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Spontaneous and radiation-induced tumorigenesis in *p53*-deficient mice

Rajamanickam Baskar, Haruko Ryo, Hiroo Nakajima, Tadashi Hongyo, Liya Li, Mukh Syaifudin, Xiao E Si, Taisei Nomura*

Department of Radiation Biology and Medical Genetics, Graduate School of Medicine, Osaka University, B-4, 2-2 Yamada-oka, Suita, Osaka, 565-0871, Japan

Abstract

To investigate the spontaneous and radiation induced tumorigenesis, tumor development and molecular changes were examined in *p53* gene-deficient mice. All of the null ($p53^{-/-}$) animals died of leukemia, sarcoma, etc. before 12 months of age, and predominantly of lymphocytic leukemia at the early age of 3–5 months, while heterozygous ($p53^{+/-}$) and wild type ($p53^{+/+}$) animals survived more than 30 and 40 months, respectively. Half of the heterozygotes developed sarcomas (osteosarcomas, rhabdomyosarcomas, hemangiomas), and some developed tumors in the lung and liver; however, a lower incidence of leukemia was found. The onset of the tumor was late in their lives in the heterozygotes, though the tumors grew rapidly. In the wild type mice, leukemia and sarcomas were rarely observed. The ^{137}Cs γ-irradiation shortened the lifespan of the heterozygotes with an earlier tumor development, though this effect was not evident in the null mice. Among the 54 spontaneous tumors studied in the heterozygotes, 12 (22.2%) showed loss of the wild type *p53* allele (loss of heterozygosity, LOH) and one functional mutation was found at exon 6 of the *p53* gene. All the LOH was found in leukemia and sarcomas, except in one breast carcinoma.

Keywords: p53 knockout; γ-radiation; Tumorigenesis; LOH; Mutation

1. Introduction

Normal cells have a number of intrinsic mechanisms that involve molecular 'gate-keepers' to protect them from rapid uncontrolled proliferation [1]. Tumor formation and

* Corresponding author. Tel.: +81-6-6879-3811; fax: +81-6-6879-3819.
E-mail address: tnomura@radbio.med.osaka-u.ac.jp (T. Nomura).

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growth are characterized by uncontrolled cellular proliferation, and it can occur when essential regulatory proteins are altered. In human cancers, one of the tumor suppressor genes closely related to ionizing radiation is *p53*. It determines the growth arrest and/or cell death following irradiation [2]. The gene *p53* appears to have a pivotal function in protecting humans from cancer, because this gene is mutated in more than 50% of human cancers [3].

The tumorigenic effects of ionizing radiation in both experimental animals and humans have been well studied. However, there is no direct evidence to demonstrate the changes in carcinogenesis or mutagenesis of *p53*-deficient tissues by radiation. To investigate the spontaneous and radiation initiated tumorigenesis, the onset and latency of tumors and their molecular changes were examined in the *p53* gene-deficient mice.

2. Materials and methods

2.1. Animals

Mice lacking one allele of the *p53* gene (C57BL/*6-p53*$^{+/-}$) [4] were provided by Prof. M. Katsuki, University of Tokyo. The mice were maintained by brother–sister inbreeding in complete barrier conditions with light from 4:00 to 18:00 at 23 ± 1 °C and 50–70% humidity, with an autoclaved mouse diet CRF-1 (Charles River Japan, Kanagawa, Japan) and acidified, chlorinated and filtrated (by Millipore) water. The animal experiments were carried out in the barrier section of the Institute of Experimental Animal Sciences following the Osaka University Guidelines for Animal Experimentation.

2.2. Identification of genotypes

The genotypes of the individual mice were determined by polymerase chain reaction (PCR, GeneAmp PCR System 9700, Perkin-Elmer, Foster City, CA, USA) from the tail DNA, using the vector specific primers and *p53* primers covering exons 6 and 7. We designated wild types as *p53*$^{+/+}$, heterozygotes as *p53*$^{+/-}$ and knockout homozygotes as *p53*$^{-/-}$.

2.3. Irradiation

At the age of 6 weeks, the mice were exposed to 0.5 Gy of ^{137}Cs γ-rays four times at 7-day intervals at a dose rate of 1.07 Gy/min.

2.4. Examination of tumor

Mice were checked 6 days a week, and allowed to live out their lifespan or were euthanatized when moribund. All animals were fully necropsied, and the tumors and normal tissues were frozen at −80 °C for molecular analysis. The tumors and selected pathological lesions were fixed in 10% neutral buffered formaldehyde solution and submitted to histological examinations to confirm diagnosis.

2.5. LOH and mutation analysis

DNA was extracted from the frozen tissue and LOH of the *p53* gene was determined by PCR. The DNA was amplified using four specific pairs of primers for the *p53* gene exons 5, 6, 7 and 5–7. The PCR amplification of the *p53* knockout allele by vector specific primers was also carried out as a control. In addition, LOH was determined based on the signal of PCR products in agarose gel electrophoresis. A very weak or no signal of PCR products of the wild type allele amplified by all the four specific primers was classified as LOH, in comparison with the standard signal by the *p53* knockout specific primers.

PCR and single strand conformational polymorphism (SSCP) was carried out to screen exons 5, 6, 7 and 8 of the *p53* gene for the presence of mutations. Single-strand conformational polymorphism (SSCP) was carried out as described previously [5]. Briefly, SSCP bands, which showed altered mobility, were extracted from the gel and reamplified by 25 cycles of PCR to enrich the mutated alleles. Direct sequencing was performed by the dideoxy chain termination method using the Big Dye terminator cycle sequencing kit (Perkin-Elmer). The sequencing primers were the same as those for PCR. After ethanol precipitation, sequencing was done using an automated DNA sequencer (Genetic Analyzer, ABI Prism 310, Perkin-Elmer).

2.6. Statistical analysis

Statistical analysis was done using the SPSS system (SPSS, Chicago, USA).

3. Results and discussion

Large and significant differences were observed in survival periods among null ($p53^{-/-}$), heterozygous ($p53^{+/-}$) and wild type ($p53^{+/+}$) mice. All unexposed null mice died of leukemia (88/104, 84.6%), sarcomas, etc. (14/104, 13.5%) before the age of 12 months. Especially, null mice died of lymphocytic leukemia at the early age of (3–5 months), while heterozygous and wild type mice developed a low incidence of leukemia (7/88, 8.0% and 2/16, 12.5%) and many of them survived more than 30 and 40 months, respectively. The heterozygotes developed various types of tumors (61/88, 69.3%), mainly in the connective tissue, at older ages. The spectrum of the observed tumors was osteosarcomas (22.7%), other sarcomas (21.6%), cancers (9.1%), reticulum cell neoplasms (4.5%) and leukemia (8.0%). The wild-type animals developed reticulum cell neoplasms (4/16, 25%) and cancers (2/16, 12.5%), which were commonly observed in the back ground strain (C57BL/6) at the end of their lives.

^{137}Cs γ-irradiation of heterozygous mice shortened their lifespan significantly, when compared to the unirradiated heterozygotes. Most of the irradiated heterozygotes had multiple tumors in different organs. In contrast, unirradiated heterozygous animals showed tumor development at older ages and only a small number of animals had multiple tumors in different organs. However, there were no substantial differences in the survival periods of the null mice between the irradiated and unirradiated groups. In the present study, the animals survived longer than those in other studies reported [6,7], probably because our

animals were maintained in the complete barrier condition. However, the spectrum of observed tumors was not different from that of the previous study [6].

We analyzed the status of the wild type allele in the tumors of unirradiated $p53^{+/-}$ mice and found that 12 out of 54 (22.2%) tumors showed LOH, while there were no LOH in 37 normal tissues examined. Among the tumors examined, LOH was found in 5 out of 8 (62.5%) leukemia and malignant lymphomas, 3 out of 13 (23.1%) osteosarcomas, 2 out of 2 (100%) fibrosarcomas, 1 out of 3 (33.3%) mammary adenocarcinomas, and 1 out of 1 (100%) neuroblastoma. Most of the LOH (5 of 12, 41.7%) were observed in leukemia and malignant lymphomas. Among the rest of the 42 tumors, which did not show any LOH, only one mutation (Tyr → Stop codon) was found in exon 6 in the reticulum cell neoplasm. The majority of the tumor samples with LOH showed a residual signal band of the wild type allele on the gel. We assume it inevitable due to the contamination of normal cells in the tumor tissue. Detailed studies of the LOH and mutations in spontaneous and radiation induced tumors are in progress.

In conclusion, the present results demonstrate that the mice lacking one or both alleles of the *p53* gene are highly susceptible to tumor induction, and irradiation of the heterozygotes enhances tumor deaths as observed in the unirradiated null mice, indicating the important role of the *p53* gene to prevent tumorigenesis.

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