Identification of Urine Biomarkers for X-Ray Radiation in Mice using NMR Spectroscopy

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Introduction

In the event of a large-scale radiological event, such as a nuclear leak or dirty bomb, many individuals may be exposed to radiation doses in the range from 0.5 to 10 Gy. Specific dosage information will be urgently needed to support triage of radiation casualties and to enable treatment decisions in a short time. Current technologies, based on cytogenetic or gene-expression approaches can only analyze a few hundred samples per day. Our approach is to develop quantitative metabolomics signatures in urine which are both sensitive and specific to radiation exposure. Once such a radiation signature is defined, existing portable mass-spectroscopy technology can be used to rapidly detect this radiation signature. Such a low-cost radiation biodosimeter, based on detection of a profile of radiation-induced small molecules, will potentially have extremely high throughput, as it is non invasive and required a measurement time of only a few seconds. In this work, we have identified several metabolomics biomarkers in urine from radiation-exposed mice by employing Nuclear Magnetic Resonance (NMR) spectroscopy. The high-throughput NMR technique enables us to analyze the large metabolomes. Subsequent informatics data analysis was performed by using a comprehensive software tool *HiRes*, which has been developed in our laboratory. Use of a mouse model allow us to define the effect of key parameters such as dose, age, time post-exposure on the biomarkers of mouse urine samples. Based on the understanding acquired in this work, we will be in a position to initiate measurements of metabolomics "fingerprints" in humans who were exposed to radiation.

Materials and methods

Groups of male C57/BL6 mice were irradiated by X-ray at two doses, 4 Gy and 8 Gy. The urine samples were collected from both irradiated and control mice in individual metabolic cages each day for seven days after irradiation. Aliquots of 300 µl urine samples were mixed with 300 µl phosphate buffer (0.2 M, pH 7.4) and any precipitate was removed by centrifugation. For each sample, 540 µl of supernatant was transferred to 5-mm NMR tube with 60 µl of sodium 3-trimethylsilyl-(2,3,3,3-D_4)-1-propionate (TSP)/D_2O/sodium azide solution (5 mM TSP and 1% wt/vol sodium azide in 100% D_2O). NMR spectra were acquired on Bruker Advance DMX 600 MHz spectrometer installed with Cryo-probe. The excitation sculpting pulse[1] was used for water suppression. Spectra were analyzed by using standard Principle Component Analysis (PCA) method in *HiRes.*

Results and discuss

The spectral analysis for both irradiated and control mice by PCA indicated that the X-ray exposure at both doses yield distinct urine metabolomic phenotypes. Furthermore, the phenotype clearly shows dose-dependence and time-dependence. The biomarkers with 4 Gy irradiation mainly include small aromatic molecules from nucleotide metabolism, such as N-methylnicotinate (NMN acid) and N-methylnicotinamide (NMN amide), whereas the biomarkers identified for 8 Gy X-ray irradiation include succinate, citrate, 2-oxoglutarate. The PCA results of the selected spectral region (including succinate, citrate, 2-oxoglutarate) for 8 Gy irradiation and corresponding 2D display of the first two Principle Components are shown in Fig. 1A and 1B, respectively. As seen in Fig.1B, in the first 24 and 48 hours of post-irradiation, the two PCs show some difference between irradiated and control mice although the trend is not very noticeable. After 72 hours or more of post-irradiation, we can easily distinguish the radiation-exposed mice from the control ones. To identify more biomarkers specific to the low X-ray dosage and earlier time, we propose acquiring spectra at 800 MHz with higher resolution and increased sensitivity. Our ultimate goal is to identify biomarkers able to determine whether a mouse has been exposed to radiation as well as estimate the general dose range.

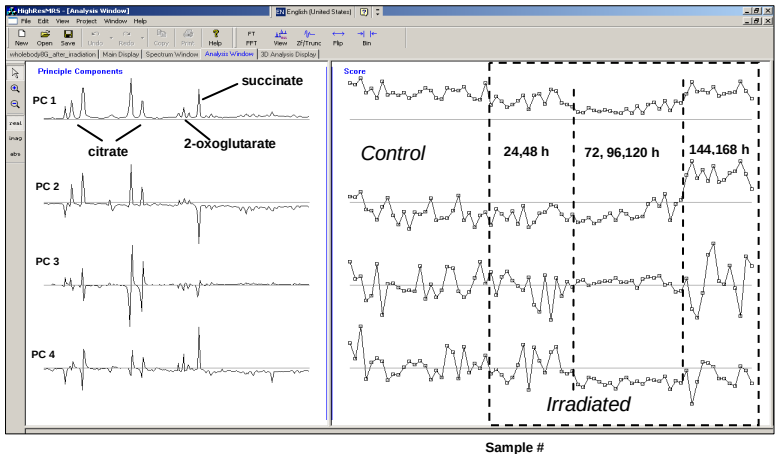

Figure 1A. The Principle Component Analysis of the selected region of NMR spectra from mouse urine samples.

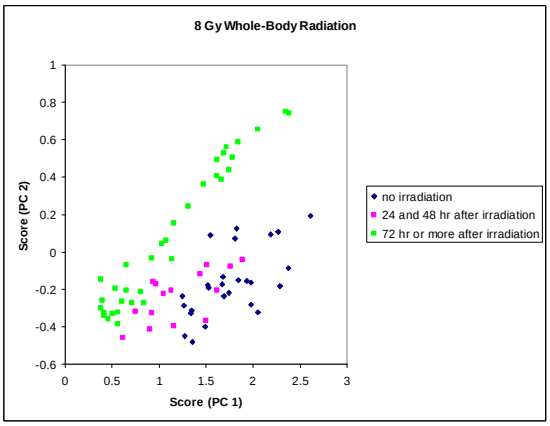

Figure 1B. The corresponding two-dimensional display of the first two Principle Components.

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References

1. T.L. Hwang and A.J. Shaka. J. *Magn. Res. A.* **112**, 275-279 (1995).