

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Neoplasms of Central Nervous System: A Diagnostic Approach

Frank Yuan Shan, Dingrong Zhong, Wanming Hu,
Nitesh Patel, Ekokobe Fonkem, Dongxia Feng,
Yilu Zhang, Jason H. Huang and Arundhati Rao

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.76294>

Abstract

Neoplasms of central nervous system accounts for approximately 1% of tumors of the human body, and they can be primary or secondary (metastatic), benign or malignant, and intra-axial or extra-axial. This chapter includes some most common brain and spinal cord tumors, like pituitary adenomas, meningiomas and gliomas, with their clinical, imaging, and histological characteristics for the diagnosis purpose, with additional treatment options and prognosis.

Keywords: brain tumors, central nervous system (CNS), World Health Organization (WHO), WHO grades, pituitary adenomas, meningiomas, gliomas, diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III), glioblastoma (WHO grade IV), oligodendroglioma (WHO grade II), anaplastic oligodendroglioma (WHO grade III), magnetic resonance imaging (MRI), computerized tomography (CT), immunohistochemical stain (IHC), isocitrate dehydrogenase-1 (IDH-1), fluorescent in situ hybridization (FISH), chromosome 1p/19q co-deletion, MGMT, molecular diagnosis, neuroimaging study, dural tail

1. Introduction

Human central nervous system (CNS) is composed of brain and spinal cord and their covers. The neoplasms of human CNS account for about 1% of all human body tumors. Location is important information for making diagnosis of brain tumors. Generally speaking, the tumor of the CNS are divided into two large categories: intro-axial and extra-axial tumors; intro-axial

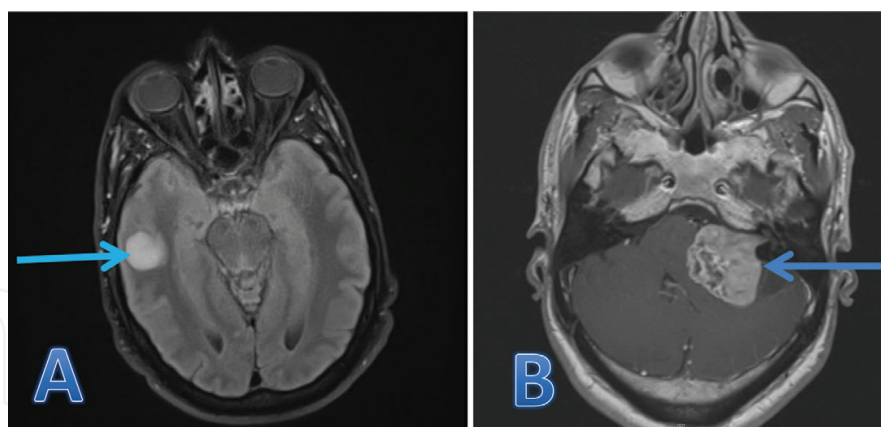


Figure 1. A well-defined right temporal intra-axial hyperintense lesion on FLAIR, see arrow (a); a heterogeneous extra-axial mass at left cerebellopontine angle (CPA) with internal auditory meatus extension on post-contrast T1WI, see arrow (B), is classic for Schwannoma/acoustic neuroma.

tumors mean those tumors that arise from the brain/spinal cord parenchyma, such as astrocytomas and glioblastomas while extra-axial tumors indicate those tumors that originate from the covers or nerve roots of brain/spinal cord, such as meningiomas and schwannomas (**Figure 1**). We include in this chapter only a few most common brain tumors [1].

Tumors of CNS include benign and malignant tumors, based on the World Health Organization (WHO) classification, brain tumors have four grades, from grade I to IV. Grade I tumor is true benign one, like pilocytic astrocytomas in children, with partial or complete surgical resection, the tumor will be cured. Grade II tumors are low-grade malignant potential with both recurrent tendency and progression to higher-grade tumor even after being completely surgically removed. Grade III is high-grade malignant tumor, it not only can be progressing to higher-grade tumor but also has greater recurrent potential, while the grade IV tumor is very high-grade malignant tumor, such as glioblastoma (GBM), even with current combined surgical, radiation and chemotherapies, patients' expected survival time is only approximately 22–24 months [1].

2. Pituitary adenomas

The anterior pituitary gland (adenohypophysis) is an important organ for human development and physiological functions, which comprises several different cell types, responsible for the synthesis and secretion of a specific hormone or group of specific hormone (plurihormonal), such as growth hormone (GH), adrenocorticotrophic hormone (ACTH), and prolactin (PRL). Each of these cell types may give rise to a discrete pituitary adenoma subtype that is either hormonal active (functional) or inactive (nonfunctional).

As one of the most common pituitary neuroendocrine tumors, pituitary adenomas constitute the overwhelming majority of tumors arising in the pituitary gland and accounts for 10–15% of intracranial neoplasms. Incidental microadenoma (smaller than 10 mm in diameter) may occur in up to 27% of pituitary glands examined at autopsy and up to one-fifth of the population has

a pituitary abnormality on magnetic resonance imaging (MRI) [10]. The monoclonal nature of the majority of pituitary adenoma subtypes was supported by data from LOH (loss of heterozygosity) analysis and by X-chromosomal inactivation analysis in female patients. Which strongly suggests the view that pituitary adenomas result from a clonal expansion of a single mutated pituitary cell [10] (**Figure 3**).

2.1. Clinical features

The clinical manifestations of pituitary adenomas can be divided into two categories: The first is the endocrine functional changes caused by pituitary adenoma; the second is the local space-occupational effect caused by tumors. They can also invade downwards into the paranasal sinus, laterally into the cavernous sinus (to compress cranial nerves leading to ophthalmoplegias) and upwards into the brain parenchyma (**Figure 2**). Endocrine changes include amenorrhea, lactation, obesity, acromegaly and gigantism, and hypopituitarism, and so on [10]. By testing six types of hormones secreted by the pituitary gland, functional pituitary adenoma can be detected. For example, among adult pituitary adenomas, PRL producing adenomas are the most common hormone-secreting tumors, in which the elevated serum prolactin (prolactinemia) level may be helpful for diagnosis. While nonfunctional pituitary adenomas are often found late and are often found when secondary symptoms of compressing adjacent surrounding tissue becomes apparent, which includes visual field defects, especially the temporal visual field defects, blindness by compressing optic chiasm, and headache, and so on. While the majority of pituitary tumors are nonmetastasizing and slowly growing adenomas, some cases (the proportion is low, but the exact percentage is unknown) will become invasive and a very small portion (approximately 0.1%) will become malignant [9]. Add more growth pattern in different directions.

2.2. Radiologic finding

Pituitary adenomas are characterized by CT low-density occupying lesions on pituitary or MRI (T1WI) low-signal occupying images (**Figure 1**). Contrast enhanced CT and MRI can

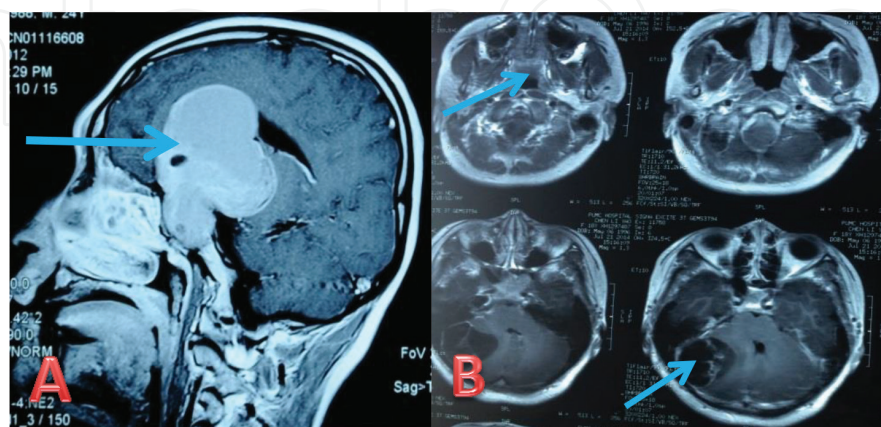


Figure 2. MRI sagittal T1 W1 shows this enhanced mass of giant pituitary adenoma, see arrow (a). MRI shows occupied masses in both sellar region and cerebellum, see arrows, based on the patients' history, the lesions most likely a pituitary carcinoma with metastasis to cerebellum (B).

significantly improve the display and diagnostic rates of pituitary adenomas, especially microadenoma. The pituitary microadenoma with diameter less than 10 mm has little effect on the surrounding bone tissue around the sellar region; however, pituitary adenomas with diameter larger than 10 mm (often referred to as macroadenomas) often result in enlargement of the sellar region and even destruction of the surrounding sphenoid bone (**Figure 2**). Pituitary adenomas with destructive behavior are often diagnosed as invasive pituitary adenomas by radiology experts [10].

2.3. Microscopic finding and diagnosis

The majority of pituitary adenomas are composed of monotonous anterior pituitary gland cells in similar size and shape with abundant small vessels and variant amount of cytoplasm (**Figure 3**). Compared with the normal histology of the pituitary gland, the reticular fibers in pituitary adenomas are significantly reduced, so the reticulin stain can also be helpful to differentiate pituitary adenoma from pituitary hyperplasia, the latter shows only expansion rather than rupture of pituitary acini demonstrated by reticulin stain. The monoclonal nature of the majority of pituitary adenoma subtypes was supported by finding that the tumor cells have the similar morphology (**Figure 3**). The pituitary adenoma subtypes secreting different hormone subtypes are slightly different in morphology and also differ in clinical biological behavior.

2.4. Immunohistochemical findings

A minimal panel of immunostains is helpful for the diagnosis of pituitary adenomas, which includes synaptophysin and low-molecular weight cytokeratin CAM 5.2. Pituitary adenomas, as most neuroendocrine tumors, are uniformly immunoreactive for synaptophysin (**Figure 4**) and variably for chromogranin A (another neuroendocrine marker) and CAM 5.2. In addition, synaptophysin immunohistochemically positive in a finely fibrillary pattern even highlights small fragments of posterior pituitary gland that can be also sometimes included in the specimen, especially in specimens being assessed for Rathke's cleft cyst.

In terms of specific anterior pituitary hormones necessary for subtyping pituitary adenomas, FSH, LH, TSH, ACTH, GH, and PRL (**Figure 4**) as a small group is highly recommended [7, 8].

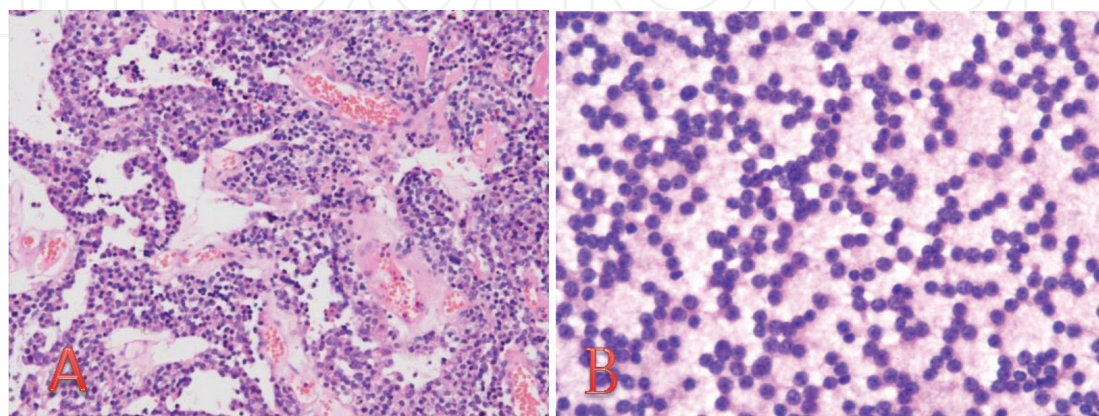


Figure 3. Monotonous tumor cells in size and shape with abundant small vessels in pituitary adenoma on H&E section (a) (H&E stain $\times 100$), and smear (B) (H&E stain $\times 200$).

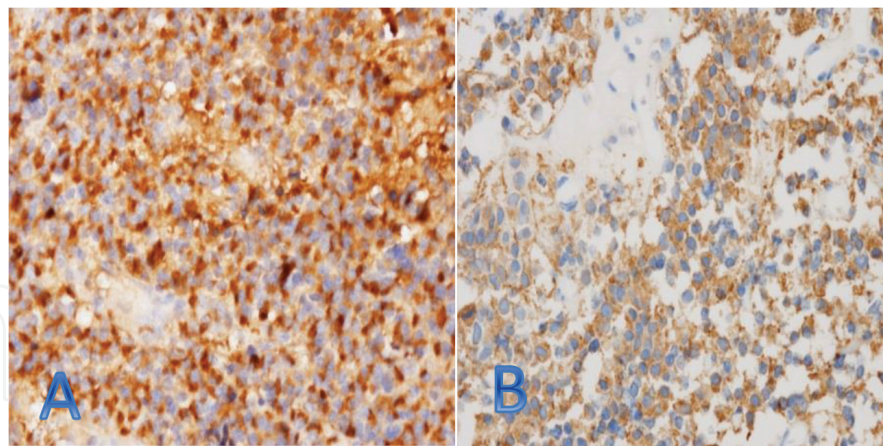


Figure 4. Tumor cells of this pituitary adenoma are immunoreactive for PRL (a), and synaptophysin (B) (immunohistochemical stains $\times 100$ (brown color indicates positive stain)).

In recent 2017 WHO classification, a major technique for tumor classification is the immunohistochemical stain, with the combination of immunostains for the main pituitary hormones (GH, PRL, ACTH, β -TSH, β -LH, β -FSH, and α -subunit of glycoproteins) in order to determine the tumor cell's lineage. In some conditions, if the immunohistochemical staining result is inconclusive, immunostains for the pituitary transcription factors (PIT-1, SF-1, T-PIT) may be necessary. In addition, immunohistochemical stains can be used for the subclassification of adenomas variants. For example, low-molecular-weight cytokeratin is very helpful in identifying fibrous bodies in sparsely granulated somatotroph and in acidophilic stem cell adenomas; cytokeratin also highlights corticotroph cell differentiation and Crooke's hyaline changes. With the combination of morphology and immunohistochemical markers, there is minimal need for ultrastructural analysis (electron microscopic) for the classification of the adenomas [9].

According to the 2017 WHO classification of tumors of the pituitary gland, **Table 1** summarizes how these pituitary neuroendocrine tumors are separated according to their clinical behavior. A large proportion of tumors are the adenomas (typical) that show low probability for recurrence. At the other extreme are the pituitary carcinomas, malignant tumors that by definition already have metastasis when diagnosed. Several tumors have higher probability

| Low probability for recurrence | High probability for recurrence | Malignant (metastatic) tumor |
|--------------------------------|---|------------------------------|
| Pituitary adenoma | Adenomas with elevated proliferative activity | Pituitary carcinoma |
| | Special subtypes (variants) of adenomas: | |
| | Sparsely granulated somatotroph adenoma | |
| | Lactotroph adenoma in men | |
| | Silent corticotroph adenoma | |
| | Crooke cell adenoma | |
| | Plurihormonal PIT-1 positive adenoma | |

Table 1. Likelihood of recurrence of pituitary neuroendocrine tumors.

for recurrence than the typical adenomas including adenomas with elevated proliferative activity and the special variants of adenomas previously mentioned [9].

In some pituitary adenomas, prominent cellular atypia can be observed but which is not indicative of malignancy. To date, there is no definitive morphological index and marker for malignant pituitary adenoma, which is consistent with endocrine tumors in other organs. Pituitary carcinoma (**Figure 5**) can be diagnosed only after a definitive metastasis of pituitary adenoma is identified [9]. Atypical pituitary adenomas with biological behavior between pituitary adenomas and pituitary carcinomas were abolished in the recent 2017 WHO classification of pituitary adenomas [9], the reason is that the exact diagnosis of atypical pituitary adenoma is difficult to be unified and quantified (the cutoff of Ki-67 varies in different studies). According to previous studies, the positive rate of Ki-67 and the mutation of P53 tumor suppressor gene are closely related to the invasiveness of pituitary adenomas. Ki-67 and P53 (**Figure 6**) may be an important marker for evaluating the risk of recurrence and invasiveness of pituitary adenomas. Many indicators have been included in the study of pituitary adenomas, such as GSP (G proteins super-family), ras, and PTTG (Pituitary Tumor Transforming Gene), CCND1 (cyclin D1 gene), MEN1, Rb (Retinoblastoma susceptibility gene), p16/CDKN2A (the cyclin-dependent kinase inhibitor p16), *p27/Kip1*, and so on, but now it seems to be of limited clinical significance. Attempts at identifying potential aggressive adenomas should be made on an individual basis by considering the histology, mitotic index, Ki-67 labeling index, and tumor invasiveness [9]. Furthermore, recognition of adenoma variants that have intrinsic substantial risk for recurrence and poor clinical behavior is imperative [9]. Therefore, more research in order to find a reliable and accurate biomarker reflecting biological behavior of pituitary adenomas will be our future goal.

2.5. Treatment for pituitary adenomas

Nonfunctional pituitary macroadenomas are typically managed with transphenoidal surgical resection followed by radiation. With the exception of prolactinomas, functional pituitary macroadenomas require surgical resection via a transphenoidal approach with subsequent radiotherapy, and some patients may also benefit from medical therapy in the postoperative period depending on the specific type of adenoma. After surgical resection and radiation therapy of growth hormone secreting pituitary adenomas, octreotide is often administered to suppress

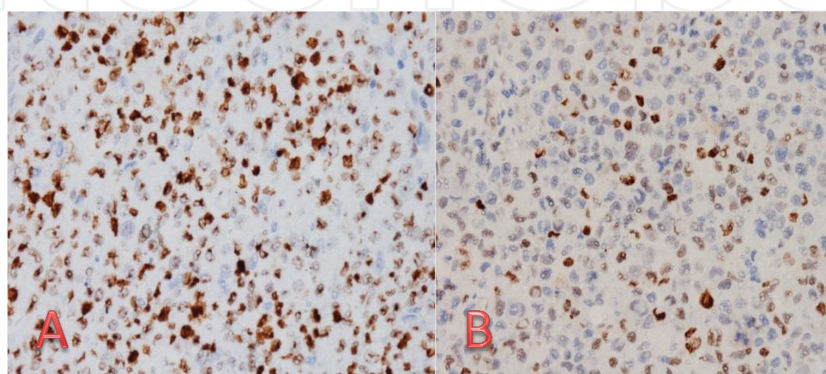


Figure 5. This picture shows the tumor from cerebellar mass of **Figure 1B** as a metastatic pituitary carcinoma, the tumor cells show larger nuclei with prominent nucleoli (arrow), indicating its malignancy (H&E stain $\times 400$).

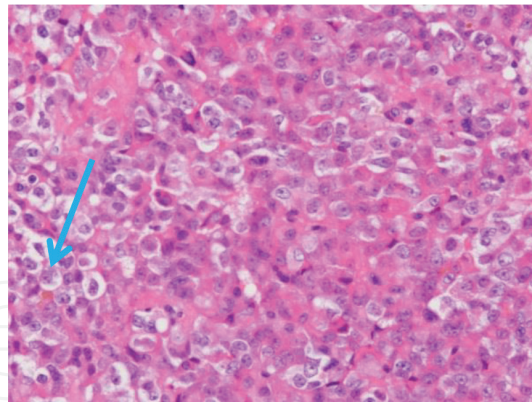


Figure 6. High expression of Ki-67 proliferation index in tumor cells (a) and approximately 30% of tumor cells express mutant P53 protein by nuclear stain (B), both suggesting an aggressive behavior of this tumor (immunohistochemical stains $\times 200$).

growth hormone secretion given that lower postoperative growth hormone levels correlate with a greater probability of remission after radiotherapy. Corticotropin secreting pituitary adenomas undergo surgery and radiotherapy, but medical therapy is only utilized in patients who fail or decline therapy; central or peripherally acting agents are available for use. Gonadotropin secreting macroadenomas are also treated with surgical resection and subsequent radiation, but medical therapy such as bromocriptine or octreotide are only administered to patients who deny surgery or radiation. Thyrotropin secreting macroadenomas respond well to octreotide after surgery and radiation and thus can be a useful adjuvant therapy [13–16].

3. Astrocytic and oligodendroglial neoplasms

Taken together, these neoplasms are also referred to as “gliomas”, which are the most common intro-axial tumors of the central nerve system (CNS). The current classification of these tumors is primarily based on the World Health Organization (WHO) classification which is continually updated and internationally accepted [1].

Based on the WHO classification, the gliomas are divided into astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas, and includes the criteria for grading of each tumor from grade II to grade IV, the highest-grade IV tumor as glioblastomas (GBM). The low-grade tumor, although only grade II, due to the diffusely infiltrating nature of the tumor, and their strong tendency toward progression and upgrading, with destruction of the normal brain architecture, the prognosis of those tumors are poor and post a great challenge to the diagnostic pathologists and oncologists.

4. Diffuse astrocytomas (WHO grade II)

4.1. Definition

The WHO grade II diffuse astrocytoma is a well-differentiated, diffusely infiltrative neoplasm [1, 2].

4.2. Clinical features

Diffuse astrocytomas most frequently affect the cerebral hemisphere of young to mid-age adult patients and the brainstem and thalamus of children. Occasionally, the tumors also occur in the cerebellum or spinal cord. Those better circumscribed (non-infiltrating) astrocytomas should be distinguished from diffuse astrocytomas, since the latter carries much worse prognosis [17–24].

Diffuse astrocytomas may produce nonspecific symptoms of mass effect, seizures, and neurologic deficits according to the lesions' size and location, and rate of growth. Seizures are more common than functional deficits due to cerebral parenchymal destruction, which occur more often in highest-grade tumors. Brainstem astrocytomas produce neurologic signs caused by dysfunction of cranial nerve nuclei and compression of the sensorimotor tracts that traverse the pons and medulla [2].

The infiltrating and diffuse forms of astrocytoma occur throughout the CNS, primarily affecting adult patients and mostly involving the cerebral hemispheres. Hypercellularity with nuclear atypia are key points for diagnosis, although mitosis is rarely identified, and necrosis and vascular proliferation should not be observed in this tumor. The tumor can progress to higher grade, like anaplastic astrocytoma (WHO grade III), and even glioblastomas (WHO grade IV). The infiltrating nature of the tumor makes completely surgical resection without damaging the functional cerebral area almost impossible and its resistance to conventional therapy making this type of tumor a medical challenge.

4.3. Radiologic finding

By magnetic resonance imaging (MRI), diffuse astrocytomas (grade II) are present as ill-defined areas of low signal intensity on T1-weighted images. Due to their content of bright edema fluid, they are even more obvious on T2-weighted or fluid-attenuated inversion recovery (FLAIR) images (**Figure 1A**). Contrast enhancing should not be observed in grade II diffuse astrocytomas [2].

4.4. Microscopic finding

Hypercellularity and nuclear atypia are the important diagnostic features for grade II diffuse astrocytomas. Those lesions are primarily located in white matter. The tumor cells widely infiltrate the gray matter. The margin of such tumors is indistinct since tumor cells lie individually dispersed within intact parenchyma and often follow fiber pathways for some distance. Such tumor spread is often more apparent in anaplastic astrocytoma (grade III) or glioblastoma (grade IV). Astrocytomas often infiltrate the overlying cerebral cortex or deep hemispheric structures. The so-called “secondary structures of Scherer” indicate the proliferation of tumor cells in subpial, perivascular, perineuronal, and subependymal zones. Such characteristic changes are usually more prominent in oligodendrogliomas, astrocytomas undergoing anaplastic transformation, or in gliomatosis cerebri [1, 2].

In addition to hypercellularity, almost all astrocytomas possess cells with enlarged, cigar-shaped, irregular and hyperchromatic nuclei. Prominent nucleoli are uncommon in grade II

diffuse astrocytomas. As well, mitosis, especially multiple mitoses, should not be observed in most classic grade II diffuse astrocytomas [2].

The cytoplasm of astrocytoma cells varies considerably both in amount and configuration. In some cases, the tumor cells' fibril-free cytoplasm is scant and devoid of processes, making it a feature of "naked nuclei", in some other cases, the tumor cells have varying quantities of fibril-containing cytoplasm and short, often asymmetric processes, which is the basis of the overtly astrocytic nature of this tumor [2]. Gemistocytes (fat astrocytes) occur in varying numbers, and only a few astrocytomas have enough glassy cytoplasm to justify the term gemistocytic astrocytoma. Most tumor cells in gemistocytic astrocytoma show a plump, glassy cell body and an eccentric corona of short, stout-to-delicate processes. Such cells, when scattered and well differentiated, can mimic reactive astrocytes. For reactive changes, the main features are their uniform distribution, minimal increase in number, and symmetric stellate configuration of long-radiating processes. Such cells are best seen in smear preparations or on immunostains for glial fibrillary acidic protein (GFAP).

It is important to know that most gemistocytic tumors fall into the category of anaplastic astrocytoma, or into the highest grade, glioblastoma, when microvascular proliferation and/or necrosis are present (see below).

Most low-grade tumor and reactive changes may contain microcysts and microcalcification, suggesting a long-term process, which are helpful features for making our diagnosis.

4.5. Immunohistochemical findings

Almost all diffuse astrocytomas are both S-100 protein and vimentin protein reactive; the tumor cells cytoplasm often is immunoreactive for GFAP. In gemistocytic tumors, GFAP reactivity is usually more prominent at the periphery of the cells. Staining for p53 protein is variable in diffuse astrocytomas; nuclei are diffusely immunoreactive in approximately one-third of tumors [1, 2].

MIB-1 (Ki67) labeling indices of grade II astrocytoma have generally been less than 2%, often even 1% or less, but there is considerable case-to-case and region-to-region variation.

Immunostains for mutated p53 protein, together with mutated IDH-1 (isocitrate dehydrogenases-1, R132) are used by some people as confirmative markers for the diagnosis of glioma, since both genes and their proteins are involved in the tumorigenesis of gliomas by current understanding (Figures 7 and 8).

4.6. Differential diagnosis

The most important differential diagnosis of glioma is reactive gliosis or reactive changes, which may occur in many conditions, such as infarct, infection, and demyelinating disease. Those lesions sometimes can mimic gliomas on imaging study and mistake pathologic diagnosis may result in unnecessary treatment for the patients and even more severe damage to patients may occur. So the diagnosis of glioma should be made cautiously with more supporting evidence ready before making the final diagnosis [2].

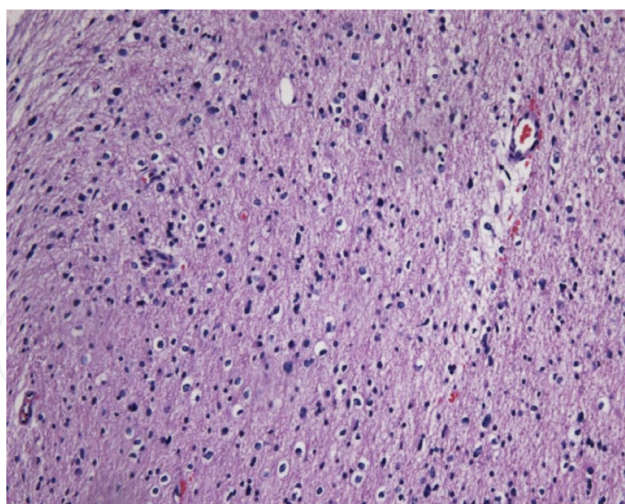


Figure 7. This histologic picture corresponds to the tumor in **Figure 1A**, with increased cellularity and atypia, but no significant mitosis, most likely a low-grade, WHO grade II infiltrating glioma (H&E stain $\times 400$).

4.7. Treatment of gliomas

4.7.1. Low-grade gliomas

Low-grade gliomas (WHO grade I and II) are typically managed with a combination of surgery, radiation therapy, and chemotherapy; however, the precise treatment is often individualized depending on the patient at hand.

The management of WHO grade II tumors, such as diffuse astrocytoma and oligodendroglioma, is more variable. In patients presenting with neurologic impairment, immediate surgical resection is utilized to relieve symptoms and obtain a definitive diagnosis. Asymptomatic patients can elect for surgical resection or choose observation. In patients who opt for careful observation, surgical intervention is indicated if tumor growth accelerates, neurologic impairment develops, or if evidence of transformation to a high-grade glioma is detected. Given that surgery is often not curative, further postoperative therapy with radiation therapy and/or

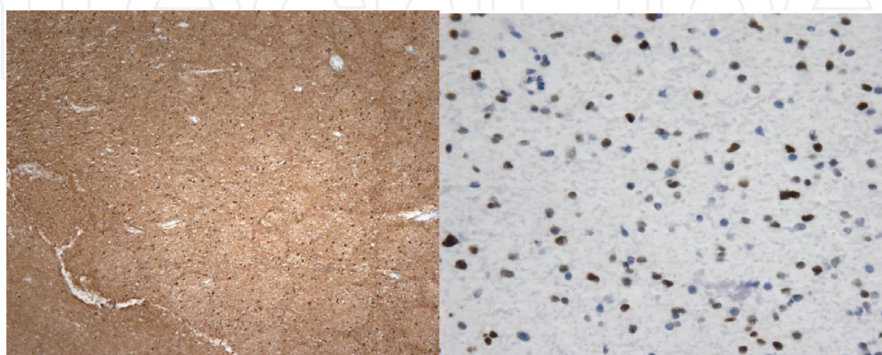


Figure 8. The tumor cells are immunoreactive for GFAP (glial fibrillary acidic protein) (immunohistochemical stain $\times 100$) and p53 (immunohistochemical stain $\times 400$), consistent with WHO grade II diffuse astrocytoma. This tumor is with IDH-1 mutation (no show).

chemotherapy is ultimately needed but the timing will depend on the patient's prognosis. If complete resection is achieved and the molecular characteristics are favorable, postoperative therapy is delayed until recurrence is detected during observation. Conversely, immediate postsurgical chemoradiation is recommended for patients with poor prognostic factors, such as neurological deficits, large tumor size, mass effect, incomplete resection, or advanced age. Conventional radiation therapy at a dose of 50–54 Gy is typically chosen for low-grade gliomas, whereas the chemotherapy regimen is individualized to the patient. PCV (procarbazine, lomustine, and vincristine) and temozolomide are the main regimens, and although no trials have compared these directly, temozolomide is being used more frequently due to improved patient tolerance and ease of administration; despite this, only PCV has proven survival benefit in a randomized control trial [11].

5. Anaplastic astrocytoma (WHO grade III)

5.1. Definition

Anaplastic astrocytoma is a WHO grade III infiltrating astrocytoma, intermediate between grade II diffuse astrocytoma and grade IV glioblastoma [1, 2].

5.2. Clinical features

Generally speaking, anaplastic astrocytomas usually occur one decade later than grade II tumors and a decade earlier than glioblastomas. The tumors in cerebral hemisphere occur most often in the fifth decade, they also appear occasionally in children [1, 2].

5.3. Radiology findings

Some lesions partially enhance on T1-weighted MRI image following administration of contrast agents, but not with the perinecrotic ring (ring enhancing) or “rim” pattern that usually typifies glioblastoma [2]. Still, a significant portion of anaplastic astrocytomas show no contrast enhancing, which makes the pathological diagnosis more informative and important [2].

5.4. Microscopic findings

Anaplastic astrocytoma has more hypercellularity, nuclear pleomorphism, and hyperchromasia; in addition, the presence of mitosis is an important diagnostic feature for grade anaplastic astrocytomas, but lack the necrosis and vascular proliferation, which are the characteristic findings in glioblastomas.

5.5. Immunohistochemical findings

The staining pattern of anaplastic astrocytomas should be the same as grade II diffuse astrocytomas, whose tumor cells are immunoreactive for GFAP and S-100 protein. Not surprisingly, reported ranges of proliferation index of Ki-67 are quite variable, in the range of 5–10% [2].

5.6. Differential diagnosis

The same as for the grade II diffuse astrocytomas, the major differential diagnosis for anaplastic astrocytomas are those of reactive type lesions. The misdiagnosis of demyelinating lesion as a grade III anaplastic astrocytoma may result in unnecessary and fatal radiation therapy to patient, which usually causes a medicolegal issue. In that case, the diagnosis should be made on strong evidence-based practice.

6. Glioblastoma multiform (GBM) (WHO grade IV)

6.1. Definition and general features

Glioblastoma is a highly malignant astrocytic glioma with WHO grade IV that appears to arise either de novo (primary GBM) or in transition from lower grade gliomas (diffuse astrocytoma or anaplastic astrocytoma, secondary GBM). It typically affects adults and is preferentially located in the cerebral hemispheres. The term of “high grade glioma” is sometimes used to describe both anaplastic astrocytoma and glioblastoma. GBM is the most frequent brain tumor accounting for approximately 12–15% of all intracranial neoplasms and 60–75% of astrocytic tumors. In most European and North American countries, the incidence of GBM is in the range of 3–4 new cases per 100,000 populations per year. GBM may manifest at any age, but preferentially affects adults, with a peak incidence at between 45 to 75 years of age. The male female ratio of GBM patients is 1.26 in USA and 1.28 in Switzerland [1, 2].

6.2. Clinical features

Affecting primarily the cerebral hemispheres of adults and the thalamus and brainstem of children, GBM is the most common malignant glioma. It is less likely to occur in the optic nerve and cerebellum and uncommon in the spinal cord. Most cases are solitary cerebral hemisphere mass lesion, but occasional examples appear radiologically separate and warrant the designation multicentric GBM. True multicentricity is difficult to establish, however, even at autopsy [1, 2].

The clinical presentation of patients with GBM are similar to those of patients with better differentiated diffuse astrocytomas, although accompanying neurologic deficits are more frequent, more abrupt in onset, and more rapid in evolution. Unlike lower grade lesions, GBMs are often expansile and edema generating. As a result, they are more likely to produce frank neurologic deficits and signs of increased intracranial pressure. Acute hemorrhage precipitates symptoms in occasional cases. Rapid growth within months or even weeks can be observed in MRI studies [2].

6.3. Radiologic findings

On MRI, GBM usually have an enhancing ring or rim in post contrast T1-weighted images and a generally broad zone of surrounding edema evident in T2-weighted or FLAIR images. The central, hypodense core of the lesion represents tumor necrosis, while the contrast enhancing ring is highly cellular neoplasm with abnormal vessels that permeable to contrast

agents. The peripheral zone of low attenuation indicates vasogenic edema containing varying numbers of isolated infiltrative tumor cells [2].

6.4. Microscopic findings

GBMs are heterogeneous tumors with multiple histological variations, and characterized by nuclear pleomorphism (different nuclear size and shape), active mitoses, necrosis, especially palisading necrosis and/or vascular proliferation. Most times, GBM have an astrocytic quality with small amount of pink cytoplasm, while others may have oligodendroglioma-like structures with prominent perinuclear halos. Small cell GBM is with monotonous small nuclei with or without halos resembling anaplastic oligodendroglioma, while at the other end is the giant cell GBM, which composed of numerous multinucleated monstrous giant tumor cells with large lipid-rich cytoplasm. The infiltrating tumor cells usually have slightly elongated nuclei [1, 2].

Vascular proliferation is characteristic for GBMs, and takes two forms; the most common is forms of globular masses resembling the glomerular tufts of the kidney. Another form of vascular hyperplasia has endothelial proliferation since it is intraluminal and consists largely of endothelial cells within small to medium-sized vessels. This latter type of endothelial proliferation is less common than glomeruloid proliferation and appears to have a more constant correlation with high-grade gliomas and poor prognosis [1, 2].

Necrosis, the second cardinal feature of GBM, takes the form of either large confluent areas of parenchymal destruction, including of vasculature, or small, often multiple serpiginous foci. On MRI scan, it is the large confluent zone of necrosis that comprises the tumor's hypotense center. Necrotic areas, particularly small foci, often feature peripheral accumulation of somewhat radially oriented cells. Such "pseudopalisading" occurs almost exclusively in high-grade astrocytomas [1, 2]. Sometimes, that necrosis is associated with small vessel thrombosis.

6.5. Immunohistochemical findings

The immunophenotype of GBM includes reactive for GFAP, and S-100 and vimentin. Reactive for vimentin is nonspecific and with no diagnostic significance. In addition, immunoreactive for IDH-1 and p53 is suggestive of a slightly better prognosis [1–3].

6.6. Molecular and cytogenetic findings

Currently, the primary GBM is considered associated with EGFR vIII (Epidermal Growth Factor Receptor variant III) amplification, which carries a poor prognosis, while the secondary GBM is associated with mutations of IDH-1 and p53 genes, which suggests a slightly better prognosis due to more sensitive to modern chemotherapy [3].

6.7. Treatment for high-grade gliomas

High-grade gliomas are also initially managed with surgical resection with the goal of gross total resection. Adjuvant chemoradiation is always required, and temozolomide (TMZ) with concomitant radiotherapy is the current regimen of choice. Patients with high-grade gliomas are also encouraged to participate in the clinical trials.

WHO grade III gliomas, such as anaplastic astrocytoma, will require maximal surgical resection if feasible followed by adjuvant therapy depending on the tumor type. If the tumor is not amenable to resection, open or stereotactic biopsy is indicated to guide subsequent adjuvant therapy. Options for adjuvant treatments include temozolomide, PCV (procarbazine, lomustine and vincristine), and fractionated external beam radiation therapy of 60 Gy. The specific combination of treatment will vary depending on the prognostic factors that were previously discussed for grade II gliomas.

Glioblastoma (WHO grade IV) is initially treated with maximal safe resection for all patients. For well-performing patients ≤ 70 years old, postoperative fractionated external beam radiation of 60 Gy is indicated, whereas hypofractionated radiation therapy at a lower dose should be considered for patients > 70 years old; concomitant temozolomide should be employed in both age groups. In poorly performing patients, monotherapy with adjuvant temozolomide is typically utilized instead of subjecting the patient to radiation therapy, but hypofractionated radiation therapy at a lower dose schedule is a potential option. For any patient on temozolomide, combination treatment with an antiangiogenic agent such as bevacizumab is an option but has not been shown to improve overall survival. Another study demonstrated significant efficacy with combination of temozolomide and TTFields [12].

7. Oligodendroglioma and oligoastrocytomas (WHO grade II)

7.1. Definition

Oligodendroglioma is a WHO grade II infiltrating glioma composed at least in part, of cells resembling oligodendrocytes [1, 2].

The patients with oligodendrogliomas have a significant better prognosis than those with infiltrating astrocytomas of comparable grade. The diagnosis of mixed glioma or oligoastrocytoma is largely due to a favorable outcome that will be attributed to the presence of an oligodendroglioma component whereas overgrowth of the astrocytic component will be used to explain cases that recur rapidly as high-grade astrocytoma [1, 2].

7.2. Clinical features

Oligodendrogliomas arise throughout the neuroaxis, but primarily affect adults' cerebral hemispheres. Manifestations of oligodendroglioma include any of the usual consequence of an infiltrating, expanding intracranial neoplasm. Seizures are common at presentation; large lesions may produce signs and symptoms of increased intracranial pressure [1, 2].

7.3. Radiologic finding

A large intracranial lesion on MRI, especially when long segments of the cortical ribbon, are affected. Oligodendroglioma is especially likely when a band of cortical mineralization undulates in a "gyriform" profile. Calcification is not detected in many small oligodendrogliomas, however, grade III lesions are large, and while variably enhancing, generally lack the ring profile so typical of glioblastoma [2].

7.4. Microscopic finding

The tumor cells of oligodendroglioma like to infiltrate the cerebral cortex, although not uniformly distributed, the tumor cells are attractive to neurons (perineuronal satellitosis) and to subpia and perivascular regions.

Oligodendroglioma is characteristic for its histology and cytologic monotony, which means the tumor is likely a uniform blueness to the section, and it is confirmed on closer view as sameness of nuclear size and shape. Round nuclei with small but prominent nucleoli, in all grades, the majority of nuclei are round, especially so in grade II lesions, and somewhat less so in intermediate to higher-grade lesions. In contrast to astrocytomas, the chromatin pattern is more open and bland, and there is often a clearly defined, solitary nucleolus. Large hyperchromatic nuclei may also be observed but not the generalized nuclear pleomorphism in many high-grade infiltrating astrocytomas [2].

Perinuclear halos producing a “Fried-egg” artifact (Figure 9). Oligodendrocytes, whether normal or neoplastic, are susceptible to the autolytic imbibition of water, resulting in the production of a clear perinuclear halo. Perinuclear halos are not specific findings, since they are present in some other tumors, such as clear cell ependymoma and neurocytoma [2].

Microcysts. Particularly common in the cortex, which are filled with protein-like fluid; again it is not specific for oligodendrogliomas, and can be observed in some other tumors.

Delicate capillaries disposed in a “Chicken-wire” pattern (Figure 9). Blood vessels in better differentiated tumors typically consist of short capillary segments arranged geometrically. This arrangement resembles the pattern angulations of “chicken-wire”. It is a “soft” finding with little diagnostic use.

Microcalcifications: Many oligodendrogliomas exhibit some degree of calcification within the tumor or in surrounding brain tissue. It is mainly intracortical, and the deposition takes the form of laminated calcospherites. Vascular walls are often affected in heavily mineralized cases.

7.5. Grading

Accounting to the 2007 WHO system, oligodendrogliomas are generally either grade II or III [1].

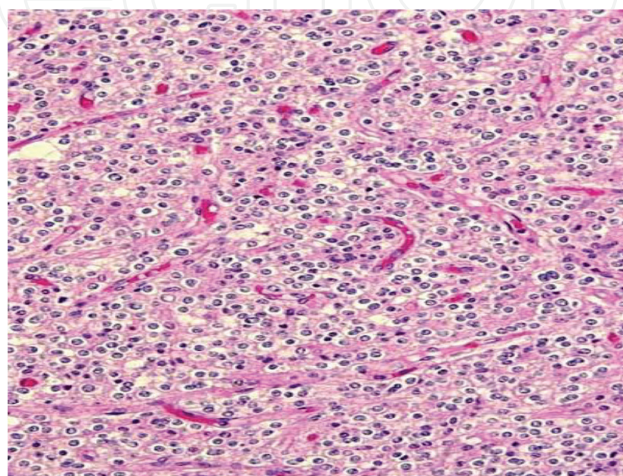


Figure 9. “Fried-eggs and chicken wire” are characteristic histology for oligodendrogliomas (H&E stain ×200).

8. Anaplastic or malignant oligodendrogliomas (WHO grade III)

This cellular tumor is with considerable nuclear hyperchromasia, brisk mitoses, and microvascular proliferation, some with necrosis, but palisading necrosis is usually not seen.

Nuclei of grade III lesions vary with the degree of anaplasia, with those of uniformly round for less anaplasia to those of histologically malignant tumor but still obviously oligodendroglial due to the roundness (more or less) and prominent nucleoli. Truly anaplastic lesions have the nuclear features of high-grade astrocytoma, although they usually retain oligodendroglioma-like round cells, at least in small numbers. Mitosis and microvascular proliferation/hyperplasia are required features in making diagnosis of WHO grade III anaplastic oligodendroglioma [2].

8.1. Immunohistochemical findings

So far, there is no a reliable immunohistochemical marker available that allows the specific and sensitive recognition of human oligodendrogliomas. However, the specific molecular marker is now available for making the diagnosis of oligodendrogliomas (see below).

The proliferation index of MIB-1 (Ki-67) is in the range of 3 to 5% for grade II oligodendroglioma, while the anaplastic oligodendrogliomas have much higher proliferation index by immunostaining for Ki67 [2].

8.2. Molecular marker

The loss of chromosome arms 1p and 19q is an established genetic hallmark of oligodendroglial tumors [3–6]. This combined loss is detected in up to 80% of oligodendrogliomas and up to 60% in anaplastic oligodendrogliomas, with decreasing frequency in mixed oligoastrocytic tumors [6]. The 1p/19q co-deletion has proven its use as both diagnostic and prognostic marker

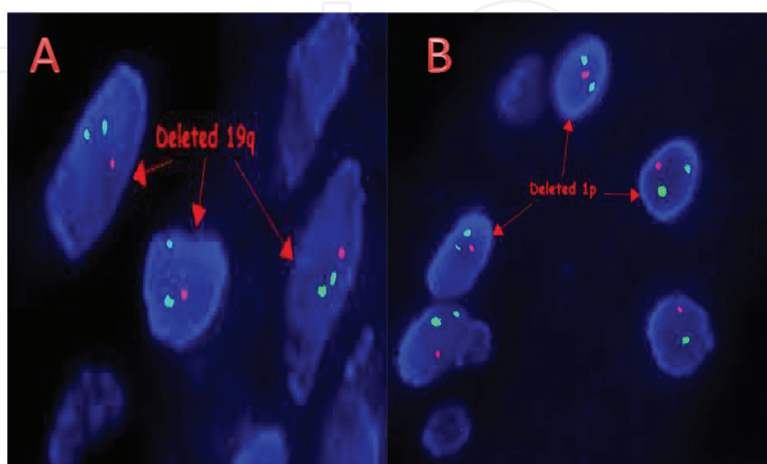


Figure 10. The FISH test shows the 1p (red in a) and 19q (red in B) co-deletion in an oligodendroglioma, the green dots in the pictures as control.

for oligodendrogliomas. The tumor with this genetic mutation has a better response to therapy and a longer survival time, regardless of using chemotherapy, radiotherapy or the combined therapy [4, 5]. Various techniques are available to detect 1p/19q deletion; however, fluorescent in situ hybridization (FISH) is often employed due to its technical ease (**Figure 10**). Another frequently utilized method is loss of heterozygosity (LOH), which is a PCR-based test that compare tumor DNA to the patients “normal” DNA, usually from peripheral blood [5, 6].

9. Meningiomas

9.1. Definition

A group of mostly benign, slow-growing extra-axial neoplasms that most likely derive from the meningotheelial cells of the arachnoid layer. People refer to it as “dura-based tumor.” They are categorized into three WHO grades (I-III) with more than 15 histologic subtypes [1, 2].

9.2. Clinical features

Meningiomas account for about 36.1% of all primary brain tumors. It is the most frequently reported brain tumor in the USA. They are most likely to be found in adults older than 60; the incidence appears to increase with age. Rarely are meningiomas found in children, but pregnant women carry an increased risk for meningiomas. For brain meningiomas, the incidence between male and female is about the same; but for spinal meningioma, it is female patient dominant, with primarily psammomatous subtype meningioma (**Figure 11**) [1, 2].

Meningiomas usually grow slowly and may reach a large size before interfering with the normal functions of the brain. The resulting symptoms depend on the location of the tumor within the brain. Headache and weakness in an arm or leg are the most common symptoms.

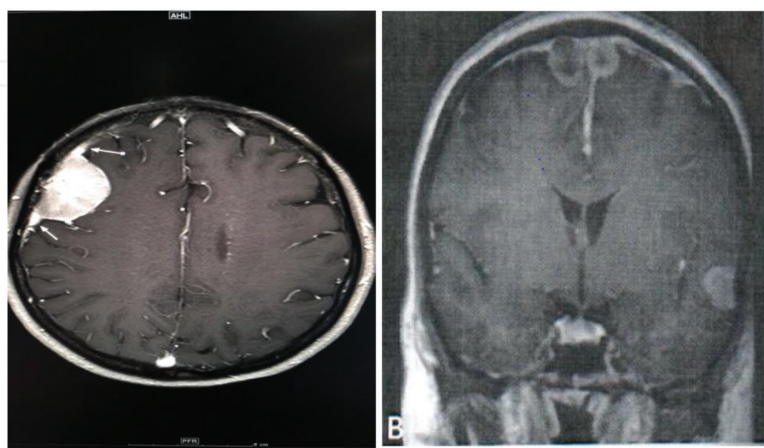


Figure 11. Left temporal intra-axial hyperintense mass with medial uncus herniation compression of the left midbrain on Flair, arrow shows dural tail sign (a). T1WI MRI demonstrates multiple dura-based enhancing mass lesions, most likely NF-2 mutated multiple meningioma (B).

However, seizures, personality changes, and/or visual problems may also occur. Patients often have subtle symptoms for a long period before the meningioma is diagnosed [1, 2].

9.3. Radiologic finding

A contrast enhanced CT (computerized tomography) and/or MRI (magnetic resonance imaging) scan could be used. Typically, it appears as extra-axial masses with a broad enhanced dural base (“dural tail sign”). Some refer to it as “mouse tail sign”, which is a radiological diagnostic marker for meningioma (**Figure 12**). They are usually homogeneous and well circumscribed, although many variants are encountered. Peritumoral edema may be seen with grade II or III tumors, secretory or adherent microcystic subtype. While MRIs are in some ways superior, the CT can be helpful in determining if the tumor invades the bone, or if it’s becoming hard like bone [2].

9.4. Microscopic finding

Histologically, meningioma cells are relatively uniform, with a tendency to encircle one another, forming meningiothelial whorls and psammoma bodies (laminated calcific concretions). As such, they also have a tendency to calcify and are highly vascularized. There are more than 10 histological subtypes of meningiomas associated with different WHO grading as listed below [2].

- **WHO grade I: 9 subtypes**
 - Meningothelial meningioma
 - Fibrous (fibroblastic) meningioma
 - Transitional (mixed) meningioma
 - Psammomatous meningioma
 - Angiomatous meningioma
 - Microcystic meningioma
 - Secretory meningioma
 - Lymphoplasmacyte-rich meningioma
 - Metaplastic meningioma
- **WHO grade II: 3 subtypes**
 - Chordoid meningioma
 - Clear cell meningioma
 - Atypical meningioma

- **WHO grade III: 3 subtypes**

- Papillary meningioma
- Rhabdoid meningioma
- Anaplastic meningioma

As to molecular pathology, between 40 and 80% of meningiomas contain an abnormal chromosome 22. The cause of this abnormality is not known. Meningiomas also frequently have extra copies of the platelet-derived growth factor (PDGFR) and epidermal growth factor receptors (EGFR), which may contribute to the growth of these tumors. TRAF7 mutations are present in about one-fourth of meningiomas. Mutations in the TRAF7, KLF4, AKT1, and SMO genes are commonly expressed in benign skull-based meningiomas. Mutations in NF2 are commonly expressed in meningiomas located in the cerebral and cerebellar hemispheres. People with neurofibromatosis type 2 (NF2) have a 50% chance of developing one or more meningiomas (**Figure 12B**) [1].

9.5. Immunohistochemical findings

The vast majority of meningiomas are positive for EMA, progesterone receptor and vimentin by immunohistochemical stain. Recently, a new marker somatostatin receptor 2A (SSTR2A) is expressed strongly and diffusely in almost all cases of meningiomas [1]. S100 protein positivity is most common in fibrous meningiomas, but is not usually diffuse, as it is in schwannomas. Other potentially useful immunohistochemical markers in selected cases include Ki-67. Studies have suggested that meningiomas with an index of >4% have an increased risk of recurrence similar to that of atypical meningioma, whereas those with an index of >20% are associated with death rates analogous to those associated with anaplastic meningioma [1, 2].

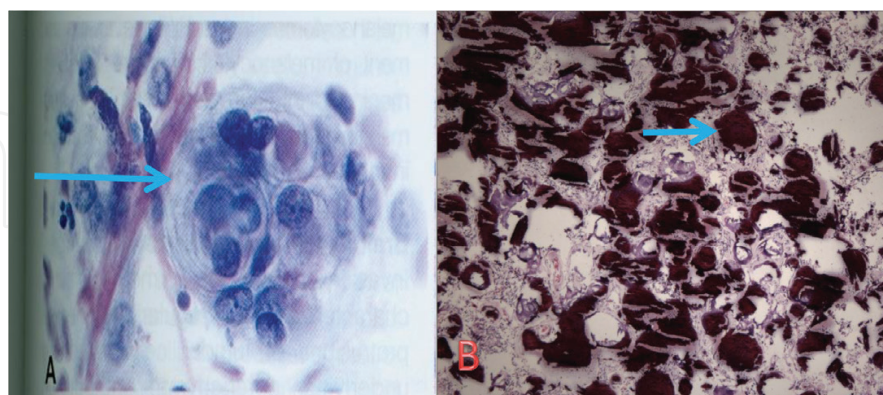


Figure 12. Meningiothelial whorl (see arrow, H&E stain $\times 400$) (a) and psammoma body (brownish blocks, see arrow, H&E stain $\times 200$) (B) are a diagnostic hallmarks for meningioma. (B) Shows a psammomatous type meningioma, it is predominantly in female patient with spinal meningiomas.

9.6. Differential diagnosis

The most important differential diagnosis of meningioma is solitary fibrous tumor/hemangiopericytoma (SFT). It was CD34 and STAT6 immunoreactive, which was not seen in meningiomas. Others were chordoma, schwannoma, superficial glioblastoma/gliosarcoma and metastatic carcinoma, and so on.

Importantly, a so-called collision tumor sometimes occurs between meningioma and metastatic carcinoma, especially metastatic breast cancer is more likely to spread into meningioma, probably due to the presence of progesterone receptor (PR) in meningioma attracts breast cancer cells. It is interesting to know that meningiomas not only occur in the system of CNS but also occur in other organs, such as lung and bone; even skin meningioma has been reported, although the pathogenesis is largely unknown.

9.7. Treatment for meningiomas

The management of meningioma is often guided by tumor size, the presence or absence of symptoms, and the WHO grade; as a result, the specific treatment varies from patient to patient. Management typically revolves around observation versus surgery and/or radiotherapy, and systemic chemotherapy is only considered in cases of malignant tumors, inoperable tumors, or exhaustion of other treatments [25–28].

In asymptomatic patients with WHO grade I meningioma less than 3 cm in size and low potential for neurologic impairment, observation is recommended; however, if symptomatic and amenable to surgery, resection is indicated. Radiotherapy alone is considered for symptomatic patients with meningioma unamenable to surgical resection [25, 26].

Meningiomas greater than 3 cm in size can be observed if asymptomatic, but surgical resection is also indicated. If the patient chooses surgery, radiotherapy is administered if there is subtotal resection of a WHO grade II meningioma or if a grade III meningioma is diagnosed. Symptomatic grade II or III meningiomas greater than 3 cm should undergo surgery followed by radiotherapy, but if inoperable, radiotherapy alone is employed and the patient is counseled on clinical trials for potential systemic therapy [26–28].

10. Conclusion

In summary, the neoplasms of the CNS are uncommon tumors, accounting for approximately 1% of tumors in human body. They can be benign or malignant; primary or secondary (metastatic) by origin; can be extra-axial or intra-axial by locations. Except WHO grade IV glioblastomas, most other CNS tumors can be successfully managed by surgery, chemotherapy and radiation therapies. An accurate diagnosis is vitally important not only for treatment options but also for prognosis. As more and more genetic information about CNS tumors become available, we are optimal to be able to cure those tumors in the near future.

Author details

Frank Yuan Shan^{1,2*}, Dingrong Zhong³, Wanming Hu⁴, Nitesh Patel², Ekokobe Fonkem², Dongxia Feng², Yilu Zhang², Jason H. Huang² and Arundhati Rao¹

*Address all correspondence to: yshan918@gmail.com

1 Department of Anatomic Pathology, Baylor Scott and White Medical Center, College of Medicine, Texas A and M University, Temple, TX, USA

2 Department of Neurosurgery, Baylor Scott and White Medical Center, College of Medicine, Texas A and M University, Temple, TX, USA

3 Department of Pathology, Peking Union Medical College Hospital, Peking Union Medical College, Beijing, The People's Republic of China

4 Department of Pathology, Sun Yat-Sen University Cancer Center, Guangzhou, Guangdong, The People's Republic of China

References

- [1] Louis DN, Ohgaki H, Wiestler OD, Cavenee WK. WHO Classification of Tumors of the Central Nervous System. Revised 4th ed. Lyon: International Agency for Research on Cancer; 2016
- [2] Burger PC, Scheithauer BW. AFIP Atlas of Tumor Pathology, Series 4, no 7. Washington, DC: American Registry of Pathology; 2007
- [3] Kresak J, Shan YF. Molecular Pathology and Diagnostics of Gliomas. In: Coppola D, editor. Molecular Pathology and Diagnostics of Cancer. Springer; 2014
- [4] Wick W, Hartmann C, Engel C, Stoffels M, Felsberg J, Stockhammer F, Sabel SC, Keoppen S, Kjetter R, Meyermann R, Rapp M, Meisner C, Kortmann RD, Pietsch T, Wiestler OD, Ernemann U, Bamberg M, Reifenberger G, von Deimling A, Weller M. NOA-04 randomized phase III trials of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *Journal of Clinical Oncology*. 2009;**27**:5874-5880
- [5] Jeuken JW, von Deimling A, Wesseling P. Molecular pathogenesis of oligodendroglial tumors. *Journal of Neuro-Oncology*. 2004;**70**:161-181
- [6] Caincross G, Jenkins R. Gliomas with 1p/19q codeletion: A.K.A. oligodendroglioma. *Cancer Journal*. 2008;**14**:352-357
- [7] Menke JR, Raleigh DR, Gown AM, Thomas S, Perry A, Tihan T. Somatostatin receptor 2a is a more sensitive diagnostic marker of meningioma than epithelial membrane antigen. *Acta Neuropathologica*. 2015;**130**(3):441-443

- [8] Shibuya M. Pathology and molecular genetics of meningioma: Recent advances. *Neurologia Medico-Chirurgica (Tokyo)*. 2014;**55**:14-27
- [9] Beatriz M, Lopes The S. World Health Organization classification of tumors of the pituitary gland: A summary. *Acta Neuropathologica*. 2017, 2017 August 18. DOI: 10.1007/s00401-017-1769-8
- [10] Delellis RA, Lloyd RV, Heitz PU, Eng C. WHO Classification of Tumors, Pathology and Genetics of Tumors of Endocrine Organs. Lyon: IARC Press; 2004
- [11] Rudà R, Trevisan E, Soffietti R. Low-grade gliomas. *Handbook of Clinical Neurology*. 2012;**105**:437-450
- [12] Ahmed R, Oborski MJ, Hwang M, Lieberman FS, Mountz JM. Malignant gliomas: Current perspectives in diagnosis, treatment, and early response assessment using advanced quantitative imaging methods. *Cancer Management and Research*. 2014;**6**:149-170
- [13] Pinar E, Yuceer N, Imre A, Guvenc G, Gundogan O. Endoscopic endonasal transsphenoidal surgery for pituitary adenomas. *The Journal of Craniofacial Surgery*. 2015;**26**(1): 201-205
- [14] Fomekong E, Maiter D, Grandin C, Raftopoulos C. Outcome of transsphenoidal surgery for Cushing's disease: A high remission rate in ACTH-secreting macroadenomas. *Clinical Neurology and Neurosurgery*. 2009;**111**(5):442-449
- [15] Wu J-S, Shou X-F, Yao C-J, et al. Transsphenoidal pituitary macroadenomas resection guided by PoleStar N20 low-field intraoperative magnetic resonance imaging: Comparison with early postoperative high-field magnetic resonance imaging. *Neurosurgery*. 2009;**65**(1):63-71
- [16] Loeffler JS, Shih HA. Radiation therapy in the management of pituitary adenomas. *The Journal of Clinical Endocrinology and Metabolism*. 2011;**96**(7):1992-2003
- [17] Omuro A. Glioblastoma and other malignant gliomas. *Journal of the American Medical Association*. 2013;**310**(17):1842
- [18] Przybylowski CJ, Dallapiazza RF, Williams BJ, et al. Primary versus revision transsphenoidal resection for nonfunctioning pituitary macroadenomas: Matched cohort study. *Journal of Neurosurgery*. 2017;**126**(3):889-896 <http://www.ncbi.nlm.nih.gov/pubmed/27203142>
- [19] Buckner JC, Shaw EG, Pugh SL, et al. Radiation plus procarbazine, CCNU, and vincristine in low-grade glioma. *The New England Journal of Medicine*. 2016;**374**(14):1344-1355
- [20] Pouratian N, Schiff D. Management of low-grade glioma. *Current Neurology and Neuroscience Reports*. 2010;**10**(3):224-231
- [21] Sanai N, Chang S, Berger MS. Low-grade gliomas in adults. *Journal of Neurosurgery*. 2011;**115**(5):948-965
- [22] Aghi MK, Nahed BV, Sloan AE, Ryken TC, Kalkanis SN, Olson JJ. The role of surgery in the management of patients with diffuse low grade glioma: A systematic review and evidence-based clinical practice guideline. *Journal of Neuro-Oncology*. 2015;**125**(3):503-530

- [23] Jakola AS, Myrmet KS, Kloster R, et al. Comparison of a strategy favoring early surgical resection vs a strategy favoring watchful waiting in low-grade gliomas. *Journal of the American Medical Association*. 2012;**308**(18):1881-1888
- [24] Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *The New England Journal of Medicine*. 2005;**352**(10):987-996
- [25] Fathi A-R, Roelcke U. Meningioma. *Current Neurology and Neuroscience Reports*. 2013;**13**(4):337
- [26] Hanft S, Canoll P, Bruce JN. A review of malignant meningiomas: Diagnosis, characteristics, and treatment. *Journal of Neuro-Oncology*. 2010;**99**(3):433-443
- [27] Aizer AA, Bi WL, Kandola MS, et al. Extent of resection and overall survival for patients with atypical and malignant meningioma. *Cancer*. 2015;**121**(24):4376-4381
- [28] Usery JB, Michael LM, Sills AK, Finch CK. A prospective evaluation and literature review of levetiracetam use in patients with brain tumors and seizures. *Journal of Neuro-Oncology*. 2010;**99**(2):251-260

