Micronuclei Induced by Low Dose Rate Irradiation in Early Spermatids of p53 Null and Wild Mice

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To obtain evidence of the dose-rate effect on induction of micronuclei in early spermatids, we observed frequencies in wild-type p53(+/+), heterozygous p53(+/-) and null p53(-/-) mice 14 days after gamma rays irradiation at a high (1,020 mGy/min) or a low (1.2 mGy/min) dose-rate. A dose- and dose-rate-related increase in micronuclei was seen in early spermatids with no difference between the different p53 status. These data were found to be best fitted by a linear-quadratic dose-response model at a high dose-rate, and by a linear dose-response model at a low dose-rate. The yields at 1.2 mGy/min were significantly lower than those at 1,020 mGy/min in the same manner, independent of p53 status. Testis weight declined significantly after 3 Gy irradiation, but did not depend on dose-rates. In our other studies, we observed the complete elimination both of malformation in fetuses and CD3–4+ mutant T-lymphocytes in p53(+/+) mice, but not in p53(-/-) mice after irradiation. This indicates that concerted DNA repair and p53-dependent apoptosis are likely to completely eliminate mutagenic damage from the irradiated tissues at low doses or dose-rates in teratogenesis and lymphocytes. In the germ cell, however, irradiation at 1.2 mGy/min was mutagenic, independent of p53 status.

INTRODUCTION

The major risks of low-level radiation are mutagenesis, teratogenesis and carcinogenesis. The p53 gene is implicated in cell cycle delay and the activation of apoptosis after exposure to DNA-damaging agents, and would therefore be expected to influence radiosensitivity. A p53 deficient environment results in increased survival of cells bearing DNA damage, thereby leading to an increased mutation frequency and ultimately predisposing malignancy and teratogenesis. We proposed that mouse embryonic or fetal tissues have a p53-dependent guardian that aborts cells with radiation-induced teratogenic damage by committing apoptosis1-3).

Wang4) reported that in the late period of organogenesis, however, the p53(-/-) embryonic mice were more sensitive to the killing effect than were the p53(+/-) and p53(+/-) mice. These results show that the function of p53 is restricted not only to specific tissues, but also to specific stages in the development of tissues.

It was also reported that p53 is important in the regulation of spermatogenesis of normal and irradiated testis, either by regulation of cell proliferation or by regulating the apoptotic process in spermatogonia5,6). The experiments reported here investigated whether the differences in sensitivity to induction of micronuclei in early spermatids after irradiation are related to differences in the p53-gene status and dose-rate.

MATERIALS AND METHODS

Animals and irradiation

Wild-type p53(+/+), heterozygous p53(+/-) and null p53(-/-) mice of 6-12 weeks of age at the time of irradiation were used, as previously described1-3). The experiments were carried out under the control of the Ethics Committee of Animal Care and Experimentation in accordance with the Guiding Principle for Animal Care and Experimentation,
Mice were exposed to 1, 2 or 3 Gy $^{137}$Cs $\gamma$-rays at a high dose-rate of 1,020 mGy/min, using a Gammacell 40 Exactor (Nordion International, Canada), and at a low dose-rate of 1.2 mGy/min, using an Exposure Instrument SK-951 (Sangyo-Kagaku, Osaka, Japan). At least five animals were used per dose and dose-rate.

**Micronucleus assay in early spermatids**

Spermatids were harvested 14 days after irradiation. This time corresponds to the treatments of cells at the developmental stage of pre-leptotene (primary spermatocytes in G1 or S stage). The spermatid harvests, staining and scoring of micronuclei in round spermatids were performed as previously described. Briefly, the testes were excised from each animal, tunicas were removed, and the seminiferous tubules were gently minced in HBSS medium. The cell suspensions were incubated in 2 mg/ml collagenase (Wako Pure Chemical Industries, Osaka) for 20 min at 33°C in a shaking water bath. The cell suspensions were filtered through a stainless-steel filter, washed, and fixed in 10% neutral buffered formalin. Cells were stained with DAPI (4’, 6-dianidino-2-phenyl indole dihydrochloride, Sigma Chemical, St. Louis, MO, USA) at a dose of 1.5 $\mu$g/ml, and the micronucleus frequency was calculated in 1000 spermatids per animal under a fluorescence microscope.

**Statistical analysis**

Student’s $t$-test was used to compare the micronucleus frequencies at each radiation dose. The two-way analysis of variance followed by the Scheffe method for contrasts was used to detect significant differences in dose-rate effect at 3Gy between p53 gene status. Differences were considered to be statistically significant when $P<0.05$.

**RESULTS AND DISCUSSION**

Spontaneous micronucleus frequency yielded no significant p53-dependent increase. The frequencies of micronuclei increased dose dependently in all p53 gene status when given at 1,020 mGy/min. These data were found to be best fitted by a linear-quadratic dose-response model. The frequencies at 1.2 mGy/min also increased dose dependently in all p53 gene status, however, these were best fitted by a linear dose-response model (Fig. 1). Similar dose-rate dependence has already observed in the frequency of hypoxanthine phosphoribosyl transferase (HPRT) deficient T lymphocytes in the spleen of the irradiated C57BL mouse. However, there are no report observed dose-rate effect on micronuclei induction in early spermatids. The yields at 1.2 mGy/min were significantly lower than those at 1,020 mGy/min ($P<0.001$) and higher than those of control ($P<0.001$) in the same manner for all p53 gene status (Fig. 1, 2).

Testis weight declined significantly ($P<0.001$) after 3 Gy irradiation but did not depend on dose-rates in each p53 gene status (Fig. 3).

A apoptosis induced in male germ cells following radiation is dependent on functional p53. Hasegawa et al showed that the p53 is induced in spermatogonia and has been shown to play a central role in DNA damage in induced spermatogonial apoptosis after irradiation. However, in p53-deficient mice spermatogonial apoptosis can still be induced by ionizing radiation. Recently it was reported that p53 independent apoptotic pathways involving p73 exist in spermatogonia, although less efficiently than the p53 route.
We have already shown that foetal irradiation with 2 Gy at 1.2 mGy/min was not teratogenic for $p53^{+/+}$ mice but teratogenic for $p53^{+/-}$ mice. This indicates that the $p53$ gene is indispensable for a threshold effect in the teratogenic risk of radiation at low doses or dose-rates. In the germ cell, however, we found a clear dose-rate effect on induction of micronuclei in early spermatids, and that was independent of $p53$ gene.

REFERENCES

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