Advances in Brief

Genetic Mapping of Lung Cancer Modifier Loci Specifically Affecting Tumor Initiation and Progression

Giacomo Manenti, Manuela Gariboldi, Antonio Fiorino, Nicola Zanesi, Marco A. Pierotti, and Tommaso A. Dragani

Division of Experimental Oncology A, Istituto Nazionale per lo Studio e la Cura dei Tumori, 20133 Milan, Italy

Abstract

Mouse inbred strains with inherited predisposition and resistance to lung cancer provide a tool for the dissection of the complex genetics of this disease. In the present report, we have crossed the BALB/c with the SWR/J strain and performed whole-genome scanning for loci affecting lung tumor development in their F2 progeny. Both parental strains carry the pulmonary adenoma susceptibility 1 (Pasi) locus, a major locus affecting predisposition to lung cancer in mice. On distal chromosome 18 and on centromere of chromosome 6, we have mapped two pulmonary adenoma resistance loci (Par2 and Par4, respectively), which reduce lung tumor multiplicity strongly, up to 15-fold. Par2 and Par4, however, do not affect lung tumor size, which is instead controlled by an additional locus that we have mapped on the central region of chromosome 4. We designated this locus as “pulmonary adenoma progression 1” (Papgl), because it specifically modifies lung tumor size but not multiplicity. The present results, therefore, provide evidence for the existence of cancer modifier loci acting on specific stages of lung tumorigenesis.

Introduction

In the last few years, progress in the genetics of an inherited predisposition to lung cancer in mice demonstrated that a major locus (Pasi) located on the distal region of chromosome 6 affects susceptibility to lung tumorigenesis (1). Other minor loci may also contribute to the susceptibility trait (2). Recently, we have reported the mapping of a Mus spretus-derived locus that strongly inhibits the lung tumorigenesis in Pasi/+ mice (3). This locus, pulmonary adenoma resistance 1 (Par1), maps on mouse chromosome 11, near the Rara locus (3). These findings provided the first evidence for the existence of a lung tumor resistance locus inhibiting the expression of an inherited predisposition to lung cancer.

To further define the genetics of predisposition and resistance to lung cancer in mice, we have considered the BALB/c strain, which shows an intermediate susceptibility that is dominant over the high susceptibility of the A/J and SWR strains (4). Given that all three strains carry the Pasi/+ allele (5), the BALB/c mouse must carry lung tumor modifier loci that decrease expression of high susceptibility to lung tumorigenesis. Therefore, a cross between the BALB/c and one of the most susceptible strains would provide a clue to map lung cancer modifier loci in a common genetic background for Pasi. Accordingly, in a cross between the BALB/c and the SWR/J mice, here we report the mapping of BALB/c-derived pulmonary adenoma resistance (Par2 and Par4) loci, on chromosomes 18 and 6. Our findings are in agreement with a recent report mapping the same Par2 locus on the distal region of chromosome 18 (6). Both loci specifically affect lung tumor multiplicity. In the same cross, we have also mapped, on chromosome 4, a locus designated as Papgl specifically affecting the size of lung tumors.

Materials and Methods

Animals and Phenotype. Male and female SWR/J (W) and BALB/c (C) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were then bred in our laboratory to obtain CWF2 mice, which are SWR/J × BALB/c progeny. CWF2 males and females were killed at 40 weeks of age. The number of tumors on the surface of the lungs were counted after tissue fixation, and the diameter of each nodule was measured. Susceptibility of each mouse to lung tumor development was estimated using quantitative parameters, as described (7).

Genetic Markers and Linkage Analysis. Primers for simple sequence length polymorphism markers were obtained from Research Genetics (Huntsville, AL). Forty mice showing extreme phenotypes were genotyped with 231 markers covering all autosomes and chromosome X. Markers located on chromosomes showing putative linkage were typed in the whole CWF2 population (218 mice). Genetic linkage maps and linkage between parameters estimating lung cancer predisposition trait and genetic markers were determined as reported (3, 8, 9). Because the susceptible phenotype showed a nonnormal distribution, the linkage methods were applied to rank-transformed data (10), although the phenotypic values reported in Table 1 refer to untransformed data.

Results

Loci Specifically Affecting Tumor Multiplicity. Whole-genome scanning of 40 extreme-phenotype mice with 231 markers showed significant linkage of a chromosome 18 region with lung tumor multiplicity (N). The lod score curve peaked at the D18Mit9 locus at 42 cM (Ref. 11; Fig. 1). The linkage between the D18Mit9 region and N was supported by a lod score of 7.9. Mean lung tumor volume (V) showed only a marginal linkage at the same locus (lod score of 2.1). ANOVA confirmed the linkage (P < 0.0001) between lung tumor multiplicity and D18Mit9, which explained ~15% of the total phenotypic variance (Table 1). Values of lung tumor multiplicity by genotypic class at D18Mit9 indicate that BALB/c mice carry the resistance allele (Par2/+), and SWR/J mice carry the susceptibility allele (Par2/−). Resistance to multiplicity of lung cancer by Par2 was partially dominant, because heterozygous CWF2 animals showed an N value lower than the expected intermediate value for genetic effect of additive alleles but higher than that of the homozygous mice for the BALB/c allele (Table 1). The use of an intercross as compared to a backcross population had the advantage of allowing analysis of locus inheritance mode (i.e., partially dominant). The weak effect of Par2 on lung tumor size (V) behaved as a recessive trait, because only mice homozygous for the BALB/c allele showed a reduced V value, and heterozygous animals were not affected (Table 1).

Therefore, Obata et al. (6) and we have identified another locus (Par2) providing inherited resistance to lung tumorigenesis. However, in our CWF2 cross, Par2 accounts for about 15% of the phenotypic variance, whereas in the (A/J × BALB/c) × A/J backcross used by
Fig. 1. Genetic linkage mapping of the pulmonary adenoma progression 1 (Papgl) locus and of the pulmonary adenoma resistance loci Par4 and Par2 on mouse chromosomes (Chr.) 4, 6, and 18, respectively. The genetic linkage panel was constituted by 218 (BALB/c X SWR/J) F2 male and female mice treated with urethane. Lod score plots for lung tumor multiplicity (N) and for lung tumor volume (V) are shown to the left of the chromosomes. The genetic distances from the centromere are shown to the right of the chromosomes and are expressed in terms of Haldane’s mapping function. Distances in cM are calculated from the most centromeric marker taken as an anchor locus.

Table 1 Lung tumor multiplicity (N) and size (V) by genotypic class at D18Mit9, D6Mit50, and D4Mit219 loci in the CWF2 mice treated with urethane.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>V</th>
<th>R² (%)</th>
<th>P</th>
<th>Lod score</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D18Mit9 (Par2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>6.1 ± 0.8 (66)</td>
<td>9.3 ± 0.8 (96)</td>
<td>16.2 ± 2.2 (55)</td>
<td>15.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V</td>
<td>1.9 ± 0.6 (66)</td>
<td>3.9 ± 1.2 (96)</td>
<td>3.0 ± 1.2 (55)</td>
<td>4.3</td>
<td>0.0095</td>
</tr>
<tr>
<td>D6Mit50 (Par4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>6.2 ± 0.8 (51)</td>
<td>7.2 ± 0.6 (107)</td>
<td>21.1 ± 2.4 (48)</td>
<td>24.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V</td>
<td>7.1 ± 2.5 (51)</td>
<td>2.1 ± 0.5 (107)</td>
<td>1.5 ± 0.3 (48)</td>
<td>1.8</td>
<td>0.15</td>
</tr>
<tr>
<td>D4Mit219 (Papgl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>11.5 ± 1.6 (62)</td>
<td>9.6 ± 0.9 (101)</td>
<td>9.3 ± 1.7 (55)</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>V</td>
<td>6.1 ± 1.5 (62)</td>
<td>2.5 ± 1.0 (101)</td>
<td>0.6 ± 0.1 (55)</td>
<td>21.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Obata et al. (6), variance explained by Par2 is ~40% (6). The difference may be either due to the cross or to the different periods of observation after initiation, i.e., 36 weeks (this report) versus 17 weeks (6).

On chromosome 6, near the centromere, we have mapped another locus linked to lung tumor multiplicity. Again, the BALB/c allele is associated with a decreased phenotype expression. The mapping was supported by a lod score of 13, and the locus accounted for ~25% of the phenotypic variance of N. We named this locus Par4, because three other lung tumor resistance loci have already been mapped (3, 6, 12). Par4 showed a stronger effect than the Par2 locus, and it specifically affected lung tumor multiplicity, because no significant linkage was found with V (Table 1). Resistance provided by the Par4 locus was dominant, because heterozygous animals showed the same phenotypical values of the mice homozygous for the BALB/c allele. Par2 and Par4 together interact with an additive effect and are associated with an up to 15-fold reduction of the lung tumor multiplicity (i.e., N = 48.5 versus N = 3.2 in animals homozygous for SWR/J and BALB/c alleles, respectively, at both loci).

Mapping of a Locus Associated with Lung Tumor Progression.

Whole-genome scanning of the CWF2 population for loci affecting the size (volume) of lung tumors showed significant linkage with the distal region of chromosome 4. Indeed, linkage of marker locus D4Mit219 (at 49.6 cM) with V was supported by a lod score of 11. Association of the same locus with lung tumor multiplicity was poor (lod score = 0.7). Therefore, we propose for this locus the name “pulmonary adenoma progression 1” (Papgl), because the locus is associated specifically with the growth (size) of lung neoplastic lesions. Papgl explained 21% of the phenotypic variance of V in this cross. Papgl susceptibility allele derived from the BALB/c strain and showed a codominant effect. This is the first locus specifically affecting tumor volume that is detectable as a main effect. Indeed, four loci associated with tumor size that have been mapped recently in recombinant congenic strains on chromosomes other than 4 show their effect only in the case of a specific mutual reciprocal interaction (13).

Discussion

The present findings indicate that lung tumor multiplicity and size may be under different genetic control. Besides the major lung tumor susceptibility locus Pas1, which affects both tumor multiplicity and size due to its putative key role in the lung cancer biology (1), other genetic elements play a more specific part in cancer development. Indeed, the Par2 and Par4 loci act as modifiers of lung tumor multiplicity, providing resistance to lung cancer, whereas the Papgl
locus acts as a modifier of lung tumor volume, stimulating the growth of lung neoplastic lesions.

In our CWF_2 cross, all animals carry the Pasl/+ allele that provides high genetic predisposition to lung cancer. This homozygosity for Pasl simplified the genetic background of the cross and allowed a more sensitive dissection of new cancer modifier loci. Therefore, it is noteworthy that, although the BALB/c mouse carries the Pasl/+ and Papgl/+ alleles stimulating development of lung cancer, this strain is much less susceptible to lung tumorigenesis as compared to other Pasl/+ strains such as the A/J and SWR/J ones. Therefore, the BALB/c-derived Par2 and Par4 alleles are able to strongly modify, reducing by up to 15-fold, genetic predisposition to lung cancer.

The Papgl locus is linked to a relatively wide region of chromosome 4, with high lod scores (>9) spanning from D4Mit15 (42.6 cM) to D4Mit203 (60 cM). In this region, the Cdkn2a (p16) gene maps and losses of heterozygosity have been reported in mouse lung tumors (14, 15), as well as in human lung cancer, in the homologous 9p21 region (16–18). The chromosomal region where Papgl maps also contains the Lmyc oncogene. The possible involvement of Lmyc is intriguing because, in agreement with our findings, genetic polymorphisms of the LMYC gene have been found to be associated with lung cancer prognosis in the Japanese and Chinese populations, although this is not confirmed in Caucasians (19–21). The present findings may suggest that the same locus (Papgl) affects lung tumor progression in both humans and mice.

In humans, individuals with cancer of the same histotype and of the same clinical stage often show a wide variability in their prognosis. Up to now, it has been thought that different somatic changes in tumors of the same stage are responsible for their different outcomes. Our study indicates that tumor progression may be determined by the genetic constitution. Accordingly, we have recently reported an association between the progression of lung cancer and genetic polymorphisms in the 12p12 chromosomal region containing the putative human PAS1 gene (22). In agreement with our findings, genetic loci associated with development of benign and malignant lesions have been reported in mouse skin tumorigenesis (23). This would open a new field of research on tumor progression and prognosis.

References

Genetic Mapping of Lung Cancer Modifier Loci Specifically Affecting Tumor Initiation and Progression

Giacomo Manenti, Manuela Gariboldi, Antonio Fiorino, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/57/19/4164

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.