Proteomic Approach for Biomarker Discovery in Radioadaptive Responses –Age-Dependent Variations of Cell Response to Low-Dose Radiation-

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Abstract

Organisms are exposed to various low-dose radiation in space. Ionizing radiation causes diverse damage, carcinogenesis, and cell death; however, low-dose radiation is reported to induce adaptive responses, different from high dose. The radioadaptive response caused by low-dose radiation is an important factor in the adaptation to life in space; therefore, we tried to identify a biomarker protein as an indicator of radioadaptive response, and to develop an index of adaptability to a stay in space. In the present review, the radioadaptive response in cultured astrocytes and the effects of aging will be described, in addition to the proteomics approach for the variation of protein expression and phosphorylation involved in the radioadaptive response. ©2009 Jpn. Soc. Biol. Sci. Space; Article ID: 092301003

Introduction

The healthcare of astronauts is most important in long-term life in space. Organisms in space are exposed to various low-dose radiation, such as high-energy particle rays, γ -ray, and x-ray; therefore, it is necessary to estimate the radiation effects on the health of astronauts, especially low-dose effects. Low-dose ionizing radiation is known to induce hormesis and an adaptive response as a stimulant. Olivieri *et al.* first reported the radioadaptive response in mammalian cells (Olivieri *et al.*, 1984). Since then, many reports have indicated that the adaptive response can be widely observed by various indexes, for example, chromosomal aberration, cell survival, apoptosis, and cell growth (Marples and Joiner, 1993; Sasaki, 1995; Korystov *et al.*, 1996; Venkat *et al.*, 1996; Wouters *et al.* 1996; Filippovich *et al.*, 1998; Suzuki *et al.*, 1998; Park *et al.*, 1999). Since radiation is known to produce reactive oxygen species (ROS) and to cause

oxidative stress in various biological systems, ROS and reactive nitrogen species (RNS), which are generated by conditioning dose, are involved in radioadaptive response (Iyer and Lehnert, 2002; Matsumoto *et al.*, 2004; de Toledo *et al.*, 2006; Matsumoto *et al.*, 2007a; Matsumoto *et al.*, 2007b; Takahashi *et al.*, 2008). Low-dose conditioning irradiation causes a regulatory imbalance in redox homeostasis and triggers redox-sensitive signaling pathways, resulting in the induction of biological defense systems, including DNA repair and antioxidant defense systems, and the acquirement of resistance to subsequent high-dose challenging irradiation. Although subsequent challenging irradiation gives rise to various types of cellular damages, the induction of radioresistance by conditioning dose reduces genotoxic or epigenotoxic damage due to challenging irradiation (Miura, 2004; Tapio and Jacob, 2007). Although the detailed molecular mechanisms or signaling pathway of the adaptive response remain unclear, the discovery of biomarkers of adaptive response would be a beneficial indicator of adaptation to life in space and to estimate the aptitude of astronauts.

Proteomic analysis offers great potential for studies on cellular protein alterations in various diseases (Hiratsuka *et al.*, 2003; Oh-Ishi *et al.*, 2003), aging (Toda *et al.*, 1998; Schoneich 2003; Sato *et al.* 2006), oxidative stress (Matsumoto *et al.*, 2001) and radiation effects (Szkanderova *et al.*, 2003; Berglund *et al.*, 2009). Radiation effects on protein profiles have been generally studied on the high-dose effects to explore clinical biomarkers of radiotherapy (Menard *et al.*, 2006) and the factors responsible for radiation resistance (Wang *et al.*, 2005), and to elucidate radiation injury caused by depleted uranium (Malard *et al.*, 2005). We focused on low-dose effects and analyzed the variation of protein expression and phosphorylation involved in the radioadaptive response using proteomics (Miura *et al.*, 2007).

Furthermore, considering that the age of astronauts ranges widely, and that the elderly might travel to space in the future, it is necessary to investigate aging effects on radioadaptive response. This review focuses on the radioadaptive response in astrocytes cultured from various aged rats, and the proteomic approach for biomarker discovery in radioadaptive responses.

Radioadaptive Responses to Low-Dose Irradiation in Glial Cell Growth and Aging Effects

The central nervous system consists of neurons and glial cells, including astrocytes, microglia, and oligodendrocytes. Because of the high metabolic demand for oxygen and many oxidizable polyunsaturated fatty acids in membrane lipids, oxidative metabolism and the generation of reactive oxygen species (ROS) play a key role in the function of the central nervous system. Glial cells, especially astrocytes, are more resistant to oxidative stress than neurons (Bolanos *et al.*, 1995; Hollensworth

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Fig. 1. Schematic illustration of adaptive response in rat astrocytes

et al., 2000) and support the survival of neurons by releasing various antioxidants and nutritional factors (Noel and Tofilon, 1998; Tanaka *et al.*, 1999); therefore, astrocytes are thought to have mechanisms that respond to various oxidative stresses, including ionizing radiation. Thus, we examined the adaptive response to low-dose irradiation and hydrogen peroxide by observing the cell growth of astrocytes cultured from various aged rats (Miura *et al.*, 2002; Miura, 2004). The conditioning dose was 0.1 Gy X-ray or 1 μ M H₂O₂, and the challenge dose was 2 Gy X-ray. In astrocytes from young rats (1 month old), pre-treatment with the conditioning dose prevented cell growth inhibition due to the challenge dose, suggesting that the conditioning dose induced adaptive response. On the other hand, in astrocytes from middle- (9 months old) and aged rats (24 months old), adaptive responses were not induced (Fig. 1). Furthermore, when the interval between the conditioning and challenge doses was 3h, an adaptive response was induced, while it was not induced with a 24 h interval, even in young rats' astrocytes.

To determine why the radiation adaptive response is suppressed with age, we tried to explore the potential proteins responsible for the radioadaptive response and performed proteomics analysis on the effects of low-dose irradiation in rat astrocytes. Furthermore, ionizing radiation is known to activate various signaling molecules (Schmidt-Ullrich *et al.*, 2000), such as protein kinase C

(PKC) (Shimizu *et al.*, 1999), mitogen-activated protein kinase (MAPK) (Shimizu *et al.*, 1999), p53 (Wang, 2001; Takahashi, 2002), ataxia-telangiectasia mutated (ATM) (Nemethova *et al.*, 1995) and DNA-dependent protein kinase (DNA-PK) (Miura *et al.*, 2002; Takahashi *et al.*, 2002), and these activations are mediated by phosphorylation; therefore, we examined the alteration of phosphoproteins due to low-dose irradiation using proteomics (Miura *et al.*, 2007).

Variation of Protein Expressions and Phosphoproteins Caused by Low-Dose Irradiation

Low dose irradiation is known to change the expression and the post-translational modifications such as phosphorylation of various proteins, for example, signaling molecules, molecular chaperones, and transcription factors (Mohan and Meltz, 1994; Suzuki *et al.*, 2001; Matsumoto *et al.*, 2007b; Takahashi *et al.*, 2008; Ahmed *et al.*, 2009). Although these proteins are considered to participate in radioadaptive response in any way, we tried to identify the cellular proteins responsible for radioadaptive response more comprehensive and widely, and

analyzed by means of proteomics. Protein extraction and 2D-PAGE were performed as previously reported (Miura *et al.*, 2005). Three cell groups ('Control', '3 h after irradiation' and '24 h after irradiation') irradiated at the same time were examined simultaneously from protein extraction to gel matching. The search for radiation-responsive protein spots and normalization of their relative abundance were performed using PDQuest software (Bio-Rad Laboratories) as described previously (Miura *et al.*, 2005). When the abundance of spots on 2D gels of irradiated cells was >2-fold or <1/2-fold than control cells, we regarded the spots as radiation-responsive proteins. The relative abundance of radiation-responsive proteins spots was normalized against that of internal standard spots, which existed close to the spot and were expressed strongly and constantly. To analyze phosphorylated protein profiles, protein spots on the gel were stained with Pro-Q[®] Diamond phosphoprotein gel stain, a specific stain for phosphoproteins. In gel digestion of selected gel spots and peptide mass fingerprinting (PMF) were also performed as described previously (Miura *et al.*, 2005). Tryptic peptides were analyzed using a MALDI-TOF mass spectrometer (AXIMA-CFR, Shimadzu Biotech) and/or MALDI-QIT-TOF mass spectrometer (AXIMA-QIT, Shimadzu Biotech). For protein identification, tryptic peptide mass data were sent to the Mascot search engine (Matrix Science, London,

Fig. 2. Typical protein (a) and phosphoprotein (b) profiles of 2D PAGE for young rat astrocytes.

Vertical axes are designated as molecular mass (kDa) and horizontal axes as pI.

a. 2D gel was stained with silver. Spot A represents 0.1 Gy irradiation-responsive protein. It was identified as EF-2 fragment. b. 2D gel was stained with Pro-Q® Diamond phosphoprotein gel stain. Spot B–D represent 0.1 Gy irradiation-responsive phosphoproteins. Spot B, C, and D were identified as EF-1 β , β -actin, and β -actin, respectively.

UK available at: <http://matrixscience.com/>) as queries for searching the Swiss-Prot protein databases.

Figure 2a shows a typical protein profile of rat astrocytes by 2D-PAGE. When comparing gels of non-irradiated cells (control) and 0.1 Gy-irradiated cells, spots that had changed >2-fold or <1/2-fold in abundance were sought. Spot A (Fig. 2a) was temporarily increased 3 h

after 0.1 Gy irradiation. In order to identify protein spot A, tryptic peptides extracted from spot A were analyzed by MALDI-TOF MS/MS. Fragment ion masses obtained by MS/MS analysis were sent to the Mascot search engine as queries. The Mascot search score was 207 and four peptides were matched, concluding that spot A was a fragment of elongation factor 2 (EF-2 fragment). Using Pro-Q® Diamond, which is a specific stain for phosphoproteins, radiation responsive phosphoproteins were examined, with the result that spot B–D (Fig. 2b) changed within 3 h after 0.1 Gy irradiation. Spot B and C decreased 3h after 0.1 Gy irradiation, while spot D was increased. To identify these proteins, tryptic peptides were analyzed by MALDI-TOF MS and database searches were performed using the Mascot search engine, concluding that spot B, C, and D were elongation factor 1 β (EF-1 β), β -actin, and β -actin, respectively. It was suggested that EF-1 β was dephosphorylated, and that β -actin was dephosphorylated (spot C) and phosphorylated (spot D) 3h after 0.1 Gy irradiation.

Aging Effects of Low-Dose Radiation-Responsive Proteins

The adaptive response in the growth of rat astrocytes was suppressed with aging and was not observed in 24-month-old rat cells. Furthermore, when the interval between conditioning and challenge irradiation was 3 h, an adaptive response was observed, while it did not occur with a 24-h interval, even in young rat cells (Miura, 2004), suggesting that any proteins responsible for the adaptive response are induced 3 h after 0.1 Gy irradiation only in astrocytes from young rats. Thus, in order to explore the proteins responsible for the radioadaptive response under the present conditions, we examined the effects of aging on protein expression and phosphorylation in astrocytes from young and aged rats. As a result, the temporary increase in the abundance of EF-2 fragment was not observed in astrocytes from aged rats, while phosphoproteins, EF-1 β and β -actin, also varied by 0.1 Gy irradiation in astrocytes from aged rats, similarly to young rats. It is suggested that alteration of the EF-2 fragment corresponded to the occurrence of a radioadaptive response; therefore, the EF-2 fragment is a candidate biomarker responsible for the adaptive response in astrocytes induced by low-dose irradiation. EF-2 plays a role in the peptide elongation step of protein synthesis (Weissbach&Ochoa 1976). If the EF-2 fragment (MW; ca. 21.5 kDa) is produced by the degradation of native EF-2 (MW; 97 kDa), protein synthesis should be temporarily inhibited by 0.1 Gy irradiation. We examined the effects of 0.1 Gy irradiation on native EF-2 expression in astrocytes from young and aged rats by Western blot and quantitative real-time RT-PCR, with the result that neither EF-2 protein nor mRNA were affected by 0.1 Gy irradiation in astrocytes from young and aged rats. This suggested that temporal up-regulation of the EF-2 fragment due to 0.1 Gy irradiation did not affect the abundance of native EF-2, that is, the function of protein

Fig. 3. Schematic illustration of the conclusion of this review

synthesis. In order to identify the EF-2 fragment as the protein responsible for radioadaptive response, further investigation is needed in relation to the generality of the alteration of the EF-2 fragment by low-dose irradiation and the function of the EF-2 fragment in the radiation adaptive response. However, it is expected that the present results will clarify the molecular mechanism of adaptive response and prevent suppression of the adaptive response due to aging, ultimately identifying a biomarker of radioadaptive response.

Conclusion

We investigated the radioadaptive response to low-dose irradiation and proteomic analysis of the proteins responsible for the radioadaptive response in astrocytes from young and aged rats (Fig. 3). It was suggested that the radioadaptive response in rat astrocytes was suppressed with aging, and that the expression of EF-2 fragment and the phosphorylation of EF-1 β and β -actin varied 3 h after 0.1 Gy-irradiation. As the temporal increase of the EF-2 fragment was suppressed with aging, while the phosphorylation of EF-1 β and β -actin was not, a possible candidate protein responsible for the radioadaptive response was the EF-2 fragment. The development of research not only for biomarkers of cellular radiation sensitivity, individual radiation

susceptibility and gene expression but also for those in radiation oncology and biodefense (e.g. radioadaptive response) may provide new opportunities for health-related assessment of radiation-exposed individuals during a long-duration travel in the space (Straume *et al.* 2008). Although further investigation is needed, it is expected that our present results will ultimately identify biomarker of radioadaptive response and contribute to the development of space medicine.

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