Bio-Assessment of RISK in Long-Term Manned Space Exploration - Cell Death Factors in Space Radiation and/or Microgravity: A Review-

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Abstract

One major concern regarding long-term manned space missions is the effect of accumulative doses of space radiation (the mean daily dose is 0.2 ~ 1 mSv). Such missions will inevitably expose astronauts to significant doses, and thus are expected to increase the risks of radiation-related carcinogenicity, neurotoxicity, and lifespan changes. These effects occur by nuclear and cytosol dysfunction, mitochondrial damage, and particular changes in signal transduction or protein synthesis. In addition, microgravity may affect cellular metabolisms, signal transduction, etc., and might lead to synergistic effects with space radiation, which could result in further risk. Because these effects will almost certainly involve cellular signaling, transcriptomics and proteomics will be powerful tools in their analysis. In order to suitably respond to all these risks, both protective measures based on physical and biological principles, and effective intra-flight assessment of the levels of radiation exposure will be required. In this article, the categories of cell death—i.e., apoptosis, necrosis and autophagy—and the recent studies on these mechanisms are reviewed. ©2009 Jpn. Soc. Biol. Sci. Space; Article ID: 092302005

Key words: Space radiation, risk assessment, DNA array, cell death

Introduction

In addition to the International Space Station (ISS), which is currently being used for long-term, manned space missions, plans to explore the Moon or visit Mars are also being developed. However, the space environment exposes humans to many stressful factors that are not present on Earth, including microgravity, confined areas, and space radiation (Fig. 1). As a risk-reduction strategy for human space exploration, NASA developed a Bioastronautics Roadmap (bio-road map) (Fig. 2) based on the 45 most important risks (<http://bioastroroadmap.nasa.gov/index.jsp>). For example, risk number 29 is listed as Acute and Late Central Nervous System (CNS) Risks (Table 1), which includes the risks posed by radiation exposure, and this risk is currently prioritized as the number 2 risk for ISS and Moon Exploration, and the number 1 risk for a Mars mission (Table 1). There is thus an urgent need for further research into the effects of space radiation. In addition, as stated on the website of the Bioastronautics Roadmap, “the combined effects of radiation and other space factors” are a crucial consideration, and this includes potential synergistic effects between microgravity and space radiation (Table 1).

Exposure of cells to radiation leads to cell death due to nuclear DNA double strand breaks. This radiation-

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Fig. 1. Space environmental conditions, space radiation research and life science studies in human space flight.

induced DNA double strand breaks is caused by the direct ionization effects, ionization of water molecules in the vicinity of intracellular DNA, and radical formation (the indirect ionization effects), all leading to attacks on DNA (Hall, 1994). These molecular mechanisms and DNA fragmentation repair mechanisms have been the focus of recent research in radiation biology (Leadon, 1996; Karran, 2000; Pfeiffer *et al.*, 2000). Recent findings have revealed that radiation causes peroxidation of DNA bases, leading to DNA damage. In addition to research targeting the cell nucleus, various cytoplasmic changes have also been reported (Kubota *et al.*, 1997;

Rosen *et al.*, 1999; Schmidt-Ullrich *et al.*, 2000). These include radiation-related changes in signal transduction, cytokine induction, lipid and protein peroxidation, and mitochondrial dysfunction (Majima *et al.*, 2006). In particular, mitochondrial DNA is more susceptible to oxidative stress than nuclear DNA (Richter *et al.*, 1988; Shigenaga *et al.*, 1994; Ames *et al.*, 1995; Yakes and Van Houten, 1997). For radiation-induced cell dysfunction, changes occur both in cell nuclei and in the cytoplasm and, consequently, cellular dysfunction can occur. In other words, the adverse consequences of radiation are not limited to effects on cellular nuclear DNA, but also involve

complex changes in intracellular oxidation–reduction (redox), antioxidation, signal transduction, and DNA transcription systems. This ultimately results in cell death (Rosen *et al.*, 1999; Schmidt-Ullrich *et al.*, 2000). Mitochondrial dysfunction and subsequent cellular malfunction are causative factors in oxidative stress-induced generation of multiple mitochondrial DNA mutations (Wei *et al.*, 1998; Lu *et al.*, 1999), aging (Linnane *et al.*, 1989; Ozawa, 1997; Wei *et al.*, 1998; Lu *et al.*, 1999; Ozawa, 1999; Melov *et al.*, 1999; Kopsidas *et al.*, 2000), and various neurological disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Melov *et al.*, 1998; Melov *et al.*, 1999; Trounce *et al.*, 2000). This suggests that there is a relationship between oxidative stress and mitochondrial dysfunction. Mitochondria are the

Fig. 2. The bioastronautics roadmap developed by NASA. (<http://bioastroroadmap.nasa.gov/index.jsp>)

Fig. 3. Electron microscopic image of apoptosis and autophagy (from Levine & Yuan, 2005)

largest source of energy production in the cell, but recent findings suggest that mitochondria also play an important role in apoptosis; this is known as mitochondria-mediated cell death (Kroemer *et al.*, 1995; Petit *et al.*, 1996; Zamzami *et al.*, 1997; Wallace *et al.*, 1997; Cavalli and Liang, 1998; Chakraborti *et al.*, 1999; Heales *et al.*, 1999). Thus, exposure to space environments, which are characterized by space radiation and microgravity, may increase oxidative stress and subsequent cell death.

In this article, the categories of cell death—i. e., apoptosis, necrosis and autophagy—and the recent studies on these mechanisms are reviewed.

Categories of Cell death

At the cellular level, when apoptosis is induced, intracellular calcium increases (Petit *et al.*, 1996; Ghafourifar and Richter, 1997; Richter *et al.*, 1997; Ghafourifar *et al.*, 1999), the mitochondrial membrane potential increases or decreases, and cytochrome C (CYTC) (Liu *et al.*, 1996; Yang *et al.*, 1997; Kluck *et al.*, 1997; Cai *et al.*, 1998; Slee *et al.*, 1999; von Ahsen *et al.*, 2000) and apoptosis-inducing factor (AIF) are released from the mitochondrial intermembrane space into the cytoplasm (Susin *et al.*, 1999; Susin *et al.*, 2000; Kroemer and Reed, 2000). This release occurs from the mitochondrial permeability transition pores (mPTP) and involves a complex of the mitochondrial outer membrane VDAC, the inner membrane ATP/ADP exchange transporter ANT, matrix cyclophilin D (cyclophilin-40; CYP-40), and intermembrane space creatine kinase. mPTPs are formed with increased mitochondrial calcium influx and they are also involved in cell apoptosis and necrosis (Halestrap *et al.*, 2002; Halestrap and Brennerb, 2003). CYTC released into the cytoplasm binds with apoptotic protease activating factor (Apaf-1), activates the proteolytic enzyme caspase 9, and forms an apoptosome complex (Riedl *et al.*, 2005; Chereau *et al.*, 2005). The apoptosome, via activation of caspases downstream from caspase 3 and caspase-activated DNase (CAD), causes chromatin aggregation and

fragmentation, leading to apoptosis. Inhibitors of apoptosis, such as Bcl-2 and Bcl-xL, inhibit mPTP, whereas inducers of apoptosis, such as Bax and Bak, promote mPTP and increase CYTC release to induce apoptosis (Vieira *et al.*, 2000; He *et al.*, 2004). In addition, mitochondrial reactive oxygen species (ROS), which increase on exposure to radiation, cause mitochondrial DNA (mtDNA) damage and are released intracellularly. This causes intracellular nuclear DNA protein damage (Solaini and Harris, 2005; Hou *et al.*, 2005; Wallace, 2005). One protective mechanism against ROS (antioxidative mechanism) involves MnSOD (SOD-2) in mitochondria, which plays an important role in the dismutation of superoxide anion ($O_2^{\cdot-}$) to H_2O_2 . We were the first research group to demonstrate that apoptosis was inhibited in cells with overexpression of mitochondrial MnSOD (Majima *et al.*, 1998). Even with radiation exposure, apoptosis is inhibited in MnSOD-overexpressing cells (Motoori *et al.*, 2001).

Table 1 Risk 29: Acute and Late CNS Risks.
(<http://bioastroroadmap.nasa.gov/User/riskDataSheetForAllMissions.jsp>)

Crosscutting Area :	Radiation Health
Discipline :	Radiation
Risk Description :	Acute and late radiation damage to the central nervous system (CNS) may lead to changes in motor function and behavior, or neurological disorders. This may be caused by occupational radiation exposure or the combined effects of radiation and other space flight factors.
Context / Risk Factors :	Radiation (space, medical diagnostic, atmospheric, experimental and nuclear sources including propulsion systems) and synergistic effects of radiation with other space flight factors may affect neural tissues, which in turn may lead to changes in function or behavior.
Justification / Rationale :	Crew health and performance in-flight may be affected. This risk will be most significant during a Mars mission, with a long travel time and no return capability.
Risk Rating :	ISS: Priority 2 Lunar: Priority 2 Mars: Priority 1
Current Countermeasures :	Polyethylene shielding Avoidance of the South Atlantic Anomaly (SAA) ALARA, and monitoring of exposure limits Vehicle altitude and attitude changes
Projected Countermeasures or Mitigations and Other Deliverables :	Anti-oxidants [CRL 1] Hydrogenous shielding [TRL 5] Pharmaceuticals [CRL 1] Autonomous monitoring [Lunar] [Mars] Improved shielding materials [Lunar] [Mars] Pharmacological cellular protectants will be required [Lunar] [Mars]

Fig. 4. Schematic model of autophagic regulation (from Shintani & Klionsky, 2004)

On the other hand, from a morphologic perspective, physiological programmed cell death observed during development can be classified into apoptosis (type 1 cell death), autophagic cell death (type 2 cell death), and cell death with intracellular organelle swelling, the appearance of vacuoles, but no lysosome involvement (type 3 cell death) (Lockshin and Zakeri, 2004; Levine and Yuan, 2005; Kim *et al.*, 2006). Type 3 cell death is also known as necrosis (Lockshin and Zakeri, 2004). Rapid advances

are being made in molecular biology research on type 2 cell death (autophagic cell death) and type 3 cell death (necrosis). Figure 3 shows changes in cell morphology observed by electron microscopy during apoptosis and autophagy (Levine and Yuan, 2005). Figures 4 and 5 depict a molecular biology signal model for autophagy (Shintani and Klionsky, 2004; Jaeger and Wyss-Coray, 2009). Mitochondria are involved in both apoptosis and autophagy (Kim *et al.*, 2006). Autophagic cell death is

Fig. 5. The autophagy pathway in mammals (from Jaeger & Wyss-Coray, 2009)

Fig. 6. The p53 network and autophagy (from Morselli *et al.*, 2009)

increased by Bcl-2 and reduced by Bax, and a related gene is beclin 1 (Shimizu *et al.*, 2004). In cancer, beclin 1, which has also been shown to play a role in inhibition of autophagy, has been shown to be monoallelically deleted in 40-75% of cases of human sporadic breast, ovarian, and prostate cancer, (Qu *et al.*, 2003). Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), like beclin 1, is a tumor suppressor gene (Chang *et al.*, 2006). PTEN catalyzes the reverse reaction of PI3K, which activates Akt, an intracellular activation factor (Fig. 4) (Shintani and Klionsky, 2004; Chung and Eng, 2005). PTEN likely plays a role in both autophagic cell death and apoptosis (Shintani and Klionsky, 2004).

Involvement of Mitochondria in Cell death

Recent findings on autophagy mechanisms have indicated that mitochondria are more involved

than previously thought (Rodriguez-Enriquez *et al.*, 2004; Morselli *et al.*, 2009). Figures 6 and 7 show that mitochondria are involved in both apoptosis and autophagy, and apoptosis related key-players such as p53 and Bax have double trigger functions for apoptosis and autophagy (Rodriguez-Enriquez *et al.*, 2004; Read, 2006; Morselli *et al.*, 2009). Data accumulated over recent years reveals that mitochondria are also subject to selective degradation, i.e., autophagy (Fig. 7) (Kissová *et al.*, 2004; Tal *et al.*, 2007; Nowikovsky *et al.*, 2007; Twig *et al.*, 2008; Todde *et al.*, 2009). Mitochondria might also play a role in inducing advancing the autophagy that ultimately kills them, perhaps by acting as a primary trigger. Majima *et al.* published the first paper on the relationship between ROS generated from mitochondria and apoptosis (Majima *et al.*, 1998). Interestingly, their paper contains an electron microscope image showing that mitochondria are present in the lysosome following nuclear degradation, whereas images of MnSOD-transfected cells reveal that they remain intact (Fig. 8). Apoptosis and autophagy might be triggered and controlled by the same mechanisms, which could explain why they occur simultaneously.

Necrosis, like apoptosis, involves permeability transmission (PT), which occurs due to a reduction in the membrane potential (Halestrap, 2005). As previously described, PT is mediated by the mPTP opening through the inner and outer mitochondrial membranes. The mPTP consists of a VDAC in the outer membrane, an ANT in the inner membrane, and involves CYP-40 (Halestrap, 2005). Apoptosis occurs when the mPTP opens and PT is induced; necrosis also occurs, but the former is ATP-dependent, whereas the latter is ATP-independent, and CypD (CYP-40) is involved (Fig. 9) (Halestrap, 2005). Apoptosis, autophagy, and necrosis might all be induced by the same stimuli (Figs. 10 and 11) (Rodriguez-Enriquez *et al.*, 2004; Fink and Cookson, 2005; Fraker, 2005), although how they are switched on or off remains

Fig. 7. Schematic presentation of autophagy processes (from Todde *et al.*, 2009)

Fig. 8. Ultrastructural analysis of cells cultured at pH 8.3 for 6 h. a: cells stably transfected with MnSOD. B: control cells; nuclear fragmentation plus focal condensation of chromosomal material, and accumulation of lysosomes with membrane debris including mitochondrion are observed internally (arrowhead). The mitochondria show focal swelling and loss of cristae (from Majima *et al.*, 1998).

unclear. Figure 12 summarizes the possible mechanisms of this switch, which may involve changes in the levels of intracellular nutrients (Fraker, 2005). Recent studies have shown that the protein kinase receptor-interacting protein3 (RIP3), an energy metabolism regulator switches TNF-induced cell death from apoptosis to necrosis (He *et al.*, 2009; Cho *et al.*, 2009; Zhang *et al.*, 2009).

Mitochondria appear to play a role in apoptosis, autophagy, and necrosis. When performing maintenance on an entire tissue or organ it is necessary to preserve homeostasis. However, there are many stimuli in bioenvironments, including redox, oxidative stress, and signal transduction. Therefore, systems that discard nonfunctional cells must exist; it seems likely that three such systems are apoptosis, autophagy, and necrosis. A failure of cell death might cause other signals to result in other types of cell death. The same stimulus might act as a trigger for different types of cell death to occur either simultaneously or in sequence.

Evaluation of Cell Signaling and Balance of

Fig. 10. Schematic model of the role of the mitochondrial permeability transition in apoptosis, necrosis and autophagy (from Rodriguez-Enriquez *et al.*, 2004).

Oxidants and Antioxidants

In order to evaluate the effects of space radiation, which occurs at extremely low doses, and microgravity, the only suitable method is measurement of changes in gene expression by a DNA microarray, due to the limitation on the smallest dose for the general experimental end points. DNA microarray analysis covers a wide range of genes and can be used to generate relevant data through data-set analysis, including clarification of signal transduction pathways.

To assess risk at the cellular level, apoptosis, autophagy, or necrosis must be considered. In addition, microgravity, which has been shown to activate apoptosis- and autophagy-related genes, may have a synergistic effect on cells in space environments. Figure 13 shows signals which may lead to cell death by space radiation and microgravity. Generation of mitochondrial ROS would be the initial action, and manganese superoxide dismutase is the first guard. Examination of each signal in this signaling pathway provides important information regarding the assessment of risk in the space environment. Knowing the intracellular antioxidant level would thus be essential for providing cellular protection against oxidative stress. Any increase in oxidative stress or decrease in the antioxidant level could increase the risk posed by additional space radiation or microgravity.

Fig. 9. Necrosis and apoptosis occur through the mitochondrial permeability transition pores (mPTP) (from Halestrap, 2005).

Fig. 11. Pathways leading to cell death (from Fink and Cookson, 2005).

Fig. 12. The major forms of cell death that may occur as a result of a nutritional imbalance (from Fraker, 2005).

However, actual experiments in space will be needed to fully clarify the effects of microgravity and space radiation. Such information will also be important for choosing materials and methods to protect against or ameliorate the adverse effects of long-term space exploration.

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Fig. 13. Hypothetical changes in the expression of cell death-related genes following space radiation and microgravity.

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