Susceptibility to astrocytoma in mice mutant for *Nf1* and *Trp53* is linked to chromosome 11 and subject to epigenetic effects

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Astrocytoma is the most common malignant brain tumor in humans. Loss of the p53 signaling pathway and up-regulation of the ras signaling pathway are common during tumor progression. We have shown previously that mice mutant for *Trp53* and *Nf1* develop astrocytoma, progressing to glioblastoma, on a C57BL/6J strain background. In contrast, here we present data that mice mutant for *Trp53* and *Nf1* on a 129S4/SvJae background are highly resistant to developing astrocytoma. Through analysis of F_1 progeny, we demonstrate that susceptibility to astrocytoma is linked to chromosome 11, and that the modifier gene(s) responsible for differences in susceptibility is closely linked to *Nf1* and *Trp53*. Furthermore, this modifier of astrocytoma susceptibility is itself epigenetically modified. These data demonstrate that epigenetic effects can have a strong effect on whether cancer develops in the context of mutant ras signaling and mutant p53, and that this mouse model of astrocytoma can be used to identify modifier phenotypes with complex inheritance patterns that would be unidentifiable in humans. Because analysis of gene function in the mouse is often performed on a mixed C57BL/6,129 strain background, these data also provide a powerful example of the potential of these strains to mask interesting gene functions.

Astrocytoma is a characteristically diffuse tumor of the central nervous system (CNS). Because of its diffuse infiltration, it often cannot be completely resected, leading to a very poor prognosis for patients. Astrocytoma, together with glioblastoma (the highest grade of astrocytoma), accounts for more than three-quarters of all gliomas, making it the most common malignant brain tumor (1). The 5-year survival rate for glioblastoma is <3%. A better understanding of the genetic risk factors associated with astrocytoma will give insight into the mechanism of astrocytoma initiation and progression and will lead to better screening methods and new targets for therapy.

Data from human populations pointing to genetic risk factors for astrocytoma are sparse. Malmer *et al.* (2) have examined the increased family risk of developing low- vs. high-grade glioma and favor the view that autosomal recessive genes affect astrocytoma risk, although the role of a common environment in familial risk cannot be excluded. Several familial cancer syndromes show an increased risk for astrocytoma, including neurofibromatosis type 1 (NF1) (3, 4) and Li–Fraumeni syndrome (LFS) (5). NF1 patients have a mutation in the *NF1* gene (6) (*Nf1* in the mouse) and are predisposed to neurofibromas and optic gliomas, with an increased risk for malignant peripheral nerve sheath tumors and diffuse astrocytoma/glioblastoma (3, 4). Studies of NF1 families have demonstrated a role for modifier genes unlinked to *NF1* in the severity of the disease with respect to the numbers of neurofibromas and the presence or absence of optic gliomas (7). Studies of NF1 patients have also shown that patients with optic glioma are more likely to develop CNS tumors such as astrocytoma (8, 9), although this observation is as likely due to the particular mutant allele of *NF1* (10) as to genetic or environmental risk factors. Astrocytomas are also frequently associated with mutations in *Tp53* in sporadic cases (11, 12) or in LFS patients carrying germline mutations in the *Tp53* gene (5) (*Trp53* in the mouse). Although there are no clear data in patients that genetic background affects the risk of particular tumors in LFS patients, data from the mouse show that the strain background can affect the incidence of teratomas and mammary tumors in *Trp53* mutant mice (13, 14). The relatively low penetrance of astrocytoma in the population makes association studies to look at genetic risk factors difficult. The identification of these risk factors is best accomplished in inbred animal models and the results then tested in human populations.

Because of the difficulty of identifying genetic risk factors in humans with astrocytoma, we have used a mouse model of the disease to examine the genetic basis of astrocytoma susceptibility. Although several mouse models now exist that recapitulate the pathology of astrocytoma (15), we have chosen the *Nf1;Trp53cis* (*NPcis*) mutant mouse model, because it can be propagated by a simple breeding strategy and is maintained on an inbred strain background to facilitate the dissection of modifier genetics. In this model, *Nf1* and *Trp53* are mutated on the same chromosome (chr) 11 of the mouse and are tightly linked so that they are inherited as a single mutation in genetic crosses. The mice on a C57BL/6J (B6) inbred strain background develop astrocytomas spontaneously with an average latency of 6 months and show loss of the WT copies of *Nf1* and *Trp53* (16). Because astrocytomas were not observed in the original characterization of *NPcis* mice on a C57BL/6J,129S4/SvJae mixed background (17, 18), we hypothesized that modifier genes affect susceptibility to astrocytoma between the B6 and 129S4/SvJae (129) strains. To address this possibility directly, we have generated this model on an inbred 129 background and examined the genetics of susceptibility to astrocytoma.

Materials and Methods

Generation of *NPcis* Mice on Different Genetic Backgrounds. *NPcis* mice on a B6 background were generated as described (16) and

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Abbreviations: NF1, neurofibromatosis type 1; SSLP, simple sequence length polymorphism; *NPcis*, *Nf1;Trp53cis*; B6, C57BL/6J; 129, 129S4/SvJae; A, A/J; DB, DBA/2J; CB, CBA/J; 129S1, 129S1/SvImJ; 129X1, 129X1/SvJ; SNP, single nucleotide polymorphism; chr, chromosome; WHO, World Health Organization; NCBI, National Center for Biotechnology Information; dbSNP, Single Nucleotide Polymorphism Database.

Data deposition: The SNP data reported in this paper have been deposited in the NCBI Single Nucleotide Polymorphism Database (dbSNP) (dbSNP ID nos. 28476647-28476655; see also Table 4, which is published as supporting information on the PNAS web site).

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maintained by crossing to WT B6 purchased from The Jackson Laboratory. The *NPcis*-B6 mice described here are backcross generation 8–11 onto B6. The *NPcis*-129 mice were generated by crossing *Nf1*+/− 129 mice (19) to *Trp53*+/− 129 mice (20) to generate *Nf1;Trp53trans* 129 mice that were then crossed to WT 129 mice, maintained as a colony at Massachusetts Institute of Technology (MIT) to generate the *NPcis* mice. F_1 progeny between *NPcis*-B6 and A/J (A), DBA/2J (DB), and CBA/J (CB) were generated by crossing *NPcis*-B6 mice to inbred strains purchased from The Jackson Laboratory. *NPcis*-B6 × 129 F_1 progeny were generated from an *NPcis*-B6 male inbred 16 generations onto B6 crossed to WT 129 females (Fig. 3, cross A). *NPcis*-129XB6 F_1 progeny were generated by crossing either male or female *NPcis*-129 mice to WT B6 (Fig. 3, cross B and C). All mice were maintained on a 9% fat diet. Twelve of the mice in this study (all F_1s between B6 and 129) were moved from MIT to National Cancer Institute–Frederick (NCI–Frederick) at ≈6 months of age to complete the aging study. We have not observed any change in phenotype of these strains at NCI–Frederick, so these mice were pooled with the mice aged entirely at MIT. All mice in this study were cared for according to the policies of the Animal Care and Use Committees of MIT and NCI–Frederick.

Genotyping of *NPcis* Mice. Mice were genotyped for *Trp53* mutations by PCR on tail-clip DNA as described (20). Mutations in *Nf1* were assayed by PCR as described (19) or by using the primers Nf1 WT 5′-TTCTGGCCTTATTGGACACC-3′, Nf1 common 5′-GCACAAAAGAGGCACTGGAT-3′, Nf1 mutant 5′-GGAGAGGCTTTTTGCTTCCT-3′, with an annealing temperature of 60°C. We added 0.1% BSA and 1% polyvinylpyrrolidone (catalogue no. PVP-40, Sigma–Aldrich) to improve specificity of the PCR reaction (21). For analysis of simple sequence length polymorphisms (SSLPs), tail DNA was amplified with ResGen MapPairs primer sets (Invitrogen) for 35 cycles and run on a 3% Metaphor agarose (Cambrex, East Rutherford, NJ), 1× Tris-borate-EDTA-buffered gel. The markers used were D11Mit20, D11Mit271, D11Mit140, D11Mit349, D11Mit5, D11Mit341, D11Mit285, and D11Mit258. Chromosomal locations of SSLP markers, *Trp53*, and *Nf1* were taken from National Center for Biotechnology Information (NCBI) Mouse Build 32, version 1 (www.ncbi.nlm.nih.gov/genome/guide/mouse).

Phenotyping of *NPcis* Mice. Mice were aged until tumors developed as described (16) and processed for hematoxylin and eosin-stained sections. The midline sagittal section and a parasagittal section through the eye were examined independently by K.M.R. and by two veterinary neuropathologists, R.T.B. and C.D.S. A consensus diagnosis was determined by K.M.R. based on the World Health Organization (WHO) grading criteria for diffuse astrocytoma (22) and given the prefix GEM to indicate that the tumors arise in genetically engineered mouse models.

Statistical Analysis of Tumor Spectrum. Statistical analysis was performed as described (16). Differences in astrocytoma incidence were compared pairwise between strains by χ^2 test in Microsoft EXCEL X.

Single Nucleotide Polymorphism (SNP) Analysis. Chr 11 SNPs with a B6 genotype were downloaded from the NCBI Single Nucleotide Polymorphism Database (dbSNP) (Build 120) (www.ncbi.nlm.nih.gov/SNP). We selected 597 SNPs located between D11Mit271 (45.4 Mb, NCBI Build 32) and D11Mit285 (82.6 Mb, NCBI Build 32) polymorphic for B6 and 129. PCR primers were designed for 161 DNA fragments containing the 597 SNPs. REVEAL ANALYSIS (Spectrumedix, State College, PA) was used to identify PCR fragments that were polymorphic between B6 and 129. Of the 89 DNA fragments found to be polymorphic or uninterpretable by REVEAL ANALYSIS, 48 fragments were chosen for sequencing. SNPs were confirmed by sequencing in both directions along the DNA strand and genotyped on the B6, 129, A, CB, and DB strains. Sequencing primers used are reported in Table 4. The Celera SNP database (www.celeradiscoverysystems.com) was searched for SNPs between D11Mit271 (44.2 Mb, release R3.6) and D11Mit285 (96.5 Mb, release R3.6). Two search criteria were used: (*i*) 129S1/SvImJ (129S1) ≠ B6 and 129S1 ≠ A and 129S1 ≠ DB and (*ii*) 129X1/SvJ (129X1) ≠ B6 and 129X1 ≠ A and 129X1 ≠ DB. Results were manually checked for genotyping ambiguities. The genotype with the higher count number was taken as the more accurate genotype. Results of the search are reported in Table 5, which is published as supporting information on the PNAS web site.

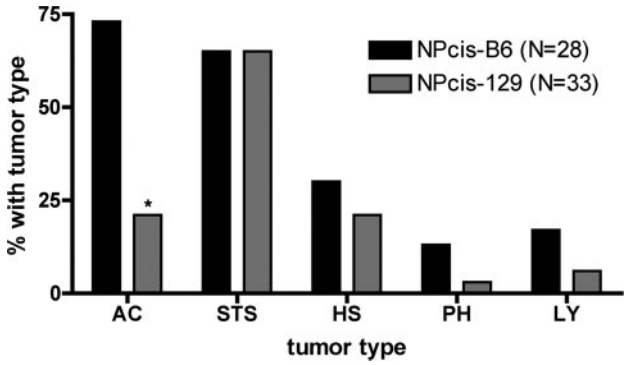

Fig. 1. Tumor spectrum of *NPcis*-B6 and *NPcis*-129 mice. The graph shows the major tumor types observed. The difference in astrocytoma incidence in the two strains is statistically significant ($P = 0.001$, indicated by asterisk). Tumor types are astrocytoma (AC), soft-tissue sarcoma (STS), histiocytic sarcoma (HS), pheochromocytoma (PH), and lymphoma (LY).

Results

To test the difference in susceptibility to astrocytoma between the B6 and 129 strains, we have regenerated the *NPcis* mice on an inbred 129 background by crossing *Nf1*+/− 129 and *Trp53*+/− 129 mice to regenerate the *cis* mutant chr (16–18). *NPcis* mice on a 129 background develop significantly fewer astrocytomas compared with *NPcis*-B6 mice (Fig. 1). Soft-tissue sarcomas were seen frequently on both the B6 and 129 backgrounds, as observed on the B6,129 mixed background (17, 18). We did not observe significant differences in the incidence of other common tumor types, lymphoma, histiocytic sarcoma, or pheochromocytoma. *NPcis*-129 mice developed fewer astrocytomas in the population than *NPcis*-B6 mice, and the astrocytomas were lower grade in *NPcis*-129 mice. On the B6 background, 89% of astrocytomas were classified as GEM WHO III, whereas 29% of astrocytomas on the 129S4/SvJae background were classified as GEM WHO III, with the remainder classified as GEM WHO II (Fig. 2). We have generated cell lines from *NPcis-129* astrocytomas that show loss of the WT copies of *Trp53* and *Nf1* (data not shown).

To determine whether the genes modifying astrocytoma formation in *NPcis* mice act dominantly in the B6 or 129 strain, we generated F_1 progeny between the B6 and 129 strains carrying the *NPcis* mutation. We found upon analyzing these F_1 progeny that susceptibility to astrocytoma does not follow simple Mendelian inheritance, in that some F_1 progeny were resistant to astrocytoma and others were susceptible (Fig. 3, Table 1). We have examined the data to look for epigenetic effects with respect to inheritance of the strain background from the mother or father, inheritance of the *NPcis* mutant chr from the B6 or 129 strain, and inheritance of the *NPcis* mutant chr from the mother or father.

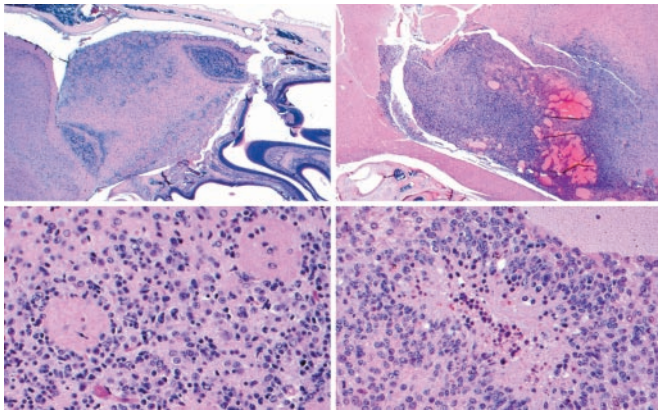

Fig. 2. Histology of astrocytoma in the resistant *NPcis*-129 strain compared with the susceptible F_1 progeny of *NPcis*-129 crossed to WT B6. (*Left*) One of the two GEM WHO III tumors found in *NPcis*-129 mice ($n = 33$). The tumor is confined to a focal area of the ventral olfactory bulb (*Upper Left*). Atypical nuclei diffusely infiltrate the olfactory bulb (*Lower Left*) and mitotic figures are rare (not shown). (*Right*) One of three GEM WHO IV tumors found in F_1 *NPcis*-129XB6 progeny ($n = 22$). This tumor is characterized by infiltrative boundaries (*Upper Right*), a high mitotic index, and pseudopallisading tumor cells around areas of necrosis (*Lower Right*).

Maternal or paternal imprinting of a modifier from B6 or 129 could alter the expression pattern of the modifier gene(s) and affect its ability to act on astrocytoma susceptibility. Similarly, strain differences inherited specifically from the mother, such as mitochondria or other cytoplasmic maternal factors, could potentially affect developmental pathways leading to indirect effects on astrocytoma susceptibility. We examined whether astrocytoma susceptibility changed when B6 alleles were inherited from the mother or father. Table 1 shows that astrocytoma susceptibility is not affected by imprinting of strain-specific genes or by strain-specific maternal factors. Mice that inherit B6 from either the maternal (Fig. 3, cross C) or the paternal side (Fig. 3, crosses A and B) show a similar incidence of astrocytoma. In both cases, F_1 progeny were significantly more susceptible than the *NPcis* 129 parental strain ($P = 0.03$ for B6 coming from the mother, and $P = 0.01$ for B6 coming from the father).

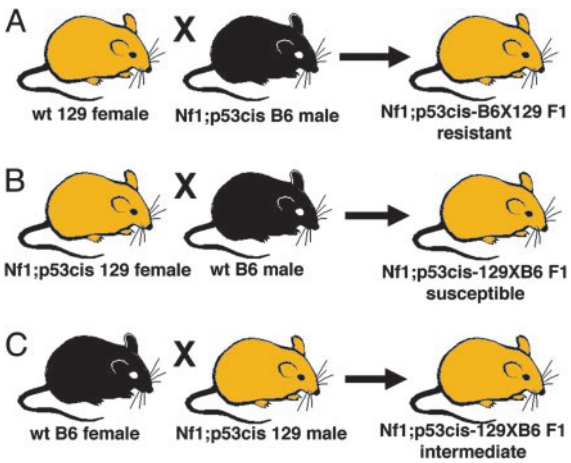

Fig. 3. F_1 crosses between B6 and 129. F_1 progeny were generated in three different crosses. (*A*) An *NPcis*–B6 male was bred to a WT 129 female to give *NPcis*-B6X129 F_1 progeny that were resistant to astrocytoma. (*B*) *NPcis*-129 females were bred to WT B6 males to give *NPcis*-129XB6 F_1 progeny that were susceptible to astrocytoma. (*C*) An *NPcis*-129 male was bred to a WT B6 female to give *NPcis*-129XB6 F_1 progeny that developed an intermediate astrocytoma phenotype.

Table 1. Inheritance of mutant chr 11 affects astrocytoma susceptibility

Cross (Fig. 3)	Cross	*n*	With astrocytoma	Without astrocytoma	*P*, χ^2
A	WT 129 female × *NPcis* B6 male	19	6 (32%)	13 (68%)	
B	*NPcis* 129 female × WT B6 male	22	16 (73%)	6 (27%)	
C	WT B6 female × *NPcis* 129 male	23	12 (52%)	11 (48%)	
A+B	129 female × B6 male	41	22 (54%)	19 (46%)	
C	B6 female × 129 male	23	12 (52%)	11 (48%)	0.9
B+C	*NPcis* 129 × WT B6	45	28 (62%)	17 (38%)	
A	*NPcis* B6 × WT 129	19	6 (32%)	13 (68%)	0.03
A+C	WT female × *NPcis* male	42	18 (43%)	24 (57%)	
B	*NPcis* female × WT male	22	16 (73%)	6 (27%)	0.02

The *Nf1* and *Trp53* mutant chr, or conversely WT chr 11, could affect tumorigenesis differently, depending on whether it is inherited from B6 or 129. For example, particular alleles of *Nf1* or *Trp53* could be less effective at blocking tumorigenesis in the heterozygous state, or the WT chr from one strain might be lost more easily than the other strain to initiate tumorigenesis, as has been shown for strain effects on $APC^{Min/+}$ mice (23). Additionally, genes tightly linked to *Nf1* and *Trp53* on chr 11 might affect tumorigenesis differently when inherited from B6 or 129. We examined the effect of inheriting the mutant chr from either 129 or B6. Table 1 shows that F_1 progeny inheriting the *NPcis* mutant chr from 129 (Fig. 3, crosses B and C) are significantly more

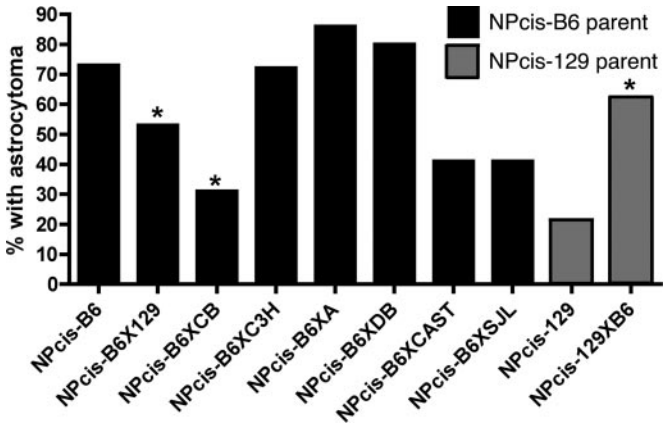

Fig. 4. Astrocytoma incidence in F_1 progeny of different inbred strains. *NPcis*–B6X129 F_1 progeny (cross A, Fig. 3) show reduction in astrocytoma incidence compared to the *NPcis*-B6 parental strain ($P = 0.02$). *NPcis*-129XB6 F_1 progeny (cross B and C, Fig. 3), show increase in astrocytoma incidence compared to the *NPcis*-129 parental strain ($P = 0.0009$). *NPcis*–B6XCB F_1 progeny show reduction in astrocytoma incidence compared to the *NPcis*–B6 parental strain ($P = 0.04$). Previously published data (16) from B6XC3H/HeJ, B6XCAST/EiJ, and B6XSJL/J are shown for comparison. Of the seven strains tested, only 129 and CB show significant resistance to astrocytoma compared to B6. Statistically significant changes relative to the parental strain are indicated by asterisks. NPcis-B6 $n = 28$, NPcis-B6X129 $n = 19$, NPcis-B6XCB $n = 26$, NPcis-B6XC3H $n = 25$, NPcis-B6XA $n = 20$, NPcis-B6XDB $n = 20$, NPcis-B6XCAST $n = 22$, NPcis-B6XSJL $n = 34$, NPcis-129 $n = 33$, and NPcis-129XB6 $n = 45$. (Adapted from ref. 16.)

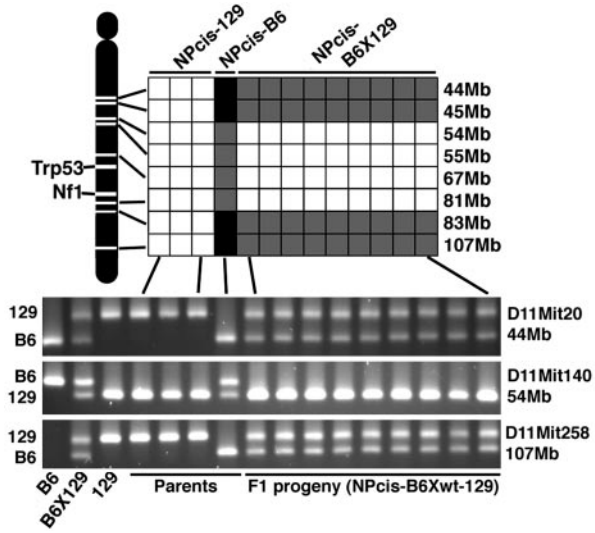

Fig. 5. SSLP characterization of chr 11 in *NPcis* parents and F_1 progeny. SSLP markers spanning the region around *Trp53* and *Nf1* were genotyped for differences between B6 and 129. (*Upper*) A summary of the results indicating homozygous 129 (white), homozygous B6 (black), and heterozygous B6X129 (gray). (*Lower*) Three SSLP examples. *Trp53* is located at 69 Mb and *Nf1* is located at 73 Mb. In the congenic region the *NPcis*-B6 parent is heterozygous for 129 and B6 sequences, and the F_1 progeny are homozygous for 129. Outside the congenic region the *NPcis*-B6 parent is homozygous for B6 sequences, and the F_1 progeny are heterozygous for 129 and B6 sequences. The first three lanes of the gel (*Lower*) are inbred and F_1 control DNA samples to show the size of the SSLP fragments for each strain.

susceptible to developing astrocytoma than F_1 progeny inheriting the mutant chr from the B6 strain (Fig. 3, cross A) ($P = 0.03$). F_1 progeny inheriting the mutant chr from 129 and the WT chr 11 from B6 are significantly more susceptible to astrocytoma than the *NPcis*-129 parental strain (Fig. 4) ($P = 0.0009$), demonstrating a dominant effect of the WT B6 chr 11 on astrocytoma susceptibility. The F_1 progeny inheriting the mutant chr from B6 and the WT chr 11 from 129 are significantly more resistant to astrocytoma than the *NPcis*-B6 parent (Fig. 4) ($P = 0.02$), demonstrating a dominant effect of the WT 129 chr 11 on astrocytoma resistance. These data suggest that the WT copy of chr 11 acts dominantly to modify susceptibility to astrocytoma.

Because the data in Table 1 point to a role of chr 11 in modifying astrocytoma susceptibility, we determined whether imprinting on this chr affects astrocytoma formation, given that one copy of chr 11 is lost during tumor initiation, leaving only the maternal or paternal copy. This is distinct from the analysis described above, in that it does not require the modifier to be polymorphic between B6 and 129, but only that it be linked to *Nf1* and *Trp53*. Table 1 shows that F_1 progeny inheriting the *NPcis* chr from the mother (Fig. 3, cross B) are more susceptible to astrocytoma than F_1 progeny inheriting the mutant chr from the father (Fig. 3, cross A and C) ($P = 0.02$). We found no significant differences in the incidence of astrocytoma between male and female F_1 progeny. It is not clear from this analysis whether the imprinting effect is at the same gene locus as the B6 or 129 modifying locus, but both loci are linked to chr 11.

To further analyze the differences on chr 11 between the different F_1 progeny, we genotyped SSLP markers along the length of chr 11. The original *Nf1* and *Trp53* mutations used to generate the *NPcis*-B6 animals were made on a 129 strain background (19, 20). During the inbreeding of mutations onto B6, the region around *Nf1* and *Trp53* is continually selected and retains 129 sequence. The *NPcis*-B6 male founder used to generate the F_1 progeny in cross A of Fig. 3 retains 129 sequence from 54 through 81 Mb on chr 11 (Fig. 5). The *NPcis* F_1 progeny in cross A inherit this congenic region and are homozygous for 129 sequence around *Trp53* and *Nf1*.

We performed a survey of six additional inbred strains to look at the effects on astrocytoma susceptibility in F_1 progeny. Three of these F_1 strain combinations (B6XC3H/HeJ, B6XCAST/EiJ, and B6XSJL/J) have been published (16) and are included in Fig. 4 for comparison. As we described previously, *NPcis*-B6XCAST/EiJ and *NPcis*-B6XSJL/J F_1 progeny develop fewer astrocytomas overall but develop astrocytomas at the same age as *NPcis*-B6 mice and show accelerated tumor latency, developing other tumors before they live long enough to develop astrocytoma. Therefore, the reduction of astrocytoma in these two F_1 groups is not necessarily due to resistance to astrocytoma. We found that the CB strain had a similar effect to the 129 strain when crossed to *NPcis*-B6 mice. In both cases, the F_1 progeny are significantly more resistant to astrocytoma than the *NPcis* parental strain. The *NPcis*-B6XCB F_1 progeny showed no significant differences in any other tumor type, developing soft-tissue sarcomas, pheochromocytomas, histiocytic sarcomas, and lymphomas similarly to the *NPcis*-B6 parental line, and there was no significant change in the survival curves between the F_1 and the B6 parental strain. Furthermore, the astrocytomas that developed in *NPcis*-B6XCB mice were delayed relative to *NPcis*-B6 mice. Thus, similar to the modifying effect observed in the 129 strain, the CB strain modifier effect is limited to increasing resistance to astrocytoma and is not seen in five other strains examined.

Because both 129 and CB can dominantly repress astrocytomas in *NPcis*-B6 mice, whereas A and DB cannot, the polymorphisms responsible for this effect are expected to be polymorphic between 129 and B6, 129 and A, and 129 and DB. If the dominant effects of 129 and CB are due to the same locus, the polymorphisms may also have a common haplotype in 129 and CB. We analyzed SNPs from both the NCBI dbSNP database and the Celera SNP database to determine whether there are SNPs within the region shown in Fig. 5 that fit these criteria. We selected 597 SNPs from the congenic region between D11Mit271 and D11Mit285 in NCBI dbSNP, contained on 161 PCR frag-

Table 2. NCBI, sequenced SNPs polymorphic for B6 and 129

ss#	Gene	N Mb*	C Mb*	B6	A	DB	129	CB
12733678	Vamp2	68.7	73.0	G	G	G	A	G
12733665	Vamp2	68.7	73.0	A	A	A	G	A
12733667	Vamp2	68.7	73.0	G	G	G	A	G
12732038	Chrnb1	69.4		C		C	T	T
5069192	Trpv1†	72.8	77.8	C	C	C	G	G

ss#, NCBI dbSNP submitted sequence identification no.
*N Mb, physical location in NCBI; C Mb, location in Celera.
†The Trpv1 SNP and one of the SNPs in Trpv3 in Table 3 are the same SNP.

Table 3. Celera, genes with >10 candidate SNPs

Gene ID	Gene	N Mb*	C Mb*	No. SNPs
mCG140133	Trim11	58.6	60.7	11
mCG23374	NcoR1	61.9	66.3	41
mCG14821	1700019I23Rik	62.5	66.9	15
mCG11237	Vamp2	68.7	73.0	11
mCG21169	Rabep1	70.4	75.4	31
mCG6663	Ankfy1	72.3	77.3	12
mCG140764	Trpv3†	72.8	77.8	11
mCG48633	Msi2h	87.9	95.0	28

*N Mb, physical location in NCBI; C Mb, location in Celera.
†The Trpv1 SNP in Table 2 and one of the SNPs in Trpv3 are the same SNP.

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ments. Of these 161 PCR fragments, 72 were found to not contain polymorphisms between B6 and 129 by REVEAL ANALYSIS of hybrid stability. Of the remaining 89, 48 were sequenced, and of 159 SNPs represented, 13 were found to be polymorphic for B6 and 129. Of the 13 SNPs, 8 were polymorphic for 129 and A, and 6 were polymorphic for 129 and DB. Two SNPs were found that met all criteria, in that they were polymorphic for 129 and B6, 129 and A, and 129 and DB, and were not polymorphic for 129 and CB (Table 2)

The Celera database was searched directly for SNPs that are polymorphic for 129 and B6, 129 and A, and 129 and DB. There are two 129 substrains represented in the Celera database, 129X1 and 129S1. Of the 46,736 SNPs within the congenic region between D11Mit271 and D11Mit285, 16,220 SNPs were polymorphic between either B6 and 129X1 or B6 and 129S1. Of the 16,220 SNPs, 854 were polymorphic for A and DB. The SNPs covered intergenic regions and 117 gene products. Identified genes varied between those with a single candidate SNP and those with as many as 41 candidate SNPs. The genes with many SNPs fitting the candidate criteria likely represent regions of common haplotypes between B6, A, and DB, polymorphic with 129. Because of potential differences between our 129 strain and 129X1 and 129S1, these 854 candidate SNPs will need to be confirmed by direct sequencing of 129 and CB. The candidate genes with >10 candidate SNPs are listed in Table 3. The complete summary of the candidate SNPs identified from NCBI dbSNP and Celera are included as Tables 4 and 5.

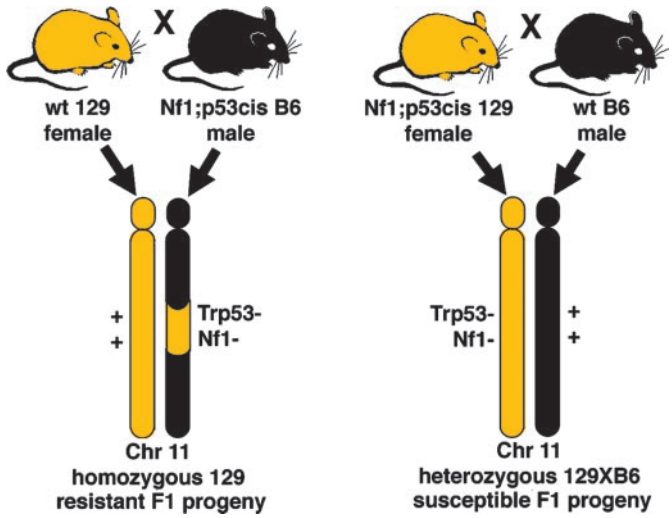

Fig. 6. Model for inheritance of resistance to astrocytoma. The F_1 progeny showing different susceptibility to astrocytoma differ in the strain background around the *Trp53* and *Nf1* loci. *NPcis*-B6 mice are inbred from 129 and carry a 129 congenic region on chr 11 (*Left*), whereas *NPcis*-129 mice are inbred 129 along the entire length of chr 11 (*Right*). F_1 progeny differ in whether they are homozygous for 129 around *Nf1* and *Trp53* or heterozygous for B6 and 129. According to this model, the B6 alleles on chr 11 confer susceptibility to astrocytoma in a dominant manner.

Discussion

The identification of modifier genes or susceptibility and resistance genes in human cancer is difficult, particularly in the case of less common cancers such as astrocytoma, where familial clustering is more difficult to find. We demonstrate here that modifier genes on mouse chr 11 affect susceptibility to astrocytoma in the presence of mutations in the ras and p53 signaling pathways, two of the major pathways mutated in human astrocytoma. Furthermore, these modifier genes show complex inheritance patterns in mice, suggesting that their identification in humans would be especially difficult. We use phenotype data from several inbred strains to identify potential candidate genes on chr 11. By identifying the genes responsible for these effects and understanding their mechanism, it may be possible to implicate them in human astrocytoma.

The data presented here show that susceptibility to astrocytoma depends on the mode of inheritance of the *NPcis* mutant chr. Because *NPcis*-B6 mice were inbred from a B6,129 background and the original mutations were engineered on a 129 background (19, 20), the *NPcis* mice inbred onto B6 carry regions of 129 sequence surrounding the two mutations on chr 11. Our data suggest that a major modifier affecting resistance to astrocytoma lies within these 129-retained regions in the *NPcis* B6 inbred mice. We have mapped the region of 129 sequence in the *NPcis*-B6 parent of our F_1 crosses and found that the region is >27 and <38 Mb in length surrounding *Nf1* and *Trp53* (Fig. 5). According to our model (Fig. 6), *NPcis*-129 mice are homozygous for 129 alleles at all loci, whereas *NPcis*-B6 mice are heterozygous for B6 and 129 at a small number of loci, including the region around *Nf1* and *Trp53* on chr 11. The B6 modifier allele(s) acts dominantly in this model to increase susceptibility to astrocytoma. In the case of the F_1 progeny inheriting the *NPcis* chr from the 129 line, the progeny inherit the modifier allele from the WT B6 parent and are susceptible (Table 1). In the case of the F_1 progeny inheriting the *NPcis* chr from the B6 line, the progeny do not inherit the dominant modifier allele and are resistant. The model predicts that B6, A, and DB all carry similar dominant susceptibility alleles at the modifier locus, whereas 129 and CB carry recessive resistance alleles. The dominant effect seen in *NPcis* B6XCB mice is due to loss of the B6 susceptibility allele and maintenance of two recessive resistance alleles at the modifier locus. Alternatively, the modifier in the CB background may be unlinked to the modifier on chr 11 but acts dominantly to suppress astrocytoma in the F_1 progeny.

We have tested whether candidate SNPs exist within the congenic region that are consistent with our phenotype data, using in silico haplotype mapping (24, 25) and sequencing. Importantly, no candidate SNPs were identified in *Trp53* or *Nf1*. Many of the candidates identified are expressed in the brain, and several are implicated in CNS stem cell biology or astrocytoma. *NcoR1* has been shown to inhibit the differentiation of neural stem cells into astrocytes (26). The *Msi2h* gene product has been implicated in the proliferation and maintenance of neural stem cells (27). Changes in neural stem cells could favor the development of astrocytoma by increasing the number of cells available to become tumorigenic. Alternatively, genes affecting neural stem cell differentiation could affect how well astrocytes maintain their differentiated state and resist transformation. *Sparc* is up-regulated in diffuse astrocytomas (28); however, because the 129, A, and DB share a common genotype, *Sparc* is not the best candidate for the astrocytoma modifier.

In addition to the observed strain-origin effect in F_1s, we have also observed a parent-origin effect of *NPcis* inheritance in F_1s (Table 1). Because the parent-origin effect is specific to chr 11 and not to the inheritance of B6 or 129, we argue that strain-specific differences in mitochondria from the mother or other cytoplasmic maternal factors are not responsible for this effect. This suggests that imprinting of genes on chr 11 modulates the susceptibility to astrocytoma. Imprinted genes have been identified on chr 11 in the mouse, supporting this possibility (29, 30). The F_1 data suggest that inheritance of the WT chr 11 from the father increases susceptibility, whereas inheritance of the WT chr 11 from the mother decreases susceptibility. It remains to be seen whether this effect is due to silencing of a maternal susceptibility allele or to silencing of a paternal resistance allele, and whether this effect acts on the same locus as the strain-origin effect. Whereas the strain-origin effect points to a locus linked to *Nf1* and *Trp53*, due to 129 sequences found in *NPcis*-B6 mice, the imprinted modifier could be anywhere on chr 11, affecting

tumorigenesis through loss or reduplication of monoallelic expression. Interestingly, Grb10 is a tyrosine kinase receptor adaptor protein imprinted on chr 11 and paternally expressed in the mouse brain (31, 32). It is likely that many imprinted genes exist along chr 11, and our experiments do not localize the imprinted modifier because large regions of WT chr 11 are likely lost during tumor initiation. It remains to be seen whether Grb10 or another monoallelically expressed gene on chr 11 can specifically alter tumor progression.

The modifier genes acting on astrocytoma susceptibility in this mouse model do not affect the development of other tumor types or tumor latency. This suggests that the modifiers act not on the overall tumor suppressor function of *Nf1* and/or *Trp53* but rather on the effect of one or both of these genes on the CNS. The modifier genes could be acting within the precursor cell to the astrocytoma, the tumor cell as it progresses, or in the surrounding normal tissue to support tumor growth and survival. Because the WT copies of *Nf1* and *Trp53* are lost during the initiation of tumorigenesis in *NPcis* mice (16, 17), modifier alleles linked to these genes on the WT copy of chr 11 may be affecting tumorigenesis during initiation steps or by acting in surrounding normal tissue to alter tumor progression. We observe that the tumors in *NPcis* 129 mice are lower grade in addition to being fewer in number; therefore, we favor an effect of the modifier on tumor progression as well as tumor initiation.

Much of the characterization of mutant phenotypes in the mouse occurs on 129 substrains or B6,129 mixed strain backgrounds. This is due to the predominant use of 129 embryonic stem cells for gene targeting. These data demonstrate the importance of examining phenotypes on multiple strain backgrounds. The role of mutations in the ras and p53 pathways in astrocytoma is well established; however, mouse models generated by mutation in these pathways fail to develop astrocytoma on the 129 inbred and B6,129 mixed backgrounds. It is only when the mutations are moved to B6 that the importance of these pathways in murine astrocytoma is appreciated.

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