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Mutations in the *p53* and *Scid* Genes Do Not Cooperate in Lymphomagenesis in Doubly Heterozygous Mice

S. Kosugi,*,† T. Miyazawa,* D. Chou,* Y. Saito,* T. Shinbo,* A. Matsuki,* H. Okano,* C. Miyaji,‡ H. Watanabe,‡ K. Hatakeyama,† O. Niwa,§ and R. Kominami*,[1]

**First Department of Biochemistry, †First Department of Surgery, and ‡Department of Immunology, Niigata University School of Medicine, Asahimachi 1-757, Niigata 951-8122; and §Radiation Biology Center, Kyoto University, Yoshida-Konoecho, Sakyou-Ku, Kyoto 606-8315 Japan*

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Analysis of double mutant mice of the *p53* and *scid* genes, which have a combination of cell cycle checkpoint/apoptosis and DNA repair defects, shows that the latter defect synergistically enhances lymphoma development with loss of the former function. These mice lack the ability to eliminate lymphocytes predisposed to neoplastic transformation resulting from faulty antigen receptor gene rearrangement. Here we examine the cooperativity in double heterozygotes of *p53* and *scid* in which normal development of lymphocytes is not impaired. MSM mice carrying a p53-knockout allele were crossed with BALB/c mice heterozygous for the *scid* locus and 129 offspring were obtained. They were subjected to γ-ray irradiation, 84 thymic lymphomas being generated. The tumors and host mice were genotyped of *p53* and *scid*. Among 42 mice developing p53-deficient lymphomas, *scid*/+ and +/+ genotypes did not provide difference in onset and latency. Besides, allelic loss of the *Scid* gene occurred at a high frequency in those lymphomas but the loss exhibited no allelic bias. The results suggest that the *scid*/+ genotype is not a modifier of loss of p53 function in the double heterozygotes. © 1999 Academic Press

Mutation of the gene for a catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), i.e. *scid* mutation, causes a generalized defect in double-strand break repair including a severely impaired ability to carry out V(D)J recombination in lymphocyte development (1–3). Analysis of the *scid* cells shows that DNA-PK has an important role in the recognition and repair of DNA damage induced by genotoxic agents such as γ-ray (4–6). Despite the repair defect, *scid* mice have only a slightly elevated incidence of spontaneous and radiation-induced tumors (1, 3). On the other hand, cells homozygous for a null *p53* allele have an inability to arrest the cell cycle and undergo apoptosis following irradiation but they have no defects in repair of DNA damages (7–10). p53 is an archetypal regulator of cell-cycle checkpoint and responds to DNA damages (11). p53-deficient mice spontaneously develop tumors and *p53*(+/−) heterozygotes are also susceptible to cancers but with a delayed onset compared to homozygotes (12, 13). Interestingly, double mutant mice of the *scid* and *p53* genes (*p53*(−/−)*scid/scid*) exhibit earlier onset and latency of lymphomas than the singly defective *p53*(−/−) or *scid/scid* mice (14, 15). Thus, a DNA-repair defect in conjunction with loss of p53 synergistically enhances lymphoma development.

p53(−/−)*scid/scid* double mutant mice lack the ability to eliminate cells with faulty or incomplete antigen receptor gene rearrangement that could produce oncogenic mutations by activating proto-oncogenes or inactivating tumor suppressor genes (14, 15). Accordingly, *p53*(−/−)*scid/scid* lymphocytes should be markedly predisposed to neoplastic transformation. Indeed, tumors in *p53*(−/−)*scid/scid* mice are derived from such aberrant lymphocytes, which would not survive in a *p53*-wild type background. Thus, synergism of loss of the p53 and DNA-PK genes in tumor development is evident in the doubly homozygous background. However, the synergism has not been examined in the mice doubly heterozygous for the two genes. In order to address this issue, we have generated double heterozygotes (*p53*(+/−)*scid*/+) and subjected to γ-ray irradiation. *Scid* heterozygotes (*scid*/+) are known to develop thymus normally as in wild-type mice (1–3). In this paper we show that the state of DNA-PKcs gene does not affect the development of p53-deficient lymphomas.

MATERIALS AND METHODS

Mice and lymphomas. MSM is an inbred strain derived from Japanese wild mice, *Mus musculus molossinus.* The details of lymphoma induction were described previously (16). In brief, the MSM

[1] To whom correspondence should be addressed. Fax: +81-25-227-0757. E-mail: rykomina@med.niigata-u.ac.jp.

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male mice with the genotype of p53(KO/+) were mated with *scid* heterozygous female mice on BALB/c background and 129 F_1 hybrid mice were obtained. The mice were subjected to γ-ray irradiation, 2.5 Gy four times at a week interval, starting at age of 4 weeks. Development of thymic lymphoma was diagnosed by the inspection of labored breathing. A total of 84 thymic lymphomas and 15 tumors of other types were obtained.

DNA and PCR analysis. Isolation of genomic DNA from lymphomas and brain was carried out by standard protocols. Polymerase chain reaction (PCR) and separation of PCR products by gel electrophoresis were performed as described previously (16).

Typing of p53 and scid loci. p53 genotyping was carried out as described previously (16, 17). One primer (F1-53) located in exon 1 of the p53 gene, a second primer (R1-53) in a region 5′ to exon 3, and the remaining one (F2-neo) in the neo gene insert. F1-53 and R1-53 amplified a region of the p53 gene to produce a fragment of 500 bp. F2-neo and R1-53 gave a 800 bp fragment comprising a part of the neo and p53 genes. Therefore, the 500 bp band and the 800 bp band represent the normal allele and the mutant allele, respectively. This primer set was also used for allelic loss analysis of lymphomas developing in p53(KO/+).

scid genotyping was performed according to the procedure described by Blunt et al. (18). Primers used for the *scid* locus were 5′-TGGTATCCACAA-CATAAAATACGC-3′ and 5′-AGTTATAAC-AGCTGGGTTGGC-3′. *D16Mit164* and *D16Mit74* microsatellite primers were synthesized according to Dietrich et al. (19).

Monoclonal antibodies and flow cytometry. Staining of thymocytes and spleen cells in young double-heterozygous mice was performed as described previously (20). FITC or PE conjugated anti-CD4(RM4-5), anti-CD8(53-6.7), anti-B220(RA3-6B2) and anti-CD3(145-2C11) antibodies were purchased from PharMingen (San Diego, CA). Cell suspensions were stained with the antibodies and analyzed by FACScan (Becton-DicVision Co. CA).

RESULTS

MSM mice carrying a p53-knockout allele were crossed with BALB/c mice heterozygous for the *scid* locus and 129 offspring were obtained. They were subjected to γ-ray irradiation at age of 4 weeks, 2.5 Gy four times at a week interval. A total of 99 tumors were obtained, 84 of which were thymic lymphomas. Other than the lymphomas, nine were subcutaneous tumors, five were leukemia, and one was hepatoma. Those tumors were excluded from the present study because of contamination of normal tissues. The irradiated mice consisted of four types of genetic constitutions: *p53*(KO/+)*scid*/+ (double heterozygotes), *p53*(+/+)*scid*/+ (heterozygous for the *Scid* locus), *p53*(KO/+)*Scid*(+/+) (heterozygous for the p53 locus), and *p53*(+/+)*Scid*(+/+) (wild-type for both loci). Genotyping of the 84 mice was carried out by detecting the presence of p53-KO allele and *scid* allele by PCR. For the *p53* genotyping, a set of three PCR primers was used for brain DNA as described previously (16, 17), in which one pair of the primers detected the presence of normal *p53* allele and the other determined the existence of the KO *p53* allele (data not shown). The analysis revealed that 57 lymphomas were derived from *p53*(KO/+) mice and 27 were from *p53*(+/+) mice. *Scid* genotyping was performed using a primer set detecting the *scid* mutation according to Blunt et al. (18). Fig. 1 shows examples of

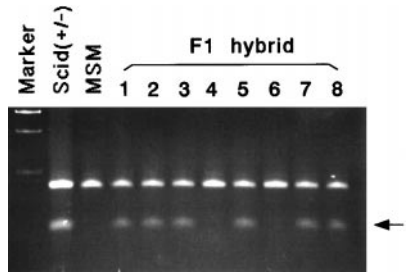

FIG. 1. *scid* genotyping of F1 hybrid mice. PCR products of 69 bp with *scid* primers were cut by *Alu*I enzyme and subjected to gel electrophoresis. The digestion produced fragments of 41 bp and 28 bp marked by an arrow (doublet) for the *scid* allele (samples 1–3, 5, 7, 8), but not for the wild-type allele (samples 4, 6). The number of tumors is given arbitrarily. Marker is *Hinf*I digests of pBR322 DNA.

that genotyping. Forty lymphomas were derived from *scid*/+ mice and 44 were from *Scid*(+/+) mice. Flow-cytometric analysis of thymocytes and spleen cells demonstrated normal T-cell and B-cell development in *p53*(KO/+)*scid*/+ mice as in wild-type mice (Fig. 2).

The *p53*(KO/+) mice developing lymphoma were divided into two groups, *scid* heterozygotes and *Scid* wild-type mice, and ordered according to the ascending age-of-onset (Fig. 3A). The latency periods of the two groups did not differ (χ^2 value: 0.51), suggesting that the presence of one *scid* allele in mice did not affect the latency of lymphoma development. Our previous analysis suggested that most of lymphomas derived from *p53*(KO/+) mice lost the wild-type *p53* allele and did not retain any function of the p53 gene (16, 17). Hence, the p53 deficiency was determined by examining the loss of *p53* wild-type allele for lymphomas developing in *p53*(KO/+)*scid*/+ and *p53*(KO/+)*Scid*(+/+) mice. Eighteen lymphomas in *scid*/+ and 24 in *Scid*(+/+) mice showed disappearance of the normal *p53* allele (Table 1). Fig. 3B shows relative cumulative lymphoma incidences for mice of those two classes. No difference was observed, either (χ^2 value: 0.80).

In order to know whether or not the *Scid* locus suffered the allelic loss, we examined 57 lymphomas carrying the p53-KO allele using the *scid* primer pair and flanking *D16Mit164* and *D16Mit74* microsatellite markers (Fig. 4). The analysis demonstrated that the loss was frequent and about a half of the lymphomas exhibited the loss. Interestingly, the loss exhibited no allelic bias and the *scid* allele and the wild-type allele were equally susceptible to the loss (Table 1). The lymphomas without the wild-type allele was expected to be deficient in the repair activity but the lymphomas without the *scid* allele was repair proficient. Consistent results were obtained for the two flanking loci in almost all lymphomas, suggesting that regions of allelic loss were large enough to cover the three loci on chromosome 16. Although there were only five mice that developed the p53-deficient *scid* lymphomas, they

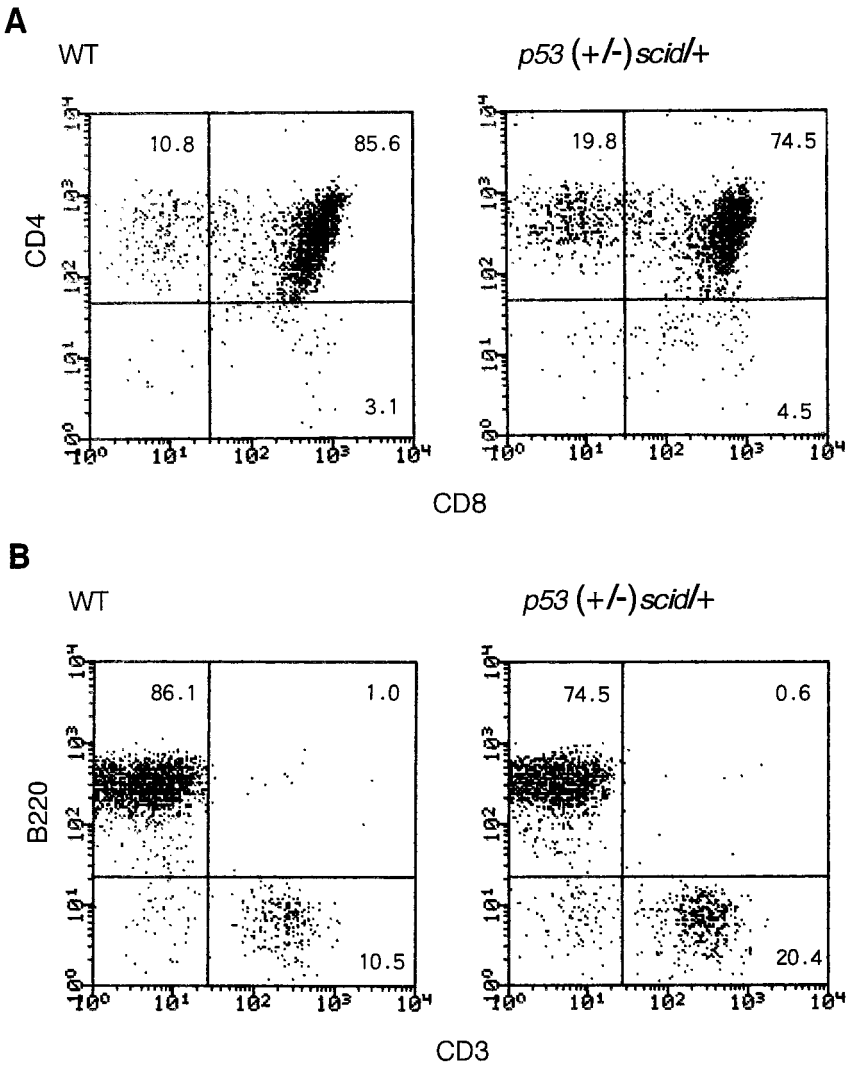

FIG. 2. Flow-cytometric analysis of thymocytes and spleen cells from 6-week old *p53*(+/−)*scid*/− mice. (A) Thymocytes from wild-type and double-heterozygous mice were double-stained with antibodies to CD4 and CD8 and subjected to flow cytometry. Percentages of cells in the boxed areas are indicated. Dead cells were excluded by forward scatter, side scatter and PI gating. (B) Spleen cells from wild-type and double-heterozygous mice were staining with antibodies to B220 and CD3 and analyzed as described above.

did not differ in latency from mice with the p53-deficient *Scid*-heterozygous lymphomas (the mean of latency for the five and the other 13 mice: 123 and 130 days, respectively). These results suggest that loss of the DNA-PK related repair function does not contribute to lymphoma development in p53(KO/+)*scid*/+ mice.

DISCUSSION

Li-Fraumeni patients with p53 gene mutations are susceptible to sarcomas and carcinoma of the breast and their outcomes range from early aggressive cancer to disease-free survival (21). This phenotypic heterogeneity may reflect the presence of low penetrance genes in the human population that can modify the impact of a given mutation in addition to environmental and epigenetic factors (22, 23). p53 heterozygous mice can be a model for Li-Fraumeni patients to study such a modifier(s) (13). Analysis of double mutant mice of the *p53* and *Scid* genes (*p53*(−/−)*scid/scid*) shows synergistic effect of the DNA-PK defect with loss of the p53 function in lymphoma development (16, 17). Acceleration of lymphomagenesis by the *scid* mutation is also found in *p53* heterozygous mice (15). DNA-PK functions in DNA repair while p53 provides a cell-cycle arrest which allows time to repair damaged DNA. p53 also functions in apoptosis that may balance the aberrant proliferation of damaged cells (7–11). Therefore, loss of p53 function is likely to contribute to tumorigenesis by permitting the propagation of premalignant cells with DNA damages. Recently, DNA-PKcs null

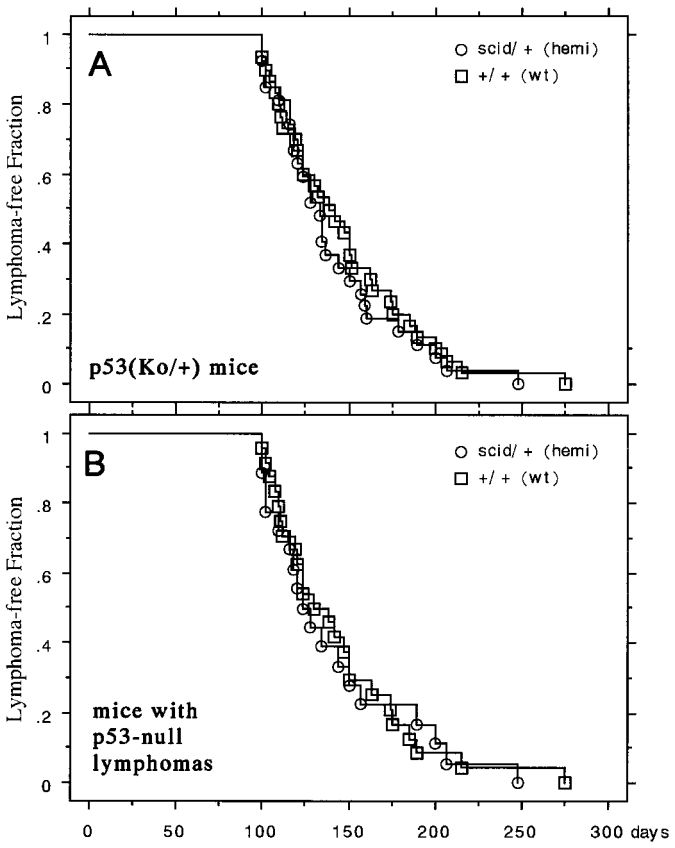

FIG. 3. Cumulative lymphoma incidences in p53 heterozygous mice (A) and mice developing p53-null lymphomas (B). One hundred twenty nine mice were monitored three times a week for development of tumors over a period of 250 days after irradiation. Moribund mice or mice with obvious tumors were killed, necropsied and some of the tumor tissues were examined by histopathology. Among 84 thymic lymphomas obtained, 57 developed in p53(KO/+) mice and 42 of the 57 lymphomas were of the p53(KO/−) genotype. The relative lymphoma incidences in A and B are displayed.

mice have been generated by insertion of a transgene into the *Scid* locus and they show complete penetrance of thymic lymphomas (24). These results suggest that *scid* mutation or possibly other mutations can be a modifier of the oncogenic phenotype conferred by the loss of p53.

Those experiments, however, address cooperativity of loss of the two genes in mice with homozygous mutation at the DNA-PKcs gene. As a result of deficiency in the DNA repair function, *scid/scid* mice yield lymphocyte progenitors undergoing aberrant rejoining events which are not excluded in the p53 deficient background. Some of these aberrant rejoinings are expected to result in oncogenesis. In contrast, our study has examined the cooperativity in doubly heterozygous mice that develop normal lymphocytes, as assayed by flow cytometric analysis (Fig. 2). The heterozygotes are probably a more valid model for Li-Fraumeni patients than the double homozygotes. Results in this study does not show synergism of the p53 deficiency and the *scid* mutation in lymphomagenesis. Among *p53*-heterozygotes developing the p53-deficient lymphomas, *scid*/+ and +/+ genotypes did not provide any difference in onset and latency (Fig. 3). Besides, no allelic preference was noted in the loss of the *Scid* locus in those lymphomas (Table 1). This suggests that the *scid*/+ genotype is not a modifier of loss of the p53 gene in the double heterozygotes. We observed five mice bearing thymic lymphomas with the *p53*(KO/−)*scid*/*scid* genotype but the mice did not differ in latency from those harboring the *p53*(KO/−)*Scid*(+/−) lymphomas. Therefore, the *scid/scid* genotype might not affect the oncogenic phenotype of the p53 deficiency in normal somatic cells and probably participates in lymphomagenesis by producing premalignant lymphocyte progenitors as described above. This is consistent with that enhancement of tumorigenesis in the double homozygotes were limited to tumors of T-cell and B-cell origins (14, 15).

We previously reported a region in the vicinity of the *Scid* locus that showed frequent allelic loss and inferred that this region harbors a novel tumor suppressor gene (16). Although the peak region of allelic loss was near the *D16Mit122* locus approximately 5 cM apart from the *Scid* locus, the DNA-PKcs gene in the *Scid* locus could be a candidate for the tumor suppressor gene. However, this study clearly demonstrates that this is unlikely, since no allelic preference was detected. The considerably high frequency of allelic

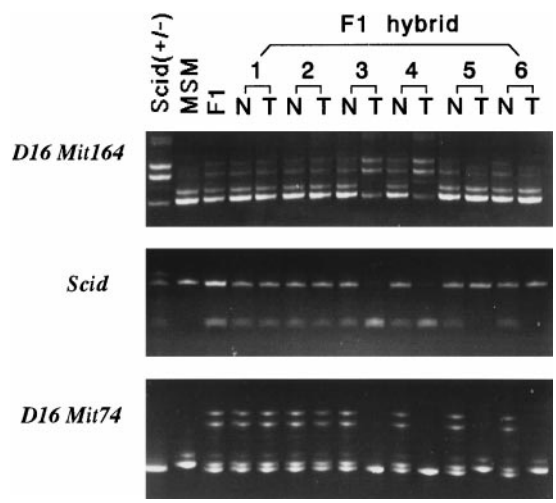

FIG. 4. Allelic loss of the *scid* locus. *Alu*I digests of the *scid* PCR products, and PCR products with the flanking polymorphic markers were subjected to gel electrophoresis. N lanes on panels represent DNA of normal tissue and T lanes represent lymphoma DNA from the corresponding mice. The numbers of mice are given arbitrarily. Samples 1 and 2 are of no allelic loss tumors; samples 3 and 4 of tumors with loss of wild-type allele; and samples 5 and 6 of tumors with loss of *scid* allele. Genetic distances between *D16Mit164* and *scid* and between *scid* and *D16Mit74* are approximately 3 cM and 1 cM, respectively (16).

TABLE 1

Summary of Loss of the *scid* Allele or the Wild-Type Allele in p53(KO/+) and p53(KO/−) Tumors

Of mice		Of lymphomas				
			Scid			
p53	*Scid*	*p53*	s/+	s	+	
Ko/+	s/+	Ko/−	6	5	7	(18)
		Ko/+	8	1	1	(10)
			+/+	c	m	
Ko/+	+/+	Ko/−	12	6	6	(24)
		Ko/+	2	1	2	(5)
			28	13	16	(57)

Note. s/+, heterozygous for *scid;* s, loss of wild-type allele; +, loss of *scid* allele; +/+, wild-type for both alleles; c, loss of MSM allele; m, loss of BALB/c allele.

loss was observed at the *Scid* locus (Table 1), but this could be ascribed to the presence of the tumor suppressor gene in the vicinity. It is probable that loss of the tumor suppressor gene accompanied loss of the *Scid* locus. Indeed, almost all of allelic loss regions found in 112 lymphomas (16) tended to be larger than 20 cM, which intervals were enough to cover the *Scid* locus.

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