Our previous study found that BALB/c mice are highly susceptible to transplanted SP2/0 tumors, whereas C57BL/6J mice are barely susceptible. This study was to detect genetic modifier loci that would influence the size of transplanted SP2/0 tumors using these two inbred mouse strains and their F2 progenies. A total of $5 \times 10^6$ SP2/0 cells were inoculated subcutaneously in the left hide legs of 208 F2 mice derived from BALB/c and C57BL/6J strains. At the 17th day since inoculation, all mice were killed, the number and weight of transplanted tumors were recorded. A whole genomic scan using 85 microsatellite markers covering all chromosomes of the mouse, and composite interval mapping analysis were conducted in 208 F2 mice. Eight loci, with the percent of the total variance explanation of ≥10% and p value of ≤0.01, were found responsible for tumor formation. They were mapped on Chr1 (D1Mit113, 55 cM and D1Mit407, 52 cM), Chr4 (D4Mit226, 41 cM), Chr9 (D9Mit302, 55 cM), Chr10 (D10Mit264, 42 cM), Chr11 (D11Mit115, 35 cM), Chr14 (D14Mit125, 45 cM) and Chr18 (D18Mit123, 31 cM). Multiple genetic variants affect individual susceptibility to transplanted SP2/0 tumors in mice. Identification of the target loci may be helpful in formation of the haplotype and understanding of the genes responsible for tumor susceptibility or resistance.

“Cancer directly affects at least one-third of the human population.”¹ An individual’s susceptibility to cancer is determined by both environmental exposures and the combination of inherited cancer susceptibility and resistance, that is, modifier genes. However, it is difficult to detect these genetic variants with low penetrance⁴,⁵ in human because of the genetic heterogeneity and variable etiology of carcinogenesis.

Mouse models offer an important approach to investigate tumor modifier genes. The discovery of genetic markers, particularly simple sequence length polymorphisms or microsatellites, greatly simplifies the approach to distinguish the strain variants of alleles at the loci dispersed over whole mouse genome. This technical advance, in turn, enables genetic linkage experiments aimed at whole genome scanning for tumor modifier loci. Essentially, these experiments are based on the cross of two parental strains that differ in their susceptibility to a particular tumor, followed by backcrossing or intercrossing to produce the segregation of strain-specific alleles, including those affecting the phenotypes of interest. Phenotypic and extensive genotypic analyses of the backcross or intercross populations enable the chromosomal mapping of tumor modifier loci.

Tumor modifier loci have more or less relationship with carcinogenesis. That is to say, they are concerned with cancer susceptibility and resistance. However, there is much to learn about relations between polymorphism of modifier loci and their functions. For scrutinizing the genetic predisposition to transplanted tumors in mice, we studied F2 progeny derived from two mouse strains with susceptibility and resistance to transplanted SP2/0 tumors by composite interval mapping analysis to search tumor linkage loci in mouse chromosomes, and related linkage data.

**Materials and Methods**

**Animals.** Inbred mouse strains C57BL/6J (B6) and BALB/c (C) were obtained from Laboratory Animal Center of Nantong University. These two mouse strains and their F1 and F2 progenies were bred in a moisture, light/dark alternation and specific pathogen-free (SPF) system at 22°C. F1 mice were produced by crossing both males and females of the inbred strains, that is, C (female) x B6 (male) and B6 (female) x C (male). F2 progenies were generated by random mating of F1 mice that came from different litters. The age of all mice was six weeks. No spontaneous tumors were observed during up to six months of age in mice of both parental strains and their offspring.

**Cell culture.** SP2/0 cell line was obtained from Shanghai Cell Line Bank of Chinese Academy of Sciences. The cells were cultured in DMEM (Gibco BRL, Gaithersburg, MD, USA), pH 7.4, supplemented with sodium bicarbonate (24 mmol/L), penicillin G (100 μg/mL), streptomycin (100 μg/mL) and 10% fetal bovine serum (FBS, Sangon Co., Shanghai), and were incubated in 100-mm culture dishes at 37°C in a 5% CO₂ air-humidified incubator.

**Tumor formation.** A total of $5 \times 10^6$ SP2/0 cells were inoculated subcutaneously in the left hide legs of the mice of each inbred strain (both 12) and F2 progeny (totally 403: 201 from B6 x C, and 202 from C x B6). The right hide legs of all experimental mice were left...
untreated for negative controls. The mice were observed every 2 days. Phenotype analysis was performed when the largest tumor reached a critical point before ulcer. As we learned from our pretest, the critical time point for killing mice is the seventeenth day since inoculation.

**Tumor confirmation by light microscopy.** To confirm that those masses formed in the left hind legs of the mice were really transplanted tumors, we examined them by light microscopy. The tissue samples were fixed, dehydrated and embedded in paraffin wax; 5-μm sections were prepared, dyed with HE staining, and observed under light microscope.

**Phenotype analysis.** On the 17th day since inoculation, body weight of the mice of each inbred strain and F2 progeny was measured, and then the mice were anesthetized by sodium pentobarbital. The spleens were removed and kept at -70°C for extraction of genomic DNA. For quantitative assessment of subcutaneous transplanted tumors, tumor weight was recorded individually.

**Genotyping analysis by PCR.** For whole genome scanning, 85 microsatellite markers that located on 20 chromosomes (1–19, and X) of the mouse with an average interval length of 16 cM were selected.3 Primers were synthesized by Sangon Co., Shanghai. The markers are distributed over the genome (Fig. 1).

A total of 208 F2 mice (98 from B6 x C, and 110 from C x B6) with phenotypic extremes (that is, with high or low trait scores)4,5 were selected for genotyping. Genomic DNA was extracted from the spleen of each mouse using DNA extraction kit produced by Fermentas Co., (No. K0512, www.fermentas.com). The concentration of DNA samples was adjusted to 50 ng/μL.

Spleen genomic DNA was used as template in polymerase chain reaction (PCR). Each system of PCR contained DNA (3 μL), upstream primer and downstream primer (25 pmol/L of each), 10 x PCR buffer (2.5 μL), MgCl2 (1.5 mmol/L), dATP, dTTP, dGTP, and dCTP (0.2 mmol/L of each), Taq DNA polymerase (MBI) (1 unit). Total volume of each PCR reaction system was 25 μL. Reaction system was pre-denatured at 94°C for 3 min and then entered 2 thermo-cycles: at 94°C for 30 s, T1 for 30 s and 72°C for 30 s for 17 cycles; at 94°C for 30 s, T2 for 30 s and 72°C for 30 s for 17 cycles. Then the reaction system was incubated at 72°C for 5 min. T1 and T2 were annealing temperatures varied according to primers used in PCR. T1 was the higher melting temperature (Tm) in one of the double primers, and T2 was the lower Tm in another primer. Amplified fragments were separated on 1.5% agarose gels with ethidium bromide staining, or on 6% non-denaturing polyacrylamide gels with silver staining. DNA ladder DL2000 (TaKaRa Biotechnology Co., Dalian, China) was used as a standard marker.

**Linkage analysis.** The presence and relative position of potential loci were detected by composite interval mapping using computer package Map Manager QTX20 (http://mapmgr.roswellpark.org/mmQTX.html). The results included likelihood ratio statistics (LRS), percent of the total variance explanation, additive effect, dominance effect and interval mapping curve. Permutation test was performed in F2 mice for 10,000 times.

**Results**

**Tumor multiplicity.** The susceptibility of the mice of parental BALB/c and C57BL/6J strains and F2 progeny to transplanted...
tumors was variable. No C57BL/6J mice developed tumors at day 17 after SP2/0 cells were inoculated. In contrast, all BALB/C mice developed tumors in the left hind legs at the seventeenth day after inoculation. The tumors weighted 0.20–2.17 g, with a mean of 0.87 g. In the 403 F2 mice, 111 (49 from B6 x C, and 62 from C x B6) developed tumors at the 17th day after inoculation. The tumor weighted 0.02–7.30 g, with a mean of 1.27 g. The difference in sex distribution among all the tumor-bearing mice was not significant.

Morphology of transplanted SP2/0 tumors. The morphology of tumor cells in the left hind legs of the mice was accordant to that of SP2/0 cells. With low power lens, we could see that these tumor cells were arranged in nests or clusters separated by connective tissues (Fig. 2A), with obvious bleeding and necrosis (Fig. 2B). Then through further observation with high power lens, we could see nuclear and cellular pleomorphism of these tumor cells (Fig. 2C). Tumor cells were round or ellipse shaped with darkly stained nuclei and relatively little cytoplasm. Arrow 1 points to a tumor giant cell containing multiple nuclei, while arrow 2 points to a tumor cell at pathologic mitotic phase (HE x600).

Genotyping and linkage analysis. Part of genotyping results are shown in Figure 3. Each allele was recorded as B/B, B/C, and C/C (B represents the C57BL/6J allele, and C represents the BALB/c allele). Homozygous type shows one strap, while heterozygous type shows two straps on gels.

In Figure 3, part of genotyping results with 1.5% agarose gel electrophoresis. Amplified fragments from F2 progeny may display 3 types, including homozygous type originates from parental BALB/C strain (B/B), homozygous type originates from parental C57BL/6J strain (C/C), and heterozygous type (B/C). Homozygous type shows one strap, while heterozygous type shows two straps on gels.
chromosomes 1, 4, 9, 10, 11, 14 and 18, respectively (Table 1 and Fig. 4). These loci were considered as major loci.

In addition, 9 weak peaks were also observed near D3Mit120, D3Mit116, D4Mit18, D10Mit198, D13Mit147, D13Mit293, D17Mit16, D18Mit68 and D19Mit68, with p values between 0.01 and 0.05. These loci were considered as minor loci.

**Discussion**

Inoculation of tumor cells seems to pose a serious health hazard to BALB/c mice. Some efficient mechanisms, such as tumor modifier loci, may be involved in tumorigenesis caused by tumor cell inoculation. Therefore, we mapped some genetic loci controlling tumor susceptibility using intercrossing progenies derived from BALB/c x C57BL/6J mice in this study.

A total of 17 genetic modifier loci affecting susceptibility to transplanted SP2/0 tumors were revealed. Of the 17 loci, 8 locate on Chr1 (D1Mit113 and D1Mit407), Chr4 (D4Mit226), Chr9 (D9Mit302), Chr10 (D10Mit264), Chr11 (D11Mit115), Chr14 (D14Mit125) and Chr18 (D18Mit123), with the percent of the total variance explanation of ≥ 10%, p value of ≤ 0.01 and LRS of > 10.0. These major loci are responsible for the formation of transplanted tumors in mice. Genes controlling susceptibility to transplanted tumors in mice should be around these loci.6‑8 In addition, nine weak peak loci were also observed. There may be some minor genes in these regions. These “minor” loci should be confirmed by further investigation in case of “ghost loci” appear in the experiment. Different intercrossing populations in this study showed different quantitative trait loci (QTLs) for tumor susceptibility. There is no satisfactory explanation to this phenomenon yet. It is one of the approaches to avoid losing important QTLs for complex traits mapping by using different populations.

Generally, genetic modifier loci from F2 populations locate in a very large region of a chromosome, vary from 10 cm to 40 cm. The size of these confidence intervals (CIs) is, to a large degree, the consequence of limited numbers of recombination events in small chromosomal regions in F2 populations.9 Therefore, it is difficult to identify candidate genes and clone them from a large region of chromosome. To pinpoint the locations of the loci to smaller intervals, a number of very sophisticated genetic tools for narrow span and further analysis of complex genetic traits are proposed, including recombinant inbred strains, recombinant congenic strains,10,11 intra- or interspecific backcrosses, consomic strain,12 genome-tagged mice,13 transgenics, knockouts, knockins14 and loss of heterozygosity (LOH).12 Using such approaches, a large number of genes or regions of chromosome with resistance or susceptibility to some common cancers, including lung, colon, skin and laryngeal carcinoma, have been mapped.10,15‑18

Inherited determinants of cancer risk remain largely unknown. Mouse models of human cancers can help us to understand the complex trait of these diseases.1 Tumor modifier loci affect tumorigenesis of almost all types of tumors in mice, with some loci acting on the entire tumorigenic process, whereas others acting on specific stages, for example, tumor initiation or tumor growth and progression.19 Using inbred mouse strains and their intercross or backcross offspring derived from susceptible and resistant strains, whole genome scanning can help to map modifier loci on chromosomes.

BALB/c mice are susceptible to a broad spectrum of carcinogens, and are widely used as experimental animal for cancer research.20‑23 Our present study found that SP2/0 cell inoculation would result in transplanted tumor in BALB/c mice after 17 days. It is an ideal model for the study on tumorigenesis of transplanted SP2/0 tumor.

Genetic predisposition to cancer is a complicated multigenic trait. In mice, more than 100 resistant or susceptible loci for cancers have been identified.19 Mice develop cancers that look remarkably similar, in many instances, to human tumors, and in so doing, they acquire mutations in a similar spectrum of protooncogenes.24 It seems that at least some of the loci, containing tumor susceptible or resistant genes that have been mapped using many different mouse models of cancers, will be proven to be relevant to the human situation.

**References**


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**Table 1** **Quantitative trait loci (QTL) for susceptibility to transplanted SP2/0 tumors in F2 mice**

<table>
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<tr>
<th>Mouse</th>
<th>Chr</th>
<th>Microsatellite</th>
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<th>Dominant</th>
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LRS, likelihood ratio statistics.
Figure 4. Linkage map that shows modifier loci affecting susceptibility to transplanted SP2/0 tumors in F2 mice. These modifier loci were mapped on chromosomes 1, 4, 9, 10, 11, 14 and 18, respectively. (A) F2 mice derived from C (female) x B6 (male); (B) F2 mice derived from B6 (female) x C (male).
Genetic modifier loci of transplanted SP2/0 tumors in mice


