Putative Tumor-suppressor Gene Regions Responsible for Radiation Lymphomagenesis in F1 Mice with Different p53 Status

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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 F1 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 F1 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 induced in radiation-induced lymphomas from (STS/A X MSM/Ms)F1 mice and (STS/A X MSM/Ms)F1-p53KO/+ 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)F1-p53KO/+ 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 lymphomagenesis might considerably differ due to the p53 status.

INTRODUCTION

Whole-body fractionated irradiation effectively induces thymic lymphomas in mice1). 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 animals3,4). 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-
genesis. Analyses of allelotype in radiation-induced
tumors from F1 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-sup-
pressor gene regions.

Extensive allelotype analyses have substantially
furthered our understanding of the molecular mecha-
nism of radiation lymphomagenesis. Santos et al.
(1996) suggested the existence of two tumor-suppres-
sor 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) F1 mice.6
They also mapped a third region (TLSR3) within the
area defined by Mom-1 and D4Mit68, and found two
additional tumor-suppressor gene loci at the proximal-
mid part of mouse chromosome 4 in T-cell lympho-
mas, 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)F1 [ (C x M)F1] 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
neuroblastomas10, advanced colorectal carcinomas11,
bladder cancers12, ovarian carcinomas13,14, endome-
trial carcinomas15, and renal cell tumors16. Physical
delineation of this region has shown that a putative
tumor-suppressor gene exists within a 35 kb interval17
and close to D12Mit233.18 The Ikaros gene on cen-
tromorphic 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)F1 [ (C x S)F1] 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 mecha-
nism.

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

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**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. Allele loss frequency of genome-wide 81 polymorphic microsatellite loci in 24 radiation-induced lymphomas from (STS/A X MSM/Ms) F_{1-p53KO/+} mice.

| Chr | Loci      | Position | LOH (%) | Chr | Loci      | Position | LOH (%) |
|-----|-----------|----------|---------|-----|-----------|----------|---------|
| Chr.1 | D1Mit1    | 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      |       |           |          |         |

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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/
Ms mice to generate (S x M) F1 and (S x M) F1-p53KO/+ offspring. The conditions for breeding were described previously.

**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.

**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. 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) F1-p53KO/+ mice**

A LOH analysis was first carried out for 24 thymic lymphomas from (S X M)F1-p53KO/+ 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 Znfn1al (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).

| Chr  | 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       |
| Chr.20| D19Mit123| 51    | 0       |
Examination of LOH in thymic lymphomas from (S X M) F1-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) F1-p53KO/+ mouse lymphomas (Table 2 and Fig. 3). LOH was observed less frequently in lymphomas from (S X M) F1-p53+/+ mice than from (S X M) F1-p53KO/+ 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 p53KO/+ mice (Table 1, 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 p53KO/+ 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) F1 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) F1 mice rather increased similar to that (2/24: 8%) in the lymphomas of (C X S) F1 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) F1-p53KO/+ mice and (S X M) F1-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

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**Fig. 3.** Genome-wide search for LOH in 20 radiation-induced thymic lymphomas from (STS/A X MSM/Ms)F1-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).
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)F1-p53KO/+ 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 F1-p53+/+ mice with different parental backgrounds

The frequency of LOH on chromosomes 4, 12 and 19 in (S X M)F1 hybrid mice (Table 2, Fig. 3) was compared with data described previously (Fig. 4). Allelic loss on these three chromosomes in (C X S)F1 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)F1 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 F1 hybrid mice, they were located quite near to each other. The frequency on chromosome 4 in (S X M)F1 and (C X S)F1 hybrid mice, one of the parents of which was STS/A, was high compared with that in (C X M)F1 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)F1 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)F1 hybrid mice was markedly high compared with that in (S X M)F1, and in (C X M)F1 mice one of the parents of which was MSM/Ms.

### 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) F1 mice with p53KO/+ or p53+/+.

| Chr | Loci (cM) | 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 χ² 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) F1-p53KO/+ mice.
**** P<0.001 compared to STS/A allele loss in lymphomas from (STS/A X MSM/Ms) F1-p53KO/+ mice.
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. 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 F1 hybrid mice.

The two hybrids, (S X M) F1-p53+/+ and (S X M) F1-p53KO/+, used in this study, differed considerably in the latent period of lymphoma development. X-irradiated female (S X M) F1-p53KO/+ 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) F1-p53+/+ 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 p53KO/+ mice. Thus, p53 heterozygous deficiency shortened the latent period of tumor development. The shortening of this period in (S x M)F1-p53KO/+ 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)F1-p53KO/+ 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. We previously found STS/A-specific preferential allelic loss on chromosome 4 in (CXS) F1 where the lymphoma resistance locus has been suggested to exist by analyzing CXS recombinant inbred strains. A susceptibility locus for the lymphomagenesis was recently reported using the same BALB/cHeA and STS/A mice. An analysis of the underlying genes for susceptibility to ionizing radiation is relevant for radiation oncology. 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)F1-p53-KO/+ mice, no bias in the loss of alleles was found at this locus. In (C x M)F1-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 allele9). 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, respectively30). 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 neuroblastomas10), advanced colorectal carcinomas11), bladder cancers12), ovarian carcinomas13,14), renal cell tumors16) and endometrial carcinomas15). 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 mice31). 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)F1-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/MMAC132–35) and MXI-136). PTEN, a protein tyrosine phosphatase gene, of human chromosome 10q23.3, is mutated at a considerable frequency in brain, breast, and prostate cancer32,33). Mutations and deletions of MMAC1 at chromosome 10q23.3 were observed in multiple advanced cancers, such as glioma, prostate, kidney and breast cancers34). Mice lacking Mxi1 (Mad) (10q24-q25) exhibit increased susceptibility to tumorigenesis either following a carcinogen treatment, or when also missing Ink4a36). Human chromosome 11q13 contains the multiple endocrine neoplasia type-1 (MEN1) gene, which is frequently mutated in familial MEN1 tumors and some sporadic endocrine tumors37). The LOH frequency on chromosome 19 was low in F1 mice containing MSM-derived genetic background (Fig. 4). The reason is unknown.

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