American Journal of Pathology, Vol. 147, No. 5, November 1995

NF2 Gene Analysis Distinguishes Hemangiopericytoma from Meningioma

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The histogenesis of dural-based or "central" hemangiopericytomas (cHPCs) remains controversial. Some authors consider these tumors variants of meningiomas while others consider them akin to peripheral hemangiopericytomas (pHPCs). Meningiomas frequently have mutations in the neurofibromatosis 2 (NF2) gene, providing a molecular marker for meningiomas and other NF2-related tumors. We therefore analyzed the NF2 gene in cHPCs, pHPCs, and meningiomas to determine whether cHPCs are more similar at the molecular genetic level to meningiomas or pHPCs. Using paraffin-embedded archival material from 28 cHPCs (including three primary and recurrent tumors), 10 pHPCs, and 26 meningiomas, we scanned all 17 exons of the NF2 gene and flanking intronic sequences for mutations with single strand conformation polymorphism analysis and DNA sequencing. No NF2 mutations were found in either cHPCs or pHPCs, whereas 35% of meningiomas had NF2 gene alterations (P < 0.001). The NF2 gene mutations in meningiomas were all truncating mutations, consistent with previous studies. Our findings suggest that cHPCs are distinct from meningiomas at the molecular genetic level and support prior clinicopathological data that distinguish these tumor entities. (Am J Pathol 1995, 147:1450–1455)

The nosological position of the dural-based or "central" hemangiopericytomas (cHPCs) has been a subject of debate for many years, particularly among neuropathologists. Some authors have argued that cHPCs are variants of meningioma and have termed cHPC the "hemangiopericytic" subtype of "angioblastic" meningioma.[1,2] Observations supporting a common origin of cHPCs and meningiomas include 1) mixed tumors with features of both hemangiopericytoma and meningioma,[1] 2) meningioma-like whorls[3] and cytoplasmic interdigitations[2] in cultured cHPCs, 3) cases of "angioblastic" meningiomas or cHPCs immunoreactive for epithelial membrane antigen,[4] and 4) ultrastructural features characteristic of HPCs (eg, basal lamina and dense bodies) in some meningiomas.[1]

Other investigators, however, consider cHPC distinct from meningioma and more similar to peripheral hemangiopericytoma (pHPC).[5–7] Pathological findings supporting this view include 1) the histological similarity of cHPCs and pHPCs, 2) immunoreactivity of meningiomas but not cHPCs for epithelial membrane antigen and sometimes cytokeratin,[8] 3) immunoreactivity of most cHPCs but not meningiomas for CD34,[9,10] and 4) ultrastructural absence of meningothelial features (interdigitating membranes and desmosomes) in primary cHPCs.[5,7]

Patients with neurofibromatosis 2 (NF2) inherit a predisposition to meningiomas, suggesting that the *NF2* gene on chromosome 22q is involved in meningioma tumorigenesis.[11–13] As predicted, the *NF2* gene is frequently mutated in sporadic meningiomas and mutations have now been identified in all of the

Supported by generous funds from the Brigham and Women's Hospital Department of Pathology, and by NIH CA57683, NIH NS24279, NIH CA51410 and a U.S. Army grant.

Accepted for publication July 13, 1995.

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common variants of meningioma.[13–16] In addition, although *NF2* gene mutations also occur in other NF2-related tumors such as schwannomas and ependymomas, they are rare in most human neoplasms.[13] *NF2* gene mutations thus provide a molecular signature for certain tumors, including meningiomas. We therefore hypothesized that, if cHPCs were variants of meningioma, cHPCs should also harbor *NF2* gene mutations. To evaluate this possibility, we studied all 17 exons and flanking intronic sequences of the *NF2* gene in DNA from archival cHPCs, pHPCs, and meningiomas, using single strand conformation polymorphism (SSCP) analysis and DNA sequencing.

Materials and Methods

Paraffin blocks of 28 cHPCs from 25 patients were obtained from Brigham and Women's Hospital (Boston), Massachusetts General Hospital (Boston), M. D. Anderson Cancer Center (Houston), Case Western Reserve Hospital (Cleveland), and University of Würzburg Hospital (Würzburg, Germany). For three cases, we used samples from both the primary and recurrent tumors for analysis. Paraffin blocks of 10 pHPCs and 26 sporadic meningiomas were obtained from Brigham and Women's Hospital (Boston) and Massachusetts General Hospital (Boston). All cases were reviewed by at least three neuropathologists (J. T. J., W. P., D. N. L.), and the cHPCs and meningiomas were classified according to standard current criteria.[5–7] When available, the light microscopic diagnoses were always supported by immunohistochemistry for vimentin, epithelial membrane antigen, and CD34, and by electron microscopy. Of the meningiomas, 14 were fibroblastic, 5 transitional, 1 meningothelial, 1 secretory, 4 atypical, and 1 malignant. None of the meningiomas came from patients with known NF2. The patients with meningiomas showed a female predominance (9 male, 17 female), with a mean age of 58 years, while the cHPCs were almost evenly divided between men and women (12 male, 13 female), with a mean age of 44 years. The pHPCs came from 7 men and 3 women, with a mean age of 55 years.

DNA was extracted from paraffin-embedded tissues using a modification of a published protocol.[17,18] Sections were scraped into 1.5 ml plastic tubes and vortexed for 30 seconds with 1 ml of xylene, followed by centrifugation at 14,000 rpm at 4°C for 25 minutes. The resultant pellet was washed in graded ethanols and then centrifuged at 14,000 rpm, 4°C, for 15 minutes. The pellets were dried and resuspended in 200 to 300 μl of 10 mmol/L Tris pH 8.0, 1 mmol/L EDTA, 0.5% Tween 20, and 1/10 volume of 50 mg/ml of proteinase K, then incubated for 1 to 3 days at 50°C, and boiled for 10 minutes to inactivate the proteinase K.

SSCP was performed as previously decribed,[18,19] with some modifications. The polymerase chain reaction (PCR) mixes for SSCP and for the initial sequencing amplification contained 1 μl of template DNA, 0.05 mmol/L each of dATP, dTTP, dGTP, and dCTP, 1 pmol of each primer, 20 mmol/L Tris pH 8.4, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 1 unit of *Taq* polymerase. For SSCP, however, 0.1 μCi [α^{32}P] dCTP was added to the reaction mix. For some cases, optimal amplification was achieved by a 10-fold reduction in the concentration of cold dCTP or template DNA. The primers used in the PCR reactions and DNA sequencing have been previously reported.[20] Exon 12 was amplified in two separate, overlapping amplicons because of its relatively large size. PCR was performed in a thermal cycler (MJ Research) using 30 cycles of 95°C for 1 minute, 58 to 60°C for 1.5 minutes, and 72°C for 30 seconds. For some exons and some cases, amplification was further optimized with a "touchdown" protocol of 36 cycles, decreasing over the first 15 cycles from an initial 30-second annealing temperature of 62°C to a final 30-second annealing temperature of 56°C. The SSCP products were run on 6% or 8% polyacrylamide gels containing 10% glycerol at 5 to 10 W overnight (16 to 20 hours). The gels were dried and visualized autoradiographically. DNA from NF2 and non-NF2 individuals were run as controls in selected assays, and negative controls, consisting of no template DNA, were run for all reactions. Direct DNA sequencing was performed on all cases that displayed a shift on SSCP analysis. After reamplification using the above conditions, the amplicon was subject to asymmetric PCR to amplify one strand preferentially, and the resultant single strand was sequenced using standard dideoxy termination methods with [γ^{35}S] ATP. These sequencing methods have been detailed elsewhere.[20]

Results

We tested all 17 exons and flanking intronic sequences of the *NF2* gene in 18 assays (two separate assays for exon 12) for each of the 64 cases from 61 patients. Successful amplification was achieved in 91% of the assays (1043 products in 1152 assays), a percentage similar to other PCR studies from archival tissues.[18] Amplification was most successful in

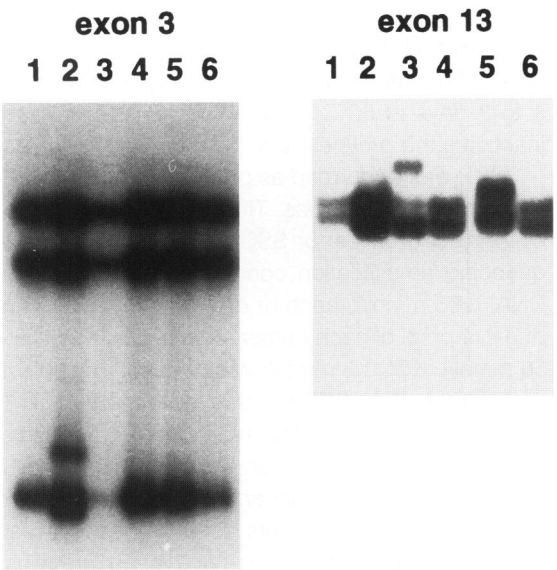

Figure 1. *Representative SSCP data.* (Left) *A shift in exon 3 of the* NF2 *gene in meningioma case 1444 is noted in lane 2.* (Right) *Shifts are present in exon 13 of the* NF2 *gene in meningioma cases 1434 and 1458, as evidenced by upward shifts of the top bands in lanes 3 and 5, respectively.*

the cHPC group (94% of assays), followed by the meningiomas (90%) and finally by the pHPCs (84%). For the cHPCs with recurrences, all exons were successfully amplified in one or both of the pairs.

Eleven SSCP shifts were noted in 10 meningiomas; case 1458 had two shifts. Representative SSCP data are illustrated in Figure 1. DNA sequencing of those 11 exons that displayed shifts on SSCP revealed nine mutations. These are catalogued in Table 1 and illustrated in Figure 2. As discussed below, these were all frameshift or nonsense mutations, presumably leading to grossly truncated proteins. In case 1458, which had two shifts, a C to T transition producing a stop codon was detected in exon 13. In exon 12 from this case, a large deletion of ~100 base pairs was noted on ethidium bromide-stained agarose gels of the exon 12 amplicon, but the precise borders of the deletion could not be determined by direct DNA sequencing. For the remaining SSCP shift, in exon 2 of case v-16, DNA sequencing of the entire amplicon failed to reveal a mutation. Because the initial SSCP shift in this case presumably reflected a technical artifact, the tumor was not included as a mutation.

Possible SSCP shifts were initially noted in two cHPCs and two pHPCs. These shifts, however, could not be replicated on subsequent SSCP assays, and DNA sequencing of the entire amplicons did not reveal mutations. We therefore also considered these initial SSCP shifts to be artifacts. No SSCP shifts were detected in any of the other cHPCs or pHPCs. The difference between *NF2* gene mutations in meningiomas (9 of 26) and the cHPCs (0 of 25) was significant ($P < 0.001$, Fisher exact test).

Discussion

The *NF2* gene on chromosome 22q is widely expressed in most human tissues.[11,12] The predicted protein product of the *NF2* gene, named merlin, is a member of the highly conserved protein 4.1 family of cytoskeleton-associated proteins. While members of this family probably have a number of functions, their primary role may be in mediating communication between the extracellular milieu and the cytoskeleton, by acting as a link between integral membrane proteins and the scaffolding proteins of the filamentous submembrane lattice.[21] Such a link may be functional in growth inhibitory signaling. As a result, inactivation of a merlin-mediated, cell membrane-cytoskeletal link may disrupt growth inhibition and facilitate the neoplastic phenotype.

Analyses of the *NF2* gene have revealed numerous somatic mutations in meningiomas, often with loss of the remaining copy of chromosome 22q, supporting the hypothesis that *NF2* functions as a tumor suppressor gene in meningeal tumorigenesis.[11–16] The described mutations have largely been inactivating genetic alterations, such as frameshift and

Table 1. *NF2 Gene Mutations in Meningiomas*

Case	Subtype	Exon	DNA sequence change	Codon change	Consequence
1424	Fibroblastic	3	339–341 ins 1 bp (A)	Thr 114 → 128X	Frameshift
1444	Transitional	3	257–259 del 1 bp (T)	Val 86 → 122X	Frameshift
1426	Atypical	4	388–391 del 4 bp (AAGA)	Lys 130 → 173X	Frameshift
1446	Transitional	4	151–164 del 14 bp	Tyr 144 → 151X	Frameshift
1432	Atypical	5	479–480 del 2 bp (GG)	Arg 160 → 201X	Frameshift
v-17	Atypical	8	783 del 1 bp (C)	Arg 262 → 295X	Frameshift
1438	Atypical	10	919–921 del 1 bp (T)	Phe 307 → 308X	Frameshift
1458	Meningothelial	12	Deletion of ~100 bp		Frameshift or deletion
		13	1357 C → T	Gln 453 → Stop	Nonsense
1434	Fibroblastic	13	1408 C → T	Gln 470 → Stop	Nonsense

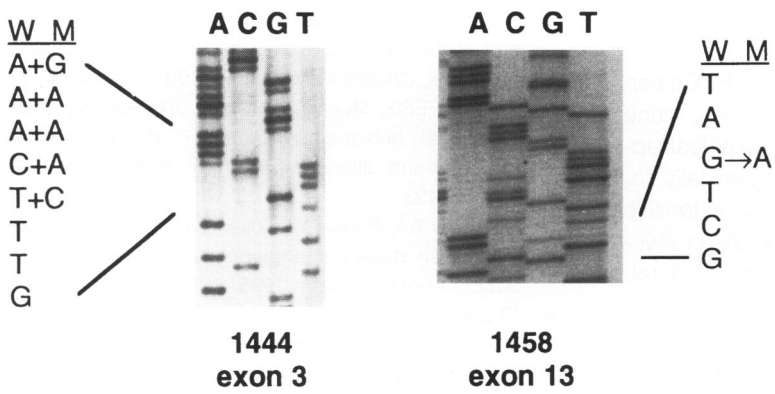

Figure 2. *Representative sequencing data.* (**Left**) *Exon 3 from case 1444 (see SSCP shift in Figure 1) has a single base deletion of a T that creates a frameshift and a premature stop at codon 122.* (**Right**) *Exon 13 from case 1458 (see SSCP shift in Figure 1) has a C to T transition in exon 13, which changes a glutamine codon (CAG) to a stop codon (TAG)* (**right**)*; sequencing of the antisense strand is illustrated).*

nonsense mutations that result in a grossly truncated protein product, although rare missense mutations have also been detected.[13] Our data support these previous findings, showing frequent *NF2* mutations in meningiomas. Of the 10 mutations in 9 tumors, 6 were small deletions leading to frameshifts, one was a small insertion also resulting in a frameshift, two were nonsense mutations caused by C to T transitions, and one was a large, ~100-base deletion that may either produce a frameshift or inactivate the protein by deleting a functional portion of the protein product. All of the mutations predict a truncated, and presumably inactivated, product. As in prior studies, there were no particular mutational "hot spots," and mutations were not detected in the two alternatively spliced exons, 16 and 17 (Table 1). The two previous studies that screened the entire *NF2* gene in meningiomas showed a tendency for mutations to occur in the first half of the gene, in the region of highest homology to the protein 4.1 family members moezin, ezrin, and radixin.[15,16] Six of our nine mutations also fell in this region, with only three mutations in exons 12 and 13, which encode the α-helical domain.

cHPCs and pHPCs did not have detectable *NF2* gene mutations. Although SSCP may not detect certain genetic alterations, such as deletions of exons and mutations in noncoding regions, such mutations have been exceptionally rare in other studies of the *NF2* gene.[13] Given the above findings, therefore, if cHPCs were variants of meningioma, *NF2* gene analysis should have revealed at least some cases with mutations. The data therefore argue that meningiomas and cHPCs are genetically distinct. A number of cytogenetic studies further support this distinction by implicating genomic regions in both cHPCs and pHPCs that are different from those involved in meningioma. In particular, rearrangements of chromosome 12q13 are common in hemangiopericytoma,[22–24] suggesting that an oncogene or tumor suppressor gene at this locus is important in HPC formation. A number of oncogenes reside in this region, including MDM2, CDK4 and CHOP/GADD153. Interestingly, CHOP/GADD153 amplification and constitutive expression has been noted in at least one pHPC.[25] A variety of other chromosomal regions are less consistently altered cytogenetically in HPCs, including 19q13, 6p21, and 7p15.[22–24] Most of these changes, particularly the common rearrangements of 12q13, occur in both cHPCs and pHPCs. Significantly, an identical reciprocal (12;19) (q13;q13.3) translocation has been reported in both a cHPC and a pHPC.[23,26] Such cytogenetic data support the hypothesis that cHPCs are closely related to pHPCs. Our data on cHPCs and pHPCs, although negative in nature, show that both cHPCs and pHPCs do not contain *NF2* gene mutations.

In contrast, cytogenetic alterations of chromosomes 12q13, 19q13, 6p21 and 7p15 are not common in meningiomas. Meningiomas frequently display monosomy 22, reflecting inactivation of the remaining copy of the *NF2* gene.[27,28] To our knowledge, only one probable HPC has been reported with a chromosome 22q alteration, a tumor from the thigh of a 28-year-old woman.[29] As discussed by these authors, however, the diagnosis of HPC may be difficult and the tumor was not studied either immunohistochemically or ultrastructurally to confirm the diagnosis. The tumor had a balanced reciprocal translocation, t(13;22) (q22;q11), but the chromosome 22q breakpoint appeared to be proximal to the NF2 locus at 22q12. Finally, molecular genetic studies have shown allelic loss of a few other chromosomal regions in meningiomas, particularly 1, 9q, 10, and 17p, but these alterations are primarily restricted to atypical and malignant meningiomas.[30,31] Alterations of the regions involved in HPCs, chromosomes 12q, 19q, 6p, and 7p, have not been documented in meningiomas. Thus, with the possible exception of a single case of probable pHPC with a proximal 22q11 breakpoint, the cytogenetic and mo-

lecular data on meningiomas and HPCs suggest distinct genetic abnormalities.

In most clinicopathological situations, cHPCs can be readily distinguished from meningiomas, since most cHPCs have a characteristic histological appearance, do not stain immunohistochemically for epithelial membrane antigen, and have extensive basal lamina detectable by reticulin staining or electron microscopy. Nonetheless, rare cases of dural-based tumors with features of both cHPC and meningioma may present diagnostic difficulties. Our study raises the possibility that *NF2* gene mutations may serve as a molecular marker to aid in this differential diagnosis. While the absence of an *NF2* mutation would not be of particular help, because some meningiomas do not harbor *NF2* mutations, the presence of a mutation would strongly favor a diagnosis of meningioma. Unfortunately, current screening methods are not sufficiently rapid, cost-effective, or sensitive to be of practical diagnostic use. For instance, SSCP and other genomic DNA-based analyses require at least 17 separate PCR assays to screen the entire *NF2* gene, and none of these methods is 100% sensitive.[32] Nonetheless, the advent of more rapid and sensitive screening techniques, such as the recently described method utilizing bacteriophage resolvases,[33] will no doubt facilitate molecular diagnostic approaches to screening genes. Such advances may allow *NF2* gene analysis to play a role in the sometimes difficult differential diagnosis of cHPC and meningioma.

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