Special Issue: Biological effects of space radiation -Part II-

Study of the Effects of Space Radiation on Mouse ES cells

Kayo Yoshida¹, Shuhei Yoshida¹, Kiyomi Eguchi-Kasai², Takashi Morita³

¹Osaka City University, Graduate School of Medicine, Department of Molecular Genetics, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585 Japan.
²Radiation Effects Mechanism Research Group, National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage-ku, Chiba 263-8555 Japan.

Abstract

As long-term human space flight is now required for cosmic exploration, the influence of space radiation and microgravity on the human body is an issue of high priority. We plan to launch frozen mouse embryonic stem (ES) cells into space, to expose them to space radiation. After returning to the ground, we will microinject the ES cells into fertilized eggs to produce mice, and evaluate the influence of space radiation on their development and on their descendents. ©2010 Jpn. Soc. Biol. Sci. Space; Article ID: 102401002

Introduction

The construction of the International Space Station (ISS) has been completed, and long-term stays in space have become a reality. However, space has higher levels of cosmic radiation, including Galactic Cosmic Rays (GCR) and Solar Particle Events (SPE). Therefore, it is extremely important to analyze the influence of space radiation on the human body and protect humans from their deleterious effects. In particular, the effect of space radiation on mammalian development and germline cells becomes a serious issue as stays in space become longer (Norimura et al., 1996; Adiga et al., 2007). We plan to examine the influence of space radiation on germ cells, which could result in infertility, miscarriage, and/or birth defects.

To elucidate the influence of space radiation on animals, model animals can be shipped on space launches and remain in space for various times. The effects of space radiation can be determined by examining the animals on the ground, after their return. However, breeding mice for long periods in space is technologically very difficult. We therefore designed an alternative method to examine the effects of radiation on mouse development and germ cells. We plan to use ES (embryonic stem) cells, because they can be microinjected into early mouse embryos to generate adult mice deriving partly from the ES-cells. ES cells have advantages for the genetic modification of animals, by introducing mutations, especially DNA repair genes, into the ES-cell chromosome using homologous recombination techniques.

We are presently performing irradiation experiments on mouse ES cells using an accelerator (HIMAC) at the National Institute of Radiological Sciences (NIRS, Chiba, Japan) to examine the effect caused by DNA damages (Marchetti et al., 2007; Derijck et al., 2008; Filion et al., 2009; Momcilovic et al., 2009; Solozova et al., 2009). In addition to these experiments, we think it is indispensable to verify the effect of mixed cosmic rays combined with the influence of microgravity in an actual space environment.

Outline of the space experiment

ES cells will be collected and frozen in stock medium at −80°C, and transferred to the launching base by airplane (Fig. 1). The ES cells will be shipped on a scheduled space mission to the ISS. Half of the samples will return to the ground with astronauts after just a short stay in the ISS. They will be kept at −80°C on the ground and used as the unirradiated control. The rest of the samples will be stored in the MELFI (Minus-Eighty Laboratory Freezer for ISS) in the ISS at −80°C to −95°C for longer periods, to be exposed to space radiation. Several months or years later, the ES cells will be returned to the earth. They will then be unfrozen in their medium and microinjected into fertilized 8-cell-stage embryos. Some embryos will be cultured in vitro, and their development examined. At the same time, other microinjected embryos will be transplanted into pseudo-pregnant mice, and their development will be followed. The resulting adult mice will be tested for chromosomal aberrations and sterility. Thus, we will use ES cells to evaluate the biological effects of space radiation on the animal body. Moreover, green fluorescent protein (GFP)-expressing ES cells can be used to visualize the effects of space radiation on development.

Preservation of ES cells at −80°C

As a pilot experiment, to examine whether storing ES cells at −80°C for several months or years causes damage to their growth or developmental capacity, we stored the mouse ES cells at −80°C on the ground for 13 months. The ES cells were then thawed, microinjected into 8-cell-stage embryos, and transplanted into the uterus of pseudo-pregnant mice. The results confirmed that we could obtain 10 chimeric mice from 13 pups from embryos with ES cells that had been stored for 13 months at −80°C, demonstrating that this treatment did not alter their multi-potency. We further confirmed the ability of cells derived from these frozen and thawed ES cells at −80°C to generate germ lines and verified that...
the transportation of ES cells on dry ice did not affect subsequent development. Finally, because the ES cells may need to be transported from the ISS to the ground in a −30˚C portable freezer for 2-3 days, we also tested the effect of transferring ES cells from −80˚C to −30˚C for 2 days. Our results confirmed that the temperature-shifted ES cells could still be used to produce chimeric mice (Fig. 2).

**Fig. 1. Outline of the space experiment.** ES cells, iPS cells, or ES cells that are sensitized by genetic modification will be frozen at −80˚C and transferred to a launching base. They will stay in space, and half of the samples will be returned to the ground after only a short stay in the ISS. These will be the unirradiated control. The rest of the samples will be stored in the ISS at −80˚C. After exposure of the ES cells to space radiation in the ISS, they will be returned to the ground and microinjected into 8-cell-stage embryos. Their development and descendents will be analyzed to examine the effects of space radiation.

**Fig. 2.** Multipotency of frozen ES cells. The ES cells frozen at −80˚C during 1 year were moved to −30˚C for 2 days (once or twice) and they were microinjected into mouse 8-cell embryos followed by transplantation to pseudopregnant mouse uterus. The chimeric mice were born after 2 times of temperature shift, demonstrating that the temperature rise to −30˚C according to temporary transportation did not impair the multipotency of ES cells.

**Sensitivity of ES cells to ionizing radiation**

We irradiated mouse ES cells cultured at 37˚C with X-rays, carbon (C) ion beam (290 MeV/u), or iron (Fe) ion beam (500 MeV/u) at doses of 0.1-5 Gy. The irradiated cells were cultured in medium at 37˚C, the number of colonies was counted (Fig. 3), and the survival rate of the cells was calculated. The results indicated that the Fe ion beam had the strongest effect, followed by the C ion beam, and then X rays. Similar results were obtained using ES cells that had been frozen at −80˚C and then
thawed before being subjected to radiation. Cells that were irradiated 1-5 Gy while they were frozen were much more resistant to the radiation than cultured cells. In case of Fe ion; 2.1-fold of increase in resistance was observed.

To detect DNA double-strand breaks in the irradiated ES cells, we collected protein samples from the ES cells 30 minutes and one hour after irradiation, and examined them for phosphorylated histone H2AX (γH2AX), which was induced by double-strand breaks, by Western blot. The phosphorylation of histone H2AX increased in samples receiving 0.5 Gy radiation or more from the Fe ion beam and X ray. The increase persisted at the 1-hour timepoint when the cells were irradiated with 1-10 Gy of Fe ion (Fig. 4). This result showed that the Fe ion beam elicited more serious damage to the DNA in ES cells at higher doses, and that the damage was more difficult to be repaired over longer periods, compared with X-rays.

Influence of irradiation on mouse embryogenesis

To examine the developmental capability of X-irradiated mouse ES cells, we microinjected the irradiated cells into mouse 8-cell embryos and cultured the embryos in vitro. The 20 embryos that survived to develop into a morula and blastocyst were then transplanted into the uterus of pseudo-pregnant mouse. After 20 days, we examined the neonates for abnormalities (Fig. 5). Mouse ES cells that were exposed to 0-0.5 Gy of X-rays resulted in chimeric mice, as indicated by their coat color. At 3-5 Gy of radiation, Caesarean birth became necessary, as normal births decreased, and abnormal embryos and traces of placenta increased. Thus, the X-irradiation of the ES cells seriously and dose-dependently affected the later development of embryos in this microinjection and transplantation assay. These results indicate that our in vivo development assay can be applied to assess the effects of other radiation sources, such as Fe and C ion beams, and to evaluate the effects of space radiation on mammalian development.

GFP-expressing ES cells for evaluating the effects of radiation on mammalian embryogenesis.

The microinjection and transplantation of irradiated ES cells works well as an in vivo assay. However, since the contribution of ES cells is determined from the coat color, there is a considerable waiting period involved, while the chimeric embryos develop and are born. We therefore applied ES cells expressing GFP to the same analytical system. Using GFP-ES cells, we can observe the developmental process of the ES cells by fluorescence microscopy (Fig. 6). This technology also makes it possible to examine several hundreds of microinjected ES cells, making it easier to obtain statistically significant data on the biological effects. The
more microinjections we perform, the more sensitively we may detect the damage to ES cells by the low doses of long-term irradiation in space.

Shortening the time of exposure to radiation by using sensitized ES cells with a modified DNA-repair gene

Another advantage of the ES-cell system is that their sensitivity to radiation can be increased by modifying the genes involved in DNA damage signal transduction and DNA repair. The histone H2AX gene is involved in double-strand DNA break repair; therefore, mice from its knockout (KO) line are more sensitive than wild-type mice to ionizing radiation. We made KO mice of the histone H2AX gene, and are planning to establish ES cells from their homozygous embryos and to examine their sensitivity to X rays and heavy ion beams. Since such H2AX-modified ES cells should also be more sensitive to space radiation, we should be able to detect its biological effects on ES cells after a much shorter time in space. Our findings will also provide insight into DNA repair in response to space radiation.

Future tasks

Research on how space radiation influences biological systems will become more and more important as people stay in space for longer periods, as will be required for future projects, such as making a base on the moon or going on a mission to Mars. Our purpose is to evaluate the influence of space radiation on humans during a long stay. Therefore, it is indispensable to have a good correlation between the results from using mouse ES cells and the effects of space radiation on the human body. We plan to carry out the irradiation experiment described above for mouse ES cells using mouse and human iPS cells as well. Comparison and extrapolation of the acquired data from mouse and human iPS cells with those from ES cells should enable us to estimate the influence of space radiation on the human body without having to perform experiments on human subjects.

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References


