Journal of Environmental Radioactivity 181 (2018) 94–101

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

journal homepage: www.elsevier.com/locate/jenvrad

Transcriptome-based biological dosimetry of gamma radiation in *Arabidopsis* using DNA damage response genes

Tae Ho Ryu[a,b], Jin Kyu Kim[a,c], Jeong-Il Kim[b], Jin-Hong Kim[a,c,*]

[a] Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, 29 Geumgu-gil, Jeongeup-si, Jeollabuk-do 56212, Republic of Korea
[b] Department of Biotechnology, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju 61186, Republic of Korea
[c] Department of Radiation Biotechnology and Applied Radioisotope Science, University of Science and Technology, 217 Gajeong-ro, Yuseong-gu, Daejeon 34113, Republic of Korea

ARTICLE INFO

Keywords:
Phytodosimetry
Arabidopsis thaliana
Transcriptome
DNA damage response

ABSTRACT

Plants are used as representative reference biota for the biological assessment of environmental risks such as ionizing radiation due to their immobility. This study proposed a faster, more economical, and more effective method than conventional cytogenetic methods for the biological dosimetry of ionizing radiation in plants (phytodosimetry). We compared various dose-response curves for the radiation-induced DNA damage response (DDR) in *Arabidopsis thaliana* after relatively "low-dose" gamma irradiation (3, 6, 12, 24, and 48 Gy) below tens of Gy using comet (or single-cell gel electrophoresis), gamma-H2AX, and transcriptomic assays of seven DDR genes (*AGO2*, *BRCA1*, *GRG*, *PARP1*, *RAD17*, *RAD51*, and *RPA1E*) using quantitative real time PCR. The DDR signal from the comet assay was saturated at 6 Gy, while the gamma-H2AX signal increased up to 48 Gy, following a linear-quadratic dose-response model. The transcriptional changes in the seven DDR genes were fitted to linear or supra-linear quadratic equations with significant dose-dependency. The dose-dependent transcriptional changes were maintained similarly until 24 h after irradiation. The integrated transcriptional dose-response model of *AGO2*, *BRCA1*, *GRG*, and *PARP1* was very similar to that of gamma-H2AX, while the transcriptional changes in the *BRCA1*, *GRG*, and *PARP1* DDR genes revealed significant dependency on the dose-rate, ecotype, and radiation dose. These results suggest that the transcriptome-based dose-response model fitted to a quadratic equation could be used practically for phytodosimetry instead of conventional cytogenetic models, such as the comet and gamma-H2AX assays. The effects of dose-rate and ecotype on the transcriptional changes of DDR genes should also be considered to improve the transcriptome-based phytodosimetry model.

1. Introduction

The environmental risks of radioactive substances have long attracted public attention in many countries, especially those with nuclear power plants, after the major nuclear accidents at Chernobyl and Fukushima. The non-human biota of ecosystems that have been contaminated by radionuclides after such nuclear accidents may result in human exposure via transport through the food chain (Dinis and Fiúza, 2007). Therefore, international organizations such as the International Atomic Energy Agency (IAEA) and International Commission on Radiological Protection (ICRP) have stated that it is necessary to pay more attention to environmental protection and to develop a dedicated risk assessment system based on the relationship between radiation dose and its effects using reference animals and plants (IAEA, 2005; ICRP, 2009). When determining the risks to non-human biota, the most representative endpoints are mortality and the incidence and reproduction of an organism (Nakamori et al., 2009). Reproduction is considered the endpoint most sensitive to radiological exposure (Hinton et al., 2007). However, the poor dose-response relationship with relatively low-dose ionizing radiation below tens of Gy as revealed by the different organs and developmental stages of plants demonstrates the need for better biological endpoints at the molecular and physiological levels (Biermans et al., 2014, 2015).

For non-human biota to be used as indicators of ionizing radiation exposure, many studies will need to examine a variety of reference animals and plants. In this respect, plants are considered better reference biota for assessing environmental stresses or risks due to their immobility compared with animals (Bartholomew, 1964; Howarth, 1991). The biological effects of ionizing radiation have been studied in various plant species. Ionizing radiation increased somatic mutations, chromosomal aberration, and homologous recombination in *Tradescantia* sp. (Nayar and Sparrow, 1967; Sparrow and Sparrow, 1976;

* Corresponding author. Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, 29 Geumgu-gil, Jeongeup-si, Jeollabuk-do 56212, Republic of Korea.
E-mail addresses: jhongkim@kaeri.re.kr, jhongkim71@gmail.com (J.-H. Kim).

https://doi.org/10.1016/j.jenvrad.2017.11.007
Received 12 August 2017; Received in revised form 20 October 2017; Accepted 6 November 2017

Chebulska-Wasilewska, 1992), *Allium cepa* (Paradiz et al., 1992), *Arabidopsis* (Kovalchuk et al., 1998, 2000), and *Capsicum baccatum* (Scaldaferro et al., 2014). Moreover, post-irradiation transcriptome analysis demonstrated a substantial difference in the plant response following acute or chronic exposure to ionizing radiation (Kovalchuk et al., 2007). Therefore, biological dosimetry (biodosimetry) of ionizing radiation has analyzed chromosomal aberration to establish a reliable dose-response curve since the 1960s (IAEA, 2011). Dicentric chromosome and micronucleus assays are the gold-standard methods for quantifying the dose-response based on chromosomal aberration (Rodrigues et al., 1997). Dicentric chromosomes and micronuclei can be detected long and sustainably after exposure to ionizing radiation (Pernot et al., 2012). Alternatively, single-cell gel electrophoresis (comet) and gamma-H2AX assays are frequently adopted for the biodosimetry of ionizing radiation at the cellular level to quantify the DNA damage response (DDR), the main cause of chromosomal aberration (Garaj-Vrhovac et al., 2002; Horn et al., 2011). However, most of these methods are based on cytogenetic experiments and are time-consuming for biodosimetry due to fixation and microscopic observation, or are often difficult to apply to plant biodosimetry (phytodosimetry) due to hardness of plant cell walls. Moreover, antibody-based cytogenetic methods such as gamma-H2AX assay are relatively costly as well as time-consuming for microscopic observation or immunoblot. These limitations necessitate the development of fast, economical, and effective biodosimetry systems based on molecular and physiological methods.

The sensitivity of plant or animal cells to ionizing radiation is associated with the early inducible expression of specific genes, such as poly (ADP-ribose) polymerase 1 (*PARP1*) and *PARP2* (Doucet-Chabeaud et al., 2001). In general, the expression of radiation-responsive genes is affected by the developmental stage and post-irradiation time, as well as by acute or chronic exposure to different radiation doses (Kovalchuk et al., 2007; Kim et al., 2013a). However, several radiation-responsive genes are considered reliable, reproducible genetic markers, such as *At2g30360*, *At4g19130*, *At4g22960*, and *At5g24280* (Kim et al., 2013b). The induced expression of these genes is more sustainably and easily detected by quantitative real time PCR (qRT-PCR) analysis than is DNA damage with gamma-H2AX or comet assays (Wang et al., 2016). The qRT-PCR-based gene expression analysis takes about 6 h, while the gamma-H2AX and comet assays need more than two days. Moreover, anti-gamma-H2AX antibody is too expensive to be comparable with qRT-PCR reagents. Although no relationship has been found among these different methods, transcriptome analysis of reliable radiation-responsive marker genes can be utilized as a fast, economical, and effective method for assaying radiation sensitivity or biodosimetry to quantify the DDR.

Arabidopsis thaliana, a small herbaceous dicot model plant, has been widely used in molecular and cellular plant biology for decades because of its short lifecycle, flexible growth conditions, discernible phenotypes, small genome size, and plentiful genetic information (Poole, 2007; Smeets et al., 2008). The DDRs of plants to ionizing radiation have also been investigated using *A. thaliana* (Ricaud et al., 2007; Gicquel et al., 2012; Manova and Gruszka, 2015). Moreover, *A. thaliana* is distributed extensively on beaches, rocky slopes, riverbanks, roadsides, and the periphery of agricultural areas worldwide (Huey et al., 2002). Therefore, *A. thaliana* can be considered one of the most valuable plants for evaluating the environmental risks of ionizing radiation at the molecular and cellular levels. In this study, we investigated the dose-response curves of several well-known radiation-induced transcriptomes for phytodosimetry in *A. thaliana* following relatively low-dose gamma irradiation below tens of Gy and compared them with those of the gamma-H2AX and comet assays. The possibility of using transcriptome-based biological dosimetry to evaluate the environmental risks of ionizing radiation is discussed in terms of its relative advantages over established cytogenetic methods.

2. Materials and methods

2.1. Plant materials and gamma irradiation

Arabidopsis thaliana seeds of ecotypes Columbia-0 (Col-0), C24, Lendsberg *erecta*-1 (L*er*-1), and Wassilewskija-2 (Ws-2) were surface sterilized for 1 min with 70% ethanol and 5 min with 20% bleach solution, and rinsed five times with sterile deionized water. Plants were grown on 1/2 Murashige and Skoog (MS) medium with 1.5% sucrose and 0.65% Phyto agar under a 16 h light/8 h dark cycle at 23 °C. The 14-day-old plants were irradiated using two gamma-irradiation facilities at the Korea Atomic Energy Research Institute: a gamma-irradiation facility with a 3 kCi ^{60}Co source and a gamma-phytotron with a 400 Ci ^{60}Co source at the Advanced Radiation Technology Institute. The gamma-ray dose rates applied were 0.75, 1.5, 3, 6, or 12 Gy h^{-1} for 4 h in the former facility. In the gamma-phytotron, plants were intermittently irradiated with 6 Gy for 60 h at a dose rate of 100 mGy h^{-1} and 800 mGy day^{-1} for 7.5 days, or continuously irradiated with 6 Gy for 120 h at a dose rate of 50 or 120 mGy day^{-1} for 5 days. The whole seedlings were harvested in liquid nitrogen and stored at −80 °C, immediately or 24 h after gamma irradiation. In the latter case, the plates were placed under a 16 h light/8 h dark cycle at 23 °C for 24 h after gamma irradiation.

2.2. Gamma-H2AX assay using immunoblot

The seedlings were harvested 30 min after irradiation and frozen immediately with liquid nitrogen. Three grams of tissue were ground with a mortar and pestle and resuspended in nuclear isolation buffer (0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 15 mM PIPES pH 6.8, 0.8% Triton X-100, 1 mM PMSF) with protease inhibitor cocktail (cOmplete ULTRA Tablets, Roche Diagnostics, Mannheim, Germany) and phosphatase inhibitor (50 mM Na_3VO_4 and 30 mM NaF). The suspension was filtered twice through Miracloth and the filtrate was centrifuged at 10,000 × *g* for 20 min at 4 °C. The pellet was resuspended in 0.4 M H_2SO_4 and left on ice for 1 h. The acid extracts were centrifuged at 15,000 × *g* for 5 min at 4 °C and the soluble proteins were precipitated from the supernatant with 12 vol of acetone at −20 °C. The precipitate was collected by centrifugation at 7000 × *g* for 15 min at 4 °C and the pellet was resuspended in 4 M urea.

The protein samples were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electro-transferred to polyvinylidene fluoride membranes. Immunoblotting was performed as previously described (Song and Bent, 2014). Rabbit anti-human gamma-H2AX antibodies (1:1000; Sigma-Aldrich, St. Louis, MO, USA) were used to detect gamma-H2AX. The band intensities on Western blots were determined using ImageJ 1.49v (NIH, Bethesda, MD, USA).

2.3. Comet assay

Approximately 100 mg of seedlings were frozen in liquid nitrogen at −80 °C for the comet assay. Plant tissues were sliced with a razor blade in 400 μl 1 × phosphate-buffered saline supplemented with 50 mM EDTA on ice. The released nuclei were separated from the cell debris by centrifugation and the 200 μl suspension was thoroughly mixed with 200 μl of warm 1% low melting point agarose at 40 °C. This mixture was dropped on microscope slides precoated with 1% normal melting point agarose and covered with a cover slide. The cover slides were removed carefully and the nuclei were subjected to unwinding in high alkaline buffer (0.3 M NaOH, 5 mM EDTA pH > 13.0) for 5 min. Electrophoresis in the same solution was performed for 10 min at 21 V (0.7 V cm^{-1}), 300 mA, and this was followed by neutralization in 100 mM Tris-HCl for 3 min. The slides were dipped into 1% Triton X-100 for 10 min, and then 70% and 96% ethanol for 2 × 5 min. Air-dried slide samples were stained with propidium iodide solution (2.5 μg ml^{-1}).