Measuring Radiation Exposure in Human Blood using Gene Expression

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Abstract

Mammalian cells are known to express genes that are associated with repairing damaged DNA. The transcript *CDKN1A* is one of several cell cycle regulator genes expressed in response to cell damage by ionizing radiation (IR). In this study, male and female lymphocytes; previously exposed ex vivo to IR, were used to demonstrate linear gene expression responses that may vary between genders. We used qRT-PCR to generate response curves for *CDKN1A*. No differences were identified for the endogenous control gene *GAPDH*. *CDKN1A* expression demonstrated average fold changes well above three fold for three of the four healthy patient donors at 24 hours after 2, 3, and 4 Gy exposures. Doses 2 and 3 Gy were significantly upregulated at 24 hours. No significant difference was seen between genders for *CDKN1A*. Our data confirms that genes involved in DNA repair, cell cycle arrest, and apoptosis can be used as biomarkers of exposure to IR. Because of growing concern of IR exposure through different mechanisms; either by nuclear catastrophe or medical radiation, gene expression analysis is a promising method for identification and estimation of IR exposure.

Background

- Ionizing radiation (IR) can be particularly harmful to biological systems and can cause damages in DNA such as cross-links, bulky lesions, and double and single strand breaks.
- Multiple transcription pathways; including cell cycle arrest, DNA repair, and apoptosis, are involved in the repair of IR induced DNA damage.
- Because IR is simultaneously carcinogenic and used as a treatment for cancer, there is a great need for the use of biodosimetry to identify and understand gene responses for individuals receiving therapeutic treatment and in case of a nuclear terrorist attack.

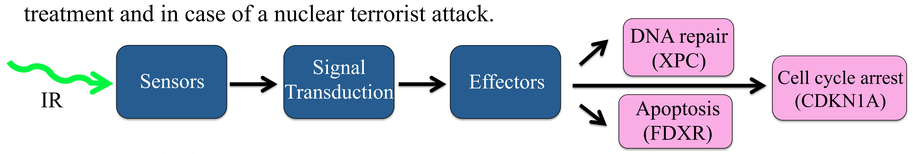

Figure 1. Cell responses to ionizing radiation with an example of one of several genes involved at each transcriptional mechanism.

Methods

RNA isolation (RNeasy Micro Kit)

1. Lyse cells with RLT buffer
2. Alter binding with ethanol
3. Add RNA to RNeasy MinElute spin column membrane for adherence
4. Discard digested DNA
5. Wash membrane bound RNA with RW1 Buffer and then wash with elutant
6. Use Nanodrop to determine RNA quantity

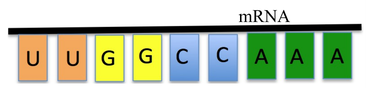

Figure 2. Isolated mRNA from white blood cells.

RNA to cDNA transcription (High Capacity DNA Archive Kit)

1. Prepare Master Mix
2. Add cDNA master mix and mRNA to tubes
3. Set Thermocycler for reverse transcription

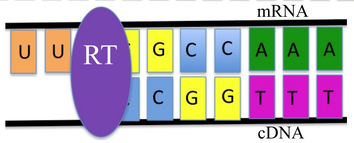

Figure 3. Reverse Transcriptase (RT) synthesizes cDNA from mRNA.

qRT-PCR (TaqMan and Sharkataq Universal PCR Master Mix)

1. Dilute cDNA
2. Add qRT-PCR master mix and cDNA to tubes
3. Set qRT-PCR instrument to appropriate settings and add loaded array plate to instrument

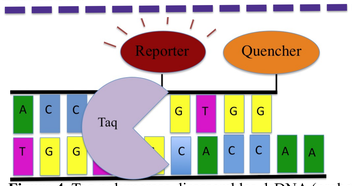

Figure 4. Taq polymerase disassembles dsDNA (probe) and replaces all nucleotides causing reporter to fluoresce.

Analysis

1. Descriptive statistics

$$\Delta C_T = C_{T,\ Time\ x} - C_{T,\ Time\ 0}$$

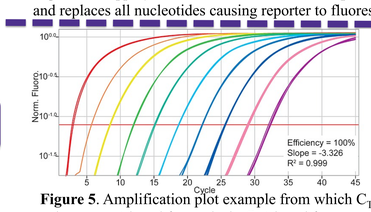

Figure 5. Amplification plot example from which C_T values are retrieved for analysis. Retrieved from www.kapabiosystems.com.

Results and Discussion

1

Table 1. RNA concentrations and cycle threshold (C_T) values for two males and two females exposed to differing doses of ionizing radiation.

Sample (Gender)	Dose (Gy)	RNA quantity (ng/µl)	GAPDH x̄ C_T value	CDKN1A x̄ C_T value
1-(Female)	0	498	30.1	33.9
	2	670	30.2	32.1
	3	740	30.6	32.0
	4	172	31.7	32.8
2-(Male)	0	310	31.1	35.0
	2	145.6	30.5	32.5
	3	143.3	31.1	32.6
	4	384	34.4	35.5
3-(Male)	0	47.9	32.0	35.4
	2	199.6	28.7	30.3
	3	139.7	28.9	30.2
	4	133.7	29.1	29.9
4-(Female)	0	113.5	26.1	29.3
	2	49.4	27.0	29.1

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Transcription Response for Individual Patients

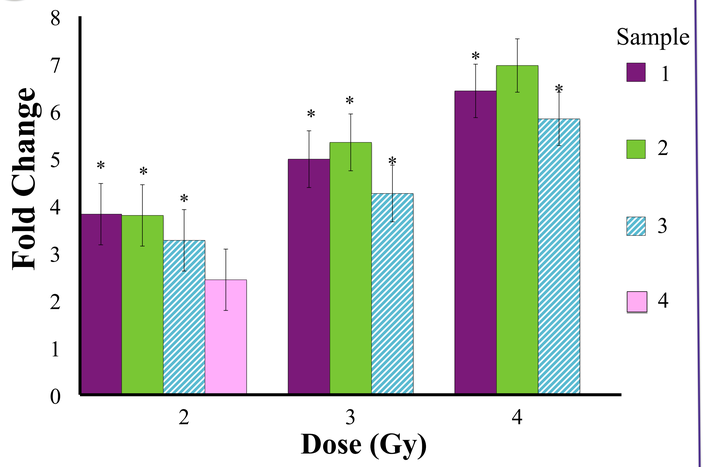

Figure 6. *CDKN1A* fold change for sample 1 (female), sample 2 (male), sample 3 (male) and sample 4 (female) at 2, 3 and 4 gray doses of low dose ionizing radiation (± standard error) at 24 hours. *CDKN1A* fold changes were higher for every patient with increasing dose. Significant differences (*p < 0.05) were found amongst individual patients, while sample 2 did not have a significant response at 4 Gy and sample 4 at 2 Gy.

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The Gene *CDKN1A* shows a Linear Dose Response

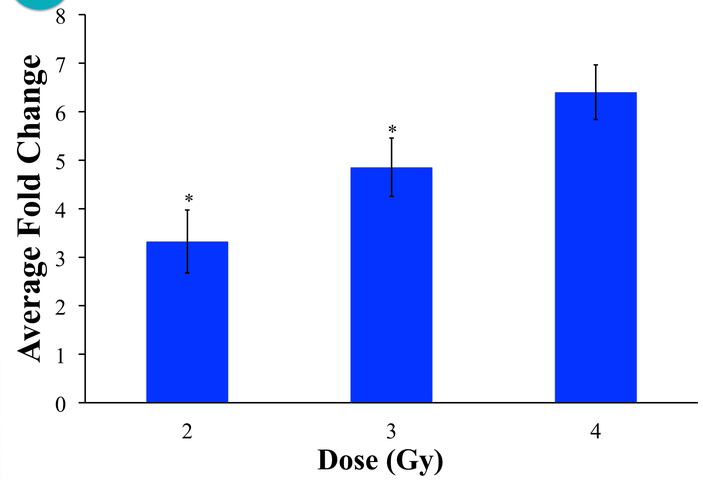

Figure 7. Average transcription fold change for four patients exposed to ionizing radiation doses of 2, 3, and 4 gray (± standard error) at 24 hours. *CDKN1A* showed linear response for the measured doses. Significant responses (*p < 0.05) were found for 2 and 3 Gy.

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Expression of *CDKN1A* across Multiple Cell Types

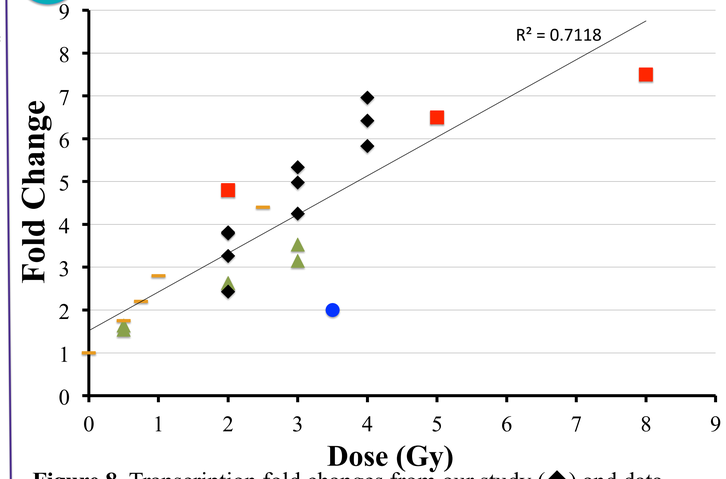

Figure 8. Transcription fold changes from our study (◆) and data taken from Paul and Amundson, 2008 peripheral blood (■), Rodnigen et al., 2005 subcutaneous fibroblasts (●), Grace and Blakely, 2007 whole blood (—), and from lymphoblastoid cell lines at LLNL (▲). For this figure we show that our data is similar to data from other previously published studies using different cell types.

Next Steps

- Confirmation of fold changes for the following genes:

Response	Genes
Cell Cycle Arrest	*FDXR, BAX, BBC3*
DNA repair	*LIG1, XPC, DDB2, POLH, RAD51*
Apoptosis	*GADD45a, CCNG1, CHK2, PCNA*

References

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Paul, S. and S. A. Amundson. 2008. Development of gene expression signatures for practical radiation biodosimetry. International Journal of Radiation Oncology Biology Physics Journal 71: 1236-1244.

Rodnigen, O. K., J. Overgaard, J. Alsner, T. Hastie, A. Borresen-Dale. 2005. Microarray analysis of the transcriptional response to single of multiple doses of ionizing radiation in human subcutaneous fibroblasts. Radiotherapy and Oncology 77: 231-240.

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