

## **Improvement of Radiation Dose Assessment Using Multiple-Protein Expression and Hematological Profiles**

**N.I. Ossetrova,<sup>1</sup> G.D. Ledney,<sup>1</sup> A.M. Farese,<sup>2</sup> T.J. MacVittie,<sup>2</sup>  
D.J. Sandgren,<sup>1</sup> S. Gallego,<sup>1</sup> and W.F. Blakely<sup>1</sup>**

<sup>1</sup>Armed Forces Radiobiology Research Institute (AFRI)  
8901 Wisconsin Ave., Bethesda, MD, 20889-5603 USA

<sup>2</sup>Marlene and Steward Greenebaum Cancer Center  
Bressler Research Building, Room 7-039  
University of Maryland-Baltimore  
655 West Baltimore Street, Baltimore, MD 21201 USA

e-mail: [ossetrova@afri.usuhs.mil](mailto:ossetrova@afri.usuhs.mil)

There is a present need to rapidly identify severely irradiated individuals that require prompt medical treatment in mass-casualty incidents, as well as to distinguish exposed vs. non-exposed individuals (Blakely et al. 2005). Early treatment of populations exposed to ionizing radiation (MacVittie et al. 2005; Waselenko et al. 2004) requires accurate and rapid biodosimetry with a precision as high as possible to determine an individual's exposure level and risk for morbidity and mortality. The early medical-management situation in the Chernobyl nuclear power accident was made difficult because for several days after the incident the doses to individuals were not known with precision (Guskova et al. 2001). The development of accurate methods for rapid individual dose assessment possesses some challenges. A major source of uncertainty is individual variability in radiation response.

Hematological biomarkers of exposure to ionizing radiation are well characterized and used in medical management of radiological casualties (Dainiak et al. 2003). Measurements of lymphocyte depletion kinetics (Baranov et al. 1995; Goanz et al. 1997) and time- and dose-dependent changes in neutrophil cell numbers observed after irradiation (Fliedner et al. 2001) provide clinical information soon after exposure. However, because of large variation in lymphocyte and neutrophil counts among normal individuals, it necessitates repeated measurements over a prolonged period. Normalization of the inter-individual variations in the ratio of neutrophils to lymphocytes has been evaluated and used along with lymphocyte depletion kinetics to get an enhanced discrimination index of radiation exposure (Zhang et al. 2004; Blakely et al. 2007).

Proteomics is an area offering hope for potential new biological indicators of radiation exposure. Radiation responsive proteins have considerable potential as biodosimeters. Evaluation of specific changes in radiation-induced protein profiles will likely identify sentinel responsive targets and hence provide a practical means to measure tissue- and organ-systems radiation injury. A proteomic approach may evaluate an individual's responses to radiation exposure, since the individual's characteristic and dynamic protein expression profile will reflect their unique biological system. Tissue specific protein biomarkers detected in peripheral blood can provide diagnostic information of organ specific radiation injury. Proteomic analyses may also be applicable for triage purposes, providing rapid estimation of individual exposure doses (Marchetti et al. 2006). The advancement in this type of research might also provide a powerful tool for the accurate assessment of an individual's radiation risk response, hence, determine appropriate pre- as well as post-exposure interventions.



We recently reported results from a study in a nonhuman primate (*Macaca mulatta*) total-body irradiation model and showed that a multiple protein expression profile (i.e., p53, p21 WAF1, IL-6, salivary  $\alpha$ -amylase, and CRP) measured in blood of 10 animals irradiated to 6 Gy 250-kVp x-rays (0.13 Gy/min) and 8 animals to 6.5 Gy  $^{60}\text{Co}$   $\gamma$ -rays (0.4 Gy/min) analyzed with use of multivariate discriminant analysis established very successful separation of samples from exposed animals vs samples from the same animals before irradiation. An enhanced separation was observed as the number of biomarkers increased (Ossetrova et al. 2007a). We also recently presented results from on-going murine (Balb/c) *in vivo* irradiation studies and demonstrated for the first time that a protein expression profile can be developed not only to predict radiation exposure in mice but also to distinguish the level of radiation exposure, ranging from 1 to 7 Gy (0.1 Gy/min). The SAS-based multivariate statistical procedures algorithm was established for dose assessment and dose-dependent discrimination of study animal groups. We showed that for individual biomarkers there is considerable individual variability in response to radiation which makes their diagnostic utility limited, but still feasible when analyzed according to a multiple biomarkers pathway (Ossetrova et al. 2007b, 2007c).

Here we present results from on-going murine (Balb/c) and nonhuman primate (*Macaca mulatta*) *in vivo* studies demonstrating that a panel of protein biomarkers, selected from distinctly different pathways, each with different radiation responses, coupled with peripheral blood cell counts, may provide more accurate radiation dose assessment as well as an enhanced discrimination index of radiation exposure. These results also demonstrate proof-in-concept that proteomics shows promise as a complimentary approach to conventional biodosimetry for early assessment of radiation exposures and coupled with peripheral blood cell counts provides early diagnostic information to effectively manage radiation casualty incidents. This approach, with additional refinement, could provide a method for practical application of a rapid screening test for the diagnosis of radiation exposure. [AFRRI supported this research under projects BD-10, GIB250-01, and RAB3AG.]

## References

- Blakely WF, Salter CA, and Prasanna PG. 2005. *Health Physics* 89(5):494–504.
- Blakely WF, Ossetrova NI, et al. 2007. *Radiation Measurements* 42: 1164–1170.
- Dainiak N, Waselenko JK, et al. 2003. *Hematology/American Society of Hematology Education Program*: 473–496.
- Fliedner TM, Friesecke I, Beyrer K. 2001. The British Institute of Radiology, London.
- Goans RE, Holloway EC, Berger ME, and Ricks RC. 1997. *Health Physics* 72:513–518.
- Guskova AK and Gusev IA. 2001. *Medical Aspects of the Accident at Chernobyl. Second Edition Medical Management of Radiation Accidents*. CRC Press. P.195–210.
- MacVittie TJ et al. 2005. *Health Physics* 89(5):546–55.
- Ossetrova NI, Farese AM, MacVittie TJ, Manglapus GL, and Blakely WF. 2007a. *Radiation Measurements* 42:1158–1163.
- Ossetrova N.I., Sandgren D.J., and Blakely W.F. (2007b) 13<sup>th</sup> International Congress of Radiation Research, San Francisco, California, July 8–12, 2007.
- Zhang A, Azizova TV, Wald N, and Day R. 2004. 49th Annual Meeting of the Health Physics Society, Health Physics Society, McLean, VA, Abstract. P8, p. 17.
- Waselenko JK et al. 2004. *Ann Intern Med*, 140(12):1037–51.