modulated biomarkers

Several genes have previously been considered as potential biomarkers for radiation exposure, including most prominently γH2AFX, TP53, and CDKN1A. Phosphorylation of H2AFX has been used in assays to determine radiation exposure due to its role in DNA double-stranded break repair [10–14] while TP53 is known to function as a transcription factor which is radiation-modulated [15–20] and CDKN1A is a downstream target of TP53 which regulates progression through the cell cycle [21–23]. A plot of the dose-dependent changes in expression of these three biomarkers (Fig. 1) illustrates that two of the three may not be the best to use at a transcriptional level, due to the lack of measurement of protein modifications of H2AX as well as low detectable changes of TP53 which may still affect downstream targets [16]. Taken together, this illustrates the potential value of this dataset in detection of either independent or sets of biomarkers for ionizing radiation exposure at low, mid, and high radiation levels.

Conflict of interest

The authors declare they have no conflict of interest.

Acknowledgements

Funding was provided by the National Aeronautics and Space Administration (NASA) contract NNX10AJ36G (BHF, ECR, JK, JWE, PKP, PS, RE and RSK); the National institutes of Health (NIH) grants P20RR016481 (DL, ECR, RMF and SJW), 3P20RR016481-0051 (ECR, RMF and SJW), and P20GM103436 (ECR and SJW). The article contents are solely the responsibility of the authors and do not represent the official views of the funding organizations, which were entirely uninvolv in the data generation or manuscript preparation.

References

[1] T. Barrett, S.E. Wilhite, P. Ledoux, C. Evangelista, I.F. Kim, M. Tomashevski, K.A. Marshall, K.H. Philippy, P.M. Sherman, M. Holko, et al., NCBI GEO: archive for functional genomics data sets—update. Nucleic Acids Res. 41 (Database issue) (2013) D991–D995.
[2] R. Edgar, M. Domrachev, A.E. Lash, Gene Expression Omnibus: NCBi gene expression and hybridization array data repository. Nucleic Acids Res. 30 (1) (2002) 207–210.
[3] R. Edgar, J. Domrachev, M.A. Lash, R. Lu, T. Lu, R. Edgar, K.I. Lu, et al., Genome Biology 5, Genome Biol. 5 (10) (2004) R80.
[4] B.S. Carvalho, L.A. Irizarry, A framework for oligonucleotide microarray preprocessing. Bioinformatics 21 (2005) 2363–2367.
[5] R. Edgar, M. Domrachev, A.E. Lash, Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30 (1) (2002) 207–210.
[6] R. Edgar, M. Domrachev, A.E. Lash, Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30 (1) (2002) 207–210.
[7] B.M. Bolstad, R.A. Irizarry, M. Astrand, T.P. Speed, A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19 (2) (2003) 185–193.
[8] J.G. Smyth, Limma: linear models for microarray data analysis. Bioinformatics and Computational Biology Solutions Using R and Bioconductor, Springer 2005, pp. 397–420.
[9] R.M. Flight, B.J. Harrison, F. Mohammad, M.B. Bunge, L.D. Moon, J.C. Petruska, E.C. Rouchka, CategoryCompare, an analytical tool based on feature annotations. Front. Genet. 5 (2014) 98.
[10] J.P. Banath, S.H. Macphail, P.L. Oliva, Radiation sensitivity, H2AX phosphorylation, and kinetics of repair of DNA strand breaks in irradiated cervical cancer cell lines. Cancer Res. 64 (19) (2004) 7144–7149.
[11] M. Borras, G. Armenegol, M. De Cabo, J.F. Barquiner, L. Barrios, Comparison of methods to quantify histone H2AX phosphorylation and its usefulness for prediction of radiosensitivity. Int. J. Radiat. Biol. 1–10 (2015).
[12] L.J. Chappell, M.K. Whalen, S. Gurai, A. Ponomarev, F.A. Cucinotta, J.M. Pluth, Analysis of radiation sensitivity, H2AX phosphorylation, and kinetics of repair of DNA strand breaks in irradiated cervical cancer cell lines. Cancer Res. 64 (19) (2004) 7144–7149.
[13] H. Furloong, C. Motherriss, F.M. Lyng, O. Howe, Apoptosis is signalled early by low doses of ionizing radiation through a radiation-induced bystander effect. Mutat. Res. 741–742 (2013) 35–43.
[14] T.P. Lu, Y.Y. Hsu, L.C. Lai, M.H. Tsai, E.Y. Chuang, Identification of gene expression biomarkers for predicting radiation exposure. Sci. Rep. 4 (2014) 6293.
[15] R. Mirzayans, B. Andrais, A. Scott, Y.W. Wang, D. Murray, Ionizing radiation-induced responses in human cells with differing TP53 status. Int. J. Mol. Sci. 14 (11) (2013) 22409–22435.
[16] E.C. Rouchka, Radiation risk prediction and genetics: the influence of the TP53 gene in vivo. Dose-Response 3 (4) (2005) 519–532.
[19] Z. Vilasova, M. Rezacova, J. Vavrova, A. Tichy, D. Vokurkova, F. Zoelzer, Z. Rehakova, J. Osterreicher, E. Lukasova, Changes in phosphorylation of histone H2AX and p53 in response of peripheral blood lymphocytes to gamma irradiation. Acta Biochim. Pol. 55 (2) (2008) 381–390.

[20] N. Whisnant-Hurst, S.A. Leadon, TP53 is not required for the constitutive or induced repair of DNA damage produced by ionizing radiation at the G1/S-phase border. Radiat. Res. 151 (3) (1999) 263–269.

[21] M. Artuso, A. Esteve, H. Bresil, M. Vuillaume, J. Hall, The role of the Ataxia telangiectasia gene in the p53, WAF1/CIP1(p21)- and GADD45-mediated response to DNA damage produced by ionising radiation. Oncogene 11 (8) (1995) 1427–1435.

[22] K. Brzoska, M. Kruszewski, Toward the development of transcriptional biodosimetry for the identification of irradiated individuals and assessment of absorbed radiation dose. Radiat. Environ. Biophys. 54 (3) (2015) 353–363.

[23] D.M. Gadbois, H.A. Crissman, A. Nastasi, R. Habbersett, S.K. Wang, D. Chen, B.E. Lehnert, Alterations in the progression of cells through the cell cycle after exposure to alpha particles or gamma rays. Radiat. Res. 146 (4) (1996) 414–424.