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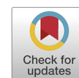
Data Article

Dataset for dose and time-dependent transcriptional response to ionizing radiation exposure



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ABSTRACT

Exposure to ionizing radiation associated with highly energetic and charged heavy particles is an inherent risk astronauts face in long duration space missions. We have previously considered the transcriptional effects that three levels of radiation (0.3 Gy, 1.5 Gy, and 3.0 Gy) have at an immediate time point (1 hr) post-exposure

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Keywords:
Radiation exposure
Microarrays
Space flight
Gene expression
Lab-on-a-chip

[1]. Our analysis of these results suggest effects on transcript levels that could be modulated at lower radiation doses [2]. In addition, a time dependent effect is likely to be present. Therefore, in order to develop a lab-on-a-chip approach for detection of radiation exposure in terms of both radiation level and time since exposure, we developed a time- and dose-course study to determine appropriate sensitive and specific transcript biomarkers that are detectable in blood samples. The data described herein was developed from a study measuring exposure to 0.15 Gy, 0.30 Gy, and 1.5 Gy of radiation at 1 hr, 2 hr, and 6 hr post-exposure using Affymetrix® GeneChip® PrimeView™ microarrays. This report includes raw gene expression data files from the resulting microarray experiments representing typical radiation exposure levels an astronaut may experience as part of a long duration space mission. The data described here is available in NCBI's Gene Expression Omnibus (GEO), accession GSE63952.

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Specifications Table

Subject	Biochemistry, Genetics and Molecular Biology
Specific subject area	Cellular transcriptional response to radiation exposure
Type of data	Microarray gene expression data
How data were acquired	Affymetrix® GeneChip® PrimeView™ Arrays
Data format	Raw; CEL files
Experimental Factors	0.15 Gy vs. 0.0 Gy at 1 hr post-exposure 0.15 Gy vs. 0.0 Gy at 2 hr post-exposure 0.15 Gy vs. 0.0 Gy at 6 hr post-exposure 0.30 Gy vs. 0.0 Gy at 1 hr post-exposure 0.30 Gy vs. 0.0 Gy at 2 hr post-exposure 0.30 Gy vs. 0.0 Gy at 6 hr post-exposure 1.50 Gy vs. 0.0 Gy at 1 hr post-exposure 1.50 Gy vs. 0.0 Gy at 2 hr post-exposure 1.50 Gy vs. 0.0 Gy at 6 hr post-exposure 0.15 Gy vs. 0.0 Gy (dose-dependent) 0.30 Gy vs. 0.0 Gy (dose-dependent) 1.50 Gy vs. 0.0 Gy (dose-dependent) 2 hr vs. 1 hr (time-dependent) 6 hr vs. 1 hr (time-dependent)
Experimental Features	Gene expression profiling of radiation exposure using: 1.0 Gy, 1 hr post-exposure (control; n = 10); 1.0 Gy, 2 hr post-exposure (n = 10); 1.0 Gy, 6 hr post-exposure (n = 5); 0.15 Gy, 1 hr post-exposure (n = 10); 0.15 Gy, 2 hr post-exposure (n = 10); 0.15 Gy, 6 hr post-exposure (n = 5); 0.30 Gy, 1 hr post-exposure (n = 10); 0.30 Gy, 2 hr post-exposure (n = 10); 0.30 Gy, 6 hr post-exposure (n = 5); 1.50 Gy, 1 hr post-exposure (n = 10); 1.50 Gy, 2 hr post-exposure (n = 10); 1.50 Gy, 6 hr post-exposure (n = 5).
Data source location	Louisville, Kentucky USA
Data accessibility	Repository name: Gene Expression Omnibus (GEO) Data identification number: GSE63952 Direct URL to data: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63952

Value of the Data

- Few high-throughput studies regarding ionizing radiation exposure are publicly available. This dataset provides novel time- and dose-dependent transcript abundance data based on typical ionizing radiation exposure faced by astronauts at 0.15 Gy, 0.30 Gy, and 1.50 Gy.
- Evaluation of time since exposure is possible with this dataset at immediate time points (1 hr and 2hr post-exposure) as well as an intermediate time point (6 hr post-exposure). Used together, these results allow for the design of appropriate transcript biomarkers used to determine radiation exposure events, the amount of radiation exposed, and the time since the event. As a result, this data can allow for design of appropriate treatment and mitigation.
- The determination of appropriate biomarkers for general radiation exposure, dose-dependent markers, and time-dependent markers within blood plasma samples makes it possible to design light-weight, portable, lab-on-a-chip diagnostic tests for measuring radiation exposure. Such diagnostic instrumentation can be incorporated on long-term space flights in order to properly address radiation exposure events.

1. Data

Transcript abundance for total RNA was compared between radiation exposed (0.15 Gy, 0.30 Gy, and 1.50 Gy) and untreated leukocytes at various time points post-exposure (1 hr, 2 hr, and 6hr). The raw and processed microarray data is available in the Gene Expression Omnibus (GEO) database at NCBI (GSE63952). The gene expression platform used was the Affymetrix® GeneChip® PrimeView™ Human Gene Expression Array (GEO platform GPL15207). The sample information is provided in Table 1. Tables 2 and 3 show the dose- and time-dependent contrast results, respectively. Fig. 1 and Fig. 2 show the respective dose- and time-dependent transcript abundance changes for three previously described radiation biomarkers, H2AFX, TP53, and CDKN1A. These results show that these biomarkers are not sensitive at the transcript level, illustrating the value of this data set in providing additional novel transcript-based biomarkers for radiation exposure.

2. Experimental design, materials and methods

2.1. 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). This study was designed to measure typical low to moderate ionizing radiation exposure astronauts are likely to encounter on long duration space flights at various time points post-exposure. Samples were taken from blood drawn from ten volunteers. Blood from each volunteer set was exposed to various levels of radiation including 0.0 Gy, 0.15 Gy, 0.30 Gy, and 1.50 Gy. Each of these sets was then measured at 1 hr and 2 hr post-exposure. Half of the samples were further selected for measurement of transcriptional responses at 6 hr post-exposure. A total of 100 samples result in the data set presented here, including 10 at 1 hr post-exposure for each radiation level, yielding a total of 40 samples; 10 at 2 hr post-exposure for each radiation level, yielding a total of 40 samples; and 5 at 6 hr post-exposure for each radiation level, yielding a total of 20 samples. A description of the sample preparation follows in section 2.2.

2.2. Sample preparation

Whole blood was drawn from ten (10) volunteers using a Safety Winged IV blood draw set (Exel International, St. Petersburg, FL) in 2-ml green topped vacutainer tubes containing 30 USP units of sodium heparin. Blood samples were then subsequently stored at 37 °C in an Engel MHD13F-DM portable incubator (Engel USA, Jupiter, FL).

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 seconds (control – 0.0 Gy exposure), 1.5 seconds (0.15 Gy exposure), 3 seconds (0.30 Gy exposure), or 16 seconds (1.5 Gy exposure). Vacutainer tubes containing whole blood were incubated at 37 °C for either 1 hr, 2 hr, or 6 hr after being radiated.

Table 1
Sample information.

Volunteer	Time	Dose	Sample Name	GEO Sample ID	Dose	Sample Name	GEO Sample ID
1	1 hr	0 Gy	SAMPLE_0.00Gy_1h-1	GSM1561166	0.30 Gy	SAMPLE_0.30Gy_1h-1	GSM1561216
2	1 hr	0 Gy	SAMPLE_0.00Gy_1h-2	GSM1561167	0.30 Gy	SAMPLE_0.30Gy_1h-2	GSM1561217
3	1 hr	0 Gy	SAMPLE_0.00Gy_1h-3	GSM1561168	0.30 Gy	SAMPLE_0.30Gy_1h-3	GSM1561218
4	1 hr	0 Gy	SAMPLE_0.00Gy_1h-4	GSM1561169	0.30 Gy	SAMPLE_0.30Gy_1h-4	GSM1561219
5	1 hr	0 Gy	SAMPLE_0.00Gy_1h-5	GSM1561170	0.30 Gy	SAMPLE_0.30Gy_1h-5	GSM1561220
6	1 hr	0 Gy	SAMPLE_0.00Gy_1h-6	GSM1561171	0.30 Gy	SAMPLE_0.30Gy_1h-6	GSM1561221
7	1 hr	0 Gy	SAMPLE_0.00Gy_1h-7	GSM1561172	0.30 Gy	SAMPLE_0.30Gy_1h-7	GSM1561222
8	1 hr	0 Gy	SAMPLE_0.00Gy_1h-8	GSM1561173	0.30 Gy	SAMPLE_0.30Gy_1h-8	GSM1561223
9	1 hr	0 Gy	SAMPLE_0.00Gy_1h-9	GSM1561174	0.30 Gy	SAMPLE_0.30Gy_1h-9	GSM1561224
10	1 hr	0 Gy	SAMPLE_0.00Gy_1h-10	GSM1561175	0.30 Gy	SAMPLE_0.30Gy_1h-10	GSM1561225
1	2 hr	0 Gy	SAMPLE_0.00Gy_2h-1	GSM1561176	0.30 Gy	SAMPLE_0.30Gy_2h-1	GSM1561226
2	2 hr	0 Gy	SAMPLE_0.00Gy_2h-2	GSM1561177	0.30 Gy	SAMPLE_0.30Gy_2h-2	GSM1561227
3	2 hr	0 Gy	SAMPLE_0.00Gy_2h-3	GSM1561178	0.30 Gy	SAMPLE_0.30Gy_2h-3	GSM1561228
4	2 hr	0 Gy	SAMPLE_0.00Gy_2h-4	GSM1561179	0.30 Gy	SAMPLE_0.30Gy_2h-4	GSM1561229
5	2 hr	0 Gy	SAMPLE_0.00Gy_2h-5	GSM1561180	0.30 Gy	SAMPLE_0.30Gy_2h-5	GSM1561230
6	2 hr	0 Gy	SAMPLE_0.00Gy_2h-6	GSM1561181	0.30 Gy	SAMPLE_0.30Gy_2h-6	GSM1561231
7	2 hr	0 Gy	SAMPLE_0.00Gy_2h-7	GSM1561182	0.30 Gy	SAMPLE_0.30Gy_2h-7	GSM1561232
8	2 hr	0 Gy	SAMPLE_0.00Gy_2h-8	GSM1561183	0.30 Gy	SAMPLE_0.30Gy_2h-8	GSM1561233
9	2 hr	0 Gy	SAMPLE_0.00Gy_2h-9	GSM1561184	0.30 Gy	SAMPLE_0.30Gy_2h-9	GSM1561234
10	2 hr	0 Gy	SAMPLE_0.00Gy_2h-10	GSM1561185	0.30 Gy	SAMPLE_0.30Gy_2h-10	GSM1561235
1	6 hr	0 Gy	SAMPLE_0.00Gy_6h-1	GSM1561186	0.30 Gy	SAMPLE_0.30Gy_6h-1	GSM1561236
2	6 hr	0 Gy	SAMPLE_0.00Gy_6h-2	GSM1561187	0.30 Gy	SAMPLE_0.30Gy_6h-2	GSM1561237
3	6 hr	0 Gy	SAMPLE_0.00Gy_6h-3	GSM1561188	0.30 Gy	SAMPLE_0.30Gy_6h-3	GSM1561238
4	6 hr	0 Gy	SAMPLE_0.00Gy_6h-4	GSM1561189	0.30 Gy	SAMPLE_0.30Gy_6h-4	GSM1561239
7	6 hr	0 Gy	SAMPLE_0.00Gy_6h-7	GSM1561190	0.30 Gy	SAMPLE_0.30Gy_6h-7	GSM1561240
1	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-1	GSM1561191	1.50 Gy	SAMPLE_1.50Gy_1h-1	GSM1561241
2	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-2	GSM1561192	1.50 Gy	SAMPLE_1.50Gy_1h-2	GSM1561242
3	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-3	GSM1561193	1.50 Gy	SAMPLE_1.50Gy_1h-3	GSM1561243
4	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-4	GSM1561194	1.50 Gy	SAMPLE_1.50Gy_1h-4	GSM1561244
5	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-5	GSM1561195	1.50 Gy	SAMPLE_1.50Gy_1h-5	GSM1561245
6	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-6	GSM1561196	1.50 Gy	SAMPLE_1.50Gy_1h-6	GSM1561246
7	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-7	GSM1561197	1.50 Gy	SAMPLE_1.50Gy_1h-7	GSM1561247
8	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-8	GSM1561198	1.50 Gy	SAMPLE_1.50Gy_1h-8	GSM1561248
9	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-9	GSM1561199	1.50 Gy	SAMPLE_1.50Gy_1h-9	GSM1561249
10	1 hr	0.15 Gy	SAMPLE_0.15Gy_1h-10	GSM1561200	1.50 Gy	SAMPLE_1.50Gy_1h-10	GSM1561250
1	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-1	GSM1561201	1.50 Gy	SAMPLE_1.50Gy_2h-1	GSM1561251
2	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-2	GSM1561202	1.50 Gy	SAMPLE_1.50Gy_2h-2	GSM1561252
3	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-3	GSM1561203	1.50 Gy	SAMPLE_1.50Gy_2h-3	GSM1561253
4	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-4	GSM1561204	1.50 Gy	SAMPLE_1.50Gy_2h-4	GSM1561254
5	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-5	GSM1561205	1.50 Gy	SAMPLE_1.50Gy_2h-5	GSM1561255
6	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-6	GSM1561206	1.50 Gy	SAMPLE_1.50Gy_2h-6	GSM1561256
7	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-7	GSM1561207	1.50 Gy	SAMPLE_1.50Gy_2h-7	GSM1561257
8	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-8	GSM1561208	1.50 Gy	SAMPLE_1.50Gy_2h-8	GSM1561258
9	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-9	GSM1561209	1.50 Gy	SAMPLE_1.50Gy_2h-9	GSM1561259
10	2 hr	0.15 Gy	SAMPLE_0.15Gy_2h-10	GSM1561210	1.50 Gy	SAMPLE_1.50Gy_2h-10	GSM1561260
1	6 hr	0.15 Gy	SAMPLE_0.15Gy_6h-1	GSM1561211	1.50 Gy	SAMPLE_1.50Gy_6h-1	GSM1561261
2	6 hr	0.15 Gy	SAMPLE_0.15Gy_6h-2	GSM1561212	1.50 Gy	SAMPLE_1.50Gy_6h-2	GSM1561262
3	6 hr	0.15 Gy	SAMPLE_0.15Gy_6h-3	GSM1561213	1.50 Gy	SAMPLE_1.50Gy_6h-3	GSM1561263
4	6 hr	0.15 Gy	SAMPLE_0.15Gy_6h-4	GSM1561214	1.50 Gy	SAMPLE_1.50Gy_6h-4	GSM1561264
7	6 hr	0.15 Gy	SAMPLE_0.15Gy_6h-7	GSM1561215	1.50 Gy	SAMPLE_1.50Gy_6h-7	GSM1561265

At the end of the incubation period, blood samples were transferred to 50ml conical tubes containing 30ml of NH_4Cl red blood cell (RBC) lysis buffer (1:15 v/v dilution), pre-warmed at 37 °C in order to isolate leukocytes. The tubes were agitated 5 minutes on a rocker platform and centrifuged for 5 minutes at 1500 RPM at room temperature. Cells were suspended in 10 ml of phosphate-buffered saline (PBS) and centrifuged again twice for 5 minutes at 1500 RPM. White blood cells (WBC) were suspended in 2 ml PBS, equivalent to the initial volume of the whole blood. WBC were centrifuged 5 min at 1500 RPM. Supernatant was discarded and cell pellets were suspended in 600 μl RLT lysis buffer

Table 2
Radiation dependent differentially expressed genes measured as the number of differentially expressed probes detected by Limma at $p \leq 0.05$ for low (0.15 Gy vs. 0.0 Gy), mid (0.30 Gy vs. 0.0 Gy) and high (1.5 vs. 0.0 Gy) radiation levels at early (1 hr), mid (2 hr), and late (6 hr) as well as combined time points. Arrows indicate the number of up (↑) or down (↓) regulated probesets.

Time Point	Comparison		
	0.15 Gy vs 0 Gy	0.30 Gy vs 0 Gy	1.5 Gy vs 0 Gy
Early (1hr)	879 (635↑, 244↓)	1462 (898↑, 564↓)	2278 (1423↑, 855↓)
Mid (2 hr)	905 (446↑,459↓)	1521 (763↑,758↓)	3575 (2115↑,1460↓)
Late (6 hr)	841 (510↑,331↓)	1461 (776↑,685↓)	1884 (1177↑,707↓)
Combined	805 (496↑,309↓)	1913 (970↑,943↓)	3289 (1815↑,1474↓)

(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 RNAeasy Mini Kit (Qiagen). Optional on-column DNase digestion was performed to eliminate genomic DNA contamination. Total RNA was eluted in 50 μl 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).

Biotinylated cRNA were prepared according to the standard protocol for Affymetrix® 3′ IVT Express Reagent Kit (Affymetrix® Inc., Santa Clara, CA) from 100 ng total RNA. Following fragmentation, 10 μg of cRNA were hybridized for 16 hr at 45 °C to Affymetrix® GeneChip® PrimeView™ Human arrays (GEO platform GPL15207) according to the GeneChip® 3′ array Hybridization User Manual from Affymetrix®. The arrays were washed and stained in an Affymetrix Fluidics Station 450.

2.3. Data acquisition

GeneChips® were scanned using GeneChip® Scanner 3000 7G (Affymetrix) and the GeneChip® Command Console® software version 4.0 (Affymetrix®). A total of 100 CEL files representing the raw intensity values were generated, which were subsequently submitted to GEO (Table 1). The CEL files, they were then processed in RStudio (version 0.98.501) [3] using R (version 3.0.1 2013-05-16 “Good Sport”) [4] and Bioconductor [4]. Pre-processing and normalization of the CEL files was performed in R using the oligo package [5] and robust multichip averaging (RMA) [6].

CEL files were organized into nine categories of comparisons: 1) dose-dependent, time-independent; 2) time-dependent, dose-independent; 3) early time, radiation-dependent; 4) mid time, radiation-dependent; 5) late time, radiation dependent; 6) no radiation, time-dependent; 7) low radiation, time-dependent; 8) mid radiation, time-dependent; and 9) high radiation, time-dependent. Early time was defined as 1 hr; mid time as 2 hr; and late time as 6 hr. Low radiation was defined as 0.15 Gy; mid radiation as 0.30 Gy; and high radiation as 1.5 Gy.

Differentially expressed genes (DEGs) (defined as Affymetrix® probesets) were determined using Limma [7]. Levels of differential expression for all contrasted conditions for all genes (regardless of p-value) were saved individually. Genes passing a p-value level of 0.05 or less for the radiation dependent results or an adjusted P-value of 0.05 (allowing for FDR) and a $\log_2$ Fold Change ≥ 1 or ≤ -1 (2 fold difference) were stored separately. Tables 2 and 3 list the major contrasts considered as well as the number of differentially expressed probes found (out of a total of 49,495 probes). These differentially expressed probe sets were then analyzed, and differentially expressed Entrez gene IDs [8] were determined for further analysis with categoryCompare [9]. Note there is not a 1-1 correspondence between probesets and Entrez IDs, since there may be multiple probesets from a single gene, and since each probeset may actually correspond to multiple transcripts/genes. The resulting differential expression analysis of this dataset will provide value in allowing for the detection of time-dependent and radiation-dependent biomarkers.

2.4. Expression of radiation-modulated biomarkers H2AFX, TP53 and CDKN1A

In our previous study of the immediate response to ionizing radiation [1,2], we looked at the response of three prominent genes commonly used to measure radiation exposure, including γH2AFX,

Table 3
Time dependent differentially expressed genes measured as the number of differentially expressed probesets detected by Limma at $p \leq 0.05$ and a log fold-change cutoff of 1 for mid (2 hr vs. 1 hr) and late (6 hr vs. 1 hr) time points measured with no radiation (0 Gy), low radiation (0.15 Gy), mid radiation (0.30 Gy) and high radiation (1.5 Gy) as well as all radiation levels combined. Arrows indicate the number of up (↑) or down (↓) regulated probesets.

Radiation Level	Comparison	
	2 hr vs 1 hr	6 hr vs 1 hr
No (0 Gy)	126 (117↑, 9↓)	1086 (630↑, 456↓)
Low (0.15 Gy)	194 (170↑,24↓)	1288 (751↑,537↓)
Mid (0.30 Gy)	222 (194↑,28↓)	1367 (771↑,596↓)
High (1.5 Gy)	272 (236↑,36↓)	1414 (800↑,614↓)
Combined	185 (168↑,17↓)	1285 (723↑, 562↓)

TP53, and CDKN1A. In that study, it was shown that two of these, H2AFX and TP53, were not suitable biomarkers for the radiation levels (0.3 Gy, 1.5 Gy and 3.0 Gy) and time (1 hr) studied in part due to the inability to measure changes in phosphorylation of H2AFX into γH2AFX as well as the fact that changes in TP53 can be subtle, yet cause cascading changes of downstream targets [10]. Examination of the dose-dependent effects of the transcript abundance of these three genes indicate that CDKN1A transcript levels increase substantially in a dose-dependent fashion regardless of time since exposure, while H2AFX and TP53 transcript counts are not significantly affected (Fig. 1). In terms of time, it appears as though CDKN1A and H2AFX transcript abundance spikes at an early time point (here measured at 2 hr) regardless of radiation level, after which the transcript level for H2AFX returns to a level slightly above pre-exposure while CDKN1A decreases, but is still significantly changed from its pre-exposure level. At the same time, the level of TP53 transcript counts shows a slight linear decrease

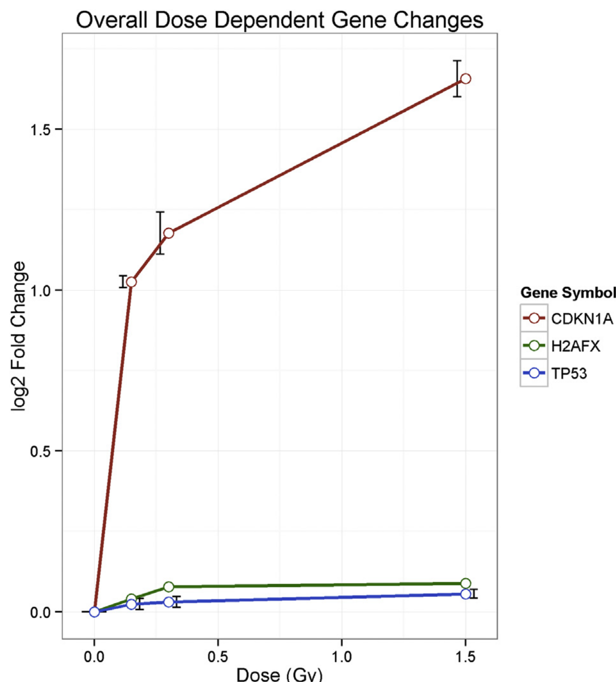

Fig. 1. Dose-dependent gene expression patterns for CDKN1A, H2AFX, and TP53 at 2hr post-exposure. Shown are the changes in transcript levels for three radiation marker genes, CDKN1A, H2AFX, and TP53 at 2hr post-exposure at varying radiation doses. Only CDKN1A shows a response, indicating that H2AFX and TP53 are not appropriate transcriptional candidates. Similar results were found at 6hr (not shown).

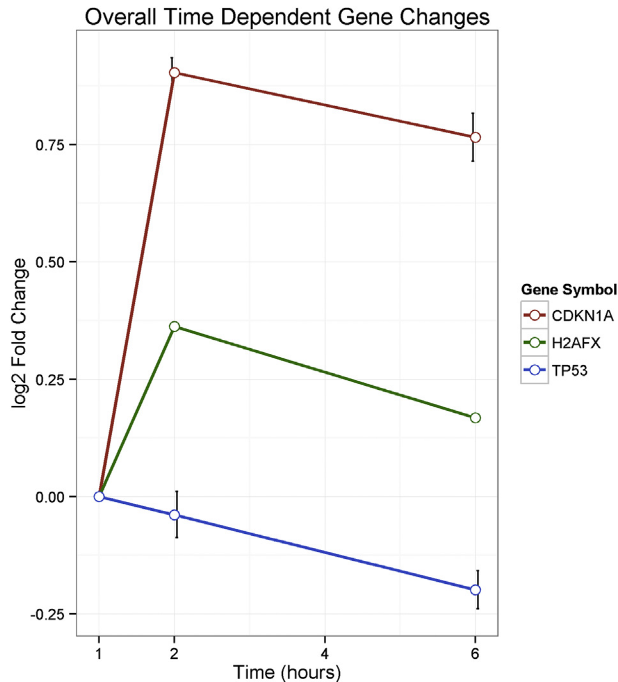

Fig. 2. Time-dependent gene expression patterns for CDKN1A, H2AFX, and TP53 at 1.5Gy radiation. Shown are the changes in transcript levels for three radiation marker genes, CDKN1A, H2AFX, and TP53 over time at 1.5Gy. While CDKN1A shows a small time-dependent response, the transcript changes for H2AFX and TP53 over time are non-significant. Thus, H2AFX and TP53 are not appropriate transcript markers for radiation exposure over time. Similar results were found at 0.15 and 0.3 Gy (not shown).

(Fig. 2). This preliminary data suggests that H2AFX and TP53 are not suitable transcript biomarkers, even when looking at a more comprehensive level of radiation exposure and time since exposure. The dataset described here thus contains valuable information on potentially novel sensitive and specific transcript biomarkers that can be used independently or as a group for detecting ionizing radiation events on a more accurate level than three widely assayed gene markers.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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