ABSTRACT

To fill the need for rapid, high-throughput radiation biodosimetry in the event of a large-scale radiological incident, we are developing a fully automated integrated biochip system based on gene expression. We have used Affymetrix whole genome microarrays to profile the gene expression response of human blood to ionizing radiation. We measured gene expression profiles of ex vivo irradiated human peripheral blood from healthy donors. This study spanned doses from 0.5 to 8 Gy, and monitored gene expression at 24 hours after irradiation. Real-time PCR of CDKN1A revealed a biphasic response, with linear kinetics up to 2 Gy and further linear increases through the highest dose used. A dose-response relationship was further evident within the microarray data. Preliminary analysis yielded 109 genes that distinguish between untreated controls and four different doses of irradiation.

We have also used blood samples from patients undergoing total body irradiation (TBI) as a model to test gene expression signatures in vivo. Interestingly, in vivo CDKN1A expression after a single TBI fraction (1.5 Gy) showed induction ratios similar to the ex vivo 2 Gy samples. When pre- and post-TBI samples were tested against the preliminary ex vivo signature, the same gene set was able to discriminate control from irradiated patient samples, although differences from the healthy donors were also evident.

In order to make such gene expression signatures useful for triage, we are developing cartridges to take a blood sample and automatically perform a chemo-luminescence based gene-expression assay. The cartridges contain all necessary reagents, pumps, valves and control electronics, do not rely on molecular amplification methods such as PCR, and deliver highly consistent results (~<1%). We have developed a handheld, microprocessor-controlled prototype for sample preparation, and are modifying a commercial chemo-luminescence reader to read the microfluidic cartridges.

This biodosimetry concept was tested at the Coyote Crisis Campaign 2006, a disaster preparedness exercise in Scottsdale, Arizona. While additional studies are needed, our current findings strongly support the usefulness of gene expression signatures and our biochip approach for radiation biodosimetry.

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CONCLUSIONS

- Microarray analysis of human peripheral blood irradiated ex vivo irradiated 240 genes that distinguished between un-irradiated controls and 4 different doses of radiation by their level of transcription at both 6 and 24 hours after irradiation.

- Two thirds of these genes were significantly up-regulated and one third down-regulated.

- Two quantitative RT-PCR confirmed the dose-response behavior of 5 selected genes (CDKN1A, FDXR, PP1F1, BCC1 and SESMT).

- A self-contained microfluidic cartridge incorporating the quantitative nucleic acid protection assay provides a realistic platform for radiation triage.

- Testing of an overall concept for use in the field is an important component of biodosimetry development, and should be incorporated at an early stage.