Ocular Pathologic Findings of Neurofibromatosis Type 2

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Objective: To gain insight into the pathogenesis of neurofibromatosis type 2 (NF2) by investigating the ocular manifestations of this disease.

Methods: Using standard histologic techniques, immunohistochemistry, and electron microscopy, we described the ocular pathologic findings of a 34-year-old woman who died from complications of NF2.

Results: We identified 3 types of NF2-associated lesions: juvenile posterior subcapsular cataracts, epiretinal membranes, and an intrascleral schwannoma.

Conclusions: Our analysis indicated that dysplastic lens cells accumulate just anterior to the posterior lens capsule in juvenile posterior subcapsular cataracts and that dysplastic Muller cells may be a major component of NF2-associated epiretinal membranes.

Clinical Relevance: Our findings suggest that a subset of glial cells with epithelial features (Schwann cells, ependymal cells, and Muller cells) may be particularly sensitive to loss of the NF2 gene. Understanding the molecular basis for this sensitivity may lead to novel strategies for treating NF2.

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The patient was a 34-year-old woman who died from complications of neurofibromatosis type 2 (NF2). She had multiple skin tumors noted in childhood. At the age of 15 years, she began to notice hearing loss and tinnitus in both ears. At 17 years of age, a computed tomographic scan showed bilateral vestibular schwannomas, leading to the clinical diagnosis of NF2. There was no family history of NF2. Exon scanning and sequencing of DNA isolated from a vestibular schwannoma, a meningioma, and peripheral blood revealed a 1-base pair (bp) insertion in the splice donor site of intron 12 of the NF2 gene (1340 + 1 ins 1 bp (t)), consistent with a new germline mutation.

When the patient was 10 years of age, she failed an eye test. Her parents were told she was blind in the left eye with premacular fibrosis. At 28 years of age, the patient was first evaluated in a neuro-ophthalmology clinic for epiphora. On examination, she had a visual acuity of 16/25 OD and 16/200 OS. There was subnormal closure of her eyelids with ectropion and poor punctual apposition. After pupillary dilation, a small axial, subcapsular cataract could be viewed in the right lens. A more subtle but similar-appearing cataract was present in the left lens. Both fundi had epiretinal membranes (ERMs) (Figure 1). The patient's ophthalmologic findings remained stable after eyelid procedures to protect her corneal epithelium from exposure.

At 25 years of age, she began a slow decline due to her increasing meningioma and schwannoma tumor burden. At 34 years of age, she elected to forego additional surgical intervention, entered hospice, and died at home.

HISTOPATHOLOGIC FINDINGS

The right and left globes were obtained at autopsy. Microscopically, small groups and individual displaced lens cells were seen just anterior to the posterior lens capsule and within the posterior lens cortex bilaterally (Figure 2A). Rare Wedl and bladder cells were identified in the left posterior lens cortex (Figure 2B).

On the right was a thin ERM comprising of spindleled and cuboidal cells (Figure 3). These cells were focally positive for S100 protein and glial fibrillary acidic protein and were negative for pan-keratin and MB-1, consistent with glial origin. The underlying retina appeared well organized.

On the left in the region of the macula was a well-demarcated area of retina that appeared raised on gross examination...
(Figure 4). Histologically, this ERM comprised cords of predominantly cuboidal cells with well-defined cytoplasmic borders. These cells formed occasional lumens and were embedded within basement membrane material. A focal area of the lesion contained spindled cells.

The cuboidal cells of the left ERM were diffusely positive for S100 protein (Figure 5A), focally positive for glial fibrillary acidic protein, epithelial membrane antigen, and pankeratin (Figure 5B-D), and negative for CD68, MART-1, HMB-45, claudin, and MIB-1. Electron microscopy of these cells revealed intermediate filaments, consistent with glial cell origin, as well as epithelioid features, including short microvilli, adherens-type cell-cell junctions, and abundant basement membrane material (Figure 6A-C). Definite pigment granules were not identified. These findings are most consistent with a Muller cell origin of the cuboidal cells.

The underlying retina on the left was mildly distorted and wrinkled but otherwise well organized. Periodic acid–Schiff staining revealed small gaps in the internal limiting membrane adjacent to the left ERM (Figure 6D).

On the posterior aspect of the left globe within the sclera, there was a 2-mm, well-circumscribed tumor comprising spindled cells with oval, elongated nuclei arranged in fascicles (Figure 7A-C). The tumor cells were diffusely positive for S100 protein (Figure 7D), consistent with schwannoma.

**COMMENT**

Neurofibromatosis type 2 is an autosomal dominant disorder characterized by neoplasms of the central and peripheral nervous system (meningiomas, ependymomas, and schwannomas). The NF2 gene product, merlin, is closely related to the ezrin-radixin-moesin proteins. The ezrin-radixin-moesin proteins link plasma membrane proteins to the actin cytoskeleton and have been implicated in a variety of processes that take place at the membrane-cytoskeletal interface. Recent work has identified 2 important functions of merlin: (1) stabilization of adherens junctions at sites of cell-cell contact and (2) down-regulation of the Rac signaling pathway. These functions may be interdependent, as transient Rac activation is required for the proper assembly of adherens junctions. Defects in cell-cell adhesion and cell morphology secondary to abnormal adherens junctions may underlie the developmental or hamartomatous and neoplastic lesions of NF2.
A variety of ocular manifestations of NF2 have been documented, including juvenile posterior subcapsular cataract,\textsuperscript{6} juvenile peripheral cortical cataract,\textsuperscript{7} retinal hamartoma and combined pigment epithelial and retinal hamartoma,\textsuperscript{8} ERM,\textsuperscript{9} optic nerve menigioma, optic disc glioma,\textsuperscript{10} intraocular schwannoma,\textsuperscript{11} and neurotrophic keratopathy. Loss of heterozygosity for the \textit{NF2} gene has been demonstrated in dysplastic or hamartomatous lesions of the retina and optic disc.\textsuperscript{12}

Juvenile posterior subcapsular cataracts occur in 80% of patients with NF2.\textsuperscript{7} In one case report of the pathologic findings of juvenile posterior subcapsular cataract, Crawford\textsuperscript{13} described focal liquefied cortex just in front of the posterior capsule. In our case, the most striking feature was the presence of small groups and individual displaced lens cells just anterior to the posterior lens capsule and in the posterior lens cortex. One possible explanation for the pathogenesis of juvenile posterior subcapsular cataracts is that, owing to

\[ \text{Figure 3. Right epiretinal membrane.} \]
A and B, The right epiretinal membrane comprises spindled cells and cuboidal cells (more prominent in B) (hematoxylin-eosin). Cells are focally positive for S100 protein (C) and glial fibrillary acidic protein (D).

\[ \text{Figure 4. Left epiretinal membrane.} \]
A, The left macular epiretinal membrane is slightly raised. B, The lesion extends along the inner surface of the retina, and the underlying retina is slightly distorted (arrowheads indicate the size of the lesion) (hematoxylin-eosin). C and D, The lesion comprises cords of predominantly cuboidal cells, forming lumens (arrowheads) and embedded within basement membrane material (C, hematoxylin-eosin; D, periodic acid–Schiff).
abnormal adherens junctions, NF2-deficient posterior lens vesicle cells are unable to vertically elongate to form primary lens fiber cells and therefore accumulate in front of the posterior capsule.

Although not described until 1992, ERMs are a common finding in patients with NF2. Epiretinal membranes have been observed in patients with NF2 as young as 4 years of age, suggesting that they are likely congenital. The pathologic findings of 3 cases of NF2-associated ERM have been described. In the first case, Kaye et al9 showed a thin glial fibrillary acidic protein–positive glial ERM covering an intraretinal glial hamartoma. In the second and third cases, Crawford13 observed numerous defects in the internal limiting membrane, and extending through the defects were spindled cells, likely of glial origin. Examination of the ERMs in our case revealed a variable mixture of cuboidal and spindled cells, most consistent with a mixture of Müller cells and astrocytes. We cannot exclude the possibility that the

Figure 5. Cuboidal cells of the left epiretinal membrane are diffusely positive for S100 protein (A) and focally positive for glial fibrillary acidic protein (B), epithelial membrane antigen (C), and pankeratin (D). C, Inset, Epithelial membrane antigen staining is also observed along the external limiting membrane in regions of unaffected retina, corresponding to the apical surface of Müller cells. Arrowhead indicates location of the external limiting membrane.

Figure 6. Electron microscopy of the left epiretinal membrane reveals that cuboidal cells have intermediate filaments (A), short microvilli (B), and cell-cell junctions (B, inset). C, Cuboidal cells are embedded in basement membrane material. D, Periodic acid–Schiff staining highlights a gap (between arrowheads) in the internal limiting membrane adjacent to the left epiretinal membrane.
ERMs contain retinal pigment epithelial cells that have transdifferentiated into glial cells; however, to our knowledge, this phenomenon has only been observed in culture.15

Müller cells are the predominant glial element of the retina.16 Whereas astrocytes are largely confined to the ganglion cell and nerve fiber layers, Müller cells span the entire thickness of the neural retina, providing critical mechanical support. Müller cells ensheath and separate photoreceptor cells except at synapses. Through Müller cell–Müller cell and Müller cell–photoreceptor cell adherens junctions, the Müller cells form the outer boundary of the retina (external limiting membrane) at the level of the inner rod and cone segments. The outer or apical surface of the Müller cells is covered by short microvilli. The Müller cells are also responsible for producing the basement membrane material of the inner boundary of the retina (internal limiting membrane). This membrane is thought to prevent cell migration into the vitreous body.

We hypothesize that NF2-associated retinal and epiretinal lesions may be directly related to dysplastic Müller cells. Owing to abnormal adherens junctions, Müller cells that have lost their wild-type NF2 allele may not be able to elongate and establish radial polarity, leading to their epiretinal accumulation. Müller cell dysfunction may also result in a local decrease in basement membrane material, leading to gaps in the internal limiting membrane through which astrocytes can migrate, as well as loss of mechanical support causing retinal disorganization, as is seen in retinal hamartomas and combined pigment epithelial and retinal hamartomas. Our hypothesis that Müller cell dysfunction underlies NF2-associated retinal and epiretinal lesions is consistent with the proposal by Schachat et al17 that combined pigment epithelial and retinal hamartomas and ERMs have a common underlying pathogenesis.

Müller cells, like 2 of the other main cell types affected in NF2 (Schwann cells and ependymal cells), are glial cells with epithelial features, suggesting that this subset of glial cells is particularly sensitive to NF2 loss.

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