The Effects of Radiation Exposure: An *in vivo* vs *in vitro* Biomarker Study Analysis

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Abstract

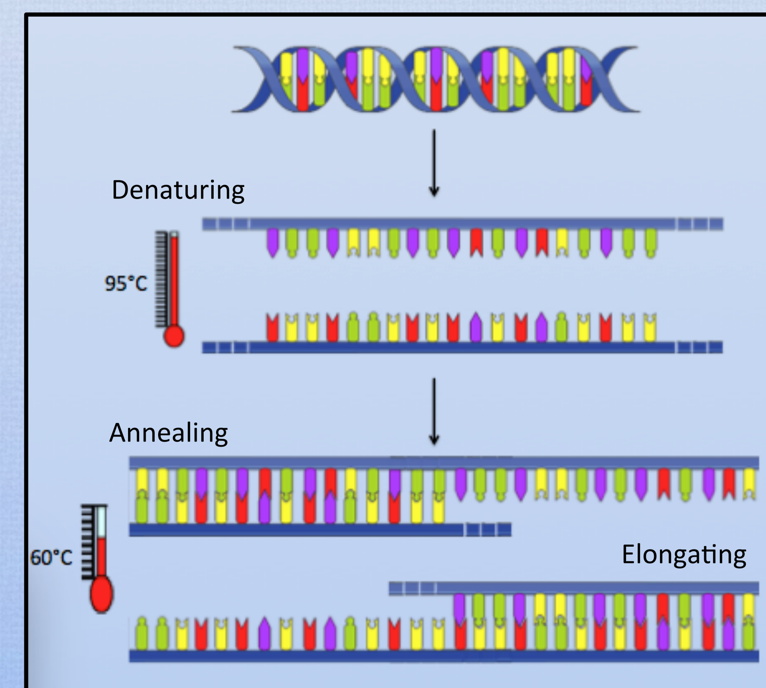
Cells contain many genes that encode proteins which dictate the cell function. The thousands of genes expressed in a particular cell determine what the cell can do. When a cell is damaged, there are powerful mechanisms to repair damaged genes. Here, we investigated different cell lines that have been exposed to ionizing radiation (IR). To understand the effect of genes to different doses of IR and their correlations to up-regulated and down-regulated genes, a biomarker study has been performed on the following different cell types: *In vivo* lymphocytes from Metaiodobenzylguanidine (MIBG) isotopic radiation treated neuroblastoma patients and *in vitro* lymphoblastoid as well as fibroblast cells externally exposed to radiation. Results of the study concluded that qRT PCR analysis can be used to illustrate different trends between irradiated samples. The externally radiated *in vitro* lymphoblastoid and fibroblast samples showed positive fold change differences for the majority of transcripts studied. In addition, the fibroblast cells showed the highest expression of transcripts compared to the other two studies. This knowledge can be applied to accidental radiation exposures and other biodefense mechanisms. It allows us to understand the process of molecular changes, predict the outcome, and monitor radiation progression.

Background

Ionizing radiation is a major DNA damaging agent that has chronic effects to the human body by breaking chemical bonds and resulting in mutations. One of the first parts of the body to suffer the effects of IR are the blood and skin. In this study, a biomarker analysis was used to determine the effects of radiation on the genes involved in multiple biological processes. This includes the genes CDKN1A, FDXR, BAX, GADD45A, BCL2L1, BCL2, and DDB2. The expression of these genes can be used as a biodosimeter that will allow us to determine how much radiation someone has been exposed to in case of a biological threat or natural disaster.

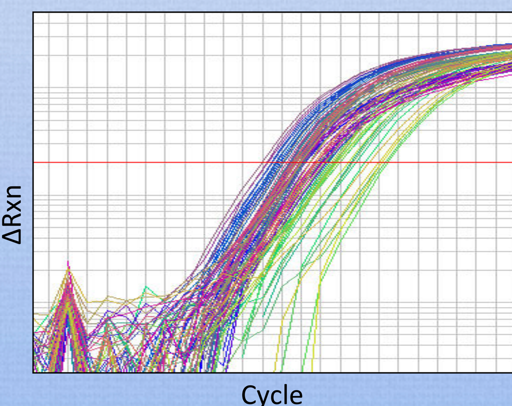
Gene	Gene Name	Function
CDKN1A	Cyclin-Dependent Kinase Inhibitor 1A	• Plays a regulatory role in S phase DNA replication and DNA damage repair. • Regulator of cell cycle progression at G1.
FDXR	Ferredoxin Reductase	• Initiates electron transport for cytochromes p 450 receiving electrons from NADPH.
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase	• GAPDH represents an optimal choice of a housekeeping gene and/or loading control
BAX	BCL2 Associated X Protein	• Functions as an apoptotic activator.
GADD45A	Growth Arrest and DNA damage Inducible Alpha	• Promotes nucleotide-excision repair, cell arrest, and apoptosis. • The protein encoded by this gene responds to environmental stresses by mediating activation of the p38/JNK pathway via MTK1/MEKK4 kinase.
BCLXL/ BCL2L1	BCL2 like 1	• Act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities. • Has a critical role in tumor progression, development and-or radioresistance.
BCL2	B-Cell CLL/Lymphoma 2	• This gene encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells such as lymphocytes. • Regulates cell death.
DDB2	Damage Specific DNA Binding Protein 2	• This gene encodes a protein that is necessary for the repair of ultraviolet light-damaged DNA. • Facilitates the cellular response to DNA damage.

Methods



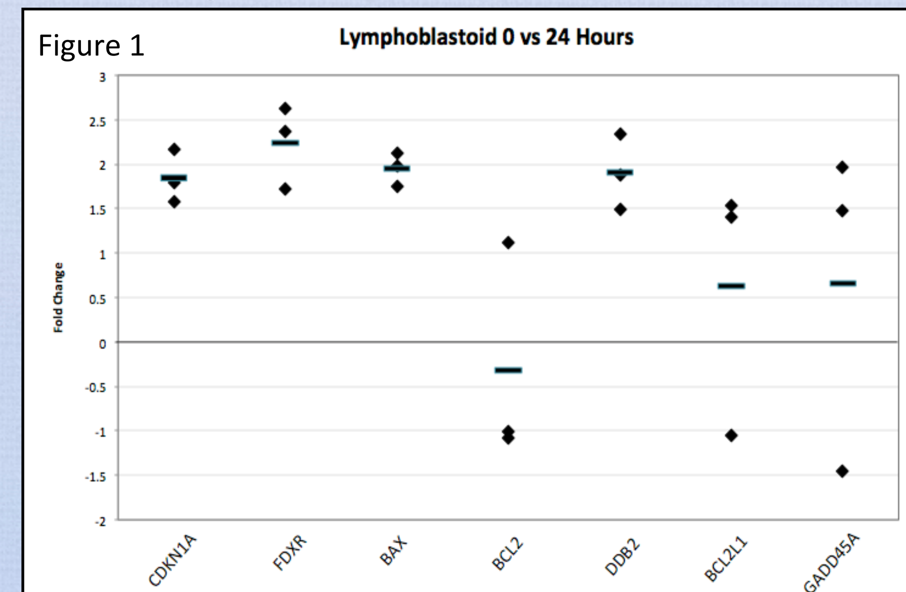
Quantitative Real Time PCR (qRT PCR) is the amplification of DNA with a polymerase chain reaction monitored in real time.
The process undergoes several cycles:

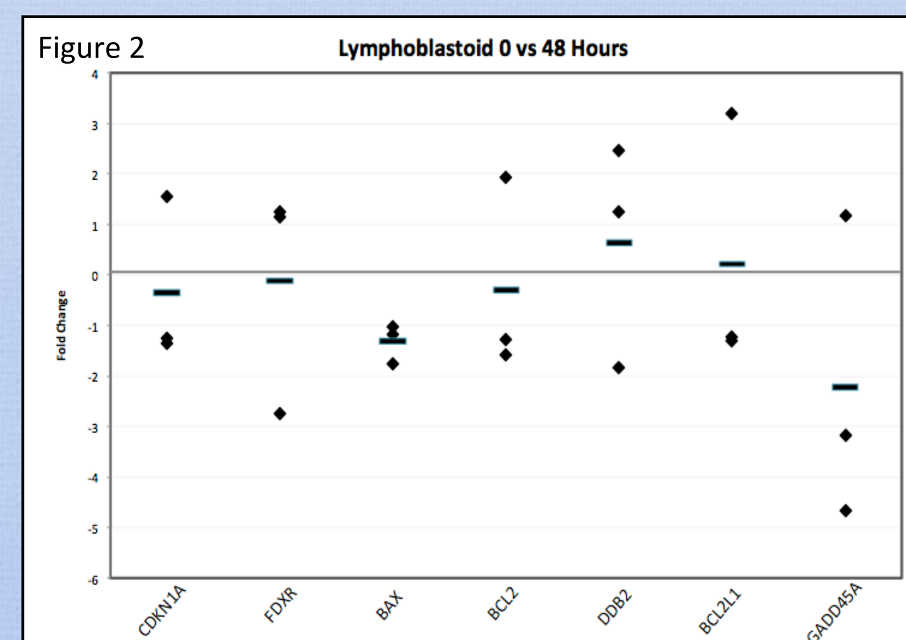
1. Heat up to break apart the DNA strands (95°C)
2. Anneal specific gene primers
3. Elongate with new dNTPs (DNA nucleotides)



Results

Lymphoblastoid cells illustrate up-regulation of transcripts at 24 hours after exposure that return to baseline at 48 hours later

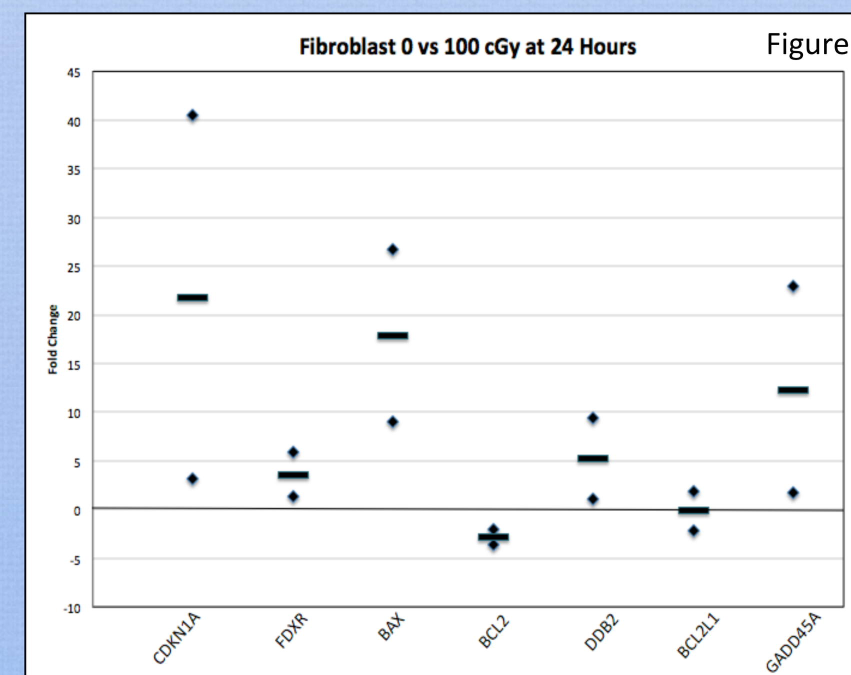




Figures 1 and 2 show the fold change differences between lymphoblastoid cells irradiated at 24 hours and 48 hours after 200 cGy IR exposure. Each dot represents one patient analyzed in triplicate. The bar represents average fold change among the three individual patients. Figures 1 and 2 indicate that most of the targeted genes had a higher fold change at 24 hours compared to 48 hours. All genes are compared to GAPDH an endogenous control transcript.

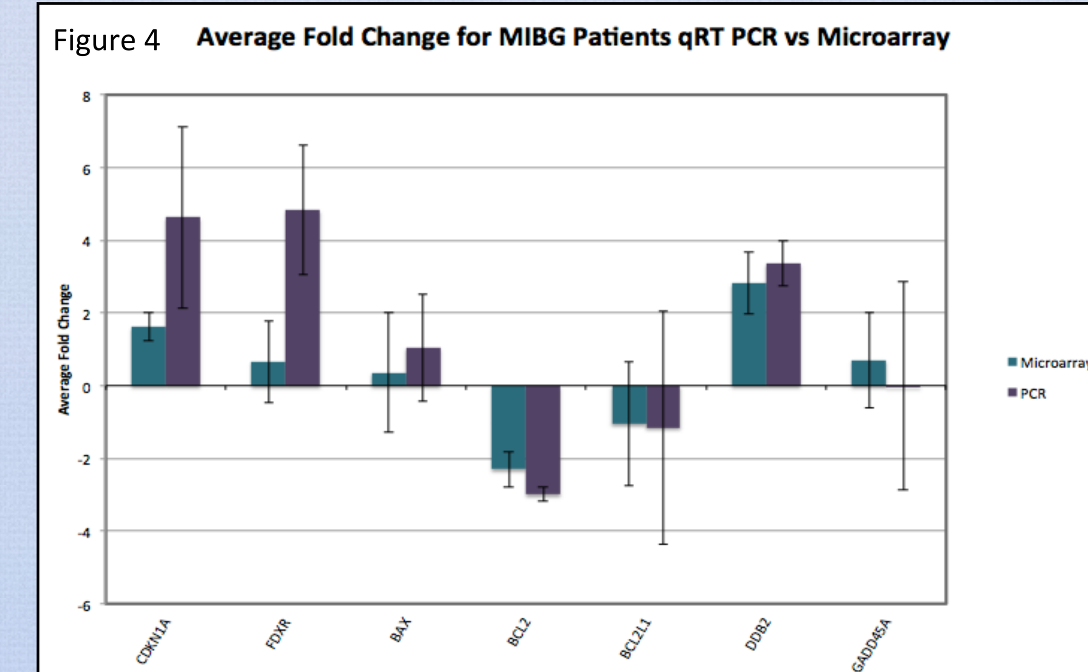
Fibroblast also demonstrate an up-regulation of transcripts at 24 hours after exposure

Figure 3 displays the average fold change across two independent patients at 24 hours post treatment. The bar represents the average fold change of both patients combined. Overall, the fibroblast fold change differences were much higher than lymphoblastoid samples.



Internalized I^{131} radiation demonstrates up-regulation of the same selected transcripts at 72 hours in radiotherapy patients

The average fold change of all radiotherapy patients using qRT PCR and Microarray under 72 Hours with standard deviation. Figure 4 demonstrates a trend in gene regulation in both methods.



Average transcript alterations for both in vitro vs. in vivo exposed samples show similar trends

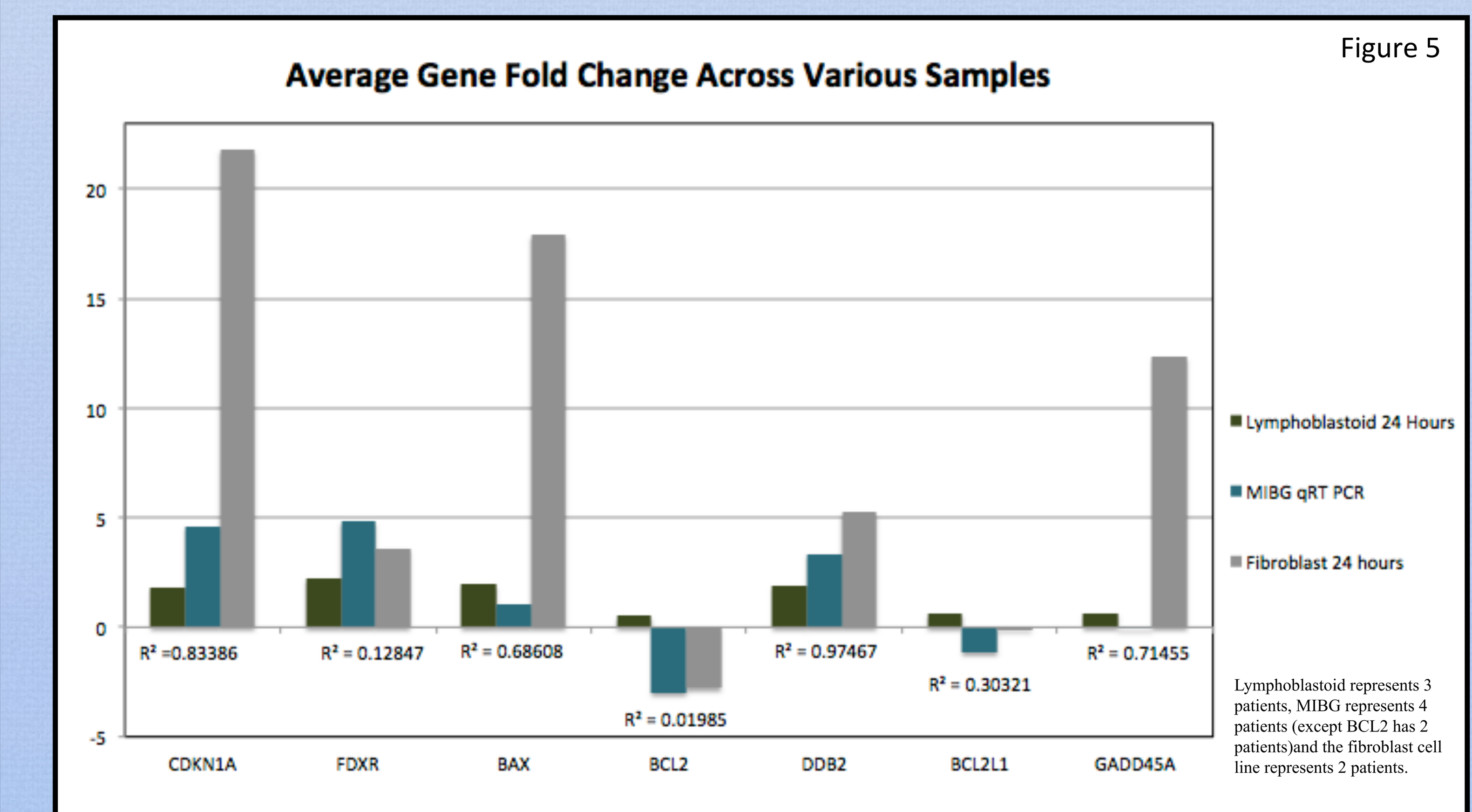


Figure 5 demonstrates the average fold changes across all patients: Internalized I^{131}(MIBG) qRT PCR at 72 hours treatment, as well as externalized 200cGy lymphoblastoid cells at 24 hours, and 100cGy fibroblast cells at 24 hours after exposure. In general, several DNA damage and repair genes are up-regulated, whereas others, such as BCL2 and BCl2L1, show down-regulated trends.

Conclusion

- *In vitro* radiation showed the greatest fold change differences 24 hours after exposure. *In vivo* Internalized I^{131} transcript levels show up-regulated and down-regulated trends beginning at 72 hours.
- Biomarker trends serve as a starting point to infer the dose of radiation one may have received.
- We can predict the molecular response to radiation treatments, especially for individuals undergoing radiation therapy.
- Can be used as a biodosimeter/biomarker for natural disasters / biological threats.
- Most transcripts demonstrated up-regulation, often involving apoptotic signaling or DNA damage repair.

Bibliography

Budworth, H., Snijders, A. M. Marchetti, et al (2012). DNA repair and cell cycle biomarkers of radiation exposure and inflammation stress in human blood. Plos One, 7: 1-11

Brzóska, K., & Kruszewski, M. (2015). Toward the development of transcriptional biodosimetry for the identification of irradiated individuals and assessment of absorbed radiation dose. Retrieved July 01, 2016, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4510913/

Farber, D. (n.d.). MIBG Therapy for Neuroblastoma. Retrieved July 01, 2016, from http://www.danafarberbostonchildrens.org/innovative-approaches/mibg-therapy.aspx

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