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HIGH GRADE ASTROCYTOMA IN THE ADULT

**BIOLOGY,
PATHOLOGY,
DIAGNOSTICS,
AND TREATMENT**

PART 1: BIOLOGY AND PATHOLOGY

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GRADE ASTROCYTOMA

The definitive classification of primary brain tumors is histologic and based on the normal cell of origin within the central nervous system. While more than one cell type may be present, the predominant cell type found within a tumor is used to define the tumor. Even with the great advances made in histological and biochemical techniques, it remains difficult to identify the cell type of origin in some tumors. In the central nervous system, a tumor may be composed of a mixture of different neoplastic cell types, but the tumor is considered to be mixed only when a significant component of each cell type is found within the tumor.⁵ Histopathological features of the tumor determine malignancy or anaplasia.

This article focuses on the high-grade adult tumor to provide a deeper understanding of the biology, diagnostics, treatment options and outcomes associated with high-grade astrocytomas and, specifically, glioblastoma multiforme.

Biology of the glioma

One critical principle of oncology is that an understanding of the normal biology of the cell of tumor origin provides information necessary to comprehend the oncogenesis of a tumor cell and potential treatment options. In order to survive, any cell needs the following:

- adequate blood supply
- adequate nutrients
- protection and communication through an intact and properly functioning cell membrane
- production and exportation of growth factors.

In the brain, there is an added need for protection against wastes or organisms that may travel in the blood stream. This protection must be in a "gated" form, able to open to nutrients and oxygen and to close for protection from potentially harmful substances. As described in the treatment section, the ways a tumor survives are also methods by which the tumor can be attacked.^{4,5}

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glial cells

The term glial evolves from the Greek word meaning glue. Glial cells are non-neuronal cells of the central and peripheral nervous systems. There are 10 glial cells for every neuron, approximately 100 billion total glial cells, providing both structural and metabolic support for the neurons. In the central nervous tissue, types of glial cells include oligodendroglial cells, astrocytes, ependymal cells, and microglial cells. In the peripheral nervous system, the satellite cells of the ganglia and the Schwann cells around peripheral nerve fibers can be interpreted as the oligodendroglial cells.

Glial cell differentiation

How do glial cells begin? Undifferentiated neural epithelium serves as progenitor to a number of differentiated structures (Figure 2). Precisely how this task is accomplished remains unknown, but the basic mechanism has been outlined. A progenitor cell produced by the stem cell is reduced to a bipotential cell that produces two cell lineages, neurons and glial cells. The process, under DNA control, requires a specific sequence of gene activation and deactivation that occurs in sequential phases:

- differentiation of the neuroectoderm^{3,9,10,15}
- formation and segmental patterning of the neural tube^{5,9}
- determination of neuronal and glial phenotypes.^{3,5,7,9,29,30,33,35}

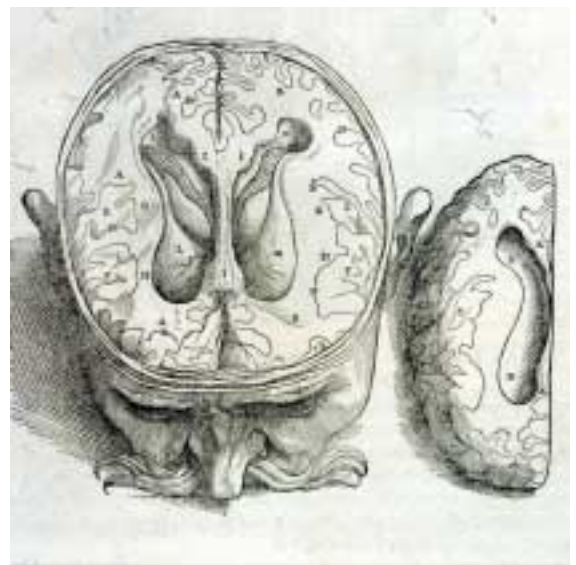
This brief description focuses on the glial lineage. These precursor cells arise from an undifferentiated, multipotential glial-cell progenitor. The multipotential glial-cell progenitor can produce some precursor cells that are only capable of producing an astrocyte. Under the proper environmental conditions, this cell will produce an (A1) astrocyte. Other progenitor cells are capable of producing both astrocytes and oligodendroglial cells.^{3,5,9,10,15}

Another route to astrocyte formation may also be simple and direct—a multipotential glial-cell progenitor to a bipotential intermediate cell to an (A1) astrocyte. However, the bipotential precursor cell can also produce an immature oligodendroglial cell depending on

environmental conditions. If the immature oligodendroglial cell is located in a cellular environment in which (A1) astrocytes are present, a mature oligodendroglial cell develops. If (A1) astrocytes are not present, the immature oligodendroglial cell becomes an (A2) astrocyte.

Normal cell cycle

The cell cycle (Figure 3) is too complex to describe in detail. However, it is important to understand that, under normal conditions, it is the precisely sequenced and timed series of intracellular actions that result in controlled cell growth and reproduction. In dividing cells, the end product of a cycle is two daughter cells that



are clones of the originating cell. Nondividing cells are not considered part of the cell cycle.

The phases in the cell cycle are:

- (G1) Gap one:
- (S) S phase
- (G2) Gap two
- (M) Mitotic phase

G1 represents a period of time when the cell is growing larger and preparing for DNA replication, and when genes are expressed and protein synthesis occurs. Extracellular factors, such as hormones or electrical stimulation, regulate this phase. While the cell cycle has many checkpoints,

FIGURE 1

Brain plate—
National Library
of Medicine.

there is a “go/no-go” point near the end of G1. If G1 is completed successfully, the cell is prepared to move to the next phase.

During the S phase, DNA is replicated and chromosomes are duplicated. G2 is the second phase of protein synthesis and growth. The M phase represents the four stages of mitosis and is regulated by multiple growth factors (genes), such as cytoplasmic cyclins (eg DNA), cyclin-dependent kinases (CDK 2, 4, and 6) and a complex that promotes anaphase. The points at which these substances must activate or deactivate other mechanisms in the cycle are the key points of vulnerability in the processes of oncogenesis (the formation of tumors).

In essence, all cancer results from some type of malfunction in the cell cycle that makes the cell act outside the normal rules for growth and reproduction. More information in a readily available format can be found at The Biology Project at Arizona University (www.biology.arizona.edu) and at the Cancer Genetics organization Web site (www.cancer-genetics.org). More academically oriented information is available at PubMed at the National Library of Medicine Web site. (Click on “Related Organizations” on the AST Web site.)

Glial cell function

Until recently, glial cells were believed to have relatively limited functions and were viewed as sup-

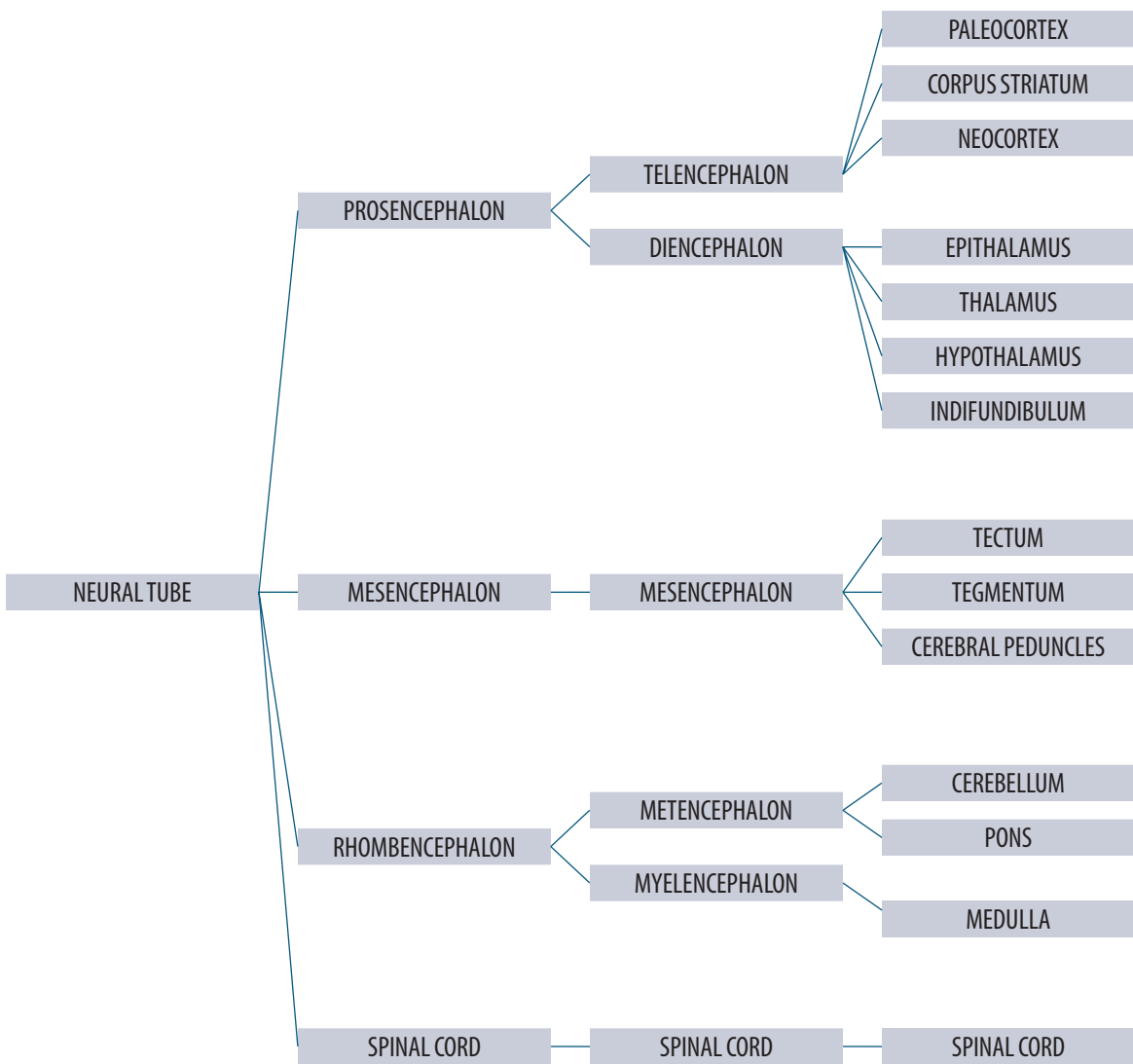


FIGURE 2

Cell differentiation process in the central nervous system—neuron, astrocyte and oligodendroglia.

Adapted from Carlson BM. Human Embryology and Developmental Biology. Mosby, St Louis. 1999.

porting cells, hence the concept as the glue of the nervous system. However, glial cells have important metabolic functions, since they are invariably interposed between neurons and the blood vessels supplying the nervous system. They are responsive to electrical input, produce substances that may function as neurotransmitters, and possibly play a role in the memory process.

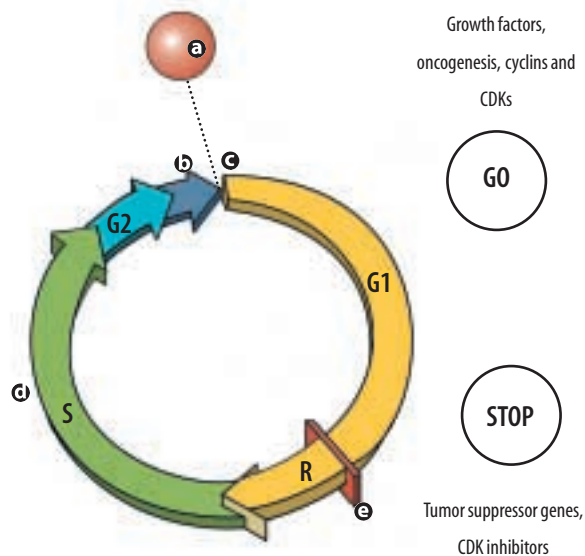
Glial cell oncogenesis

The term cancer can be applied to more than 100 diseases. In the last 25 years, tremendous advances have been made in understanding the mechanisms of cancer formation and therapeutic approaches, but much remains unknown. More-

ancestral cell that initiates this aberrant behavior. Depending on the location and type of cell, the proto-oncogene of the primary ancestral cell mutates and initiates the program of inappropriate reproduction that is carried forward by the cells of its familial line.

For a cell to become cancerous, then, a number of events are required:

- multiple genetic mutations in select genes^{5,18,36,37}
- activation of a proto-oncogene
- inhibition of tumor suppressor genes
- deactivation of the executive cell-cycle clock
- successful defense against apoptosis.



over, in many instances, what is known in the research community is yet to be translated into clinical application.

FIGURE 3

Cell cycle.

- Ⓐ New daughter cell
- Ⓑ Mitosis (cell division)
- Ⓒ Begin cycle
- Ⓓ Synthesis (doubling of DNA)
- Ⓔ Restriction point (point of no return)

There are some 30 trillion cells in the normal human body, most of which follow a very strict reproductive code. Reproduction is inhibited or facilitated by on-going local communication between cells. Cancer cells violate this rule. These cells function outside the normal controls and follow an internal, but altered, genetic agenda. Cancer cells are, in a human analogy, sociopathic. In most of the body, cancer cells are not restricted to local growth but have the ability to migrate from their site of origin to invade nearby tissues. Malignant masses begin from a common

Oncogenes and suppressor genes

There are two gene classes that coordinate the life cycle of the cell and, specifically, the sequence of events governing cell growth and reproduction. Under normal conditions, a balance exists between the proliferation and suppression messages. Proto-oncogenes encourage growth and reproduction; whereas, tumor suppressor genes (the second class of genes) inhibit growth and reproduction. These two gene classes account for much of the normal growth of cells or, when mutated, the uncontrolled proliferation of cells.

Proto-oncogenes

Proto-oncogenes encode a form of protein that functions as part of a genetic relay team to move stimulating signals from the cell's external environment to its internal environment. Cancer cell development requires deregulated cell growth that results when a proto-oncogene continuously energizes proteins, which function as growth factors to act on nearby cells. More importantly, in the case of cancer cells, the new cells also turn back and drive the proliferation of parent cells. It is possible that the growth factor involved is manufactured in normal amounts, but the proteins produced are overly active. In either case, the growth-stimulation pathway is active when it should be inactive. Some known growth factors that present in primary brain-tumor formation are listed in Table 1.

Table 1. Growth factors related to brain tumor formation

GROWTH FACTOR	FACTOR PRESENT ON AND SECRETED BY	SUSPECTED FUNCTION
Epidermal growth factor	Astrocytoma	Autocrine stimulation of astrocytoma cell growth
Fibroblast growth factor	Astrocytoma & meningioma	Autocrine stimulation of astrocytoma cell growth, migration and invasion
Insulin-like growth factor I	Normal brain, meninges and choroid plexus Meningiomas	Autocrine growth regulation in meningiomas
Insulin-like growth factor II	Autocrine stimulation of astrocytoma cell growth	Autocrine growth regulation in meningiomas
Platelet- driven growth factor AA	Astrocytoma	Autocrine stimulation of astrocytoma cell growth
Platelet- driven growth factor BB	Astrocytoma	Paracrine endothelial proliferation
Transforming growth factor- α	Astrocytoma & meningioma	Phenotypic transformation of normal cells
Transforming growth factor- β	Astrocytoma & meningioma	Inhibition of growth of astrocytoma cells
Vascular endothelial growth factor	Astrocytoma	Paracrine stimulation for endothelial proliferation

Note: autocrine designates a process of cellular self-stimulation through the production of both a growth factor and a specific receptor for it, whereas paracrine refers to a type of hormone function that is restricted to the local environment.

Adapted from Greenberg HS, Chandler WF, Sandler HW (1999). *Brain Tumors*. New York, Oxford University Press: Figure 2-1, pg 37.

Tumor suppressor genes

Cells have a number of mechanisms to combat cancer development, including tumor suppressor genes (RB and TP53) that function as brakes on unwarranted growth and reproduction. However, in the case of cancer, the tumor suppressor genes, instead of being activated, are inhibited or suppressed and are unable to stop the runaway cellular division.

G1 and point R

Most of the activities of the cell during a trip through the cell cycle are determined by intracellular factors. An exception occurs during the G1 phase prior to passing the point referred to as R (restriction point, a go/no-go decision). Part of the decision-making process is governed by autocrine growth factors. Other factors communicate with the extracellular environment and provide feedback to help regulate the cell cycle. A “go” decision allows the cell to move to the S phase, while a “no-go” decision stops the cycle and leads to cell death.

P53 and apoptosis

Under normal conditions, there is another mechanism that allows cells to regulate themselves—even when a proto-oncogene overstimulates production and the tumor suppressor cells are inhibited. Cells have the natural ability to commit suicide. The executive decision-

maker in the cell, the cell-cycle clock, regulates the cycle and, thereby, reproduction. A failure in this cycle stops the cell’s normal cell life span.

Even then, cells have one last opportunity to avoid becoming cancerous—apoptosis (cellular suicide), but there are restrictions. Developing cancer cells have several defense strategies they can employ to avoid apoptosis. A nuclear protein known to act as a tumor suppressor, called p53, is “the guardian of the genome,” and performs three critical tasks:

- a major role in the transcription of DNA
- regulation of cell growth
- regulation of cellular proliferation.

In the cell cycle, p53 functions as an emergency break and appears to be involved in apoptosis. Mutation in this protein results in the loss of its ability to block abnormal cell growth. (Interestingly, this mutation switches the protein’s role from a suppressor to a stimulator of cell division and a promoter of cancer development.) A p53 mutation occurs in nearly one half of all human cancers, including the more aggressive cancers of the breast, cervix, colon, lung, liver, bladder, and skin. P53 is mutated in most astrocytomas and almost universally in glioblastomas. This mutation is important to treatment success, because the effectiveness of radiotherapy and some chemotherapy depend on triggering cell suicide.

When the p53 protein mutates, tumors are more difficult to treat, reducing the likelihood of a successful response to therapy.^{5,11,16,20,27}

There are two basic mechanisms that control cell death: apoptosis and necrosis. Apoptosis is characterized by morphological changes in the nucleus and cytoplasm. The ability to kill a cell serves as a balance to mitosis and helps regulate cellular growth.^{5,11,16,20,27}

Essential to life, apoptosis governs which cells are programmed to die given certain physiological and developmental stimuli. P53 encodes a transcription factor that mediates cellular response to environmental damage, halts cell division so the cell can repair itself, or triggers apoptotic death. Several genes turn on the apoptotic process, but researchers are still looking for the underlying explanations.

Other genes also trigger the essential “off” message. For instance, cells in the immune system identify and kill “non-self” T-cells. In the brain, neural cells are connected by a process that “chips away” unwanted cells to create the desired synaptic pathway, much like a sculptor creating form from a piece of marble.

Select astrocytoma cell characteristics

Astrocytoma cells demonstrate several abnormal characteristics including:

- changes in DNA (chromosomes) and gene expression
- production of growth factors
- changes in kinase receptors.

This article includes only a brief discussion of each with select examples that do not represent the complex genetic and biochemical processes of the astrocytoma cell.

Chromosomal mutations

The astrocytoma shows a series of sequential changes that includes both the amplification and loss of genetic material as the tumor develops and the malignancy evolves. Early in development, there is a loss of genetic material on chromosomes 6, 13, 17, and 22. P53 is located on chromosome

17 and, in 75% of the cases of astrocytoma, has mutated. The effects of this mutation are probably exerted during early changes in the cell as it evolves from a normal to an abnormal cancer cell.

Cancer cell activities

Some cancers are capable of forming mass lesions well beyond the regional site. Malignant brain tumors do not metastasize to other areas of the body. However, high-grade gliomas are capable of aggressively invading surrounding tissue, crossing to the opposite hemisphere, and seeding the spinal cord with cancer cells.⁵

Angiogenesis

Although foreign in normal adult cells, angiogenesis is a natural part of embryonic development and requires two-way intracellular communication.^{5,9} Tumor cells need to create a new arterial supply to support their high-nutrient needs, and growth factors are produced in tumor cells that are not present in normal glial cells. In some instances, these growth factors may be correlated to the malignancy grade (eg a low-grade glioma may produce small amounts of the growth factor, while the high-grade glioblastoma produces large amounts). It is important to see that the fast-growing cell needs the blood supply, but the increasing blood supply makes it easier for the cell to grow and divide, promoting uncontrolled growth.

Glioma types and staging

The earliest system of histologic classification for gliomas was created by Bailey, a neuropathologist, and Cushing, a neurosurgeon, in 1926. In the introduction to their book on the subject the authors state the problem of the day.^{4,5,6}

“The impression in both laboratory and clinic that the microscopical examination of a specimen removed at operation will not serve to predict, with any degree of certainty, the future course of development of a true tumor of the brain substance. These lesions, which are commonly grouped together as gliomas, represent about forty per cent of all intracranial neoplasms. They exhibit a bewildering variety of microscopical structure, and

Table 2. Select tumors of the neuroepithelium

1	Astrocytoma
2	Anaplastic astrocytoma
3	Glioblastoma multiforme
4	Gliosarcoma
5	Pilocytic astrocytoma
6	Anaplastic pilocytic astrocytoma
7	Oligodendroglioma
8	Anaplastic oligodendroglioma
9	Oligo-astrocytoma
10	Anaplastic oligo-astrocytoma
11	Ependymoma
12	Anaplastic ependymoma
13	Choroid plexus tumors

*existing classifications have served to do little more than add confusion to a complicated subject.*⁵

Thus stated, they began the search for a histologically based classification system. The intent was to answer three questions:

1. What is the basis of the structural variability shown by gliomatous tumors?
2. What, if any, is the clinical significance of the various structures?
3. Can the histologic variability account for longer-than-expected survival periods following debulking of the tumor?

While techniques have changed radically, the basic questions remain. Bailey and Cushing produced a hierarchical system with 13 tumor types (Table 2) and a prognosis for each. Kernohan and Sayre offered a different system that defined five subtypes—astrocytoma, oligodendroglioma, ependymoma, gangliocytoma, and medulloblastoma.²⁷ More importantly, they added a grading system. The system used today was initially developed by neuropathologists under the oversight of the World Health Organization (WHO) and published by Zulch.³⁷ This effort was intended to clarify issues of the day and provide a single international grading system. New questions naturally arose, so Kleihues, Burger, Scheithaueuer produced a revised version for WHO following two international meetings in the late 1980s.²⁴ The

primary question during the late 1980s was whether grading systems were helpful. The usefulness of a grading system for brain tumors seems to have depended on viewpoint. The scientific community found grading systems generally unhelpful, while the clinicians found them helpful. The revision was something of a compromise. It allows for a grading system because of its clinical usefulness, but it did not find a grading system helpful for histological identification and research. If used for these purposes, the grading system was required to be identified.

Grades

Once tumors were graded, some new issues arose. For instance, is a grade IV astrocytoma equivalent to a GBM, or is there a clinically identifiable distinction between the two (Table 3). Other questions that arise with grading are:

1. Does the grade assigned affect treatment strategy?
2. Does grade assignment correlate to prognosis?

There are some common features used in grading neuroepithelial tumors: (1) nuclear atypia, (2) cellular pleomorphism, (3) mitotic activity (4) angiogenesis or vascular proliferation, and (5) development of necrotic areas. The more features that are present, the more malignant the tumor.

The astrocytoma group

Pilocytic astrocytomas are benign, typically occur in children and young adults, and are frequently located in the thalamus or other important subcortical locations. The histologic borders are usually defined accurately by MRI contrast enhancement. Surgical removal is usually total and produces excellent results.

Grade 2 astrocytomas exhibit pleomorphism, an increase in the number of cells and show no indications of mitotic activity or necrosis, demonstrate hypodensity on CT and prolongation of T1 and T2 on MRI. Typically they do not show any contrast enhancement.^{5, 8, 20, 31, 38}

Frequently referred to as malignant astrocytomas, Grade 3 and 4 astrocytomas show contrast

enhancement on imaging studies. The contrast enhanced mass may be surrounded by an area of hypodensity on the CT scan or prolonged T1 and T2 on the MRI. This area consists of edematous brain parenchyma that has been infiltrated by isolated tumor cells. Commonly, Grade 3 astrocytomas are called anaplastic astrocytomas and Grade 4 astrocytomas, glioblastomas.^{5,8,20,31,38}

Anaplastic astrocytoma

The cells of Grade 3 or anaplastic, astrocytomas, are abnormal in appearance. Mitotic evidence also appears in some cells. Some cells may infiltrate individually into normal brain tissue; other malignant cells continue to divide, destroy

summary, CT and MRI scans show a contrast enhancing mass with a necrotic center which is surrounded by a zone of hypodensity on CT and prolonged T1 and T2 on MRI correlating to the infiltrated brain tissue.^{5,8,13}

Prognostic indicators

In general, it is clear that several factors correlate to length of survival. The greater the number of indicators present in any given tumor, the shorter the life expectancy.^{4,5,12,17} However not all indicators carry equal weight in a given tumor type and grade. Prognostic indicators for anaplastic astrocytomas include the presence of endothelial vascular proliferation and/or mitotic activity, both of which reduce survival time considerably.¹⁵ Age is a strong prognostic indicator for patients in their early 50s and younger, living significantly longer than those over 60 at the time of diagnosis.^{4,5,12,17}

Table 3. Select glioma types correlated to grades

TUMOR TYPE	GRADE 1	GRADE 2	GRADE 3	GRADE 4
Astrocytoma	NA	Yes	NA	NA
Anaplastic astrocytoma	NA	NA	Yes	NA
Glioblastoma multiforme	NA	NA	NA	Yes
Gliosarcoma	NA	NA	NA	Yes
Pilocytic astrocytoma	Yes	NA	NA	NA
Anaplastic pilocytic astrocytoma	NA	NA	Yes	NA
Oligodendroglioma	NA	Yes	NA	NA
Anaplastic oligodendroglioma	NA	NA	Yes	NA
Ependymoma	Yes	Yes	NA	NA
Anaplastic ependymoma	NA	NA	Yes	Yes
Choroid plexus carcinoma	NA	NA	Yes	Yes

Grade 1=benign; Grade 2=low grade malignancy; Grade 3=malignant; Grade 4=highly malignant.

the brain parenchyma at the site of origin, become attached, and form a mass lesion.^{5,17}

Glioblastoma multiforme

Grade 4 astrocytomas are commonly called glioblastoma multiforme, the most malignant type of brain tumors. Infiltrated areas of brain tissue may also have areas of mass lesion within them. Mitotic signs are frequently seen. These tumors grow so fast that they have a lot of angiogenic activity associated with them and readily enhance on diagnostic scans. Even with this angiogenic activity, the tumor often outgrows its blood supply. This results in cell death and areas of necrosis that show on diagnostic scans. In

Epidemiology of gliomas

Approximately 16,000 people were diagnosed with a primary brain tumor in 1991. In this group, 35% to 45% were diagnosed with a form of malignant astrocytoma. The average incidence for glioma in adults ranges between 5 and 5.4 per 100,000 annually. Of all gliomas diagnosed, 65% are malignant astrocytomas.

Age is a significant risk factor with the rate increasing from 2 per 100,000 at ages 35 to 44 years of age to 17 per 100,000 for ages 75 to 84. Looking only at the elderly (over 70 years) of Kumamoto, Japan, Kuratsu and Ushio (1997) researchers found that primary intracranial tumors were diagnosed in 271 cases with 155 being confirmed histologically. In Kumamoto, this produces an incidence rate of 18.1 per 100,000, with females having the greater overall risk, 20.3, with the average at 15.2 per 100,000. Interestingly, the 70-74 age group ran a higher risk than the older groups. Slightly more than 50% were meningiomas with the next highest type being malignant gliomas (all sub-types combined). These two were followed in order by pituitary adenomas, schwannomas, malignant lymphomas and benign astrocytomas.

In the United States, gender and race are also risk factors for malignant astrocytoma. Both anaplastic astrocytoma and glioblastoma multiforme are more common in men than women, with the ratio varying between 1.06 and 2.1. However, in a British study, Hopewell, Edwards, and Wiernik noted that it is not until one reaches the 45-49 age group that males show a significantly higher risk than females. In the African-American community only 37% of primary brain tumors are malignant astrocytomas, while in the Caucasian community astrocytoma and malignant astrocytoma occur more than 50% of the time.⁵ Genetics also appear to play a role in some types of primary brain tumor.

Increasing rate of incidence?

The incidence of primary malignant brain tumor in the industrialized countries has been reported to have grown dramatically over the past 25 years. This increase has been reported to be as high as 40% in the general population and 100% in those older than 65. The validity of this data is under current debate, with some researchers arguing that better diagnostic imaging techniques have produced the increase and not a true rise in per capita primary brain tumors.

In Rochester, MN, Radhakrishnan, et al (1995) reviewed the incidence of intracranial tumors from 1950 to 1990. The researchers concluded that the increased rate of primary brain tumors was due to a statistically significant increase in pituitary adenomas and better imaging techniques. They did not find a significant change in occurrence of other tumors. This study, too, has been challenged and debate continues. It seems likely two factors affect the overall increase in primary brain tumors: better imaging techniques and the increasing number individuals over 65 years of age.

About the author

Bob Caruthers received both his bachelor's and doctoral degrees from the University of Texas at Austin. His interest in neurology has persisted since his first scrub on a craniotomy in 1970. Dr

Caruthers has published several articles in the Surgical Technologist Journal.

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