Meningiomas are primary tumors arising from arachnoid cap cells of the arachnoid villi of the meninges and account for 15% of intracranial and 25% of intraspinal tumors. They occur most commonly in the patient’s fifth and sixth decades of life and affect twice as many females as males. Although primarily benign, well-circumscribed tumors amenable to curative surgical excision, some meningiomas have malignant characteristics with cerebral cortical invasion, multiple recurrences, and a fatal outcome. Almost all cases of familial meningioma occur in association with NF2. Familial meningioma in isolation from NF2 (sporadic) is exceedingly rare, with only 14 reports since 1959. The authors report the existence of a family lacking any stigmata of NF2, in which two members had spinal meningiomas. Tumor specimens were subjected to immunocytochemical analysis for the NF2 protein product Merlin, which has been implicated in the tumorigenesis of meningioma. Merlin immunoreactivity was present in both tumor specimens, implying that the NF2 tumor suppressor gene was not deleted in these tumors. This supports the hypothesis that a second tumor suppressor gene locus, other than NF2, acts in the formation of familial sporadic meningioma. The results are discussed in the context of putative oncogenic mechanisms of familial meningiomas.

Key Words • meningioma • protooncogene • growth factor • familial tumor • tumor-suppressor gene • neurofibromatosis type 2 • tumorigenesis

Familial meningioma: analysis of expression of neurofibromatosis 2 protein Merlin

Report of two cases


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Meningiomas are primarily benign brain tumors thought to arise through multistep tumorigenesis, involving both the activation of oncogenes and the loss of tumor suppressor genes. The recently isolated neurofibromatosis 2 (NF2) tumor suppressor gene has been found to be mutated in a large proportion of meningiomas. Almost all cases of familial meningioma occur in association with NF2. Familial meningioma in isolation from NF2 (sporadic) is exceedingly rare, with only 14 reports since 1959. The authors report the existence of a family lacking any stigmata of NF2, in which two members had spinal meningiomas. Tumor specimens were subjected to immunocytochemical analysis for the NF2 protein product Merlin, which has been implicated in the tumorigenesis of meningioma. Merlin immunoreactivity was present in both tumor specimens, implying that the NF2 tumor suppressor gene was not deleted in these tumors. This supports the hypothesis that a second tumor suppressor gene locus, other than NF2, acts in the formation of familial sporadic meningioma. The results are discussed in the context of putative oncogenic mechanisms of familial meningiomas.
Protooncogene and growth factor overexpression, particularly of insulin-like growth factor-1 and -2,\(^1\) KRAS,\(^4\)\(^,\)\(^6\) erbB/epidermal growth factor,\(^23\)\(^,\)\(^44\) transforming growth factor–α,\(^2\)\(^,\)\(^3\) ROS1,\(^48\)\(^,\)\(^2\)\(^5\) sis/platelet-derived growth factor–β,\(^2\)\(^3\) and cyclin D1\(^2\)\(^4\) have been reported in a high proportion of meningiomas. Both progesterone and androgen receptors have also been shown to be overexpressed in meningiomas and may act in tumorigenesis via interaction with protooncogenes and tumor suppressor genes.\(^2\)\(^6\)

The \(NF2\) tumor suppressor gene has become somewhat of a paradigm, together with the \(RB1\) and \(p53\) genes, for the role of tumor suppressor genes in tumorigenesis.\(^3\)\(^3\)\(^\,\)\(^3\) The loss of the tumor suppressor gene \(NF2\), deleted in 30\(^\sim\)60%\(^4\)\(^6\) of sporadic meningiomas, is thought to be an early event in the development of this tumor.\(^7\)\(^,\)\(^3\)\(^9\) The estimated frequency of inactivating mutations in vestibular schwannomas, a bilateral tumor in \(NF2\), exceeds 50%.\(^4\)\(^2\) The \(NF2\) tumor suppressor gene has recently been cloned and shown to encode a membrane cytoskeleton protein called Merlin or schwannomin.\(^3\)\(^3\)\(^,\)\(^4\)\(^1\) Recent evidence, however, suggests that the familial (non-\(NF2\) or sporadic) meningioma tumor suppressor gene locus may in fact be distinct from the \(NF2\) locus.\(^7\)\(^,\)\(^1\) The purpose of this study was to investigate the involvement of the \(NF2\) tumor suppressor gene in the pathogenesis of familial meningioma.

We report the cases of two members of a family afflicted by meningiomas, together with determination by immunocytochemistry of expression of the \(NF2\) protein Merlin.

Case Reports

**Case 1**

**History.** This 31-year-old woman, the first of three affected family members (see Fig. 1 for family pedigree), presented at our institution with a 6-week history of subacute onset of low-back pain. Two to 3 weeks later she began to experience pain extending into the right thigh with bilateral leg discomfort and spasms. She denied any motor or sensory loss and had not had any bowel or bladder dysfunction.

**Examination.** The patient was in acute distress from low-back pain and had significantly reduced range of motion in the lumbar area and decreased right straight leg raising. She had mild weakness of her hip flexors bilaterally and minimal weakness of the right quadriceps muscles. Her adductor muscles were strong. She was diffusely hyperreflexic, although plantar responses were flexor. The results of sensory testing were normal. There were two to three beats of clonus at the right ankle. A lumbosacral magnetic resonance (MR) image revealed an intradural extramedullary enhancing lesion filling most of the spinal canal at the L-3 level consistent with a meningioma (Fig. 2 left).

**Operation.** The patient underwent L2–4 laminectomies and an intradural lesion, 2 x 1.5 x 1.5 cm in size, consistent with a meningioma, was entirely removed. No point of dural attachment was observed and the lesion appeared to be arising from the posterior L-3 nerve rootlet on the right. This nerve had to be sacrificed to achieve a complete excision.

**Pathological Examination and Postoperative Course.** The tumor was reported to be a meningioma of the clear-cell type. The patient tolerated the procedure well and experienced a minimal postoperative sensory deficit in the right foot and leg.

![Fig. 1. Family tree. Roman numerals denote generation. Cases reported: daughter (1), son (2), mother (3). c = cranial meningioma; f = spinal meningioma. Circles = females; cross-hatch = dead; squares = males.](image)

**Fig. 2.** Left: Case 1. Gadolinium-enhanced sagittal T_1-weighted lumbosacral MR images showing a brightly enhancing L-3 meningioma. Right: Case 2. An enhanced sagittal T_1-weighted MR image showing an L-5 meningioma.
Case 2

History. The second patient, the 24-year-old brother of the first patient, presented with a 1-month history of left leg pain and tingling associated with decreased muscle strength.

Examination. The patient had decreased muscle mass in both legs with decreased (4/5) strength bilaterally. No sensory loss was observed and tendon reflexes were normal bilaterally. Lumbosacral MR imaging revealed an enhancing intradural extramedullary mass at L-5 consistent with meningioma (Fig. 2 right).

Operation. An L4–5 laminectomy was performed and removal of a 3 × 1.5 × 1.5–cm meningioma was achieved.

Pathological Examination and Postoperative Course. The tumor was reported to be a transitional meningioma with an unusually large proportion of clear cells. The patient experienced an uncomplicated postoperative course and was discharged with minimal leg weakness and no sensory deficits.

Additional Family History

The 61-year-old mother of the aforementioned patients has undergone two intracranial and one spinal meningioma resections at another institution. Her first meningioma (intraspinal) occurred in the 1970s and was resected without difficulty. In 1980, a left frontal convexity meningioma was excised. It recurred and was subsequently resected in 1981. In 1992, a left cerebellopontine angle meningioma was diagnosed and totally removed. No additional pathological data were available for these lesions. Postoperatively she has a right-sided hemiparesis with a plegic right arm. She is able to walk with the help of a cane.

Immunocytochemical Studies

Specimens obtained from the lumbar meningiomas in the patients in Cases 1 and 2 were collected at the time of surgery. Paraffin-embedded tumor tissue samples were sectioned and deparaffinized after being heated at 60°C for 1 hour and were rehydrated by serial washes (2 × xylene, 2 × 100% ethanol [Sigma Chemical Co., St. Louis, MO] and finally 2 × 95% ethanol). The 8-μm sections were then washed three times in phosphate-buffered saline and boiled for 15 minutes in 0.01 M citrate buffer (sodium citrate plus citric acid at pH 6) in a microwave oven for antigen recovery. Endogenous peroxidase activity was quenched with 0.3% H₂O₂ in methanol and reacted with the primary NF2 antibody by using a Vectastain ABC-DAB kit (Vector Laboratories, Burlingame, CA). The specific antiserum used in these studies was rabbit polyclonal antibody to NF2 protein product Merlin/schwannomin.²² The specificity of the immunostaining was determined by replacing the primary antibody with preimmune sera, which produced negative results. Preadsorbed control antiserum also led to negative results.

Western Blot Analysis

Protein was extracted from fresh-frozen tumor tissue by using a Dounce homogenizer and 200 μl of NP-40 buffer as previously described.²² The extracted protein was quantified against a bovine serum albumin standard. Equal amounts of protein were loaded in duplicate on 8% denaturing polyacrylamide gels and run at 100 mV for 1 hour. One gel was stained with Coomassie dye and the other was blotted onto a nitrocellulose membrane for 1 hour at 4°C. The gel was checked by means of Ponceau S staining to ensure that the protein had transferred. The conditions for anti-Merlin binding have previously been described.²² The detection method used was the alkaline phosphatase secondary antibody (1:1,000) and nitro-blue-tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate reaction (Sigma). A prestrained sodium dodecyl sulfate–polyacrylamide gel electrophoresis standards (low-range) ladder was used for size determination: bovine serum albumin corresponded to 83 kD and ovalbumin to 50,600 kD.

Results

Immunocytochemical Analysis for NF2 Merlin Protein

Immunocytochemical analysis for Merlin was performed in tumor samples obtained in both patients (Fig. 3). Strong Merlin positivity was seen scattered among meningioma cells in both samples from Cases 1 (Fig. 3A and 2) and 2 (Fig. 3C). A membranous disposition was noted for the staining pattern, consistent with the known function of the Merlin protein linking cytoskeleton to plasma membrane. Replacement of the primary antibody with preimmune sera produced no staining in tumor specimens in either Case 1 (Fig. 3B) or Case 2 (Fig. 3D). The Merlin peptide-preadsorbed antiserum control showed no staining in either of these tissues (Fig. 4A [Case 1] and B [Case 2]).

To eliminate the possibility of immunoreactivity arising from nonmeningiomatous cells such as macrophages, samples from both specimens were stained with antibodies to macrophages (KP1) and CD4 helper T lymphocytes (anti-T3–10). Control glioblastoma tissue showed rich infiltration of all cell types (Fig. 5A and B), whereas tissue from both meningioma specimens showed no such infiltration of these cells (Fig. 6A–D).

Western Blot Analysis for NF2 Merlin Protein

To provide both positive and negative controls, Western blot analysis of Merlin protein expression was performed in tumor samples known to have NF2 gene deletions and therefore no Merlin protein expression (negative control) and in tumors without NF2 gene deletions with consequent Merlin protein expression (positive control) (Fig. 7). The protein ladder with the 80-kD Merlin protein is denoted by L. Lanes 1 and 2 contain glioblastoma samples showing strong expression of the Merlin protein; Lanes 3 and 4 contain meningioma specimens obtained in NF2 patients in whom there was a deletion of the NF2 gene and consequently no Merlin expression; Lanes 5 and 6 contain acoustic neuroma specimens in which there were NF2 gene deletions and no Merlin protein synthesis.

Discussion

We describe a family lacking any stigmata of NF2, two
members of which had spinal meningiomas. The mother of these patients had undergone resections of two intracranial and one spinal meningiomas but no tissue was available for analysis. Familial meningiomas (such as those described here) in the absence of NF2, so-called sporadic familial meningiomas, are extremely rare. This study is a report of the first analysis of NF2 Merlin expression in the tissue samples of two family members harboring familial meningiomas.

The demonstrable expression of NF2 Merlin protein in both samples implies that, in these specimens of familial meningioma at least, deletion of the NF2 tumor suppressor gene locus did not occur. Complete absence of the NF2 gene, and therefore of the Merlin protein product, would have been expected with loss of the NF2 allele, leading to loss of heterozygosity (LOH). Positive control specimens of glioblastoma in which NF2 is very infrequently mutated showed strong expression of the 80-kD NF2 Merlin protein (Fig. 7). Negative control specimens including two meningiomas obtained in patients with NF2 and two acoustic neuroma specimens obtained in NF2 pa-

patients were negative for Merlin protein expression as assessed by Western blot analysis; this is consistent with the NF2 tumor suppressor gene deletion in these tumors.

Evidence suggests that truncated Merlin product arising from missense mutations of the NF2 gene may be unstable at the protein level. Inframe and missense point mutations are rare, accounting for approximately 10% of NF2 mutations. These unstable mutated proteins are probably degraded and would result in no signal when reacted with the antibody to the COOH terminus of NF2 protein Merlin used here. However, although one cannot exclude the possibility that the antibody used does detect inframe or missense mutations of the Merlin protein, it is thought unlikely (G Rouleau and S Pulst, personal communication, 1997). Given these caveats, the data presented here support the hypothesis that a second tumor suppressor gene locus, other than NF2, acts in the tumorigenesis of familial meningioma.

The NF2 gene product, Merlin, is homologous to the highly conserved band 4.1 family of proteins that connect the cytoskeleton to components of the plasma mem-

Fig. 3. Photomicrographs showing the results of immunocytochemical analysis of the NF2 protein Merlin in two familial meningiomas. A and B: Case 1. C and D: Case 2. Strong staining of Merlin is shown in A and C. Replacement of primary antibody with preimmune sera produced negative results shown in B and D.
brane. Members of this band 4.1 superfamily of proteins, which have not been implicated previously in tumorigenesis, include protein band 4.1, moesin, ezrin, and radixin, as well as proteins that may subserve growth factor–directed cell division by acting as tyrosine kinase substrates. Serving as focal contact points between the cytoskeleton and the plasma membrane, these proteins may play a role in determining cell shape, motility, and growth.

The role of the NF2 gene as a potent tumor suppressor has been demonstrated by a finding that constitutes the acid test for putative tumor suppressor genes, that transfection into NIH 3T3 cells resulted in growth suppression. Furthermore the converse experiment, treatment with blocking antisense oligodeoxynucleotides to Merlin, led to suppression of its synthesis, decreased cell adhesion with morphological changes, and increased cell proliferation.

Allelic loss of chromosome 22 has also been reported in ependymoma, a tumor which is also found in association with meningioma as well as within the context of NF2. This implies that the NF2 tumor suppressor gene may be active in the pathogenesis of ependymoma as well as vestibular schwannoma. A broader role for NF2 in tumorigenesis is supported by the finding of occasional NF2 gene mutations in breast and colon carcinoma with a much higher incidence in malignant melanoma and mesothelioma.

Previously, LOH studies showed absence of chromosome 22 in approximately 60% of sporadic meningiomas with recent confirmation of concomitant inactivation of the NF2 gene in approximately 30% to 60% of meningiomas. Wellenreuther, et al., also detected differences among histopathological variants in terms of frequency of NF2 gene mutation, which ranged from a rate of 70 to 83% in fibroblastic and transitional meningiomas to only 25% of cases of meningothelial meningiomas exhibiting NF2 mutation. These findings suggest alternate molecular pathogenic pathways for histological variants of meningioma. Similarly, the longstanding controversy over the histogenesis of hemangiopericytomas may have been resolved by the conclusion that they are distinct from meningiomas, because the former have been shown to lack the NF2 mutation characteristics of the latter.

Multiple meningiomas occur commonly in the setting of NF2 but more infrequently in sporadic meningioma. A controversy has existed about whether they represent independent tumors or arise from a single progenitor cell. A recent study has provided evidence for monoclonal spread in meningiomas.

**Fig. 4.** Photomicrographs of the NF2 Merlin peptide–preabsorbed antiserum control showing no staining in either of these tissues. A: Case 1. B: Case 2.

**Fig. 5.** Photomicrographs showing the results of immunocytochemical analysis of macrophage and T cell infiltration in control glioblastoma specimens. Control glioblastoma tissue shows rich infiltration of both macrophages (A) and CD4 T cells (B).
It is now believed that multiple meningiomas develop from the uncontrolled subarachnoid spread of a single progenitor cell. Based on the absence of LOH in chromosome 22 in 40% of sporadic meningiomas and the finding that familial cases of meningioma are not linked to the \(NF2\) locus,\(^{31}\) together with the findings reported in this study, other tumor suppressor gene(s) have been hypothesized to act in the pathogenesis of meningioma.

Other chromosomal regions that are often found to be deleted in meningioma and are thought to harbor putative tumor suppressor genes include chromosomes 10 and 1p.\(^{20,32}\) Loss of heterozygosity for loci on chromosome 10 and the tumor suppressor gene \(p53\) have been associated with morphologically malignant meningioma progression.\(^{32,45}\) The recent cloning of another tumor suppressor gene, \(MN1\), in meningioma has also been reported.\(^{19}\)

Our findings confirm earlier reports that the \(NF2\) gene locus is not involved in familial meningioma and that a second tumor suppressor gene locus is responsible.\(^{31}\) This provides evidence that meningiomas can arise from a multiplicity of pathways involving the loss of different tumor suppressor genes and the activation of various protooncogenes. It is apparent that the loss of \(NF2\) and other tumor suppressor genes, in concert with the activation of protooncogenes, is necessary for the neoplastic transformation of arachnoid cap cells to meningiomas. However, no single gene, be it tumor suppressor or protooncogene, has been found to effect tumorigenesis in vivo. Continuing study of the molecular genetic basis of meningiomas will identify new candidate genes that, together with those already characterized, comprise vital points of growth control whose abrogation contributes to neoplastic transformation.
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