

AN 80-YEAR EXPERIENCE WITH OPTIC NERVE GLIOMA CASES AT THE ARMED FORCES INSTITUTE OF PATHOLOGY: EVOLUTION FROM MUSEUM TO MOLECULAR EVALUATION SUGGESTS POSSIBLE INTERVENTIONS IN THE CELLULAR SENESCENCE AND MICROGLIAL PATHWAYS (AN AMERICAN OPHTHALMOLOGICAL SOCIETY THESIS)

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ABSTRACT

Purpose: To determine whether p16, a molecular marker of cellular senescence, and CD68, a microglial marker, are detectable in optic nerve glioma tissue stored for decades, thus providing potential targets for pharmacologic intervention.

Methods: Cases were retrieved from the Armed Forces Institute of Pathology Registry of Ophthalmic Pathology. Clinical information was tabulated. In specimens with sufficient tissue, a tissue microarray was constructed to conduct molecular studies.

Results: Ninety-two cases were included: gender distribution was in a ratio of one male to 1.6 females, and age range was 2 months to 50 years (average age, 10.8 years). Neurofibromatosis type 1 was identified in 10 cases (10.8%). The majority presented with decreased vision and exophthalmos. Forty-eight cases were studied by a tissue microarray construction. Glial fibrillary acidic protein, a control for immunoreactivity, was positive in 46 cases (96%). Immunoreactivity for p16 protein was seen in 36 cases (75%) and CD68-positive cells in 34 (71%). Limitations include referral bias, limited clinical information, limited amount of tissue, and extended period of tissue preservation.

Conclusions: Optic nerve glioma is a tumor of the visual axis in young individuals, which is generally indolent but with a variable clinical course. Traditional histopathologic techniques have not been reliably predictive of clinical course. This microarray contains tumors with representative demographic, clinical, and histologic characteristics for optic nerve glioma. Immunoreactivity for p16 protein and CD68 is positive in the majority. These findings suggest a possible explanation for the variable clinical course and identify therapeutic targets in the cell senescence and microglial pathways.

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INTRODUCTION

Advances in molecular genetics have entered the armamentarium of the diagnostic pathologist and serve as logical extensions for traditional diagnostic methods. The availability of a large series of archival cases of optic nerve glioma (ONG) at the Armed Forces Institute of Pathology (AFIP) has provided the unique opportunity to study surrogate protein markers of underlying genetic events with therapeutic potential. Optic pathway glioma is a subcategory of astrocytoma with a low but distinct mortality. The clinical course is often indolent but may be associated with extensive loss of vision from intrinsic involvement by the tumor in the optic pathway at any site from the lamina cribrosa of the eye to the posterior cortical tracts of the brain. Exophthalmos may be extensive enough to cause optic nerve compression and corneal decompensation. Unlike with most tumors, there are no reliable histopathologic factors predicting outcome in any particular case. Studies of basic biochemical factors controlling cell cycling and survival (molecular biology) are now being used to identify prognostic factors at a subcellular level. The hope is that these basic factors will assist in the understanding of tumor behavior and offer avenues of tumor control.

The AFIP closed permanently on September 15, 2011¹ (Figure 1). The AFIP was established at the height of the American Civil War by the Lincoln administration to improve military medicine in the United States.² Initially the AFIP was called, and functioned as, the Army Medical Museum (AMM), collecting, analyzing, and displaying artifacts, but it evolved into the preeminent referral center from not only military sources but also domestic and foreign civilian institutions.

Ophthalmology played an important role in the development of AMM consultation and research activities. In 1910 US medicine in general, and medical education specifically, was totally reorganized because of the publication of the Flexner report.³ In 1911 50% of the medical schools in the United States were closed because of the report, and the remainder were drastically reorganized. In 1917 Edward Jackson, the president of the American Academy of Ophthalmology and Otolaryngology (AAOO), expressed his concern about graduate medical education in ophthalmology in an article entitled "Defects in education for ophthalmic practice."⁴ He proposed that a consortium of the AAOO and the AMM be established. The financial sponsors were the AAOO, the Section of the American Medical Association, and the American Ophthalmological Society. Harry S. Gradle, a practicing ophthalmologist in Chicago, represented the AAOO. His goal was "to provide a centralized standardized department of eye pathology for all the academically unattached ophthalmologists of the United States."⁵

In 1920 George R. Callender was the first practicing military pathologist to be assigned as Curator of the AMM. Dr Callender reestablished the original 1862 scientific mission of the AMM. Up to that time most tissue removed at the time of surgery was simply discarded. Dr Callender founded the Registry of Ophthalmic Pathology, the first registry of 27 subspecialty registries to be established at the AMM (later known as the AFIP).

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FIGURE 1

The Armed Forces Institute of Pathology (AFIP) as it appeared in April 2011. All of the pathologists had left, and the entire contents of the buildings were being dispersed to other facilities or discarded. The AFIP officially closed on September 15, 2011.

The first published scientific study of the AMM was a novel clinicopathologic investigation linking histologic findings of cases of malignant uveal melanoma with long-term follow-up. This was Dr Callender's classic study of uveal malignant melanoma: "Malignant melanotic tumors of the eye: a study of the histologic types in 111 cases."⁶ This study was one of the very first reports that emphasized that the future course of a malignancy could be estimated by the cytologic characteristics of the tumor. Uveal melanomas composed primarily of spindle-shaped cells were likely to have a better outcome than those with discohesive or necrotic cells. Although modified slightly in 1978 by McLean, Zimmerman, and Evans,⁷ the classification remains in use to this time.

This thesis reports the findings of what is the final AFIP study. The subject of ONG was chosen because of the potential availability of tissue, the availability of expertise in both ophthalmic pathology and neuropathology, and the preoccupation of the staff with this tumor over the lifetime of the Registry. The first case was accessioned in 1928, most likely by Dr Callender. The specimens were accumulated at a rate of approximately one or two cases per year until 1996, when the last specimen was accessioned. During the interval, many of the paraffin blocks had been cut to exhaustion from investigating any type of stain or cytologic finding that might emulate the work of Dr Callender. In fact, no helpful histologic criteria were found. In a letter written in March 1973 to his coauthor, Myron Yanoff,⁸ Dr Lorenz E. Zimmerman, the chairman of the Department of Ophthalmic Pathology, wrote: "I have gone over the 12 fatal gliomas of the optic nerve.... So far, I have not been able to detect any significant cytologic difference between these fatal gliomas and the non-fatal tumors."

Optic nerve glioma, also known as pilocytic astrocytoma, is a neoplastic proliferation of fibrous astrocytes that form well-demarcated tumors in many locations in the brain, including the optic nerve and the cerebellum.^{9,10} Most pilocytic astrocytomas of the optic nerve (ONGs) arise in the first two decades of life; however, this type of tumor is rarely clinically evident at birth. Indolent growth characterizes the natural history of ONGs, with periods of inactivity alternating with periods of growth. Spontaneous tumor regression has also been observed.¹¹ Adverse outcome is often associated with location within the central nervous system; those tumors that are surgically accessible tend to have a better outcome.

Optic nerve glioma is associated with neurofibromatosis type 1 (NF1) in approximately 10% of cases, representing an additional risk for systemic tumors. Optic nerve glioma arising in association with NF1 tends to present in patients younger than 10 years of age and to progress more slowly than sporadically occurring tumors.^{8,12} Study of the biologic character of ONGs has been hampered by infrequent occurrence (less than 1000 cases have been reported), limited amount of tissue (observation rather than surgical removal is now a more standard treatment strategy), and the lack of cytologic, ultrastructural, and immunohistochemical clues regarding the ultimate degree of aggressiveness of individual tumors. Treatments by surgery, radiation, and conventional chemotherapy have not been effective.¹³

Recent investigations into the fundamental molecular aspects of cell proliferation, particularly in hematologic tumors, have led to more precise methods of diagnosis and have suggested what have become novel and effective treatments.¹⁴ Molecular studies of aggressive astrocytomas that occur in the brain proper have indicated that alterations of intracellular signaling systems, particularly the mitogen-activated protein kinase (MAPK) and the mammalian target of rapamycin (mTOR) pathways, may have a profound influence on tumor cell replication and cell survival.¹⁵

In order to use available tissue in an economic and efficient manner, a tissue microarray (TMA) of the available AFIP cases was constructed. A TMA is composed of 1-mm cores of tumor tissue embedded in a specific pattern in a paraffin block. Thus as many as 50 cases can be cut and stained simultaneously.

Molecular control abnormalities leading to the development of cancer generally occur in specific compartments of a cell: genetic damage in the nucleus; alterations of promotion or suppression enzymatic pathways of cell division in the cell cytoplasm; and abnormalities in the microenvironment of the cell.¹⁶

Early studies by our group have found that ONG demonstrates molecular alterations typical of pilocytic astrocytomas, including the universal presence of either serine/threonine-protein kinase B-Raf (BRAF) gene duplication or NF1 association and common MAPK pathway activation. Also, a MAPK pathway signaling, mTOR, has been found in pilocytic astrocytoma, suggesting a therapeutic approach to ONG.¹⁷ This study was conducted to test the hypothesis that pathways involving cell senescence or the microglial cell microenvironment may or may not be involved in the pathogenesis of ONG, providing potential therapeutic targets for the treatment of ONG.

METHODS

This retrospective analysis and tissue evaluation project was approved by the Institutional Review Board of the AFIP, Washington, DC, on July 6, 2010, and bears the file number 365620-3. All files coded as ONG, optic nerve pilocytic astrocytoma, and optic nerve tumors not otherwise specified were obtained from the AFIP Registry of Pathology. All clinical information, correspondence, glass slides, and paraffin blocks were obtained and surveyed. The physical condition of each block was inspected, and those blocks with paraffin abnormalities (eg, heat damage) were re-embedded. All existing slides were reviewed, and slides with cracked mounting media were repaired and all cases with faded stains were restained or recut and restained. All information in each case was reviewed by at least two staff pathologists, and the diagnosis of pilocytic astrocytoma was confirmed by at least three staff pathologists.

These cases of ONG had been submitted to the AFIP in consultation for diagnosis of the histologic findings. In the majority of cases, only glass slides were submitted; however, occasionally wet tissue was available. The quantity and quality of accompanying clinical information were variable and often limited to demographic information. Images in the form of clinical photographs, radiographs, or digital images were rarely contributed with the case except for those cases that had been presented at ophthalmic pathology meetings. Extensive efforts were made on the part of the AFIP to obtain follow-up information; however, there was only the occasional response. During the interval of the study, most written information had been transferred to microfilm media or had been photocopied on first-generation copying equipment and was of poor quality. All of the available information in any form was reviewed and recorded. The following information was tabulated: source of the case (civilian, military, university, not stated), age, gender, race, presence of exophthalmos, presence of vision loss, duration of symptoms, presence or absence of NF1, whether or not the chiasm was involved, and the type of treatment. Separate tables of data were created for the entire patient population identified, the patients included in a TMA (described below), patients aged 10 years and younger, and patients aged 11 and older.

Elisabeth Rushing, MD and Iren Horkayne-Szakaly, MD identified 48 cases with sufficient tissue for preparing the TMA. Three pathologists reviewed each case slide simultaneously, and representative areas of the tumor were identified. Each area of the confirmed tumor was marked with a felt-tipped pen on the glass slide. The glass slide was superimposed on the paraffin block from which the slide had been cut, and the corresponding areas of the tissue within the paraffin block were sampled. The microarrays were constructed by extracting 1.0-mm cylindrical core biopsies from the paraffin donor blocks displaying representative tissue, then re-embedding these into a prepunched hole on a single recipient paraffin block at defined array coordinates. A TMA machine (Beecher Manual Arrayer MTA-1, Beecher Instruments, Inc, Sun Prairie, Wisconsin) was used to create the TMA blocks. Using this technique and following the manufacturer's protocol, previously selected tumor regions were extracted and prepared as a TMA with three blocks, each with 50 tissue cores. The TMA blocks were then cut with a microtome (3- μ thickness), and serial sections were placed on Fisherbrand SuperFrost Plus slides (ThermoFisher Scientific Inc, Waltham, Massachusetts).

The tumor tissue had been collected from many sources. The exact type of fixative used and the method of tissue embedding and staining were not known. Some of the tissue samples had been stored in paraffin for over 80 years. To determine whether or not the tissue would yield reliable information, TMA preparations were submitted for analysis to an established molecular research and molecular diagnostic laboratory. TMA analysis was performed under the direction of Dr Charles Eberhart, Director of the Laboratory of Ophthalmic Pathology of the Johns Hopkins Medical Institute. With the assistance of Dr Fausto Rodriguez, the TMA samples of ONG were subjected to tissue analysis by immunohistochemistry (IHC) for glial fibrillary acidic protein (GFAP) to act as a positive control (Figure 2), p16 (p16^{Ink4A}) to investigate the cell senescence pathway, and CD68 to evaluate for microglial activity. The samples were stained in the Johns Hopkins Hospital Pathology Department Central Laboratory according to standard clinical protocols, including use of prediluted p16^{Ink4A} antibody (MTM Laboratories, #CMA802).

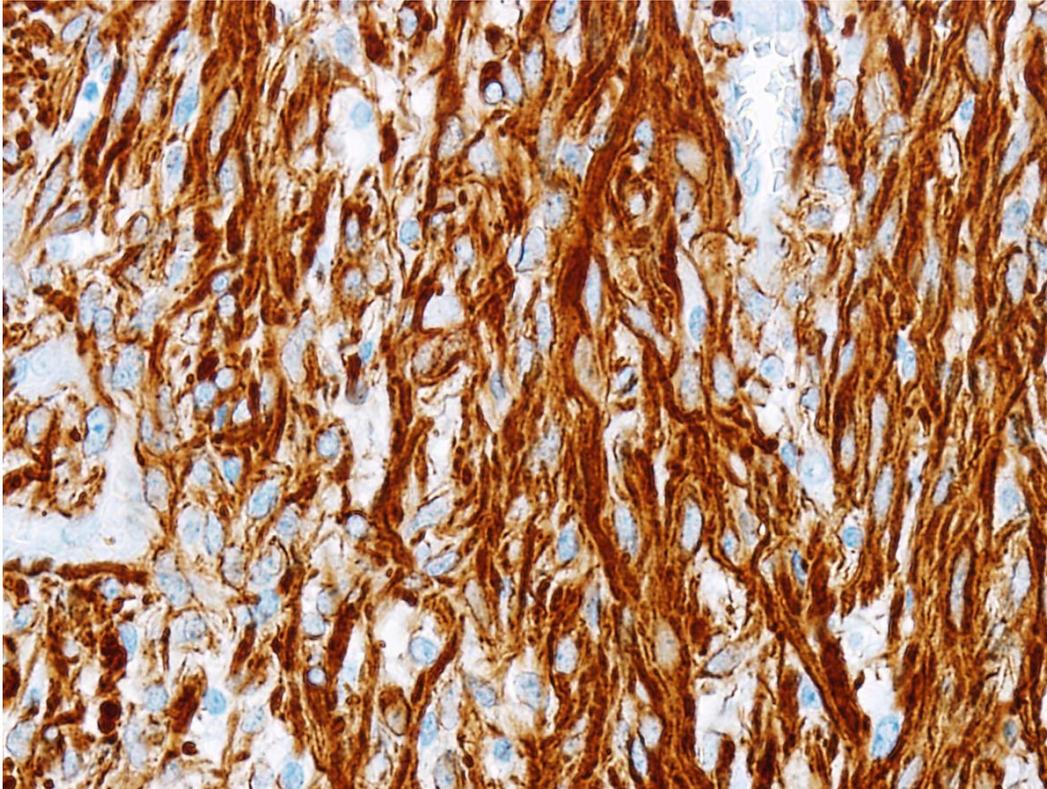


FIGURE 2

TMA analysis of optic nerve glioma tissue specimens. Sections were stained with glial fibrillary acidic protein (GFAP) stain as a control to demonstrate intact reactivity of tissue after prolonged storage (GFAP stain, original magnification $\times 40$).

RESULTS

The service life of the AFIP was from 1921 to 2011. The first case of ONG was submitted in 1928, and the last case was submitted in 1996.

The diagnosis of World Health Organization (WHO) grade I pilocytic astrocytoma was confirmed in 92 cases, with 48 cases identified as having sufficient tissue for inclusion in the TMA study.

DEMOGRAPHIC FEATURES

The core group of the study consisted of 92 cases (Table 1). The sources of the cases were 49 (53.3%) civilian, 22 (23.9%) military, and 18 (19.6%) from university teaching hospitals. In 3 cases, the submitting agency could not be determined from the available records. The average age of the overall study group was 10.8 years (range, 2 months to 50 years). Gender was distributed as 35 (38.0%) male and 56 (60.9%) female; no information was available in one case. Racial origin was 67 (72.8%) white, 12 (13.0%) black, and 9 (9.8%) other races; in 4 cases (4.4%) race was not stated.

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF 92 CASES OF OPTIC NERVE GLIOMA INCLUDED IN THE STUDY

AGE		GENDER		RACE	
Minimum	2 months	Male	35 (38.0%)	White	67 (72.8%)
Maximum	50 years	Female	56 (60.9%)	Black	12 (13.0%)
Average	10.8 years	Not stated	1 (1.0%)	Not stated	13 (14.2%)

The TMA study group comprised 48 cases (Table 2); average age was 10.0 years (range, 2 months to 50 years). Gender distribution was 20 (41.7%) male and 27 (56.3%) female; no information was available in one case. Racial origin was 34 (70.8%) white, 6 (12.5%) black, and 5 (10.4%) other races; in 3 cases (6.3%) race was not specified. Tables 3 and 4 compare patients aged 10 and younger to those aged 11 and older.

TABLE 2. DEMOGRAPHIC CHARACTERISTICS OF 48 CASES OF OPTIC NERVE GLIOMA INCLUDED IN THE TISSUE MICROARRAY

AGE		GENDER		RACE	
Minimum	2 months	Male	20 (41.7%)	White	34 (70.8%)
Maximum	50 years	Female	27 (56.3%)	Black	6 (12.5%)
Average	10.0 years	Not stated	1 (2.1%)	Not stated	8 (16.7%)

TABLE 3. DEMOGRAPHIC CHARACTERISTICS OF 60 CASES AGED 10 YEARS AND YOUNGER

AGE		GENDER		RACE	
Minimum	2 months	Male	21 (35.0%)	White	67 (72.8%)
Maximum	10 years	Female	38 (63.3%)	Black	12 (13.0%)
Average	4.8 years	Not stated	1 (1.7%)	Not stated	13 (14.2%)

TABLE 4. DEMOGRAPHIC CHARACTERISTICS OF 32 CASES AGED 11 YEARS AND OLDER

AGE		GENDER		RACE	
Minimum	11 years	Male	14 (43.8%)	White	22 (68.8%)
Maximum	50 years	Female	18 (56.3%)	Black	6 (18.8%)
Average	22.2 years	Not stated	0 (0 %)	Not stated	4 (12.5%)

CLINICAL FEATURES

The most common presenting symptoms in the core group (Table 5) were vision loss in 76 (82.6%) and exophthalmos in 75 (81.5%). Pain and abnormal ocular motility were reported infrequently, generally in cases with large tumor volumes. The average duration of symptoms was 1.9 years (range, 1 week to 20 years). Neurofibromatosis was confirmed in 9 cases (9.8%) and stated to be absent in 24 (26.1 %). In 59 cases (64.1%), information about the presence or absence of NF1 was not available. Treatment included one or more than one of the following procedures: orbitotomy 52 (56.5%), enucleation 40 (43.5%), and craniotomy 37 (40.2 %). The optic nerve chiasm was found to be involved in 24 cases (26.1%). Outcome was noted to be fatal for 10 patients for an overall mortality rate of 10.9%. In 3 of the fatal cases, patients were treated with exenteration; one case was suspected to represent recurrent retinoblastoma but was found to be ONG. Two cases from Africa were not included in the analysis because the treatment was not described and there is no follow-up information.

TABLE 5. SYMPTOMS OF 92 CASES OF OPTIC NERVE GLIOMA INCLUDED IN THE STUDY

DURATION		VISION LOSS		EXOPHTHALMOS	
Minimum	1 week	Present	76 (82.6%)	Present	75 (81.5%)
Maximum	20 years	Absent	14 (15.2%)	Absent	14 (15.2%)
Average	1.9 years	Not stated	2 (2.2%)	Not stated	3 (3.3%)

As in the overall group, the main presenting symptoms in the TMA subgroup were exophthalmos in 40 (83.3%) and vision loss in 39 (81.2 %). Pain and abnormal ocular motility were found only in an occasional case of large tumor volume. The average duration of symptoms was 2.0 years (range, 1 week to 20 years). NF1 was positively identified in 7 cases (14.6%) and stated to be absent in 11 (22.9%); no information was available in 30 cases (62.5%). Treatment may have included more than one of the following procedures: orbitotomy 25 (52.1%), enucleation 21 (43.8%), and craniotomy 21 (43.7%). The optic nerve chiasm was found to be involved in 11 cases (22.9%). Outcome was fatal for 4 patients for an overall mortality rate from all causes of 8.3%.

In the subgroup of individuals aged 10 years or younger, the presenting symptom was exophthalmos in 52 (86.7%). Additionally, vision loss was reported in 46 (76.7%). The average duration of symptoms was 1.7 years (range, 1 week to 7.5 years). NF1 was positively identified in 8 (13.3%) and stated to be absent in 14 (23.3%). No information about the presence or absence of NF1 was found in 38 (63.3%). Treatment may have included one or more than one of the following procedures: orbitotomy in 37 (61.7%), enucleation in 26 (43.3%), and craniotomy in 24 (40.0%). The optic nerve chiasm was found to be involved in 14 (23.3%). Outcome was fatal for 8 patients for an overall mortality rate from all causes of 13.3%.

In the subgroup of individuals 11 years or older, the presenting symptom of exophthalmos was found in 23 (71.9%). Additionally, vision loss was reported in 30 (93.8%). The average duration of symptoms was 3.3 years (range, 1 week to 20.0 years). NF1 was positively identified in one case (3.1%) and stated to be absent in 10 (31.3%). No information about the presence or absence of NF1 was found in 38 (65.6%). Treatment may have included one or more than one of the following procedures: orbitotomy in 15 (46.88%), enucleation in 14 (43.8%), and craniotomy in 12 (37.5%). The optic nerve chiasm was found to be involved in 12 cases (37.5%). There was a fatal outcome for 2 patients for an overall mortality rate from all causes of 6.3%.

CASE ILLUSTRATIONS

The first case submitted in 1928 was a 10-year-old boy with a 2-year history of exophthalmos and loss of vision. An orbitotomy and enucleation were performed. The patient was known to have survived at least 20 years following surgery.

In the first three cases accessioned, initial tissue diagnosis was incorrectly identified as neurofibroma, oligodendroglioma, or ganglioglioma.

Two benign features simulating apparent ONG growth were identified among the first five cases accessioned. Intralesional accumulation of mucoid material was identified as a noncellular component of an otherwise indolent ONG in a case submitted in 1939. Macroscopically the tumor was heterogeneous grayish white, soft, and translucent and blended into surrounding normal optic nerve tissue.¹⁸ In 1945 and 1946, two cases of ONG (in an 8-year-old girl and a 12-year-old boy) were misdiagnosed as optic nerve meningioma. This diagnostic problem was created by reactive arachnoid glial hyperplasia misinterpreted as optic nerve meningioma.¹⁹

In several cases exophthalmos was recognized at birth. The mother of a baby girl noted proptosis at birth, but the child was not evaluated until age 7½ years, when the girl accidentally noted decreased vision in the affected eye. She was initially treated with enucleation and ultimately with a craniotomy 3 years later. Evaluation of the surgical specimen indicated that the intracranial portion of the tumor was incompletely resected; however, the patient is known to have survived without recurrence for an additional 7 years. In 1965 another child was described with signs of proptosis noted at birth. At age 5 years she was noted to have exotropia associated with a visual acuity of 20/100. At craniotomy the tumor was found to be too extensive for complete surgical extirpation. Accordingly, tantalum clips were placed for subsequent radiation therapy. She was evaluated 2 years later, and only minor residual proptosis and ptosis were noted.

Optic nerve gliomas are usually confined to the dural sheath; however, exceptions are sometimes reported. For example, in 1956, a 9-year-old boy presented with limitation of gaze. At the time of surgery, extensive invasion of the meninges was accompanied with early invasion of the orbit.

The association of glial neoplasms and neovascular proliferation is well known. Central retinal vein occlusion was reported in 1948 in a 47-year-old woman with progressive exophthalmos to the point of no light perception. In 1949 a “vascular mass” that obscured the optic disc at the posterior pole was described in a 6-month-old baby girl. There was associated iris neovascularization, vitreous hemorrhage, and aplasia of the internal retina. In addition, there was marked infiltration of the arachnoid by the astrocytic tumor (Figures 3 and 4). In 1959 a 3-year-old child was diagnosed with central retinal vein occlusion and iris neovascularization, which was caused by a tumor that rapidly expanded over a 3-month interval. In 1966 a 14-year-old boy developed central retinal vein occlusion and neovascular glaucoma after radiation therapy for an incompletely resected ONG.

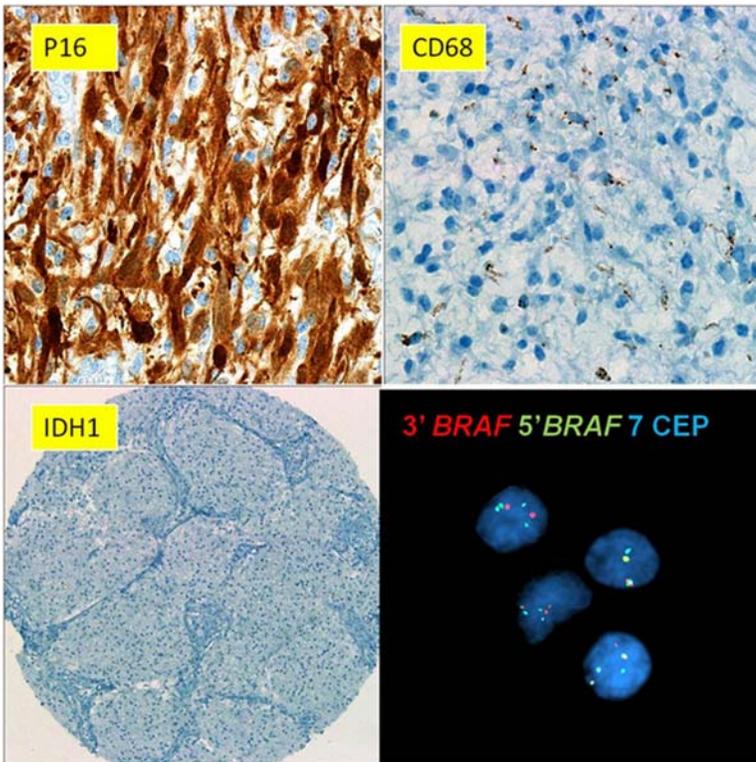
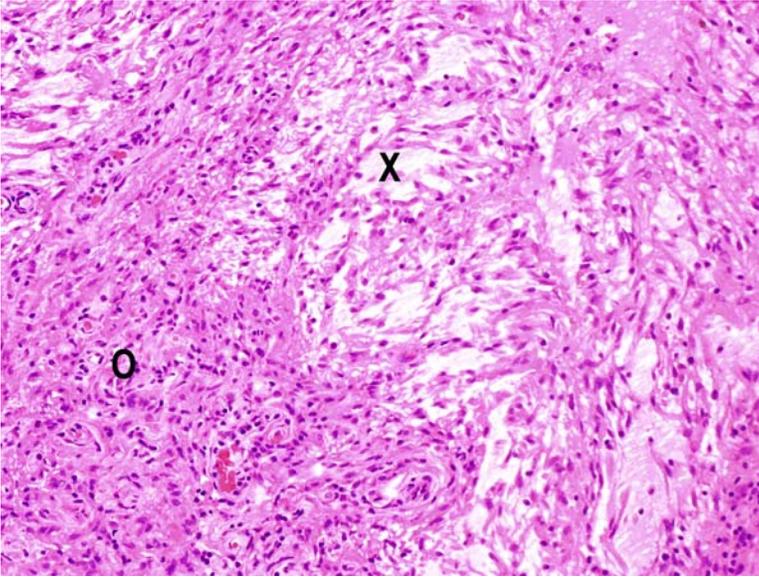


FIGURE 3

Examples of staining of TMA tissue samples. The p16 stain (upper left, original magnification $\times 40$) was used to evaluate the cellular senescence pathway. The CD68 stain (upper right, original magnification $\times 40$) was used to evaluate microglial cells. The isocitrate dehydrogenase 1 (IDH1) stain (lower left, original magnification $\times 20$) was used to differentiate from other types of astrocytoma. In this case the stain is negative. The fluorescence in situ hybridization technique (lower right, original magnification $\times 100$) was used to evaluate for genetic abnormalities (pinpoint yellow-orange spots).

**FIGURE 4**

An example of neoplastic glial cells in an optic nerve glioma. Generally there is a biphasic pattern with area of compressed cells (O) and loose areas (X). Astrocytes such as these have no distinguishing features from reactive or normal astrocytes (hematoxylin-eosin stain, original magnification $\times 20$).

Cases of documented clinical recurrence have been infrequent. In 1950 an orbital recurrence of a tumor initially diagnosed as retinoblastoma was identified in a 15-year-old girl. She was treated with enucleation at the age of 9 years (no histologic evaluation is available) but was noted to experience increasing orbital volume at age 15. Subsequently, she underwent exenteration; the orbital mass proved to be a pilocytic astrocytoma. In 1955 a 4-year-old girl developed proptosis 2 years after enucleation for what was thought to represent an ONG. The tumor was found to be an orbital encephalocele. In another case, a 26-year-old woman developed rapidly progressive proptosis during her first pregnancy. She was found to have an orbital glioma that extended to the chiasm. Because the lesion could not be completely resected, she was treated with radiation therapy, which resulted in significant visual field loss in the remaining eye. Interestingly, she was able to deliver three additional children without signs of recurrence. In 1959 a 3-year-old girl presented with mild proptosis and enlargement of the ipsilateral optic canal. At orbitotomy tumor was not identified; however, rapid progression of orbital volume occurred over the following 6 months. At that point, a large intraconal tumor was found and treated with resection including the globe. At gross inspection, the tumor was found to be incompletely excised. No follow-up information is available. In a similar case, reported in 1966, a 3-year-old girl presented with exophthalmos, in which there was no evidence of tumor at craniotomy. Over the following 9 months there was marked enlargement of the intracranial tumor, which prompted a second surgery. Gross thickening of the orbital and intracranial optic nerve was seen, which proved to be due to proliferation of arachnoidal tissue accompanied by necrosis of the dural sheath with extension of the tumor into orbital soft tissues. In 1964 a 16-year-old girl treated by a physician at a missionary hospital experienced proptosis leading to phthisis bulbi, which had been relatively stable until sudden progressive exophthalmos occurred. At orbitotomy, extensive areas of degeneration and hemorrhage were observed within the remaining tumor. The conclusion following microscopic examination was that the clinical enlargement was attributable to secondary changes rather than active cellular proliferation of the neoplasm.

In 1957 there was a single instance of ONG occurring in a 2½-year-old twin. It was not stated whether the twin was identical or fraternal or whether there was a history of NF1. Her sibling did not develop any signs or symptoms of ONG.

The earliest reported fatality in the younger age-group was that of an 8-year-old girl who in 1936 developed a 10×20-mm retrobulbar tumor that extended to the chiasm. The girl died during the craniotomy procedure. A second fatality, reported by Ida Mann in 1953,²⁰ was that of a 4-year-old girl from Australia who died of a soft tissue sarcoma thought to be secondary to radiation treatment. In 1956 a 6-week-old infant with multiple congenital abnormalities presented with seizures. A chiasmal glioma was identified extending into the frontal lobes. She died shortly after the craniotomy. The fourth fatality occurred in 1957 in a 5-month-old infant with an intraorbital tumor extending to the chiasm. Although the cause of death was not stated, she died 6 weeks following the completion of radiation therapy. Another child presented in 1957 at 2 years of age with bilateral blindness. The child's father, but not the patient, had confirmed NF1. At craniotomy an orbital tumor was found that extended to the chiasm and included an intracranial component. Approximately 30% to 40% of the tumor could be resected, and the remainder was treated with radiation. The patient died 6 years following treatment. At autopsy a pilocytic astrocytoma from the area of chiasm and remaining optic nerve extended into the frontal lobe. A 1-year-old girl presented with vomiting in 1973. A pilocytic astrocytoma was found to extend from the globe, via the optic chiasm, to the temporal lobe. The case is presumed to have had a fatal outcome. A child with developmental abnormalities at the time of birth in 1973 failed to thrive. She died at age 1 year and was found to have a pilocytic astrocytoma at the base of the brain at autopsy. A child born in 1972 was healthy until age 1 year, when she developed a gait disturbance. Her head circumference increased, and hydrocephalus was found, caused by obstruction from an ONG. A "huge" ONG was found extending from the optic nerve into the temporal lobe. This case is presumed to have had a fatal outcome.

The first reported fatality in the older age-group was that of a 38-year-old man who presented with pain thought to be due to sinusitis. At orbitotomy and subsequent craniotomy he was found to have a large intradural tumor of the orbital optic nerve with involvement to the chiasm. The tumor could not be completely resected, and the patient was subsequently treated with radiation. He

died 2 months following radiation therapy in 1950. The second fatality occurred in 1955 when a 22-year-old man was being evaluated by craniotomy. At autopsy he was found to have surgical damage of the pituitary.

MOLECULAR FEATURES

The p16 gene is an important regulatory protein that can be detected in cells by immunohistochemistry. The gene product is usually not detectable in normal glial cells. In tumor tissue sampled for our TMA, nuclear and cytoplasmic staining was detected in 75% of cases of pilocytic astrocytoma in our series, indicating up-regulation of the p16 gene, a marker of oncogene-induced senescence (Figure 3; upper left panel). The presence of microglial cells in tumors was confirmed by the CD68 immunostain. Microglia were identified in 71% of tumors and appeared as focal dustlike deposits interspersed among neoplastic astrocytes. CD68 immunoreactivity (Figure 3; upper right panels) showed microglia, which represent a component of the tumor microenvironment and have been shown to participate in tumor growth in murine models of optic glioma.²¹

Two additional analyses were performed in this human glioma TMA: fluorescence in situ hybridization (FISH) to detect the BRAF fusion abnormality, and mutant isocitrate dehydrogenase 1 (IDH1) immunohistochemistry. Mutant IDH1 is a tumor marker used for the identification of more aggressive types of glioma (eg, diffuse fibrillary astrocytomas and oligodendrogliomas). The tumor tissue in this study was completely negative (Figure 3, lower left panel). A positive study would be characterized by brown reaction product in the tumor cells. Some astrocytomas exhibit a chromosomal abnormality caused by a dislocation and fusion of specific DNA sequences (eg, BRAF fusion abnormality). Abnormal fragments of genetic material can be identified with colored probes, in this case red and green. A fusion is detected if the colored areas are combined to form a yellow color. In this example, the four cells in the field show an absence of the BRAF mutation (Figure 3, lower right panel).²¹

There were no strong associations between molecular findings and outcome.

DISCUSSION

This study of 92 cases of ONG strongly suggests that there are three promising avenues of investigation, primarily by molecular means, leading to treatment strategies for ONG. The three goals of investigation suggested by this and other recent studies are (1) to control the cell proliferation abnormally stimulated by the genetic defect at 17q34, (2) to selectively induce a nonproliferative state of the tumor cells by inducing cell senescence mechanisms, and (3) to stimulate microglial cells to control tumor cells in the microenvironment of ONG.

Optic nerve glioma by current convention is termed pilocytic astrocytoma WHO grade I. This tumor is at the benign extreme of the family of astrocytomas, with the most malignant form being glioblastoma multiforme. The primary cell involved is the astrocyte, and the general category of tumor is pilocytic astrocytoma. The tumor is architecturally well defined and may be found at any location within the central nervous system.²² The term juvenile pilocytic astrocytoma is no longer used.²³ In the past many other terms have been used to describe pilocytic astrocytoma, including peritheliomata, endotheliomata, gliomata, and neurocytomata.

The nonlethal gene abnormality leading to overproduction of abnormal astrocytes is thought to originate in genes that control proteins in one of several proliferation pathways in the cell, such as the MAPK and the mTOR pathways.²⁴ A hereditary aspect is indicated by a frequent association with NF1, although most cases appear to be sporadic. The affected gene product in NF1, neurofibronin, has been found to act as a tumor suppressor. Loss of the gene product leads to uncontrolled cell proliferation.²⁵

Optic nerve glioma is the most frequently recognized tumor of the visual pathway, but only approximately 1000 cases have ever been reported.^{8,26-28}

Most of the cases of ONG present in the first two decades of life, although there is a strong indication that the primary tumor-initiating events begin much earlier in life, as suggested by associated bone developmental abnormalities of the facial skeleton (enlarged optic canal diameter). Also, characteristics of astrocytes populating the optic nerve in early embryological development may be different from those in other regions of the brain.²⁹ In our series the average age was 10.8 years (range, 2 months to 50 years). Several of the young children had signs of tumor at birth; however, the children were not evaluated medically until later in life.

Gender specificity slightly favors women in most series. In our series the difference between genders was more marked. The tumor distribution was 38.0% male and 60.9% female. Because these cases were sent in consultation to a referral center for evaluation of features related to histology, and were not from a general population, this information is difficult to evaluate beyond the fact that the trend is similar.

Clinical presentation of ONG is most often vision loss and exophthalmos.

Vision loss in our series accounted for presenting symptoms in 82.6 % of cases; however, this may be underreported because vision loss may not be recognized at an early age. In one of our cases, vision loss was not recognized until the child was 7½ years old after she accidentally occluded her better eye. Cases presenting at an older age usually had experienced even greater loss of vision. When vision loss was analyzed by age, vision loss was present in 78.7% of the group aged 10 and younger and in 93.8% of the older groups.

Exophthalmos was a presenting sign in 81.5% of cases in this series. Because the tumor is often located completely within the muscle cone and because tumor progression was slow, other symptoms, such as pain, inflammation, or ophthalmoplegia, were found only in very large tumors. In this series only occasional patients with exposure keratitis or corneal perforation reported pain.

Intraocular findings were infrequent, usually associated with tumors in the optic nerve near the eye, and were limited to mild disc edema and disc atrophy. Four cases of arterial or venous occlusion of the central retinal vessels associated with opticiliary shunts and intraocular neovascularization were present. No cases of intraocular tumor extension were found.

Tumors posterior to the chiasm were usually recognized at a later age than orbital tumors. Duration of symptoms was also longer

for the older age-group (3.3 years) compared to the younger age-group (1.7 years).

Systemic abnormalities were usually limited to the cases of ONG with involvement of the hypothalamus and pituitary and those cases associated with NF1. Two patients had developmental abnormalities, which did not appear to relate to the presence of pilocytic astrocytoma.

NF1 is associated with approximately 15% (range, 10% to 44%)^{8,27,30,31} of cases of ONG. The association was first noted by Davis in 1940³² and was confirmed by Marshall in 1953.²⁷ NF1 is a common autosomal dominant disorder characterized by abnormalities in multiple tissues derived from the neural crest leading to production of benign and malignant tumors (eg, neurofibromas, malignant peripheral nerve sheath tumors).³³ There is a diverse spectrum of clinical manifestations, including neurofibromas, café au lait spots, and Lisch nodules. The *NF1* gene is one of the largest of the human genome and is associated with a high mutation rate. The NF1 gene encodes neurofibromin, a protein, with a role as a tumor suppressor in the Ras pathway, where the protein affects cell proliferation and cell differentiation.³⁴ In our series NF1 was identified by clinical findings in 8 of the younger age-group and in only one member of the older age-group. The total prevalence was 9.78%. This series includes the time before the association of NF1 and ONG was established. Lack of familiarity with the association of ONG with NF1 in the general ophthalmic community most likely contributed to what appears to be underreporting. NF1 deletions were not found in a small subset of this series.¹⁷

NF1-associated optic pathway glioma has a predilection for young children (most are younger than age 7 at presentation), as was found in this series. The tumor rarely grows or causes clinical symptoms after age 10 years. In contrast, with sporadic pilocytic astrocytoma the NF1-associated tumors tend to be located in the optic pathway or hypothalamus instead of the cerebellum. The histologic appearance of sporadic and NF1-associated pilocytic astrocytoma is identical. Only 30% of NF1-associated tumors require treatment, whereas sporadic tumors more frequently exhibit aggressive clinical behavior requiring treatment.^{35,36}

Imaging studies have become the mainstay of clinical evaluation of ONG. Enlargement of the optic foramen has been frequently identified in the presence of visual pathway glioma.^{37,38} However, a large ipsilateral optic foramen is of limited clinical value in determining tumor extent and behavior, as this finding has been noted with tumors limited to the orbit or limited to the intracranial tissues of the visual pathway.⁸ Modern imaging techniques have completely changed the accuracy of clinical diagnosis to a point that imaging is sensitive enough to determine the presence of optic pathway glioma in children with NF1.^{39,40} The current accuracy of detection and characterization, however, is not specific enough to exclude lesions such as craniopharyngioma or tuberculomas, leading to the recommendation that biopsy and tissue diagnosis should always be sought before instituting radiotherapy or chemotherapy for optic chiasmatic-hypothalamic gliomas.⁴¹ Most of the cases in our series occurred before high-resolution imaging was available. Clinical imaging information was rarely included in the information received from the contributors.

Orbital tumors most often arise between the globe and the orbital apex, producing a fusiform expansion of the optic nerve profile. Tumors may progress toward the globe or orbital apex or both. Tumors only infrequently affect intraocular structures.^{42,43} Generally, the dura remains intact and the tumor does not extend through the dura into the surrounding orbital soft tissues.^{27,44} Tumors within the posterior visual pathways, including those of the chiasm, exhibit the infiltrating features of pilocytic astrocytoma elsewhere. The optic chiasm was involved in 25.1% of cases in this series. Almost all combinations of involvement of the visual pathway have been reported, leading some investigators to speculate that the tumor is, in fact, multicentric.^{8,45,46} Intraoperative observations have noted that the character of the cut surface is variable from firm to soft with cystic zones composed of myxoid material. When the optic nerve is transected during a surgical procedure, mucinous material may extrude from an incision. Hemorrhage and necrosis at the tumor site are uncommon.⁴⁶⁻⁵⁰

One of the major guidelines for therapy for many tumors is the histologic character of the tumor. The primary proliferating cell of ONG is the astrocyte.⁴⁸ Astrocytes, oligodendroglia, and ependyma are derived from neuroectoderm. Astrocytes have an oval to round 10- μ nucleus. The cell's starlike outline is due to branching plasma membrane processes containing an evenly dispersed, GFAP-positive, pale cytoplasm (Figure 4). Protoplasmic astrocytes are found in gray matter, whereas fibrous astrocytes are found in both gray and white matter, where they generally surround blood vessels. The cells are a component of the blood-brain barrier and function in multiple biochemical support activities, including detoxification. Astrocytes have a limited role in tissue repair.^{51,52}

By light microscopy, tumor nuclei are oval with compact to moderately vesicular nuclei. Focal nuclear atypia is common, but the nuclei only infrequently contain nucleoli, and mitotic figures are rare. Differentiation between tumor cells and reactive gliosis is difficult (Figure 4).

By macroscopic examination, the tumor is often sausage-shaped with a gradual transition between normal-appearing optic nerve tissue and tumor tissue (Figure 5). The tumor is usually contained within the dura, although transdural extension may be present with larger tumors (Figure 6). Only one of our cases exhibited dural necrosis and early extension into adjacent orbital soft tissue.

Tumor cells are less organized and more densely concentrated than cells occurring in normal optic nerve tissue. The tumor may have the appearance of reactive gliosis. Myxomatous areas may be present, particularly in necrotic regions or loosely organized areas. Hemorrhage and hemosiderin may be present. The more densely populated cellular areas may be found around blood vessels and form perivascular rosettes. Arachnoid hyperplasia may be extensive and difficult to differentiate from primary optic nerve glioma, particularly if the sample size is small.^{19,53,54,55}

Three histologic patterns have been defined in pilocytic astrocytoma (coarsely reticulated, neovascular, and finely reticulated), and usually more than one type is present in an individual tumor. Tumors tend to be heterogeneous with one pattern predominating. None of these patterns has been found to be predictive of tumor aggressiveness. Rosenthal fibers are identified as tapered, corkscrew-shaped, brightly eosinophilic hyaline masses located in the cytoplasm of astrocytes. These fibers are found in conditions other than pilocytic astrocytoma (eg, reactive gliosis). Rosenthal fibers were infrequently present in this series. Neovascularization may be a prominent feature in some tumors but is not an indication of malignant transformation. Also, necrosis (nonpalisading, infarctlike),

hemorrhage, and mitotic figures may be present in lesions that have benign biologic behavior.⁸ By light microscopy, the area of cellular growth and infiltration appears to be a narrow zone immediately adjacent to the main tumor.

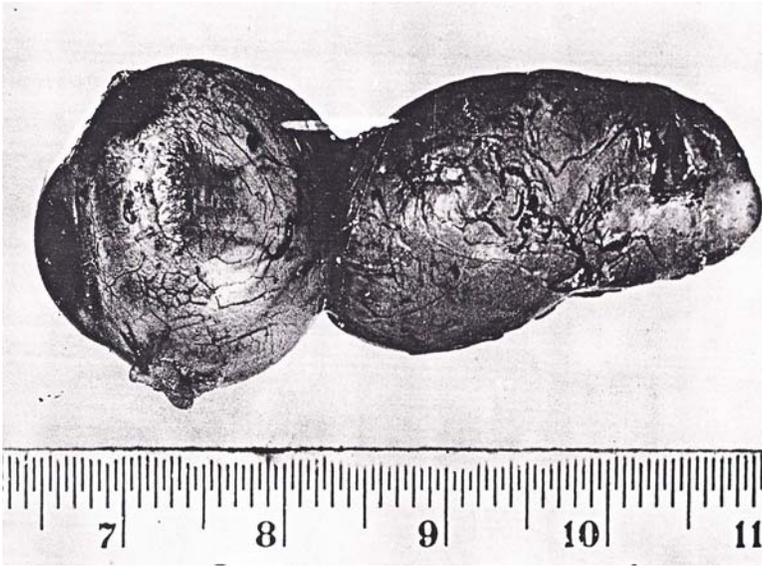


FIGURE 5

The gross appearance of an eye and optic nerve removed because of optic nerve glioma in 1949. The fusiform swelling of the optic nerve is characteristic of this tumor. The optic nerve dura appears to be intact. The tumor may extend to the surgical cut margin.

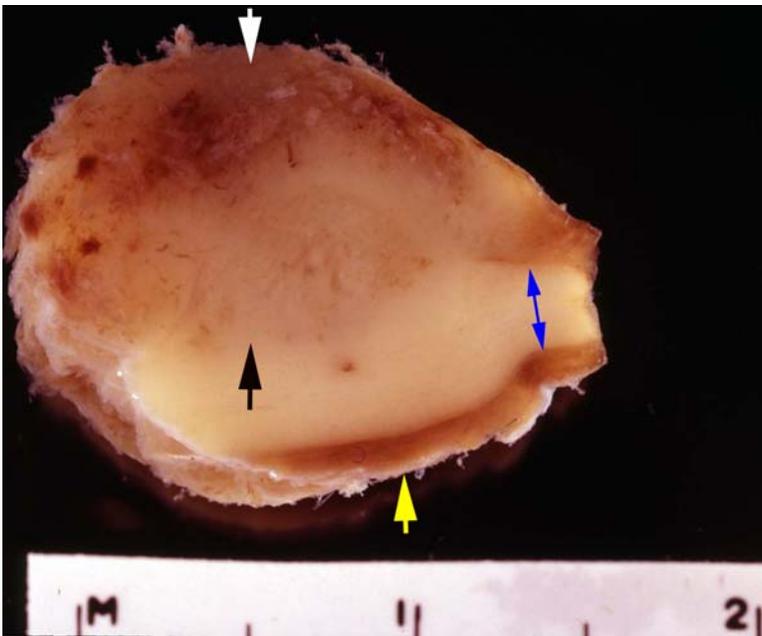


FIGURE 6

The gross appearance of an optic nerve glioma removed via orbitotomy. The optic nerve is at the right (double blue arrows). The tumor itself (between white and black arrows) has invaded the subarachnoid space associated with effacement of the overlying dura. Normal dura (yellow arrow) is present opposite the tumor.

Ultrastructural features of ONG include increased glial disorganization, disordered axonal projections, and profiles of degenerating myelin.⁵⁶ The ultrastructure of glial tumor cells does not distinguish ONG cells from native or reactive glial cells. Electron microscopy was performed on one of the cases in this series, but the results, apparently, were never reported.

Neoplasia of central nervous system tissues has several unique features: benign and malignant tumors are difficult to differentiate from each other based on cytologic characteristics; tumor margins are difficult to distinguish during surgery and even at histologic evaluation; morbidity and mortality depend more on location of the tumor than biologic activity of the tumor; and tumors may spread in the plane of the arachnoid but only rarely metastasize outside of the central nervous system. It has been noted that astrocytomas tend to become anaplastic over time.

Tumor progression is uncommon. Most optic pathway gliomas tend to remain static or slowly progressive.⁵⁷ Clinical regression of tumor volume has been observed.⁵⁸ Some investigators have even questioned whether malignant transformation actually exists,⁵⁹ although others disagree.⁶⁰⁻⁶³ It has been observed that partially resected orbital gliomas tend not to recur.^{35,54}

Routes of tumor progression include extension into the subarachnoid space, where reactive proliferation of meningotheial cells may be difficult to differentiate from the tumor itself.⁸ Tumor cells do not appear to breach the dural sheaths (except with very large tumors) and do not appear to spread via cerebrospinal fluid to distant areas of the central nervous system. Five of our cases were

evaluated for recurrence. One child initially diagnosed as having retinoblastoma was treated with enucleation; however, postoperative exophthalmos prompted a second orbitotomy, where the presence of a posterior pilocytic astrocytoma was identified. In another case enucleation was followed by the development of an encephalocele and not a recurrent tumor. Other cases are included where intratumor production of mucoid material, tumor necrosis, or intratumoral hemorrhage accounted for the increase in orbital volume. In the 10 fatal cases of this series, 4 deaths could be attributed to treatment and 6 to aggressive tumor behavior.

Optic nerve gliomas located in the tissues of the optic chiasm and adjacent optic radiations appear to be more aggressive than their more peripherally located counterparts. There are cases of diffuse infiltration into the optic pathway causing vision loss and infiltration of the hypothalamus resulting in precocious puberty. Though such events are considered rare, it was recognized early on by investigators, including Arnold Knapp in 1915,⁶⁴ that recurrences or intracranial extensions were an ever-present danger even many years after surgery.⁶⁵

Treatment of ONGs has been controversial since Antonio Scarpa first described the entity in 1816.⁶⁶ In retrospect Listernick has noted that most of the therapy for ONG in the past was unnecessary and overly aggressive.³⁵

Disagreement continues over the role of surgical therapy for ONG. Most of the early literature was centered on which surgical technique to use, not the indications for surgery.^{42,65,67} Algernon Reese pointed out the practical problem that in many cases both an ophthalmic surgeon and a neurosurgeon must be involved.⁵⁴ The frustration with surgical therapy was exemplified by the conclusion of Hoyt and Baghdassarian in 1969.⁵⁷ They noted that biopsy or surgical removal of the tumor did not affect the prognosis for vision or life and concluded that no treatment should be advocated except measures to relieve unsightly exophthalmos or obstructive hydrocephalus. Surgery is now used very selectively, which explains the very small number of surgical specimens currently submitted to pathology laboratories.

Radiation therapy in most instances also appears to be of limited value. In 1963, Bane⁵⁴ stated that radiation treatment of orbital glioma is not recommended by most experienced eye surgeons but is used in the inoperable cases in which the optic chiasm has been invaded. More recently, in 2007, Via⁶⁸ noted that radiation in children with NF1 might further deteriorate an already compromised intellect and possibly increase the risk of vascular complications and second tumor development.

Therapy is usually guided by clinical findings, imaging, and histopathologic, ultrastructural, and immunohistochemical features. In the particular situation of optic pathway gliomas, none of the information generated by these methods is uniformly helpful in predicting tumor behavior and outcome. After reviewing the fatal cases of this AFIP series, Dr Lorenz Zimmerman on March 20, 1972, wrote to Myron Yanoff: "So far, I have not been able to detect any significant cytologic difference between these fatal gliomas and the non-fatal tumors."

Molecular markers of cell proliferation offer the promise of needed guidance to identify cases of ONG with potential aggressive character and suggest opportunities for highly specific treatment of ONG. Three aspects of tumor development relevant to ONG have been investigated recently in central nervous system tumors: a chromosomal defect at 17q34 of one of the cellular proliferation pathways, a defect in the cell senescence pathway, and an alteration in the microglia found in tumor cell environment. Preliminary molecular studies were conducted on the cases of the AFIP TMA to determine if these abnormalities are detectable, particularly since these specimens were maintained in paraffin for up to 80 years.

Chromosomal abnormalities have long been recognized as an origin for cancer. Recently, treatments have been specifically designed to correct the abnormalities of the intracytoplasmic signaling system. The most impressive gains have been in hematopathology in the treatment of chronic myelogenous leukemia and cutaneous melanoma.⁶⁹ Abnormalities in the MAPK pathway have been determined to be an important molecular mechanism in the development of pilocytic astrocytoma. The origin of the abnormality in NF1-associated tumors is biallelic NFI gene activation,⁷⁰ and in sporadic tumors, tandem duplication at chromosomal region 17q34 involving the BRAF kinase domain. Several alterations of BRAF have been identified, including a fusion KIAA1549:BRAF and a point mutation BRAF(600E).⁷¹ Rodriguez and colleagues¹⁷ found by FISH studies that the BRAF duplication was present in 11 (65%) of 17 evaluable specimens including 1 case (25%) of NF1. Thus ONGs were found to contain molecular alterations typical of pilocytic astrocytoma elsewhere and suggest that inhibitors of BRAF and other pathway components being tested as treatment for other tumors, including sorafenib and AZD6244 (clinicaltrials.gov), may also be appropriately investigated for pilocytic astrocytoma.⁶⁹

Another, less well-studied pathway with a role in cell proliferation is the cell senescence pathway. This pathway does not promote cell division but is one of the mechanisms protecting against uncontrolled growth of cells with DNA damage.

Normal growth and development of cells involves a complicated balance between growth promotion and growth inhibition mechanisms embedded within the cell cycle. The cell cycle is a series of intracellular enzymatic events that prepare a cell for cell division. When all of the necessary components are produced, the cell divides (mitosis).⁷² The G1 portion of the cycle is for production of mRNA (messenger ribonucleic acid, which delivers "blueprint" information from the genes for protein production in the cell cytoplasm) and a host of proteins. Factors in the cell environment (eg, growth factors) stimulate a responsive cell by attaching to cell surface receptors. These "mitogenic" factors stimulate intracellular protein cascades to prepare for cell division. Early events of G1 can be permitted or terminated by cell control mechanisms until the R (restriction) point of G1 is reached. Beyond the R point the process is internally autonomous and can no longer be influenced by external factors. It is at the R point where the p53 gene, Rb gene, and p16 gene function as "gatekeepers" to prevent cells with damaged DNA from establishing a cancerous growth pattern. If DNA damage is detected, several outcomes are possible, including stimulating enzymes to repair DNA damage, initiating a genetically driven process of cell self-destruction (apoptosis), or allowing a cell to remain viable but incapable of reentering the cell cycle (cellular senescence). Growth factors are necessary for nutrition of senescent cells, but there is no proliferative response. Biochemical markers of cellular senescence include acidic β -galactosidase, plasminogen activator inhibitor-1 (PAI-1), and p16^{Ink2A}.

Immunohistochemical procedures can demonstrate regions of senescence-associated heterochromatic foci in the nuclei of senescent cells that are the result of chemical modification of histones within nucleosomes. These areas represent regions of the chromosome where gene expression has been silenced.^{16,72}

P16 (*CDKN2A*) is a gene located at 9p21.3 encoding protein p16^{Ink2A} that functions as a tumor suppressor. P16 deletions have been associated with high-grade pediatric gliomas.⁷³

Optic nerve glioma is characterized by initial growth that may continue to progress, may stabilize at a certain tumor volume, or may even regress. Tumors may also resume growing following a considerable period of no growth or even following apparently successful therapy. This unpredictable growth pattern makes evaluation of any treatment strategy difficult.^{31,58,74}

As previously discussed, ONG is characterized by a growth-promoting cascade containing an abnormal protein formed because of a *BRAF* mutation on 17q34. This abnormality may lead to uncontrolled growth of the cell. The proteins at the R point are designed to detect this type of threat and guard against the possibility of evolving into cancer.

In a recent series of experiments, Raabe and coworkers⁷⁵ introduced the mutation *BRAF*^{V600E} into cultured human neural progenitor cells, anticipating cologenic growth to serve as a model of ONG. However, even though there was proliferation initially, there was not sustained cell proliferation sufficient to form tumors. Thus there was no long-term proliferative advantage to the expression of *BRAF*^{V600E}. After 5 passages, cell-senescence proteins, including acidic β -galactosidase, PAI-1, and p16^{Ink2A}, were detected. Raabe and coworkers⁷⁵ also studied a microarray preparation of 77 cases from Johns Hopkins University School of Medicine. Where p16 protein was not detected (loss of tumor suppressor activity), patients were more likely female, tended to be older, and were more likely to have a fatal outcome.

Microglial cells that reside outside of the cell were also investigated. The development and maintenance of a clone of cancer cells not only depends on nonlethal genetic events within the cells, but is profoundly influenced by complex interactions with components found in the cell microenvironment. More specifically, these factors include, but are not limited to, constituent stromal cells, intratumoral vasculature, B and T lymphocytes, and members of the microglial/macrophage family.^{76,77}

Of note, CNS tumors, including gliomas, contain a variable quantity of infiltrating macrophages and recruited microglia.⁷⁸ A major cell type in the CNS microenvironment is the CD68+/CD45- microglial cell, which arises during embryogenesis and does not develop from postnatal monocyte lineage cells.⁷⁹

Microglial cells have been described in both sporadic and NF1-associated gliomas⁸⁰ and have been studied extensively in a murine model of NF1-associated glioma.²¹ In this model simple biallelic loss of the NF1 gene was not sufficient to induce glioma formation and progression. However, in the NF1+/- variant microglia were activated and increased proliferation and motility of glioma cells. The observation that microglial cells produce paracrine factors that support the growth of glial cells further supports the role of the microglial cell activation in the proliferation of glial cells. Neurofibromin regulates microglia proliferation and motility by hyperactivation of c-JUN-NH2-kinase.⁷⁹

Conversely, the control of glial cell proliferation can be achieved by microglial inactivation by minocycline⁸⁰ and ganciclovir.⁷⁹

Almost no information is available about the presence or activity of microglia in human pilocytic astrocytoma. In our series CD68, immunoreactivity showed moderate to marked numbers of microglia in 71% of samples tested. Further investigation is indicated to test whether reducing microglial activation will limit the growth of ONG.

CONCLUSION

This study of 92 cases of ONG collected over an 80-year period at the former AFIP strongly suggests that there are three promising avenues of investigation, primarily by molecular means, leading to treatment strategies for visual pathway low-grade pilocytic astrocytoma.

The clinical course of ONG is characterized by onset at an early age; proliferation of abnormal tissue to a sufficient degree to cause loss of vision, exophthalmos, and possible loss of life; episodes of apparent regression; variable and disappointing response to all forms of treatment; possible recurrence after treatment; and apparent de novo occurrence at an older age. These factors indicate that ONG is a multidimensional, multi-event process. With this degree of complexity there exists a plush opportunity for tailored, individual strategies of intervention.

The three goals of investigation suggested by this and other recent studies are (1) to control the cell proliferation abnormally stimulated by the genetic defect at 17q32, (2) to selectively induce a nonproliferative state of the tumor cells by inducing cell senescence mechanisms, and (3) to stimulate microglial cells to control tumor cells in the microenvironment of ONG.

A high priority for these avenues of investigation for this rarely occurring, indolent-appearing tumor is suggested by the possibility that these cellular events are early factors in other, more aggressive, more commonly occurring brain tumors. Also, even though the mortality for ONG is low, ONG is associated with an extremely high prevalence of loss of vision and facial physical disfigurement (exophthalmos) in children.

Optic nerve glioma is a rare tumor. Tissue for evaluation is scarce. However, there are established in vitro models of ONG; for example, Gutmann and associates' work with a knockout mouse model and Eberhart and associates' work with a human tissue model of neuromicrospheres. The work of these groups and the work of many other groups have produced a much clearer understanding of the pathophysiology of ONG. The role of this human tumor TMA and other similar collections of irreplaceable tissue is to selectively verify promising concepts.

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