Mutation Research 788 (2016) 41–49

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Implications of genotypic differences in the generation of a urinary metabolomics radiation signature

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ARTICLE INFO

Article history:
Received 10 September 2015
Received in revised form 4 March 2016
Accepted 24 March 2016
Available online 25 March 2016

Keywords:
Metabolomics
DNA repair
Urine
Ionizing radiation
Biomarkers

ABSTRACT

The increased threat of radiological terrorism and accidental nuclear exposures, together with increased usage of radiation-based medical procedures, has made necessary the development of minimally invasive methods for rapid identification of exposed individuals. Genetically predisposed radiosensitive individuals comprise a significant number of the population and require specialized attention and treatments after such events. Metabolomics, the assessment of the collective small molecule content in a given biofluid or tissue, has proven effective in the rapid identification of radiation biomarkers and metabolic perturbations. To investigate how the genotypic background may alter the ionizing radiation (IR) signature, we analyzed urine from *Parp1*$^{-/-}$ mice, as a model radiosensitive genotype, exposed to IR by utilizing the analytical power of liquid chromatography coupled with mass spectrometry (LC–MS), as urine has been thoroughly investigated in wild type (WT) mice in previous studies from our laboratory. Samples were collected at days one and three after irradiation, time points that are important for the early and efficient triage of exposed individuals. Time-dependent perturbations in metabolites were observed in the tricarboxylic acid pathway (TCA). Other differentially excreted metabolites included amino acids and metabolites associated with dysregulation of energy metabolism pathways. Time-dependent apoptotic pathway activation between WT and mutant mice following IR exposure may explain the altered excretion patterns, although the origin of the metabolites remains to be determined. This first metabolomics study in urine from radiation exposed genetic mutant animal models provides evidence that this technology can be used to dissect the effects of genotoxic agents on metabolism by assessing easily accessible biofluids and identify biomarkers of radiation exposure. Applications of metabolomics could be incorporated in the future to further elucidate the effects of IR on the metabolism of *Parp1*$^{-/-}$ genotype by assessing individual tissues.

1. Introduction

In past decades, exposure to ionizing radiation (IR) has increased, particularly for diagnostic and therapeutic purposes. One of the primary concerns, however, remains the threat of radiological terrorism, radiological incidents such as the Fukushima Daiichi accident, and accidental exposures. Rapid identification of exposed individuals with new high-throughput methods can provide early assessment of exposure, within a few hours or days, that can be further fine-tuned through classical yet more time laborious techniques, such as cytogenetics, for effective and appropriate triage

Abbreviations: IR, ionizing radiation; LC, liquid chromatography; MS, mass spectrometry; TCA, tricarboxylic acid; WT, wild type; CT, computed tomography; PARP1, poly(ADP-ribose)polymerase 1; BER, base excision repair; SIRT1, sirtuin-1; D1, day 1; D3, day 3; HMDB, the Human Metabolome Database; KEGG, Kyoto encyclopedia of genes and genomes; QC, quality control; FDR, false discovery rate; SEM, standard error of the mean; ANOVA, one-way analysis of variance; ROC, receiver characteristic curves; FMN, flavin mononucleotide.

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http://dx.doi.org/10.1016/j.mrfmmm.2016.03.003
0027-5107/

and administration of medical treatment. In accordance with the Radiation and Nuclear Countermeasures Program of the National Institute of Allergy and Infectious Diseases (NIAID), our laboratory has focused on identification and characterization of metabolic biomarkers in urine from WT rodents, non-human primates, and total body irradiated humans within the first week post radiation exposure [1–9].

However, through epidemiological studies of the atomic bomb survivors [10,11], Chernobyl, and medical exposures [12], unique populations have begun to emerge that are more radiosensitive than the general population. Females and the pediatric population in particular have been identified as radiosensitive groups [13]. Studies with diagnostic computed tomography (CT) have identified a small risk of radiation-induced cancers in children, and have thus increased awareness to reduce unnecessary medical diagnostic exposures [14,15]. Increased erythema, early after single dose irradiation or following a number of fractions, has also been observed in a number of radiotherapy patients, sometimes severe enough to halt the radiation treatment [16]. A reason for the underlying radiosensitivity is attributed to genetics and therefore, based on inherent genetic mutations and polymorphisms, 2–4% of the general population can be considered radiosensitive [17] and should be treated appropriately. Rapid identification of these individuals can provide medical personnel with appropriate information for personalized medical intervention [18] for both therapeutic planning and radiation injury assessment. To date, biomarkers associated with underlying genetic changes and models for prediction of outcome and cancer risk are not well defined or explored.

Increased radiosensitivity has been strongly associated with mutations in DNA repair associated genes. Base excision repair (BER) is an important pathway associated with IR damage, as it protects cells from oxidative damage [19]. The Poly(ADP-ribose) polymerase 1 (PARP1) protein, the most widely-expressed protein of the PARP family, recognizes not only single-strand breaks and facilitates their repair, but also recruits the BER repair machinery and catalyzes the polymerization of ADP-ribose units from donor NAD^+ molecules to target proteins [20]. With low levels of DNA damage, PARP1 can act as a survival factor, while with high levels of DNA damage, it can promote cell death [20]. *Parp1*$^{-/-}$ mice, a genetic model that is highly radiosensitive, exhibit protection against oxidative stress-induced cell death in the short-term and increase in intracellular NAD^+ with subsequent enhanced SIRT1 (NAD-dependent deacetylase sirtuin-1) activity [21,22]. In the long term, mice with this genotype exhibit increased NAD^+ availability with subsequent mitochondrial biogenesis and lipid oxidation gene expression, improved β-cell regeneration, and enhanced energy expenditure [22,23]. Less known is the mitochondrial localization of PARP1 and its function. Unlike its role in the nucleus, mitochondrial localization of PARP1 shows that the protein is a negative regulator of mtDNA transactions and repair [24,25]. Depletion of PARP1 leads to increased bioenergetics parameters, including increased activity of TCA cycle enzymes, respiratory reserve capacity, and overall tolerance to oxidative stress [25] and therefore metabolic disease. Taken together, PARP1 is not only a significant component for efficient nuclear DNA repair, but is also a major regulator of metabolism. Mutations in this gene in the human population have been linked to increased risk for cancer susceptibility [26,27].

As such, *Parp1*$^{-/-}$ mice should exhibit altered metabolic markers when compared to wild type (WT) mice, particularly following exposure to IR. In fact, radiation exposed mice are highly radiosensitive due to increased DNA damage accumulation, G2 arrest and subsequent mitotic catastrophe. This in turn can affect the overall radiation metabolic signature in biofluids and tissues, therefore allowing for the specific identification of particular radiosensitive populations. To test this hypothesis, we employed modern liquid chromatography mass spectrometry (LC–MS) techniques to assess the urinary metabolome of WT and *Parp1*$^{-/-}$ mice exposed to semi-lethal doses of gamma radiation. The results primarily show perturbations in the TCA cycle that are time-dependent between the two genotypes. These significantly altered levels of metabolites combined with other identified biomarkers, such as amino acids, demonstrate the ability of metabolomics to identify IR and genotype-specific differences in an exposed population.

2. Materials and methods

2.1. Chemicals

All chemicals were of the highest purity available and reagents were of LC–MS grade. All chemicals, utilized as internal standards and for tandem mass spectrometry, were purchased from Sigma-Aldrich (St. Louis, MO, USA), except hexanoylglycine, which was purchased from ONBIO Inc. (Ontario, Canada).

2.2. Animal studies, radiation exposure, and sample collection

Mice homozygous for the knockout mutation in *Parp1* (common name ADPRT$^-$) were obtained from the Jackson Laboratory (129S-*Parp1*^{tm1Zqw}/J) along with wild type (WT) mice from the same vendor and bred at Georgetown University. All animal breeding and radiation studies were conducted according to Georgetown University Institutional Animal Care and Use Committee (GUACUC) protocols (#13-003). Mice were bred and housed at Georgetown University, provided water and food *ad libitum*, and housed under 12 h light and 12 h dark cycle conditions. Male mice were used for the experiments, 8–10 weeks old at the time of the experiment. A radiation dose that confers an approximate equal percentage of survival vs death (40% survival) was determined to be equal to 6 Gy by Masutani et al. [28] for the *Parp1*$^{-/-}$ genotype. An approximate equitoxic dose for the WT mice was determined to be 8.8 Gy, as shown in Supplementary Fig. 1. Briefly, 8–10 mice were exposed to either 8.5 or 8.8 Gy, approximately equally distribution in groups, and observed and weighed daily for 30 days. Mice were euthanized when in distress or total body weight loss exceeded 15% of the initial weight, according to IACUC guidelines. Percent survival was calculated and graphed with a Kaplan-Meier curve through the software Prism 6 (GraphPad Software, Inc.). For further studies and comparisons, WT mice were also exposed to an equidose of 6 Gy. The numbers of mice per group are provided in Supplementary Table 1. Mice were exposed to gamma rays with ^{137}Cs source at a dose rate of ~1.67 Gy/min. Urine collection has been described in detail elsewhere [5]. Mice were euthanized at one day (D1) or three days (D3) after irradiation. Lung and kidney tissues were collected and snap frozen in liquid nitrogen. The urine samples, lung and kidney tissues were stored at −80 °C until further use.

2.3. Sample processing and data acquisition

Urine samples were analyzed as previously described [5,7]. Briefly, urine was deproteinized with 50% acetonitrile: 50% water and 2 μL were injected into an Acquity UPLC H-Class (Waters Corporation, MA) equipped with a BEH C18 column, 130 Å, 2.1 × 50 mm coupled to a Xevo G2 Time-of-Flight mass spectrometer (Waters Corporation, UK) (LC–MS). The capillary voltage was set to 2.5 kV and the source temperature to 120 °C. The column temperature was set to 40 °C with a flow rate of 0.5 mL/min. Quality controls (QC) samples were created by pooling samples, deproteinized in an identical manner as individual samples and run every twenty samples for assessment of chromatographic quality and retention time drift. Centroided data was acquired in both positive and negative ionization modes (mass range 50–1200 m/z) with MS^E function.