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 on 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 is 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 biosensor which will allow us to determine how much radiation someone has been exposed to in case of a biological threat or natural disaster.

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

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

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.

Internalized 131I radiodinated 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 131I(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 131I 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.

Acknowledgements

*This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.² Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Any mention of trade names, trademarks, or commercial products does not constitute an endorsement, recommendation, or favoring by the U.S. Government.

The authors are grateful to M. Marshak, et al. (2012) DNA repair and cell cycle biomarkers of radiation exposure and inflammation stress in human blood. PLoS ONE, 7, 1-11

Bibliography

- Angeles Mora1,2, Angela Evans2, Matthew Coleman2
- Fresno Unified School District1, Lawrence Livermore National Laboratory2
- 1Department of Biology, Madera, CA, USA, 2Fresno State University, Fresno, CA, USA
- *This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.² Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.
- The authors acknowledge the encouragement and support of Dr. Angela Evans for her continuous help during my research experience and for the wonderful conversations.
- We would like to thank Dr. M. Marshak, et al. for allowing the use of statistical analysis and for providing the data of blood samples. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.² Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.
- The authors acknowledge the encouragement and support of Dr. Angela Evans for her continuous help during my research experience and for the wonderful conversations.