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Allelic Loss Mapping and Physical Delineation of a Region Harboring a Thymic Lymphoma Suppressor Gene on Mouse Chromosome 16

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Our previous mapping of allelic loss in γ-ray induced thymic lymphomas in F_1 hybrid and backcross mice between BALB/c and MSM strains identified three regions with high frequencies of allelic loss which probably harbor a tumor suppressor gene. One region, Tlsr7, exists near the *D16 Mit122* locus on chromosome 16. This study has further localized Tlsr7 by constructing a physical map and scanning a total of 587 thymic lymphomas. The map consists of 13 overlapping BAC clones and isolation of BAC-derived polymorphic probes leads to fine mapping of allelic losses. Eleven lymphomas show informative breakpoints of allelic loss regions relative to the flanking markers on the map. Pulsed-field gel electrophoresis of *Not*I digests of the clones shows that the commonly lost region is localized within an approximately 300 kb interval near *D16Mit192*. This map is invaluable to facilitate the identification of genes in the Tlsr7 region. © 2001 Academic Press

Key Words: **tumor suppressor gene; physical mapping; allelic loss (or LOH) analysis; γ-ray induced thymic lymphoma.**

Identification of novel tumor suppressor genes is important to understand the molecular mechanism of carcinogenesis. Allelic losses in a genomic location in multiple tumor specimens has been interpreted as the presence of a tumor suppressor gene in the region (1–3). Mouse genetic systems offer a number of useful features for such allelic loss mapping, because thousands of polymorphic markers are available and genetically uniform mice can be used for breeding that provides an essentially unlimited number of tumors (4, 5). Accordingly, several extensive analyses of tumors in F_1 hybrid mice have been reported but none of them achieve physical mapping of allelic loss regions (5–8).

We previously performed allelic loss analysis for mouse thymic lymphomas that were induced by γ-irradiation in F_1 hybrid mice and N_2 backcross mice between BALB/c and MSM strains (9). Among 62 microsatellite loci examined, three loci, *D11Mit71*, *D12Mit181*, and *D16Mit122*, exhibited high frequencies of allelic loss, 40, 65, and 45%, respectively. Further allelic loss mapping of the *D11Mit71* region on chromosome 11 and subsequent positional candidate approach identified the *Ikaros* gene as a tumor suppressor gene in the development of thymic lymphomas (10). On the other hand, neither of the other two regions provided any candidates on databases. Therefore, the construction of physical maps is required for further analysis. As for the candidate region (Tlsr4) on chromosome 12, a physical map consisting of 15 BAC clones covering Tlsr4 has been made which localizes the most frequently deleted span within one BAC clone (11).

In this paper we report the construction of a physical map of the candidate region on chromosome 16 (Tlsr7). The map consists of 13 BAC clones covering an entire Tlsr7 region which contains informative breakpoints of the allelic loss region relative to the flanking markers. This physical map is indispensable for the isolation of genes in the Tlsr7 region and the subsequent identification of the tumor suppressor gene.

MATERIALS AND METHODS

Lymphomas and mice. Lymphomas were induced by fractionated doses of γ-ray irradiation, 2.5 Gy four times at a week interval, when mice were at the age of 4 weeks (9). A total of the 587 thymic

Abbreviations used: YAC, yeast artificial chromosome; BAC, bacteria artificial chromosome.

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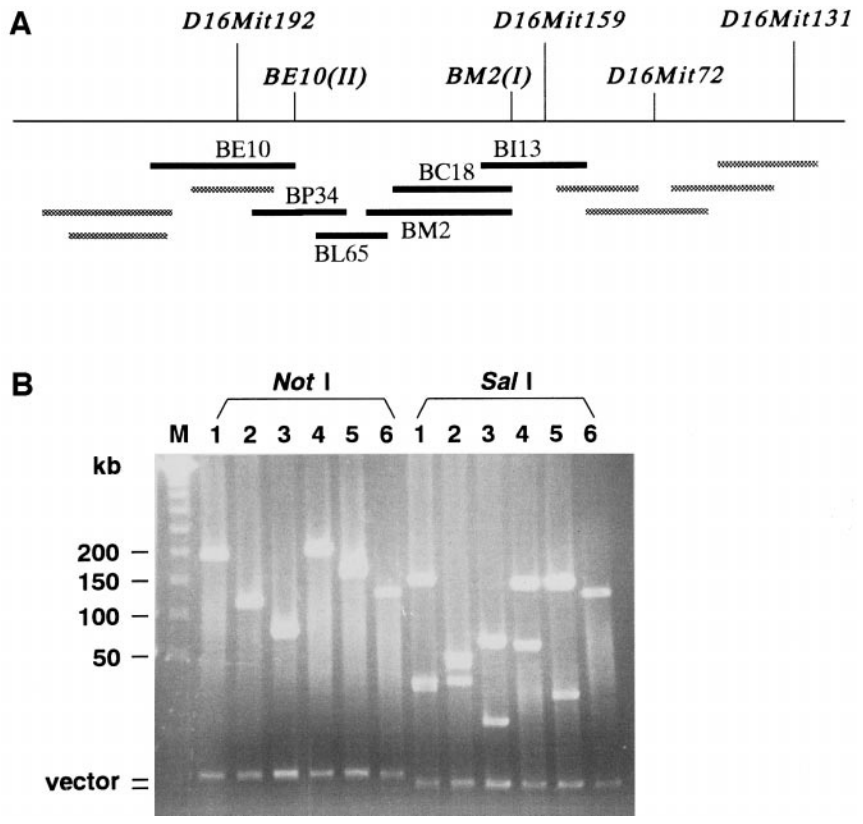

FIG. 1. Physical mapping of the Tlsr7 locus. (A) A long line marks the Tlsr7 region and the position of Mit markers and BAC-end markers is indicated above the line. Bold lines below the long line represent locations of BAC clones. (B) Gel staining pattern of *Not*I and *Sal*I digests. Six BAC DNAs marked by bold lines in (A) were digested with *Not*I and subjected to pulsed-field gel electrophoresis: 1, BE10; 2, BP34; 3, BL65; 4, BM2; 5, BC18; 6 BI13. The marker lane contained a 50 kb fragment ladder.

lymphomas were used for allelic loss analysis, 258 lymphomas of which were previously obtained from F_1 hybrid and N_2 backcross mice between BALB/c and MSM (9). In this study additional 329 lymphomas were generated from F_1 hybrid mice of the two strains, 161 of which carried a *p53*-deficient allele and the other 168 were of p53-wild-type. Presence of a *p53*-deficient allele in mice was determined using primers for *p53* and *neo* genes (9). MSM is an inbred strain derived from Japanese wild mice, *Mus musculus molossinus* (12).

PCR analysis. Genomic DNA was extracted from normal brain and thymic lymphoma, and polymerase chain reaction (PCR) was carried out by standard protocols. Aliquots of 5 μl of product were separated by electrophoresis on 8% polyacrylamide gel or on 4% NuSieve-agarose gel. Microsatellite markers studied include those reported by others (13). Other primer sequences are as follows: *BE10(II),* 5′-CTTGCTGTGAAGTGAGTTCC and 5′-TTGTGTGTACATGATGTGGG; *BM2(I),* 5′-CTGGAATACCTTGA-GTCTAGG and 5′-ACCTACTATGCCTGTCCAGC.

Isolation of BACs and analysis of their linkage. BAC clones were isolated by PCR screening of BAC libraries. The libraries were purchased from Research Genetics, Inc. The size of cloned DNA was determined by pulsed-field gel electrophoresis. Each end of BAC inserts was directly sequenced and used for synthesis of PCR primers.

Pulsed-field gel electrophoresis of DNA. DNA was prepared in agarose plugs as described (14). The plugs were digested with a restriction enzyme, *Not*I, under the manufacturer's recommended conditions, and subjected to electrophoresis using a BioRad Sheff Mapper apparatus.

RESULTS

We previously performed a genome-wide allelic loss analysis for 258 thymic lymphomas that were induced by γ-ray irradiation in F_1 hybrid and N_2 backcross mice between BALB/c and MSM strains (9). This analysis assigned the Tlsr7 (previously called TLSR16a) region exhibiting frequent allelic loss to an interval between *D16Mit9* and *D16Mit183* on mouse chromosome 16. However, this interval was not narrow enough to construct a physical map which was the first step toward positional cloning. Hence, we obtained additional 329 lymphomas from F_1 mice between the BALB/c and MSM strains, 161 lymphomas of which were derived from *p53*(KO/+) mice and the other 168 from *p53*-wild-type mice. These lymphoma samples were subjected to allelic loss analysis using five Mit markers near Tlsr7: *D16Mit9* (4.4 cM from the centromere in data base), *D16Mit192* (4.4 cM), *D16Mit72* (7.7 cM), *D16Mit122* (6.6 cM), and *D16Mit183* (7.7 cM). Results of this rough mapping revealed that *D16Mit192* showed an allelic loss frequency higher than the other four markers did (data not shown).

Database search informed us that one YAC (281-F-11) contains sequences of the centromeric four markers

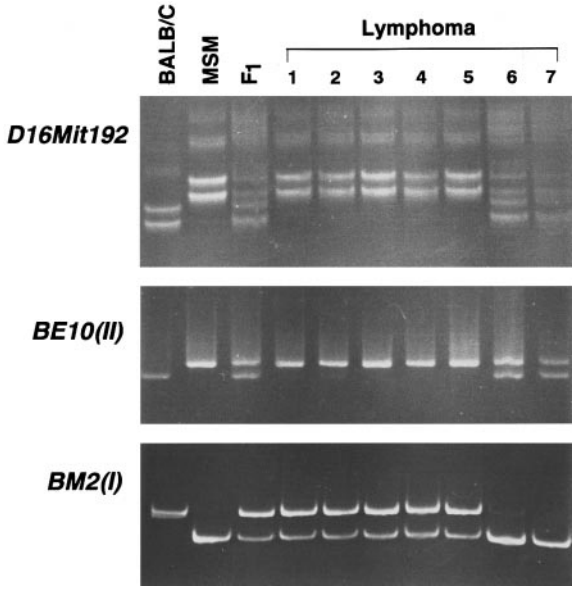

FIG. 2. Allelic loss analysis of lymphomas with *D16Mit192, BE10(II)* and *BM2(I)* markers in the vicinity of the Tlsr7 locus. The first three lanes on panels represent control DNA samples of BALB/c, MSM, and F_1 mice. The other lanes display lymphoma samples of individuals (arbitrary numbers indicated above the lanes).

(*D16Mit9, D16Mit192, D16Mit72,* and *D16Mit122*), and analysis of DNA of the YAC confirmed that information. The YAC DNA also comprised sequences of *D16Mit160* and *D16Mit159.* This indicated that the six marker loci described above were physically linked on a single YAC. Using these markers we isolated BAC clones, and their flanking clones were further obtained with sequence-tagged sites (STSs) of centromeric and telomeric ends of the BAC DNAs. This procedure led to construction of a contig of 13 BACs (Fig. 1A). Figure 1B shows pulsed-field gel electrophoresis (PFGE) of *Not*I and *Sal*I DNA digests of six clones. Only one DNA fragment ranging from 70 to 200 kb was seen in each of the *Not*I digests, indicating no *Not*I recognition site present in the region between *D16Mit192* and *D16Mit159.* Polymorphisms within the region were searched by sequencing DNA of the two strains, which were required for further allelic loss mapping. Accordingly, two polymorphic STSs of *BE10(II)* and *BM2 (I)* were found and used for positioning the critical region of allelic losses.

Allelic loss was examined for the 587 lymphomas. Fig. 2 shows gel electrophoretic patterns of *D16Mit192, BE10(II),* and *BM2(I).* Differences between the BALB/c and MSM alleles were clearly seen. The frequency of allelic loss was 198/587 (33.7%) at the *BE10(II)* locus, higher than that of the other loci. Figure 3 summarizes results. A total of 389 lymphomas retained both alleles and 187 showed allelic loss of the entire Tlsr7 region. Eleven lymphomas lost one allele at one, two, or several of the ten loci examined, and therefore these samples were useful to localize Tlsr7. Comparison of the extent of allelic loss in the ten polymorphic sites revealed boundaries of allelic losses relative to the flanking loci (Fig. 3). The result indicated that the interval between *BE10(II)* and *BM2(I)* was the only site to be consistently lost in these lymphomas. Therefore, this interval, an approximately 300 kb in length from the PFGE analysis, was the candidate for Tlsr7.

Table 1 summarizes allelic loss frequency at the *BE10(II)* locus in lymphomas that were induced in *p53*(KO/+) and *p53*-wild-type mice. The loss frequency was 51.9% in lymphomas from p53(KO/+) mice, whereas it was only 9.5% in lymphomas from the *p53*-wild-type mice. This difference ($P < 0.0001$) suggests an association between the presence of *p53*-deficient allele in mice and allelic loss around the Tlsr7 locus in lymphoma. On the other hand, no allele preference was observed in such losses.

DISCUSSION

This paper presents construction of a physical map consisting of 13 BACs in the vicinity of Tlsr7 which probably harbors a tumor suppressor gene involved in γ-ray induced mouse thymic lymphomas. Among the 587 lymphomas examined, the *BE10(II)* locus shows the peak of allelic loss (33.7%) on mouse chromosome 16 and an interval between the *BE10(II)* and *BM2(I)* is an only site showing allelic loss in all of the informative

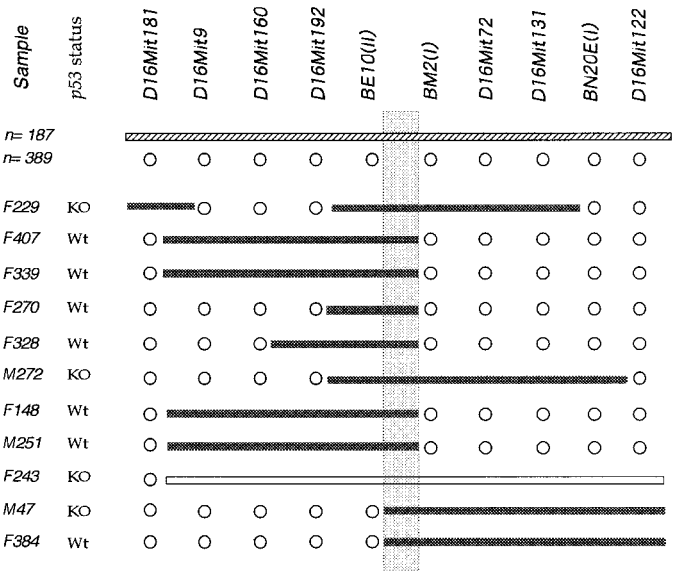

FIG. 3. Allelic loss mapping of the Tlsr7 region in thymic lymphomas induced by γ-irradiation. Open and solid bars represent allelic loss of BALB/c allele and MSM allele, respectively. Circles indicate both alleles retained. The number of lymphomas showing allelic loss of all markers in this region is 389 (shown by a striped bar) and the number of lymphomas retaining both alleles is 187. The *p53* genotype of mice bearing thymic lymphoma is shown in the second column. The commonly lost region is indicated by a vertical rectangle.

TABLE 1

Allelic Loss Frequency at the *BE10(II)* Locus in Lymphomas

Genotype[a] of mice	Number of lymphomas	Both alleles retained	C-allele loss	M-allele loss	Frequency of allelic loss
p53(KO/+)	335	161	90	84	51.9%
p53-wild-type	252	228	14	10	9.5%

[a] *p53* genotype of mice is shown which developed thymic lymphomas.

lymphomas (Fig. 3). PFGE analysis shows that the interval is an approximately 300 kb long (Fig. 1B). Database search revealed that an end sequence of a BAC comprising *D16Mit72* has homology (86%) to a region of the human BAC (Accession No. AC006075) which is assigned to human chromosome 16q13. This indicates that Tlsr7 is syntenic to the human 16q13 region, consistent with a syntenic map on data base. A human gene of *ataxin-2* binding protein 1 (15) is located by the search in the vicinity of Tlsr7 on human 16q13. However, we failed to find any candidates for tumor suppressor gene. The *scid* gene encoding a catalytic subunit of DNA-dependent protein kinase is mapped near Tlsr7. However, our previous study demonstrated that it is not the candidate (16).

Several human tumors including hepatocellular carcinomas (17), thyroid tumors (18), pancreatic cancer (19), and neoplasms of the breast (20) showed allelic losses at this region. Therefore, a human homolog of the candidate tumor suppressor gene in Tlsr7 could be involved in the development of some of those human tumors. Although the types of mouse and human tumors are different, this implication is sound because the different spectrum of tumors is observed between human and mouse carrying defects in the same gene (21, 22). There is also a difference in allelic loss frequencies of genes in human and mouse tumors of the same tissue origin (22, 23). These suggest that the physical map presented here is valuable not only for the isolation of genes in the Tlsr7 region but also for the identification of a tumor suppressor gene in the human 16q13 region.

The frequency of allelic loss of Tlsr7 differs between lymphomas induced in the *p53*(KO/+) and *p53*(+/+) mice, suggesting that *p53* deficiency is associated with generation of allelic losses in that region (Table 1). Experiments using *p53*-KO mice revealed that the presence of one *p53*-deficient allele in cells contributes to the development of thymic lymphoma. Since the remaining wild-type allele in lymphomas was lost at a high frequency which resulted in complete impairment of the p53 function (9), we infer that the effect of the *p53*-deficient allele on tumor development is attributed to the loss of *p53* function. It is possible that the elevated mutation frequency of Tlsr7 in lymphomas of the *p53*(KO/+) mice is due to genomic instability conferred by *p53* loss. One of the p53 roles is to ensure that, in response to genotoxic damage, cells arrest in G_1 and attempt to repair their DNA before it is replicated (24–26). Accordingly, p53 loss may well increase the rate of allelic loss of Tlsr7 in lymphomas.

Another possibility of their association is selection of cells with the loss of both *p53* and Tlsr7 during lymphomagenesis. p53 can induce apoptosis in a variety of cell types including mouse thymocytes (27), and hence the loss of p53 function contributes to tumorigenesis by permitting the propagation of premalignant lymphomas initiated by irradiation. Genes regulating cell growth often show such cooperativity with *p53* loss in tumor development. For instance, the *Rb*(+/−) and *p53*(−/−) mice develop a wide range of tumors at earlier ages than mice that are either *Rb*(+/−) or *p53*(−/−) alone (28, 29). Another example is *Ikaros*, a tumor suppressor gene which negatively regulates cell growth (30, 31). Allelic loss of *Ikaros* is also enhanced by loss of p53 in γ-ray induced lymphomas (10). Of interest is that there is no difference in the frequency of allelic loss of Tlsr4 on chromosome 12 between lymphomas induced in the *p53*(KO/+) and *p53*(+/+) mice (9, 11).

It may be noteworthy that lymphomas developed in the *p53*(KO/+) mice, relative to those from the *p53*(+/+) mice, tend to lose a whole or a large region of chromosome 16 (data not shown). The difference in the extent of allelic loss region is shown by that among 24 lymphomas with allelic loss that derived from the *p53*(+/+) mice seven were informative to localize the Tlsr7 interval, while only four in 174 lymphomas with allelic loss that derived from the *p53*(KO/+) mice were informative. This difference ($P < 0.0001$) may be ascribed to chromosomal instability given by *p53* loss.

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