J. RADIAT. RES., 43, 175–185 (2002)

Putative Tumor-suppressor Gene Regions Responsible for Radiation Lymphomagenesis in F_1 Mice with Different *p53* Status

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Lymphoma / Loss of heterozygosity (LOH), Tumor suppressor gene / *p53* / Mouse

Regions of allelic loss on chromosomes in many tumors of human and some experimental animals are generally considered to harbor tumor-suppressor genes involved in tumorigenesis. Allelotype analyses have greatly improved our understanding of the molecular mechanism of radiation lymphomagenesis. Previously, we and others found frequent loss of heterozygosity (LOH) on chromosomes 4, 11, 12, 16 and 19 in radiation-induced lymphomas from several F_1 hybrid mice. To examine possible contributions of individual tumor-suppressor genes to tumorigenesis in *p53* heterozygous deficiency, we investigated the genome-wide distribution and status of LOH in radiation-induced lymphomas from F_1 mice with different *p53* status. In this study, we found frequent LOH (more than 20%) on chromosomes 4 and 12 and on chromosomes 11, 12, 16 and 19 in radiation-induced lymphomas from (STS/A X MSM/Ms)F_1 mice and (STS/A X MSM/Ms)F_1-*p53*$^{KO/+}$ mice, respectively. Low incidences of LOH (10–20%) were also observed on chromosomes 11 in mice with wild-type *p53*, and chromosomes 1, 2, 9, 17 and X in *p53* heterozygous-deficient mice. The frequency of LOH on chromosomes 9 and 11 increased in the (STS/A X MSM/Ms)F_1-*p53*$^{KO/+}$ mice. Preferential losses of the STS-derived allele on chromosome 9 and wild-type *p53* allele on chromosome 11 were also found in the *p53* heterozygous-deficient mice. Thus, the putative tumor-suppressor gene regions responsible for lymphomaganesis might considerably differ due to the *p53* status.

INTRODUCTION

Whole-body fractionated irradiation effectively induces thymic lymphomas in mice[1)]. Malignant lymphoma cells are considered to develop through multi-step genetic events during latent periods after irradiation. Some of the genetic events, including non-disjunction with or without reduplication, heterozygous deletion, gene conversion and homologous or non-homologous mitotic recombination, can be detected as loss of heterozygosity (LOH)[2)]. The LOH regions often harbor tumor-suppressor genes in numerous malignancies in both human and experimental animals[3,4)]. Therefore, the search for the tumor-suppressor genes responsible for tumorigenesis has centered on LOH studies to demonstrate the molecular genetic mechanism underlying the tumori-

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genesis. Analyses of allelotype in radiation-induced tumors from F_1 hybrid mice of inbred strains have been performed by several groups, because they are very simple and all tumors are uniformly informative at a number of loci as to the location of the tumor-suppressor gene regions.

Extensive allelotype analyses have substantially furthered our understanding of the molecular mechanism of radiation lymphomagenesis. Santos *et al.* (1996) suggested the existence of two tumor-suppressor gene regions, TLSR (*Thymic lymphoma suppressor region*) 1 between D4SWsm1 and *D4Mit9*, and the more distal TLSR2, centered at the marker *D4Mit54*, each homologous to the human chromosomal regions 9p21-22 and 1p32-36, respectively, in radiation-induced lymphomas of (C57BL/6J x RF/J) F_1 mice[5]. They also mapped a third region (TLSR3) within the area defined by *Mom-1* and *D4Mit68*[6], and found two additional tumor-suppressor gene loci at the proximal-mid part of mouse chromosome 4 in T-cell lymphomas, defined by the markers *D4Mit116* (TLSR4) and *D4Mit21* (TLSR5). They then characterized a genomic DNA fragment of about 12 kb corresponding to the murine *p73* gene, and reported evidence suggesting that *p73* is the tumor-suppressor gene (TLSR2) around marker *D4Mit205b* at the distal end of chromosome 4 in T-cell lymphomas[7].

An extremely high frequency (more than 60%) of allelic loss on distal chromosome 12 was observed in radiation-induced lymphomas from (BALB/cHeA x MSM/Ms)F_1 [(C x M)F_1] mice[8,9]; interestingly, this region is syntenic homologous to human chromosome 14q32-33. Frequent LOH at human chromosome 14q has been reported in a variety of tumors, such as neuroblastomas[10], advanced colorectal carcinomas[11], bladder cancers[12], ovarian carcinomas[13,14], endometrial carcinomas[15], and renal cell tumors[16]. Physical delineation of this region has shown that a putative tumor-suppressor gene exists within a 35 kb interval[17] and close to *D12Mit233*[18]. The *Ikaros* gene on centromeric chromosome 11 has been suggested to be an important tumor-suppressor gene in mouse thymic lymphomas[19,20].

We also found frequent LOH on chromosome 19 with syntenic homology to human chromosomes 10q and 11p in addition to the LOH on chromosomes 4 and 12 in the lymphomas from (BALB/cHeA x STS/A)F_1 [(C x S)F_1] mice[21] . Santos *et al.*[22] described specific LOH on chromosome 19 in radiation-induced thymic lymphomas from (C57BL/6J x BALB/c)F1 mice, and evidence for a possible epigenetic mechanism.

In radiation-induced lymphomas, alterations of *p53* are rare[23]. However, p53-deficient mice are extremely susceptible to the induction of lymphomas[24]. Although allelic loss at the centromeric

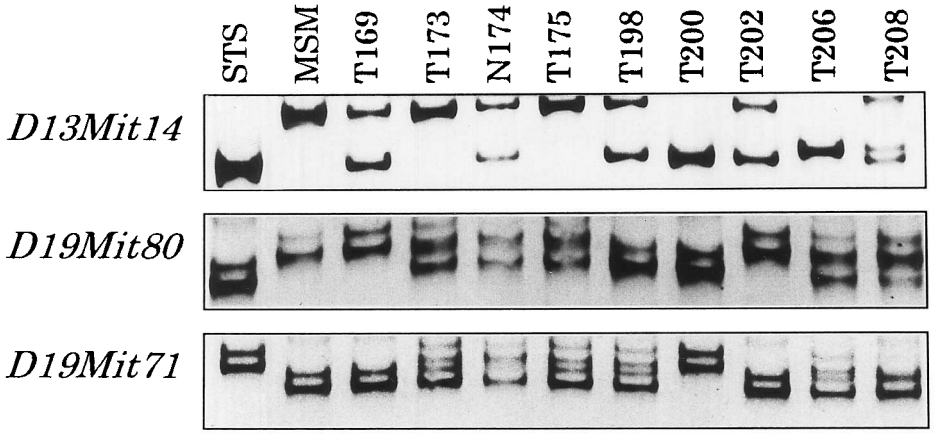

Fig. 1. Representative profiles for polyacrylamide gel electrophoresis of PCR products at microsatellite loci of DNA from normal and tumor tissues. Allelotypes at microsatellite loci *D13Mit14*, *D19Mit80* and *D19Mit71* are shown. Lanes marked STS and MSM contain normal liver DNA; Normal liver DNA (N174) and tumor DNA (T173) were obtained from the same mouse. T169 and T175 to T208, tumor DNA.

Table 1. Allelec loss frequency of genome-wide 81 polymorphic microsatellite loci in 24 radiation-induced lymphomas from (STS/A X MSM/Ms) F_1-*p53*$^{KO/+}$ mice.

Chrs	Loci	Position	LOH (%)	Chrs	Loci	Position	LOH (%)
Chr.1	D1Mlt1	8.7	17	Chr.11	D11Mit4	37	50
	D1Mit5	32.8	13		D11Mit7	44.3	42
	D1Mit9	52	13		D11Mit14	57	29
	D1Mit14	81.6	0		D11Nds7	62	25
Chr.2	D2Mit3	5	0		D11Mit10	63	25
	D2Mit8	30.5	4		D11Mit104	79	13
	D2Nds3	53	4	Chr.12	D12Mit37	1	21
	D2Mit208	76.7	17		D12Nds1	27	37
	D2Mit51	95.5	4		D12Mit3	32	44
Chr.3	D3Mit21	19.2	0		D12Mit4	34	37
	D3Mit11	49	0		D12Mit233	52	67
	D3Mit17	71.8	0		D12Mit263	58	67
Chr.4	D4Mit4	12.1	0	Chr.13	D13Mit14	10	4
	D4Mit9	44.5	4		D13Mit9	45	0
	D4Mit31	51.3	4	Chr.14	D14Mit2	5	0
	D4Mit54	66	8		D14Mit31	28	0
Chr.5	D5Mit4	20	0		D14Mit97	58	0
	D5Mit9	54	0	Chr.15	D15Mit11	10.4	0
	D5Mit51	81	0		D15Mit123	30.6	0
Chr.6	D6Mit16	30.5	4		D15Mit33	48.6	0
	D6Mit11	49.4	4		D15Mit161	69.2	0
	D6Mit15	74	4	Chr.16	D16Mit74	9.7	33
Chr.7	D7Mit25	16.00	4		D16Mit4	27.3	33
	D7Nds5	23.00	4		D16Mit7	57.7	25
	D7Mit18	26.40	4	Chr.17	D17Mit18	4	17
	D7Mit16	40.00	8		D17Mit7	32.3	8
Chr.8	D8Mit6	30	4		D17Mit4	34.3	8
	D8Mit80	41	4		D17Mit1	56.7	17
	D8Mit55	62	4	Chr.18	D18Mit12	17	4
Chr.9	D9Mit130	27	8		D18Mit49	49	4
	D9Mit10	49	13		D18Mit16	58	4
	D9Mit12	55	17	Chr.19	D19Mit32	0	13
	D9Mit24	56	17		D19Mit80	22	17
	D9Mit20	61	17		D19Mit63	35	17
	D9Mit17	62	17		D19Mit11	41	17
	D9Mit19	71	13		D19Mit10	47	17
Chr.10	D10Nds1	6	0		D19Mit123	51	17
	D10Mit15	35	8		D19Mit71	54	17
	D10Mit11	50	0	Chr.X	DXNds1	17	17
Chr.11	D11Mit1	0.25	58		DXMit1	29.01	17
	D11Mit51	18	46				

region on chromosome 16 is observed in thymomas from (BALB/cHeA x MSM/Ms)F_1 - *p53* $^{KO/+}$ [(C x M)F_1-*p53* $^{KO/+}$] mice[9], it is very rare in *p53*$^{+/+}$ mouse thymomas[8,9,21], indicating that the LOH on chromosome 16 is specific to *p53*$^{KO/+}$ mice.

The evidence on the radiation-induced thymic lymphomas mentioned above indicates that the genome-wide patterns of LOH in F_1 mice differ with the parental combinations, even if the histopathological feature of the tumor and the oncogenic treatment are the same. This makes us suspect that different tumor-suppressor genes contribute to lymphomagenesis in different genetic backgrounds. Frequent LOH is observed on chromosomes 4, 12 and 19 in (CXS)F_1 mice, but only on chromosome 12 among (CXM) F_1 mice in which STS/A (S) and MSM/Ms (M) strains, respectively, are the parents. On the other hand, in (C57BL/6 X C3H/HeJ) F_1 (B6C3 F_1) mice, LOH (more than 20%) is observed on chromosomes 4, 11, 12, 15 and 19, and highly frequent allelic losses (about 50%) on chromosomes 11 and 12[19]. In this study, an allelotype analysis was performed in tumors from (STS/A x MSM/Ms)F_1 [(S x M) F_1] and (STS/A x MSM/Ms)F_1-*p53*$^{KO/+}$ [(S x M) F_1-*p53*$^{KO/+}$] hybrid mice to examine the effects of the *p53* status and genetic background on the genome-wide features of LOH.

MATERIALS AND METHODS

Mice

STS/A-*p53*$^{KO/+}$ mice were generated by backcrossing 129 *p53*-deficient mice with STS/A mice ten times. STS/A-*p53*$^{KO/+}$ mice were crossed with MSM/

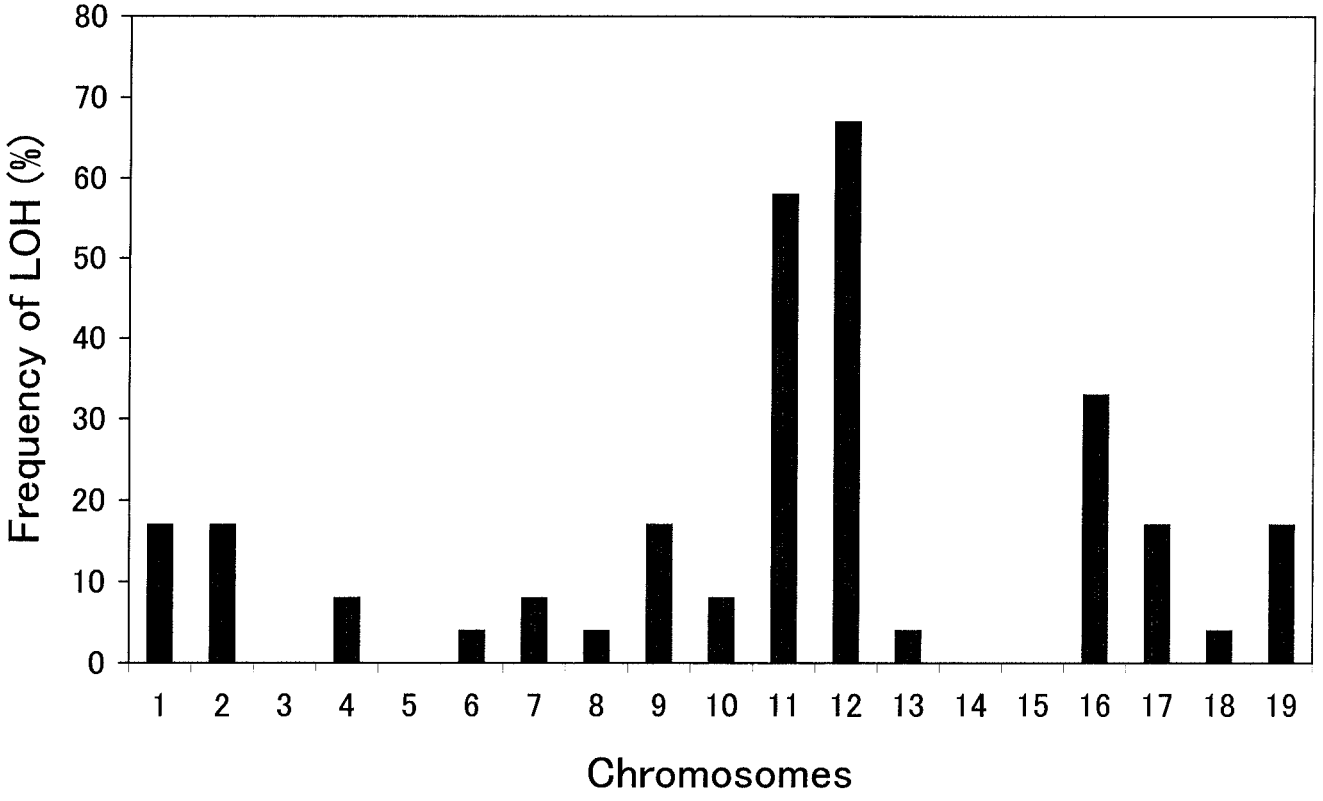

Fig. 2. Genome-wide search for LOH in 24 radiation-induced thymic lymphomas from (STS/A X MSM/Ms)F_1-*p53*$^{KO/+}$ mice. The maximum frequency obtained on each chromosome is shown. The loci at which the frequency is shown are as follows: *D1Mit1* (8 centimorgans from the centromere: 8.7 cM), *D2Mit208* (76.7 cM), *D3Mit11* (49 cM), *D4Mit54* (66 cM), *D5Mit9* (54 cM), *D6Mit11* (49.4 cM), *D7Mit16* (40.00 cM), *D8Mit80* (41 cM), *D9Mit24* (56 cM), *D10Mit15* (35 cM), *D11Mit1* (0.25 cM), *D12Mit233* (52 cM), *D13Mit14* (10 cM), *D14Mit31* (28 cM), *D15Mit123* (30.6 cM), *D16Mit4* (27.3 cM), *D17Mit1* (56.7 cM), *D18Mit49* (49 cM), *D19Mit63* (35 cM).

Ms mice to generate (S x M) F_1 and (S x M) F_1-*p53*$^{KO/+}$ offspring. The conditions for breeding were described previously[8].

Induction of thymic lymphomas

Mice were exposed four times to X-rays of 1.7Gy (0.5Gy/min) with weekly intervals starting at 4 weeks of age, and the moribund mice were examined as previously described[25].

DNA isolation and LOH analysis

Developed lymphomas and normal tissues were removed. The isolation of DNA, PCR of microsatellite markers, electrophoresis of PCR products and assessment of allelic losses were performed according to a procedure described previously[8]. Oligonucleotide primers corresponding to microsatellite loci were purchased from Research Genetics, Inc. (Huntsville, AL). The chromosomal locations of the microsatellite markers and several loci were based on the 2000 Chromosome Committee Reports in the Mouse Genome Database (Mouse Genome Informatics; Jackson Laboratory, Bar Harbor, ME). Representative profiles for polyacrylamide gel electrophoresis of PCR products at microsatellite loci of DNA from normal and tumor tissues are shown in Fig. 1.

RESULTS

Genome-wide search for LOH in thymic lymphomas from (S X M) F_1-p53$^{KO/+}$ mice

A LOH analysis was first carried out for 24 thymic lymphomas from (S X M)F1-*p53*$^{KO/+}$ mice at 81 polymorphic microsatellite loci (Table 1 and Fig. 2). Table 1 gives the frequency of LOH at each locus. Highly frequent peaks were found at *D12Mit233* (52 cM from centromere) to *D12Mit263* (58 cM), *D11Mit1* (0.25 cM) near *Znfn1a1* (*Ikaros*), *D11Mit4* (37 cM) near *p53* and *D16Mit74* (9.7 cM) to *D16Mit4* (27.3 cM); the frequencies of LOH at these regions or loci were 67, 58, 50 and 33%, respectively. LOH with a lower incidence was also observed in several regions; four lymphomas out of 24 had LOH at *D1Mit1* (8.7 cM), *D2Mit208* (76.7 cM), *D9Mit12* (55 cM) to *D9Mit17* (62 cM), *D17Mit18* (4 cM), *D17Mit1* (56.7 cM), *D19Mit80* (22 cM) to *D19Mit71* (54 cM) and *DXNds1* (17 cM) to *DXMit1* (29.01 cM) (Table 1 and Fig. 2).

Table 2. Allelec loss frequency of 26 microsatellite loci covering the LOH regions observed in table 1 in 20 radiation-induced lymphomas from (STS X MSM/Ms) F_1-*p53*$^{+/+}$ mice.

Chrs	Loci	Position	LOH (%)
Chr.1	D1Mit1	8.7	0
	D1Mit9	52	0
Chr.2	D2Mit8	30.5	0
	D2Mit208	76.7	0
Chr.3	D3Mit11	49	0
Chr.4	D4Mit9	44.5	5
	D4Mit54	66	25
Chr.5	D5Mit9	54	0
Chr.6	D6Mit11	49.4	0
Chr.7	D7Mit25	16.00	5
	D7Mit16	40.00	0
Chr.8	D8Mit80	41	5
Chr.9	D9Mit130	27	0
Chr.10	D10Mit15	35	0
Chr.11	D11Mit1	0.25	15
	D11Mit7	44.3	15
Chr.12	D12Mit37	1	0
	D12Mit233	52	60
Chr.13	D13Mit14	10	0
Chr.14	D14Mit31	28	0
Chr.15	D15Mit123	30.6	0
Chr.16	D16Mit4	27.3	10
Chr.17	D17Mit1	56.7	0
Chr.18	D18Mit49	49	0
Chr.19	D19Mit63	35	5
	D19Mit123	51	0

Examination of LOH in thymic lymphomas from (S X M) F_1-$p53^{+/+}$ mice

Allelic losses were examined for 20 thymic lymphomas from (S X M) F1 mice bearing wild-type *p53* at 26 microsatellite loci covering the LOH regions observed in (S X M) F_1-$p53^{KO/+}$ mouse lymphomas (Table 2 and Fig. 3). LOH was observed less frequently in lymphomas from (S X M) F_1-$p53^{+/+}$ mice than from (S X M) F_1-$p53^{KO/+}$ mice. Only 9 (35%) out of 26 loci were identified as having LOH in 20 lymphomas from $p53^{+/+}$ mice, while 22 (85%) out of the same 26 loci indicated LOH in 24 lymphomas from $p53^{KO/+}$ mice (Table1, 2). The averages of the total LOH numbers at the 26 loci per lymphoma were 1.5 (29 LOH / 20 lymphomas) and 3.6 (86 LOH / 24 lymphomas) for $p53^{+/+}$ and $p53^{KO/+}$ mice, respectively.

The LOH frequencies markedly decreased at *D11Mit1* (0.25 cM) and *D11Mit7* (44.3 cM) compared with the *p53* hetero-deficient (S X M) F_1 mice. The LOH on chromosome 16 also slightly decreased. In contrast, the incidence (5/20: 25%) of LOH at *D4Mit54* (66 cM) in these (S X M) F_1 mice rather increased similar to that (2/24: 8%) in the lymphomas of (C X S) F_1 mice bearing wild-type *p53*, suggesting a reverse effect of *p53* hetero-deficiency.

Allelotype analysis of the LOH regions in lymphomas from (S X M) F_1-$p53^{KO/+}$ mice and (S X M) F_1-$p53^{+/+}$ mice

To further examine the dependency of the frequency of LOH on the *p53* status and the parental bias for the allelic loss, we determined allelotypes of more tumors in the regions on chromosomes 9, 12 and 19 in which LOH was observed at several loci encompassing wide areas. As shown in Table 3, LOH on chromosome 9 was significantly more frequent in the

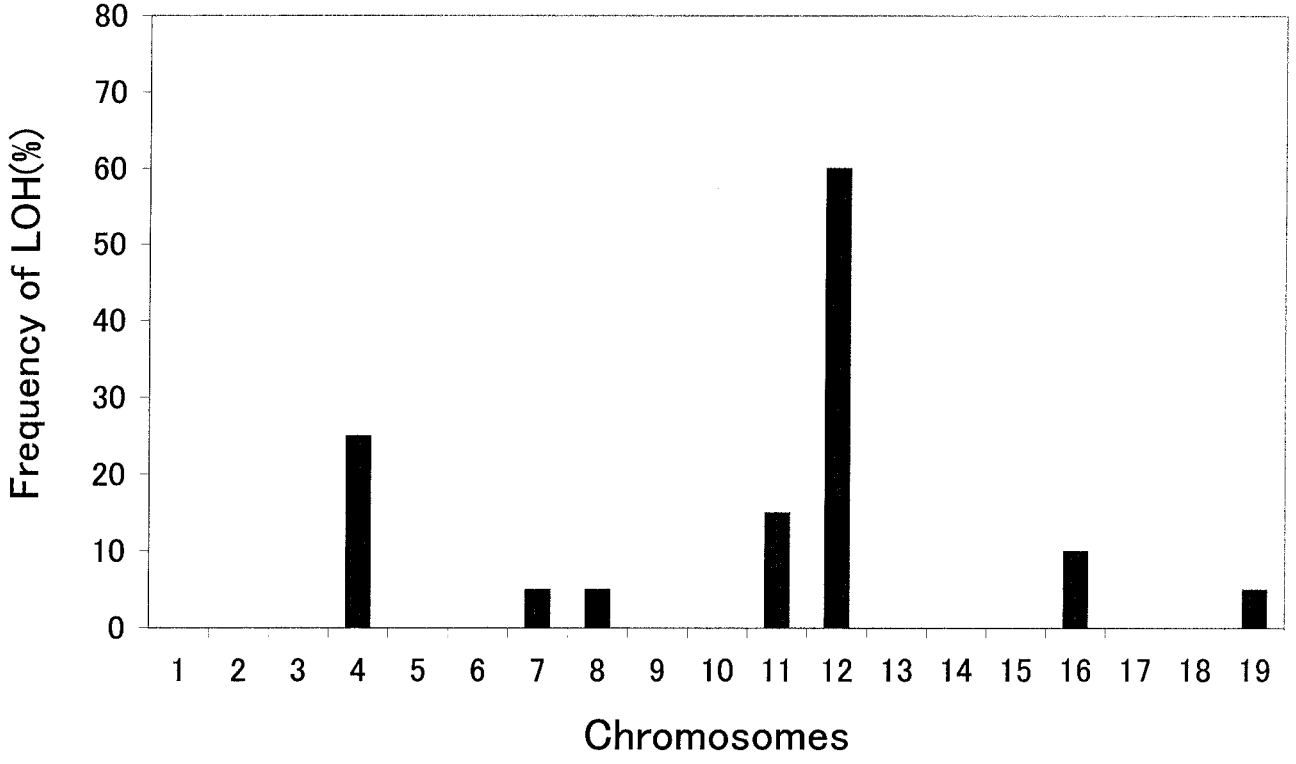

Fig. 3. Genome-wide search for LOH in 20 radiation-induced thymic lymphomas from (STS/A X MSM/Ms)F_1-$p53^{+/+}$ mice. The maximum frequency obtained on each chromosome is shown. The loci at which the frequency is shown are as follows: *D1Mit1* (8 centimorgans from the centromere: 8.7 cM), *D2Mit208* (76.7 cM), *D3Mit11* (49 cM), *D4Mit54* (66 cM), *D5Mit9* (54 cM), *D6Mit11* (49.4 cM), *D7Mit25* (16 cM), *D8Mit80* (41 cM), *D9Mit130* (27 cM), *D10Mit15* (35 cM), *D11Mit1* (0.25 cM), *D12Mit233* (52 cM), *D13Mit14* (10 cM), *D14Mit31* (28 cM), *D15Mit123* (30.6 cM), *D16Mit4* (27.3 cM), *D17Mit1* (56.7 cM), *D18Mit49* (49 cM), *D19Mit63* (35 cM).

Table 3. Frequency of LOH and origins of the lost alleles on chromosomes 4, 9, 11, 12, 16 and 19 in lymphomas from (STS/A X MSM/Ms) F_1 mice with $p53^{KO/+}$ or $p53^{+/+}$.

Chrs	Loci (cM)	$P53^{KO/+}$				$p53^{+/+}$			
		Number of tumors tested	Number with LOH (%)	STS/A allele loss	MSM/Ms allele loss	Number of tumors tested	Number with LOH (%)	STS/A allele loss	MSM/Ms allele loss
4	D4Mit54 (66)	24	2 (8)	2	0	20	5 (25)	5	0
9	D9Mit10 (49)	87	14 (16)*	13***	1	24	0 (0)	0	0
11	D11Mit1 (0.25)	24	14 (58)**	0	14***	20	3 (15)	1	2
12	D12Mit233 (52)	45	34 (76)	15	19	20	12 (60)	8	4
16	D16Mit4 (27.3)	24	8 (33)	1	7	20	2 (10)	2	0
19	D19Mit80 (22)	123	29 (24)	17	12	24	2 (8)	1	1

Statistical comparison of the incidence of LOH or allele loss was carried out by χ^2 analysis or Fisher's exact probability test.

* $P<0.05$ compared to the incidence of LOH (%) on chromosome 9 in mice with $p53^{+/+}$.

** $P<0.005$ compared to the incidence of LOH (%) on chromosome 11 in mice with $p53^{+/+}$.

*** $P<0.002$ compared to MSM/Ms allele loss in lymphomas from (STS/A X MSM/Ms) F_1-$p53^{KO/+}$ mice.

**** $P<0.001$ compared to STS/A allele loss in lymphomas from (STS/A X MSM/Ms) F_1-$p53^{KO/+}$ mice.

p53 heterozygous-deficient mice in addition to the LOH on chromosome 11 mentioned above. Of 87 lymphomas, 14 (16%) showed LOH at *D9Mit10* (49 cM) on chromosome 9 in the *p53*-deficient mice, while none of 24 lymphomas had allelic loss in wild-type mice. Although 123 tumors were examined at *D19Mit80* (22 cM) in the p53 heterozygous-deficient mice, the incidence did not increase significantly compared with wild mice. Allelic loss at *D12Mit233* (52 cM) was extremely high regardless of the *p53* status.

We compared the frequencies of the loss of the alleles derived from each parent in tumors. Preferential losses of STS-derived alleles on chromosome 9 and MSM-derived wild-type p53 alleles on chromosome 11 were found in (S x M)F_1-$p53^{KO/+}$ mice. Parental bias for lost alleles was not seen on chromosomes 12 and 19.

Comparison of the LOH frequency on chromosomes 4, 12 and 19 in F_1-$p53^{+/+}$ mice with different parental backgrounds

The frequency of LOH on chromosomes 4, 12 and 19 in (S X M)F_1 hybrid mice (Table 2, Fig. 3) was compared with data described previously (Fig. 4). Allelic loss on these three chromosomes in (C X S)F_1 hybrid mice was observed in 20 (27%), 42 (57%) and 37 (50%) of 74 lymphomas at *D4Mit31* (51.3 cM), *D12Mit17* (55 cM) and *D19Mit11* (41 cM), respectively[21]. On the other hand, the frequency in (C X M)$F_1$1 hybrid mice was 4 of 51 (8%), 83 of 125 (66%) and 2 of 25 (8%) at *D4Mit13* (71 cM), *D12Mit233* (52 cM) and *D19Mit12* (35 cM), respectively[8]. Although the polymorphic markers used for each chromosome differed among the three F_1 hybrid mice, they were located quite near to each other. The frequency on chromosome 4 in (S X M)F_1 and (C X S)F_1 hybrid mice, one of the parents of which was STS/A, was high compared with that in (C X M)F_1 mice. In this region, alleles derived from the lymphoma-resistant STS/A strain are lost with a higher frequency than those from the susceptible strain BALB/cHeA in the (C X S)F_1 hybrid mice[21]. Loss of the STS/A-derived allele on chromosome 4 may predispose the animals to lymphomas. Therefore, tumor -suppressor gene(s) modifying resistance to radiation lymphomagenesis may reside on chromosome 4. The frequency on chromosome 19 in (C X S)F_1 hybrid mice was markedly high compared with that in (S X M)F_1, and in (C X M)F_1 mice one of the parents of which was MSM/Ms.

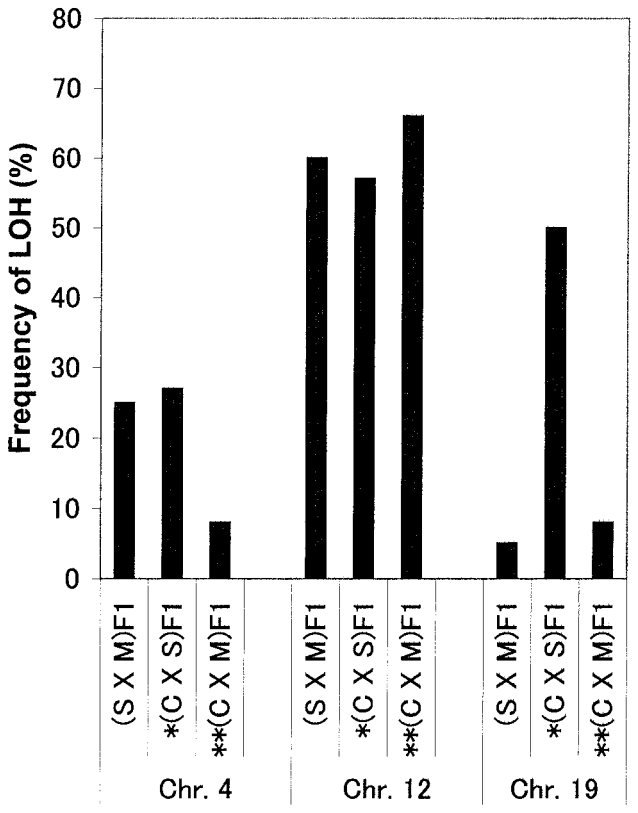

Fig. 4. Comparison of the LOH frequency on chromosomes 4, 12 and 19 in three F_1 mice of different parental backgrounds. STS/A (S), MSM/Ms (M), BALB/cHeA (C). Frequency of LOH on chromosomes 4, 12 and 19 in (S X M) F_1 hybrid mice was determined at D4Mit54 (66 cM), D12Mit233 (52 cM) and D19Mit12 (35 cM), respectively (Table 2, Fig. 3). *Data from Ref. 21. **Data from Ref. 8.

DISCUSSION

Malignant lymphomas are considered to develop through a multi-step genetic process and to be efficiently induced by genetic events brought about by irradiation. In an epidemiological study, few events are supposed to be directly involved in the leukemogenesis compared with those which occur in solid tumors[26]. To identify the genes involved in the development of leukemia/lymphoma, we studied radiation-induced lymphomas in mice. Also, to detect tumor-suppressor genes involved in the lymphomagenesis, we analyzed allelotypes in the tumors from F_1 hybrid mice.

The two hybrids, (S X M) F_1-*p53*$^{KO/+}$ and (S X M) F_1-*p53*$^{+/+}$, used in this study, differed considerably in the latent period of lymphoma development. X-irradiated female (S X M) F_1-*p53*$^{+/+}$ mice first developed thymic lymphomas about 4 months after the last irradiation, and thereafter the lymphomas were induced frequently from 6 to 10 months after the last irradiation (data not shown). The mean latent period of lymphoma development was 252±36 days (95% confidence interval by *t*–test). Incidences of the tumors reached 33% (20/60) at 1 year after the last irradiation. On the other hand, in irradiated female (S X M) F_1-*p53*$^{KO/+}$ mice, the lymphomas were first observed after about 3 months, and were induced most frequently from 3.5 to 7 months after the last irradiation (data not shown). The mean latent period was 147±12 days. The incidences of the tumors reached 32% (19/59) 1 year after the last irradiation. The *p53*$^{+/+}$ mice developed lymphomas about 3.5 months later than *p53*$^{KO/+}$ mice. Thus, *p53* heterozygous deficiency shortened the latent period of tumor development. The shortening of this period in (S x M)F_1-*p53*$^{KO/+}$ mice might be mainly due to the highly frequent and preferential loss of MSM-derived wild-type *p53* alleles (Table 3) as well as an increased incidence of LOH in several other regions (Table 1 and 2, Fig. 2 and 3).

The frequency of LOH at *D9Mit10* (49 cM) on chromosome 9 was significantly increased in the (S x M)F_1-*p53*$^{KO/+}$ mice, and a preferential loss of STS/A-derived alleles at this locus was found. The STS/A mouse is extremely resistant to radiation lymphomagenesis[27]. We previously found STS/A-specific preferential allelic loss on chromosome 4 in (CXS) F_1 where the lymphoma resistance locus has been suggested to exist by analyzing CXS recombinant inbred strains[21]. A susceptibility locus for the lymphomagenesis was recently reported using the same BALB/cHeA and STS/A mice[28]. An analysis of the underlying genes for susceptibility to ionizing radiation is relevant for radiation oncology[29]. The region lost around *D9Mit10* might also contain gene(s) that modify the resistance to radiation lymphomagenesis. *Pml* (promyelocytic leukemia) and *Mlh1* (*mutL* homolog 1) have been mapped near this region on chromosome 9. The fact that LOH on chromosome 9 increased in *p53* heterozygous-deficient mice suggests that the loss of function of the putative tumor-suppressor gene on

chromosome 9 cooperates with *p53* deficiency for lymphomagenesis.

Although LOH at *D16Mit4* (27.3 cM) on chromosome 16 was also more common in the (S x M)F_1-*p53*$^{KO/+}$ mice, no bias in the loss of alleles was found at this locus. In (C x M)F_1-*p53*$^{KO/+}$ mice, frequent allelic loss in the centromeric region (around D16Mit122/D16Mit162) of chromosome 16 has been found, and the frequency is raised by the existence of *p53*-deficient allele[9]. LOH is reported around *D9Mit355* (53 cM) and *D16Mit57* (21.5 cM) in islet cell carcinomas arising in transgenic mice and referred to as *Loh1* and *Loh2*, respectively[30]. It is also suggested that *Loh1* contributes to the progression from the angiogenic stage to a solid tumor and that *Loh2* contains an angiogenic suppressor. It is unclear whether *Loh1* and *Loh2* contain identical tumor-suppressor genes to our LOH regions.

The most frequent LOH on chromosome 12 occurred in all crosses tested, and has syntenic homology to human chromosome 14q32-33. LOH of 14q has been observed in a variety of human tumors such as neuroblastomas[10], advanced colorectal carcinomas[11], bladder cancers[12], ovarian carcinomas[13,14], renal cell tumors[16] and endometrial carcinomas[15]. Recently, Kominami reported *Rit1* coding a transcription factor, as a novel candidate for a tumor-suppressor gene at the common allelic loss region on the distal chromosome 12 of mice[31]. However, sequence information on the gene has not yet been released.

Twenty-nine (24%) of 123 lymphomas exhibited LOH at *D19Mit80* (22 cM) on chromosome 19 in (S x M)F_1-*p53*$^{KO/+}$ mice (Table 3). The region was observed to encompass *D19Mit80* (22 cM) to *D19Mit71* (54 cM) (Table 1). This wide area may contain more than one tumor-suppressor gene. Chromosome 19 is homologous to human chromosomes 10q23-q26, 9 and 11q11-q13. Human chromosome 10q23-q26 contains putative tumor-suppressor genes, such as *PTEN /MMAC1*[32–35] and *MXI-1*[36]. *PTEN*, a protein tyrosine phosphatase gene, of human chromosome 10q23.3, is mutated at a considerable frequency in brain, breast, and prostate cancer[32,33]. Mutations and deletions of *MMAC1* at chromosome 10q23.3 were observed in multiple advanced cancers, such as glioma, prostate, kidney and breast cancers[34]. Mice lacking *Mxi1 (Mad)* (10q24-q25) exhibit increased susceptibility to tumorigenesis either following a carcinogen treatment, or when also missing Ink4a[36]. Human chromosome 11q13 contains the multiple endocrine neoplasia type-1 (*MEN1*) gene, which is frequently mutated in familial *MEN1* tumors and some sporadic endocrine tumors[37]. The LOH frequency on chromosome 19 was low in F_1 mice containing MSM-derived genetic background (Fig. 4). The reason is unknown.

ACKNOWLEDGEMENTS

We are grateful to Mr. M. Ikeda and Mr. U. Ujihara for their skillful animal care. This work was supported in part by a grant-in-aid from the Japan Atomic Energy Research Institute under contract to the Nuclear Safety Research Association, and a grant-in-aid for Scientific Research (No. 13480172) from the Ministry of Education, Science and Culture of Japan.

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Received on December 10, 2001
1st. Revision on February 13, 2002
2nd. Revision on March 29, 2002
Accepted on April 15, 2002