Meningioma is a common nervous system tumor that affects older adults and particularly women, often associated with significant morbidity (Louis et al. 2000). Though most lesions are benign grade I malignancies, a significant percentage demonstrate aggressive features. In this regard, meningiomas can invade brain tissue, recur after resection, and spread along the leptomeninges to involve multiple regions (Louis et al. 2000).

Individuals with neurofibromatosis type 2 (NF2) are at significantly elevated risk for developing meningiomas (Evans et al. 1992), suggesting that the NF2 gene might play a central role in regulating leptomeningeal cell proliferation. Biallelic inactivation of the NF2 gene has been identified in 30%–70% of sporadic meningiomas, leading to loss of expression of the NF2 gene product, merlin or schwannomin (Gutmann et al. 1997). In addition, NF2 inactivation is likely an early event in sporadic meningioma pathogenesis and is observed as frequently in grade I meningiomas as it is in high-grade tumors (Perry et al. 2000).

Current animal models of meningiomas have relied on implantation of human meningioma cells in immunocompromised mice (McCutcheon et al. 2000). Grade I meningiomas grow slowly in vitro and rarely survive as explants in vivo. Only a few high-grade malignant human meningioma cell lines grow as explants in immunocompromised mice in vivo, with tremendous variability and success. Based on these limitations, the availability of an in vivo model system in which meningiomas arise from normal arachnoidal cells would be a major advance.

Although cancer prone, heterozygous NF2 mutant mice (NF2+) do not develop meningioma (McClelley et al. 1998; Giovannini et al. 2000), but rather die with osteosarcomas and other tumor types not found in humans with NF2. We demonstrated previously that NF2 inactivation is a rate-limiting step in murine Schwann cell tumorigenesis using the P0 promoter to express Cre recombinase in Schwann cells (Giovannini et al. 2000). Remarkably, meningioma was not observed in these mice, suggesting that Cre recombinase expressed from a Schwann cell-specific promoter does not affect meningioma progenitor cells. Electron microscopy and immunophenotypic studies show that meningiomas originate from arachnoidal cells of the meningeal coverings of the brain and spinal cord that are in contact with the cerebrospinal fluid (CSF) (Todma et al. 1992).

An alternative approach for the delivery of Cre recombinase into specific target tissues involves the use of a recombinant adenovirus (adCre) (Wang et al. 1996). This approach also has the advantage of targeting the inactivating genetic lesion [in this case, homozygous inactivation of NF2] to a small population of susceptible cells, which is likely to model human cancer more accurately than when all of the cells in a target tissue are mutated (Zhang et al. 2001).

In this report, we describe the first mouse model for familial and sporadic meningioma. Mice with conditional NF2 gene inactivation in leptomeningeal cells were prone to the development of meningiomas that were observed on two distinct genetic backgrounds (wild-type and heterozygous mutant p53) with no differences in tumor grade or histological appearance. Thus, we show that NF2 loss in arachnoidal cells, but not loss of p53, is rate-limiting for meningioma development, confirming the critical role of the NF2 gene as growth regulator for leptomeningeal cell.

**Results and Discussion**

**Delivery of adenoviral vectors to leptomeninges of newborn mice**

To model human NF2-related and sporadic meningioma in the mouse, we have targeted Cre recombinase to the leptomeninges by direct injection of adCre into the CSF of NF2+/(loxP/loxP) mice. To examine the distribution of virally infected cells with respect to the injection site, we used a recombinant adenovirus encoding the lacZ gene driven by the CMV promoter (adlacZ) (Stratford-Perricault et al. 1992, Fig. 1A,B). Histochromic analysis of...
Nf2flox2/flox2 deleted in leptomeningeal cells in vivo, we constructed an E1-expressing functional Cre protein could inactivate the injected pups. To investigate whether adenovirus distant from the injection site. Mortality was <1% of injected newborn mice, both in the vicinity of and good reproducibility of the spatial distribution of the ad-lacZ in newborn mice. X-Gal staining demonstrates extensive transduction and high expression levels of lacZ in the leptomeninges of the brain (transorbital and subdural). Microscopic examination of the leptomeninges demonstrates transduction of cells in the arachnoid and pia mater surrounding the cerebral cortex (A), and after subdural adlacZ infusion also in the leptomeninges covering the spinal cord [B]. Immunohistochemical analysis of leptomeninges dissected at the adCre injection site with anti-merlin WA30 polyclonal antibodies showing merlin loss in arachnoidal cells of transorbital adCre;Nf2flox2/flox2 mice (C); leptomeninges of noninjected Nf2^lox2/lox2 mice exhibit merlin staining (D).

β-galactosidase activity from tissues of pups injected by the transorbital approach revealed numerous positively stained cells throughout tissues surrounding the injection area, the leptomeninges covering the right trigeminal nerve, the arachnoid layer on the ventral face of the right cerebral frontal lobe [Fig. 1A], and bones of skull base and right orbit. After adlacZ subdural infusion, the leptomeninges (arachnoid and pia mater) covering the right frontal cerebral cortex, the skull, and surrounding the spinal cord showed positively stained cells, indicating wide spatial diffusion of adlacZ through the CSF circulation [Fig. 1B]. Despite the nonstereotactic handling, the transorbital and subdural approaches allowed good reproducibility of the spatial distribution of the adenoviral solution: After X-Gal staining, a blue precipitate was observed in all 16 (8 transorbital, 8 subdural) adlacZ injected newborn mice, both in the vicinity of and distant from the injection site. Mortality was <1% of the injected pups. To investigate whether adenovirus expressing functional Cre protein could inactivate Nf2 in leptomeningeal cells in vivo, we constructed an E1-deleted adCre recombinant adenovirus and used Nf2^lox2/lox2 mice as our in vivo system. In both transorbital- and subdural-injected adCre;Nf2^lox2/lox2 mice, the Nf2^lox2 allele was PCR-amplified in the leptomeninges proximal to the injection site [Fig. 2A]. Nf2 gene inactivation in arachnoidal cells was also confirmed by immunohistochemical analysis using specific anti-merlin polyclonal antibodies. Loss of merlin expression was observed in arachnoidal cells covering the trigeminal nerve in the proximity of the adCre [transorbital] injection site (Fig. 1C,D). Scattered regions of merlin-positive arachnoidal cells were also found in areas distant from the injection site. Altogether, these data indicate that the adCre virus transduces cells in the neuraxis by diffusion through the CSF after injection and that it efficiently induces Nf2 gene inactivation by Cre/loxP recombination.

Disruption of Nf2 in arachnoidal cells promotes meningioma development

After adCre administration, two cohorts of 17 transorbital Nf2^lox2/lox2 and 19 subdural Nf2^lox2/lox2 mice were observed and the survival compared to that of 24 noninjected and nine adlacZ-injected Nf2^lox2/lox2 mice over a period of 20 mo. The percentage of surviving transorbital- and subdural-injected adCre;Nf2^lox2/lox2 mice was significantly reduced compared to that of adlacZ-injected and noninjected Nf2^lox2/lox2 animals [log rank, P < 0.0001] [Fig. 3].

Transorbital-injected adCre;Nf2^lox2/lox2 mice developed meningeal tumors (4 of 14 mice; 29%) emanating from the meninges of the skull base at a mean age of 11 mo. Similarly, subdural-injected adCre;Nf2^lox2/lox2 mice also developed meningeal tumors (3 of 16 mice; 19%) in the vicinity of the injection site over the cerebral convexity at mean age of 14 mo [Table 1]. In contrast to human pathology, a marked female bias was not observed in meningioma development (female: male ratio of 2:5).

As in humans, the most frequent histological meningioma subtypes observed in these mice were meningothelial, fibroblastic, and transitional [Fig. 4]. Similar to human patients in whom histologically benign meningiomas may invade brain [Louis et al. 2000], one mouse tumor showed irregular groups of tumor cells infiltrating the adjacent cerebral parenchyma causing reactive astrogliosis [Fig. 4E–G].
Schwann cell hyperplasia is considered an early manifestation of biallelic Nf2 inactivation and eventually results in schwannoma formation (Giovannini et al. 2000). Similarly, the first lesion associated with murine leptomeningeal tumorigenesis in this meningioma model was meningothelial proliferation that developed in the vicinity of the injection site in 33% (transorbital plus subdural) of adCre;Nf2\(^{flox2/flox2}\);p53\(^{+/−}\) mice (Fig. 4M) and was not observed in adlacZ–injected mice. Histologically, this lesion is composed of sheaths of spindle and polygonal cells without obvious perivascular aggregates and differs from meningioangiomatosis occasionally seen in NF2 patients (Stemmer-Rachamimov et al. 1997). Meningothelial proliferation in the adCre–Nf2 conditional knockout mice was limited to leptomeninges and the cerebral cortex was not involved. No signs of inflammation were found in adlacZ–adCre–injected mice at the time of sacrifice; it is likely that neonatal adenovirus injection prevents an immune response to the virus [Kass-Eisler et al. 1994]. Hydrocephalus was observed in 43% (transorbital plus subdural) of adCre;Nf2\(^{flox2/flox2}\) mice, including the two mice with intraspinal meningioma. In contrast, only one adlacZ–injected mouse developed hydrocephalus. Thus, neither the scar at the site of injection nor a hypothetical immune reaction against adenovirus within the subdural space explains the hydrocephalus. In one case, an osteosarcoma emanating from a vertebral bone was found associated with hydrocephalus due to spinal cord compression. Meningothelial proliferation impeding CSF flow and causing progressive ventricular dilation is one possible etiology for the hydrocephalus observed in adCre;Nf2\(^{flox2/flox2}\) mice. A similar mechanism of hydrocephalus formation has been postulated for immunocompromised mice with intracranial meningioma xenografts exhibiting leptomeningeal involvement (McCutcheon et al. 2000). No tumors were found in the ventricles or in the spinal cord. In particular, no ependymomas (a less common NF2-associated tumor) were detected. Indeed, analysis of the topography of adlacZ–transduced areas did not show

### Table 1. Summary of the phenotypic consequences of adCre injection in Nf2\(^{flox2/flox2}\) and Nf2\(^{flox2/flox2}\);p53\(^{+/−}\) mice

<table>
<thead>
<tr>
<th>Phenotypic abnormality</th>
<th>adCre;Nf2(^{flox2/flox2}) t.o. (n = 14)</th>
<th>adCre;Nf2(^{flox2/flox2});p53(^{+/−}) t.o. (n = 18)</th>
<th>adCre;Nf2(^{flox2/flox2}) s.d. (n = 16)</th>
<th>adCre;Nf2(^{flox2/flox2});p53(^{+/−}) s.d. (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningioma (intracranial)</td>
<td>4(^{+}) (29%)</td>
<td>3 (19%)</td>
<td>2(^{+}) (11%)</td>
<td>0</td>
</tr>
<tr>
<td>Meningioma (intraspinal)</td>
<td>0</td>
<td>2 (13%)</td>
<td>0</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Meningothelial proliferation</td>
<td>5 (36%)</td>
<td>5 (31%)</td>
<td>4 (22%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>MPNST</td>
<td>0</td>
<td>1 (6%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Schwann cell hyperplasia</td>
<td>3 (21%)</td>
<td>0</td>
<td>1 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>Osteoma</td>
<td>10 (71%)</td>
<td>14 (88%)</td>
<td>10 (56%)</td>
<td>11 (73%)</td>
</tr>
<tr>
<td>Osteosarcoma (peripheral)</td>
<td>0</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Osseous metaplasia (trigeminal nerve)</td>
<td>4 (29%)</td>
<td>0</td>
<td>1 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Sarcoma (at injection site)</td>
<td>0</td>
<td>0</td>
<td>14(^{+}) (78%)</td>
<td>14(^{+}) (93%)</td>
</tr>
<tr>
<td>Liver tumor</td>
<td>3 (21%)</td>
<td>3(^{+}) (19%)</td>
<td>2(^{+}) (11%)</td>
<td>2(^{+}) (13%)</td>
</tr>
</tbody>
</table>

\(^{+}\)N\(^{flox2}/flox2\)(2/2);\(^{−}\)N\(^{flox2}/flox2\)(1/1);\(^{+}\)p53 LOH–;\(^{+}\)p53 LOH–;\(^{+}\)N\(^{flox2}/flox2\)(10/10);\(^{+}\)p53 LOH–;\(^{+}\)N\(^{flox2}/flox2\)(10/10);\(^{+}\)N\(^{flox2}/flox2\)(2/2);\(^{+}\)N\(^{flox2}/flox2\)(2/2);\(^{+}\)p53 LOH–;\(^{+}\)p53 LOH–;\(^{−}\)N\(^{flox2}/flox2\)(2/2);\(^{+}\)p53 LOH– t.o., Transorbital; s.d. subdural.
Meningioma development in Nf2 mutant mice

The incomplete penetrance of the meningioma phenotype suggests that additional epigenetic or genetic events are required for tumor development. At present, we have no evidence of strain-specific effects on Nf2-related tumor development (meningiomas are rarely observed in wild-type mice of any genetic background; Morgan et al. 1984), as both our schwannoma and meningioma models were generated in a mixed FVB/Nx129/Ola genetic background. Further studies will be necessary to determine if strain-specific effects modify the tumor spectrum in these Nf2 conditional mutant mice, as described for Nf1 (Reilly et al. 2000). Mutations of other tumor suppressor genes might cooperate with an Nf2 mutation to promote or accelerate tumorigenesis. Mice carrying mutations of Nf2 and p53 in cis rapidly develop multiple osteosarcomas and fibrosarcomas (McCleatchey et al. 1998), and one meningioma has been reported in a p53+/− mouse (Harvey et al. 1993). To investigate the potential cooperativity of Nf2 inactivation and a heterozygous p53 mutation in meningioma formation, Nf2flox2/flox2 p53+/− mice were injected with adCre. After administration of adCre, two cohorts of 19 transorbital Nf2flox2/flox2 p53+/− and 19 subdural Nf2flox2/flox2 p53+/− mice were observed over a period of 20 mo.

The survival of adCre,Nf2flox2/flox2 p53+/− mice was significantly reduced compared to that of adCre,Nf2flox2/flox2 and 20 adlacZ,Nf2flox2/flox2 p53+/− animals (log rank, P < 0.0001) (Fig. 3). This reduced viability was attributable to the early development [mean age, 5.5 mo] of highly aggressive sarcomas at the transorbital [14 of 18; 78%] or subdural [14 of 15; 93%] injection site (Table 1). Sarcoma development at the site of injection was presumably attributable to the local adCre extravesation during injection, resulting in Cre-mediated Nf2 inactivation and sarcomas of the fibrosarcoma, rhabdomyosarcoma, and osteosarcoma histological subtypes. All analyzed tumors displayed recombination of the Nf2flox2 alleles and loss of the wild-type p53 allele (20 of 20 tested by Southern blot analysis, data not shown). These results are in agreement with those of McCleatchey et al. (1998).
describing sarcoma formation in \( N^{f2+/−}p^{S3+/−} \) cis mice with loss of heterozygosity (LOH) for both \( N^{f2} \) and \( p^{53} \). The rate of meningioma development was similar in transorbital- (2 of 18; 11%) or subdural-injected (2 of 15; 13%) \( adCre;N^{f2lox2/lox2}p^{S3+/−} \) mice (sex ratio female: male of 1:1), which is not significantly different \( (\chi^2, P = 0.08) \) from the rate of meningioma development in \( adCre;N^{f2lox2/lox2}p^{S3+/−} \) mice (transorbital plus subdural) of \( N^{f2flox2/flox2} \) mutant mice (Giovannini et al. 1999). Meningothelial proliferation and hydrocephalus developed in 13% and 52% (transorbital plus subdural) of \( adCre;N^{f2lox2/lox2}p^{S3+/−} \) mice, respectively.

In addition, a malignant peripheral nerve sheath tumor (MPNST) emanating from the right trigeminal nerve was found in a transorbital-injected \( adCre;N^{f2lox2/lox2}p^{S3+/−} \) mouse (6.5 mo old). Synergy between \( N^{f2} \) and \( p^{53} \) mutations in development of MPNSTs was also observed in \( P^{C0}C;N^{f2lox2/lox2}p^{S3+/−} \) mice that rapidly developed multiple tumors (E. Robanus-Maandag and M. Giovannini, unpubl.).

Liver tumors [two hepatocellular carcinoma and two cholangiocarcinoma] occurred in 4 of 30 (20%) transorbital- and subdural-injected mice with a mean age of 9 mo. All liver tumors displayed recombination of the \( N^{f2} \) alleles, but not \( p^{53} \) LOH [4 of 4 tested by Southern blot analysis, data not shown], indicating that \( N^{f2} \) and \( p^{53} \) mutations do not cooperate in liver tumor development. Six tumors were found that related exclusively to \( p^{53} \) loss (data not shown): One large pituitary macroadenoma and two osteosarcomas were found at locations not related to the injection site in three \( adCre;N^{f2lox2/lox2}p^{S3+/−} \) mice. In addition, one glioblastoma and two osteosarcomas were found in three \( adlacZ;N^{f2lox2/lox2}p^{S3+/−} \) mice. These tumor types belong to the typical tumor spectrum of heterozygous \( p^{53} \) mutant mice (Donehower et al. 1992). Genetic studies have suggested that inactivation of the retinoblastoma and \( p^{53} \) tumor suppressor genes are uncommon in meningiomas [Pykett et al. 1997; Tse et al. 1998]. Our results suggest that in mice, as in humans, \( p^{53} \) gene inactivation is not a critical event for meningioma development. An additional event thought to be associated with malignant progression in meningiomas is loss of the p16\(^{INK4A} \) tumor suppressor [Tse et al. 1998]. The availability of meningioma-prone \( N^{f2} \) conditional mutant mice will greatly facilitate the elucidation of additional genetic factors that influence meningioma development and progression.

**Conclusion**

The present mouse model demonstrates a successful alternative approach for developing animal models for human tumors where tissue-specific promoters are not available. Adenoviral delivery of Cre recombinase was initially attempted in vivo to develop a model for colorectal adenoma formation [Shibata et al. 1997]. The application of this technology to central nervous system tumors has not been attempted previously. This strategy has the advantage of inducing \( N^{f2} \) inactivation in a small population of cells that are surrounded by, and must out-compete, their normal counterparts in vivo. In this respect, \( adCre \)-based models probably mimic human cancer more accurately than strains in which a cancer-associated abnormality is induced simultaneously in an entire tissue. Direct evidence that the presence of wild-type competitor cells can modulate the ability of mutant cells to induce disease emerged recently from competitive repopulation experiments using \( N^{f2−/−} \) mutant hematopoietic cells [Zhang et al. 2001]. \( adCre \) inactivation of \( N^{f2} \) was not only sufficient for meningioma formation, but also resulted in a similar spectrum of tumors originally observed in the conventional \( N^{f2−/−} \) mice on loss of the wild-type \( N^{f2} \) allele (McClatchey et al. 1998; Giovannini et al. 2000). The present mouse model, which results in the development of one of the clinically relevant tumors in \( N^{f2} \), by a mechanism that is genetically similar to that observed in the human disease, provides a powerful new tool to study meningioma formation and progression as well as to evaluate potential novel therapeutic interventions prior to trials in humans.

**Materials and methods**

**Mice**

\( N^{f2lox2/−} \) mice on a mixed 129/Ola, FVB/N background were bred to FVB/N mice for eight generations and then intercrossed to obtain \( N^{f2lox2/lox2} \) mice [Giovannini et al. 2000]. To obtain \( N^{f2lox2/lox2}p^{53−/−} \) mice, \( N^{f2lox2/lox2}p^{S3+/−} \) mice were bred to \( p^{53−/−} \) mice inbred in 129/Sv [Donehower et al. 1992], and the resulting \( N^{f2lox2/lox2}p^{S3+/−} \) mice bred to \( N^{f2lox2/lox2} \) mice.

**Injection of adenoviruses**

The Cre expression cassette was excised from pBS185 [Life Technologies] and inserted into the adenovirus shuttle vector pMA37 [gift from M. Latta-Mahieu, CNRS, Villejuif, France]. Viral stocks were prepared and titred in 293 cells according to standard procedures [Stratford-Pernicaud et al. 1992]. Mouse pups on postnatal day 2 were used for injections and anesthetized by hypothermia on ice. In the transorbital approach, a hand-held glass micropipette was gently introduced in the external corner of the right orbital cavity until the posterior bone wall was reached and perforated by application of a light pressure. In the subdural approach, the micropipette was lowered through the skin following a tangential axis in the right frontal region until perforation of the thin skull bone. Using a microinjector [Narashige, Japan], 3 μL [3 × 10\(^{10}\) pfu] of the adCre or adlacZ solutions were infused over the course of 1 min and the pipette was left in place for a further minute before removal to limit diffusion away from the site of release. Following injections, pups were kept in an isolator under an infrared heat lamp until active and showing no signs of respiratory distress.

**Histopathology and immunohistochemistry**

Mice were sacrificed at the times indicated and a complete necropsy performed as described previously [Giovannini et al. 1999]. To preserve the arachnoidal layer, the head was fixed in formalin in toto, decalcified, and sliced coronally before embedding in paraffin. Histological analysis, detection of β-galactosidase activity, and immunohistochemistry with
affinity purified rabbit polyclonal antibodies against merlin [1:500, WA30], Gutmann et al. 1997]. DAL-1 [1:500, 3A1], Perry et al. 2000], and rat monoclonal anti-GFAP [1:100, Zymed] were performed as described in Giovannini et al. (2000).

Acknowledgments

We thank L.A. Donehower for p53−/− mice, CDTA (Orléans, France) for mouse housing, F. Amira, N. Gervais, L. Legres, and N. Hedrick for histotechnical assistance, A. Courvelard, C. Degott, D. Figarella, D. Louis, A. Perry, and J. Woodruff for discussion on the tumor phenotypes, P. Berthaud for help with microdissection, K. Shannon and L. Parada for critically reading the manuscript. DAL-1 antibody was generously provided by J. Newsham. This work was supported by Grants from the U.S. Army Medical Research and Materiel Command (DAMD17-00-1-0594 to M.G.), Ligue Nationale Française contre le Cancer (M.G.), Association pour la Recherche sur le Cancer (M.G.), and by grants from the National Institutes of Health (NS35848 and NS41520) to D.H.G.

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References


Nf2 gene inactivation in arachnoidal cells is rate-limiting for meningioma development in the mouse

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Genes Dev. 2002, 16:
Access the most recent version at doi:10.1101/gad.226302