BRAIN TUMOURS: CLASSIFICATION AND GENES

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This paper aims to provide an outline of the surgical pathology of the most common tumours of the nervous system in children and adults, and briefly summarise their common genetic changes. The reader is referred to more comprehensive texts for further details about brain tumour classification and the genetic abnormalities of these tumours.[1]

CLASSIFICATION

Most recent classifications of brain tumours build on the 1926 work of Bailey and Cushing.[2] This classification named tumours after the cell type in the developing embryo/fetus or adult which the tumour cells most resembled histologically. The cell of origin of the majority of brain tumours is unknown as no pre-malignant states are recognised, as is the case in some epithelial tumour forms. In some tumours, cells may be so atypical that it is difficult to compare them with any normal cell type—hence the use of terms such as glioblastoma. Many unsound or illogical terms have remained in the classifications, as once established in a complex medical setting they are difficult to change. In this paper the terminology and definitions of the World Health Organization classification of 2000 will be exclusively used.[1] There are more than 120 entities in this classification and here we will concentrate on those that most frequently occur in adults and children. These are the pilocytic astrocytomas, ependymomas, and medulloblastomas in children, and the diffuse astrocytic tumours (including astrocytoma, anaplastic astrocytomas, and glioblastomas), oligodendrogliomas, and meningiomas in adults.

Tumours of the central nervous system often have a wide morphological spectrum and classification is dependent on the recognition of areas with the characteristic histology for a particular tumour type. Immunocytochemical methods may be required to demonstrate the expression by the tumour cells of an antigen typically expressed by a particular cell type and thus to assist in classification. Unfortunately there are no antibodies that unequivocally identify the different tumour types. The presence or absence of an antigen only adds a further piece of information helping to indicate the tumour type.

Four malignancy grades are recognised by the WHO system, with grade I tumours the biologically least aggressive and grade IV the biologically most aggressive tumours. The histological criteria for malignancy grading are not uniform for all tumour types and thus all tumours must be classified before the malignancy grade can be determined. Only one or two malignancy grades can be attributed to some tumour types. Brain tumours are well known to progress, becoming more malignant with time. Such progression will initially be focal. A patient's diagnosis is based on the most malignant part of the tumour. Thus it is of the utmost importance to sample the tumour adequately in order to determine its type and judge its malignant potential. It follows that malignancy grading on biopsies/stereotactic biopsies is always a minimum grading as more anaplastic regions may be present in non-biopsied areas.

Cytotoxic or radiation therapy before histological diagnosis may make classification and malignancy grading extremely difficult or impossible. The clinical implications of tumour classification and malignancy grading have been empirically determined. The application of objective methods of measuring cell proliferation and death in tumours to malignancy grading is conceptually attractive but have yet to be accepted and utilised in the malignancy grading of brain tumours. The MIB 1 antibody recognising the same antigen as Ki67 as well as other antibodies identifying antigens associated with proliferation (for example, Cdc6 and Mcm5) can be used efficiently on formalin fixed, paraffin embedded tissues following microwave antigen retrieval.[3 4] However, wide variations in the proliferation indices are observed in different areas of individual brain tumours and this has resulted in difficulties in defining relevant proliferation levels. The same applies to the assessment of the numbers of cells undergoing apoptosis.

The advances in neuroradiology and parallel improvements in stereotactic and surgical techniques permit the biopsy of just about any neoplastic or non-neoplastic lesion in the central nervous system (CNS). The list of potential diagnoses is thus vast. The neuropathologist may be expected to make a diagnosis on the basis of often very small and fragmented biopsies. He thus

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Table 1 Familial syndromes associated with human brain tumours

Disorder	Gene	Location	Protein function	Tumour types associated with disorder	Involved in sporadic CNS tumours	References
Li fraumeni	*TP53* (only 70%)	17p13.1	Transcription factor, apoptosis induction, etc	Many including astrocytomas	Mainly astrocytic	114, 115
Neurofibromatosis type 1	*NF1*	17q11.2	GTPase activating protein homology	Astrocytomas (brain stem optic nerve) ependymomas, PNETs and meningiomas (pheochromocytoma), etc	Unknown	5, 116
Neurofibromatosis type 2	*NF2*	22q12.2	Ezrin/moesin/radixin-like	Vestibular schwannomas, meningiomas, spinal schwannomas	Meningiomas, schwannomas	117
Familial adenomatous polyposis coli (or Turcot syndrome A)	*APC*	5q21–q22	Regulates β-catenin	Medulloblastoma	Unknown	118, 119
Hereditary non-polyposis colorectal cancer (or Turcot syndrome B)	*MLH1*	3p21.3	Microsatellite instability (MIN+)	Glioblastoma (unknown if all germline mutations are associated with glioblastoma)	Unknown; astrocytic tumours that are MIN+ occur but are uncommon	118
	MSH2	2p22–p21				
	MLH3	14q24				
	PMS1	2q31–q33				
	PMS2	7p22				
Basal cell naevus syndrome/Gorlin's syndrome	*PTCH*	9q22.3	Receptor for SHH inhibits SMO	Medulloblastoma	Medulloblastoma	119
Cowden disease (multiple hamartoma syndrome, Lhermitte-Duclos, etc)	*PTEN*	10q22–q23	Dual specificity phosphatase and Tensin homology	Astrocytomas reported but tumours in other organs more common—thyroid, breast, female genito-urinary tract	Glioblastomas	120
Melanoma-astrocytoma syndrome	*CDKN2A/p14ARF*	9p21	Cell cycle control (G1-S)/p53 level control	Astrocytomas	Astrocytic	121

PNET, primitive neuroectodermal tumour; SHH, sonic hedgehog; SMO, smoothened.

needs to know the clinical background of the case. Information must be provided: age, neuroradiological findings including location of the tumour, relevant clinical and family history, and whether the patient has received any treatment, including steroids. As can be deduced from the above, morphology combined with immunocytochemistry may only provide a differential diagnosis and the most likely diagnosis will then only be reached by considering all the information available at a multidisciplinary team meeting.

The vast majority of brain tumours are sporadic. A number of familial syndromes are well documented with an increased incidence of brain tumours (see table 1 and the references therein). However, even in the most common syndromes (neurofibromatosis type 1 and neurofibromatosis type 2), the precise relative risk is difficult to define.

COMMON CHILDHOOD TUMOURS

Pilocytic astrocytomas

Pilocytic astrocytomas most commonly occur in the cerebellum of children. However, they may occur anywhere from the optic nerve to the medulla oblongata. Patients with pilocytic astrocytomas that can be excised have a good prognosis. There is an increased incidence of pilocytic astrocytomas in NF1 patients, particularly involving the optic nerve, and these tumours in NF1 patients behave in a particularly benign fashion.[5] Pilocytic astrocytomas are generally biologically non-aggressive and are remarkable among astrocytic tumours in maintaining their grade I status over years and even decades (in contrast to the diffuse astrocytic tumours in adults). However, very occasional cases may prove more sinister and progress to more malignant tumours.[6] Pilocytic astrocytomas show a wide spectrum of morphologies, from the pilocytic, bipolar cellular areas with Rosenthal fibres (fig 1) to less cellular protoplasmic astrocytoma-like areas with eosinophilic granular bodies and clear cells. The latter are reminiscent of oligodendroglioma and in the posterior fossa can also be confused with clear cell ependymoma. The presence of features typically associated with a malignant biological behaviour (for example, vascular proliferation or mitosis) does not carry the same sinister implications as in the other astrocytic tumours. This morphological spectrum can make histopathological diagnosis extremely

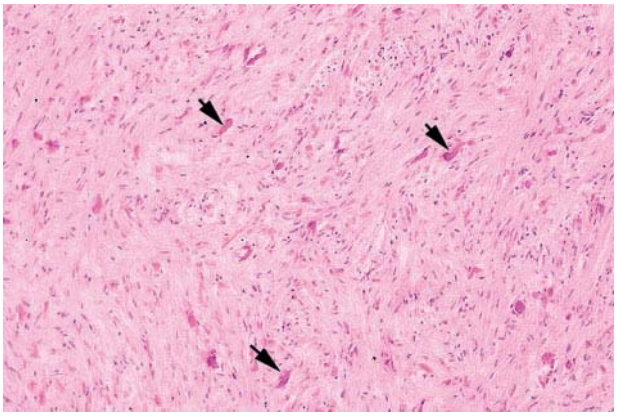

Figure 1 Pilocytic astrocytoma malignancy grade I (H&E). Note the piloid bipolar cells and Rosenthal fibres (arrows). This shows the classical morphology that is generally found somewhere in a pilocytic astrocytoma; other areas can show very different histological patterns.

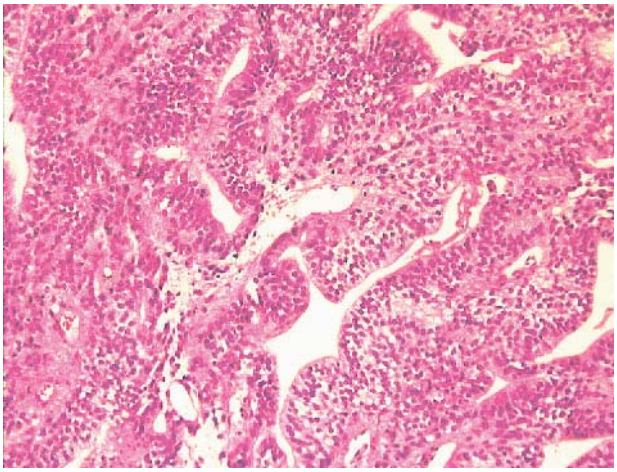

Figure 2 Ependymoma malignancy grade II, showing multiple ependymal canals (H&E).

difficult—particularly in the absence of the clinical data that must be provided to the pathologist as outlined above.

More than 100 cases of pilocytic astrocytomas have been analysed cytogenetically and many more by comparative genomic hybridisation. No consistent findings have been made. The majority show normal cytogenetic and comparative genomic hybridisation (CGH) findings.[7–10] Adult pilocytic astrocytomas have been found to show the most frequent but again variable abnormalities. Molecular genetic studies have been few and have shown allelic losses on both 17p and 17q including the TP53 and NF1 loci. Few TP53 mutations have been reported and no mutations of the NF1 locus have been reported in sporadic tumours.[11–15] Recently studies of methylation of the promoter regions of a number of genes reported to be hypermethylated in the adult diffuse astrocytic gliomas have provided somewhat inconsistent data on methylation in pilocytic astrocytomas.[16 17]

Ependymoma

Ependymomas arise at or close to ependymal surfaces and may occur anywhere in the ventricular system as well as in the spinal cord and very occasionally at extraneural sites. The most common location is in the fourth ventricle, followed by the spinal canal, lateral ventricles, and the third ventricle. Children have the highest incidence of ependymomas, but they can occur into late middle age. Ependymomas are the most frequent glioma of the spinal cord and this location is common in adults. There are a number of subtypes. The least biologically aggressive are malignancy graded as grade 1, and consist of the subependymoma (intraventricular and often symptomless) and myxopapillary ependymoma that most commonly occurs at the cauda equina. The tumour named ependymoma is malignancy graded as grade II and has a number of histopathological variants. Ependymomas show in some area(s) evidence of an ependymal cell phenotype—by the formation of ependymal rosettes and sometimes canals (fig 2). More commonly perivascular pseudo-rosettes are identified but are not specific for ependymomas. Ependymomas (malignancy grade II) are differentiated from anaplastic ependymomas (malignancy grade III) on the basis of low mitotic rate and a low level of nuclear polymorphism, but the borderline between these remains ill defined.

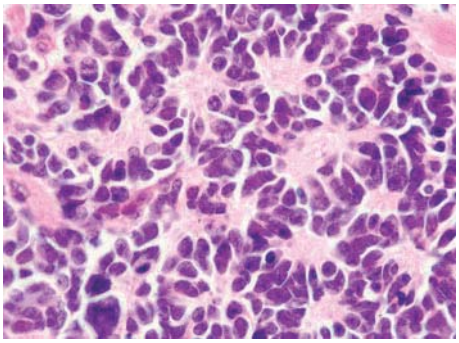

Figure 3 Medulloblastoma malignancy grade IV showing an area with typical neuroblastic rosettes (H&E).

Necrosis and microvascular proliferation do not have the same significance in this tumour type as in the adult astrocytic tumours. Most ependymomas (malignancy grade II) show immunoreactivity for glial fibrillary acidic protein (GFAP), S-100 protein, and epithelial membrane antigen (EMA).

Chromosomal copy number abnormalities detected by classical cytogenetics and CGH include chromosomes 1, 6, 7, 9, 10, 13, 17, 19, and 22. Deletions are most common with losses on chromosome 22 a frequent event in adult spinal ependymomas (over 50%) but infrequent in paediatric ependymomas. Gains have been reported for chromosome 7. These findings have been confirmed by molecular genetic data that have identified losses on 6q, 9p, 10, 11q, 13q, 17p and 19q.[18–22]

The genes targeted by these allelic losses and gains are in most cases unknown, with the exception of the loss of both wild-type copies of the neurofibromatosis type 2 (*NF2*) gene in sporadic intramedullary spinal ependymomas but not in intracranial ependymomas.[19 21 23] Single cases have been reported with loss of other wild type genes such as the *MEN1* gene.[21 24] Germ line mutations of *TP53* are uncommon in contrast to the situation in the diffuse astrocytic tumours.[25 26]

Medulloblastoma

Medulloblastoma has a peak incidence in childhood but also can occur into late middle age. Histologically childhood and adult medulloblastoma are identical, being highly cellular, malignant invasive tumours corresponding to WHO malignancy grade IV. Medulloblastomas occur in the posterior fossa. They consist of densely packed tumour cells with round to oval or carrot shaped hyperchromatic nuclei with scanty cytoplasm, high mitotic and apoptotic rates, and usually neuroblastic rosettes in some areas (fig 3). Neuronal differentiation and glial differentiation may be present. Microvascular proliferation is relatively uncommon. Tumours arise with similar frequency in the cerebellar vermis (mainly in children) and the cerebellar hemispheres (older patients), and often invade the fourth ventricle, with occasional brainstem involvement. There is a high risk of seeding through the subarachnoid space due to the tendency of the tumour to penetrate the ependymal surface. Many antigens have been identified focally in medulloblastomas (nestin, vimentin, neurofilament proteins, GFAP, retinal S-antigen, N-CAMs, Trk-A, -B, -C etc). However, most are not of any great importance in the day-to-day diagnosis of these

tumours. It is most important to differentiate medulloblastomas from atypical teratoid/rhabdoid tumours, as the latter have a very poor prognosis and do not respond to the current relatively successful treatment protocols for medulloblastomas.[27 28] In adults the possibility of a metastasis of a small cell lung cancer must often be excluded.

The most common chromosomal abnormality in medulloblastomas is iso-chromosome 17q, in which most of the short arm is lost from two chromosomes 17 and they are then fused head-to-head producing a chromosome with two centromers, little 17p and two 17q arms. This is observed in 30–50% of cases by using cytogenetic techniques.[8 29 30] These findings have been confirmed by CGH and molecular genetic studies.[11 31]

Many other chromosomal aberrations have been identified using conventional cytogenetic, CGH or molecular genetic techniques—for example, loss of 10q (35%).[32 33] In addition, several growth and transcription factors have been investigated, some reporting high expression in a subset of tumours—for example, erbB2&4.[34]

A major contribution to our understanding of medulloblastoma biology has come from the study of two genetic syndromes exhibiting a predisposition to medulloblastoma formation. Gorlin's syndrome (hereditary naevoid basal cell carcinoma syndrome) and familial adenomatous polyposis (FAP) syndrome arise from mutations in the *PTCH* (9q) and *APC* (5q) genes, respectively, and both are associated with medulloblastoma formation. The gene products of these two genes take part in two interconnected pathways that are fundamental to neural development and cell turnover. Hemizygous loss and mutations in the retained allele of *PTCH* in sporadic medulloblastomas have been shown.[35 36] However, alterations in the *PTCH* and *APC* genes as well as other genes coding for components of these two pathways are involved in the development of less than 15% of sporadic medulloblastomas.[37 38] Other genes involved in the two pathways, including *SMO*[39] and *SUFU*,[40] have been studied and also show loss of wild type in only single, isolated cases. Other genes currently being investigated for their significance in medulloblastoma biology are the myc family[41 42] and the PDGF receptors and ligands.[43 44]

COMMON ADULT TUMOURS

Diffuse astrocytic tumours

The adult diffuse astrocytic tumours include the astrocytomas (malignancy grade II), the anaplastic astrocytomas (malignancy grade III), and the glioblastomas (malignancy grade IV). The astrocytoma malignancy grade II tumours have a peak incidence between 25 and 50 years of age, while the glioblastomas have a peak incidence between 45 and 70 years. All are more common in males and most are located in the cerebral hemispheres. Glioblastomas are the most common form and are divided into those that develop from a previously diagnosed tumour of lower malignancy grade and those that appear to develop de novo.[45 46] Both clinical and molecular data support the hypothesis that these tumours may develop from the mutation of different genes but affect the same cellular pathways.[47–49] The relevance of the histologically based malignancy grading scheme is indicated by patient survival. Patients with an astrocytoma (malignancy grade II) have an average survival of approximately seven years, patients with anaplastic astrocytomas have a median survival half that time,[50] while glioblastoma patients have an average survival of between 9–11 months.[51] This is despite the best currently available treatments. The astrocytomas (malignancy grade II) and anaplastic astrocytomas have been well documented to progress to tumours of higher malignancy grade.

The tumour cells of astrocytomas (malignancy grade II) resemble astrocytes, show little nuclear atypia, and have extensions producing a loosely textured matrix (fig 4). They generally express S-100 protein and glial fibrillary acidic protein. Anaplastic astrocytomas (malignancy grade III) show increased cellularity but the tumour cells still show histological and immunocytochemical characteristics of astrocytes. The tumour cells are more pleomorphic than found in astrocytomas, show distinct nuclear atypia, and there is mitotic activity. No evidence of spontaneous tumour necrosis or abnormal microvascular proliferation is permitted in anaplastic astrocytomas. Glioblastomas (malignancy grade IV) are more cellular than the anaplastic astrocytomas. The tumour cells show a wide spectrum of morphologies, can be very pleomorphic with giant forms, but generally retain some of the phenotypical characteristics of astrocytes. Mitosis, spontaneous tumour necrosis with pseudopalisading of tumour cells, as well as florid endothelial proliferation, are inevitably found in some areas of a well sampled tumour (fig 4). A large central necrotic area with a ring-like zone of contrast enhancement, representing the viable tumour tissue, can often be identified by neuroimaging.

Before reading the following section it is essential that fig 5 is first reviewed and referred to as necessary. Cytogenetic and molecular data are limited on astrocytomas (malignancy grade II) as they are not so common.[7 52 53] Over 60% of astrocytomas (malignancy grade II) have loss of alleles on 17p, including the *TP53* locus, and the retained *TP53* allele is mutated in the majority of cases.[49 54 55] The absence of wild type p53 is therefore the most common abnormal finding in astrocytomas malignancy grade II,[49] resulting in a non-functional p53 pathway. A small percentage of tumours have mutations of one allele but retain one wild type allele. As the p53 protein is believed to function as a tetramer and as tetramers with one abnormal p53 protein may not function

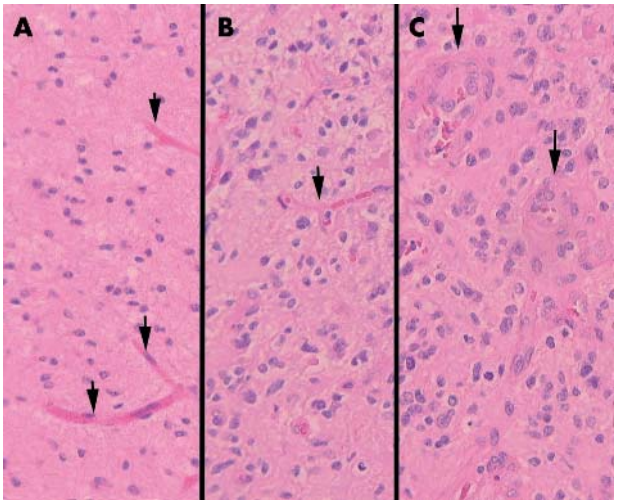

Figure 4 Tumours of the astrocytic series (H&E). (A) Astrocytoma malignancy grade II (arrows pointing to thin walled tumour capillary vessels). (B) Anaplastic astrocytoma malignancy grade III demonstrating anaplastic tumour cells but with no evidence for microvascular proliferation (arrows; compare with A and C). (C) Glioblastoma with florid endothelial proliferation (arrows).

normally, the finding of these single mutated alleles together with a wild type allele may well be significant. Other genes coding for components of the p53 pathway (fig 5), *MDM2* and *p14ARF*, have been studied in small numbers of these tumours and no abnormalities have been reported. Recent studies of the *TP53* related gene, *P73*, have not identified any mutations.[56] Other findings considered significant include overexpression of the PDGFRA gene.[57 58] Loss of alleles from 6q, 13q, and 22q occur in some astrocytomas. There is no evidence to suggest that there is mutation of the single retained tumour suppressor gene *RB1* allele at 13q14.2[59] or the *NF2* tumour suppressor gene on 22q.[60 61] Deletion mapping of chromosomes 6 shows losses on 6q in a significant number of astrocytomas.[62] The potential tumour suppressor genes in all of these regions remain unknown. There are no consistently reported amplified genes or amplified regions of the genome in astrocytomas.[59 63–66] The changes found in the astrocytomas form the baseline for progression in the adult diffuse astrocytic tumour series. Epigenetic changes such as hypermethylation of tumour suppressor gene promoters may also play an important role in transcriptional silencing of some of the genes cited above or other important cancer genes and the development of astrocytomas. This has not been studied in any detail as yet.[67]

The numbers of cases of anaplastic astrocytomas (malignancy grade III) studied are also limited. Mutations of the *TP53* gene also occur at approximately the same frequency as is found in the astrocytomas malignancy grade II.[49] Thus in the anaplastic astrocytomas the p53 pathway is also non-functional, and in the majority of cases (more than 60%) this is due to mutations of the *TP53* gene. Cytogenetics, CGH, and molecular genetic techniques all show that the losses of alleles on 6q, 13q, 17p and 22q, as seen in the astrocytoma malignancy grade II, occur at similar or higher frequencies in the anaplastic astrocytomas. With the sole exception of losses of alleles on 19q (targeted gene unknown) there are no conclusively demonstrated abnormalities specific to this malignancy grade. Around 20% of anaplastic astrocytomas show similar genetic abnormalities to those found in glioblastomas involving other components of the p53 pathway (that is, *MDM2* and $p14^{ARF}$) and lead to disruption of the Rb1 pathway (fig 5), and these are discussed in the glioblastoma section below[59]

De novo glioblastomas are common and this has ensured their study in considerable numbers. Secondary glioblastomas are less frequent and very less commonly studied.[68 69] Such patients will frequently have been treated by irradiation and/or with cytotoxic drugs. Glioblastomas show the greatest

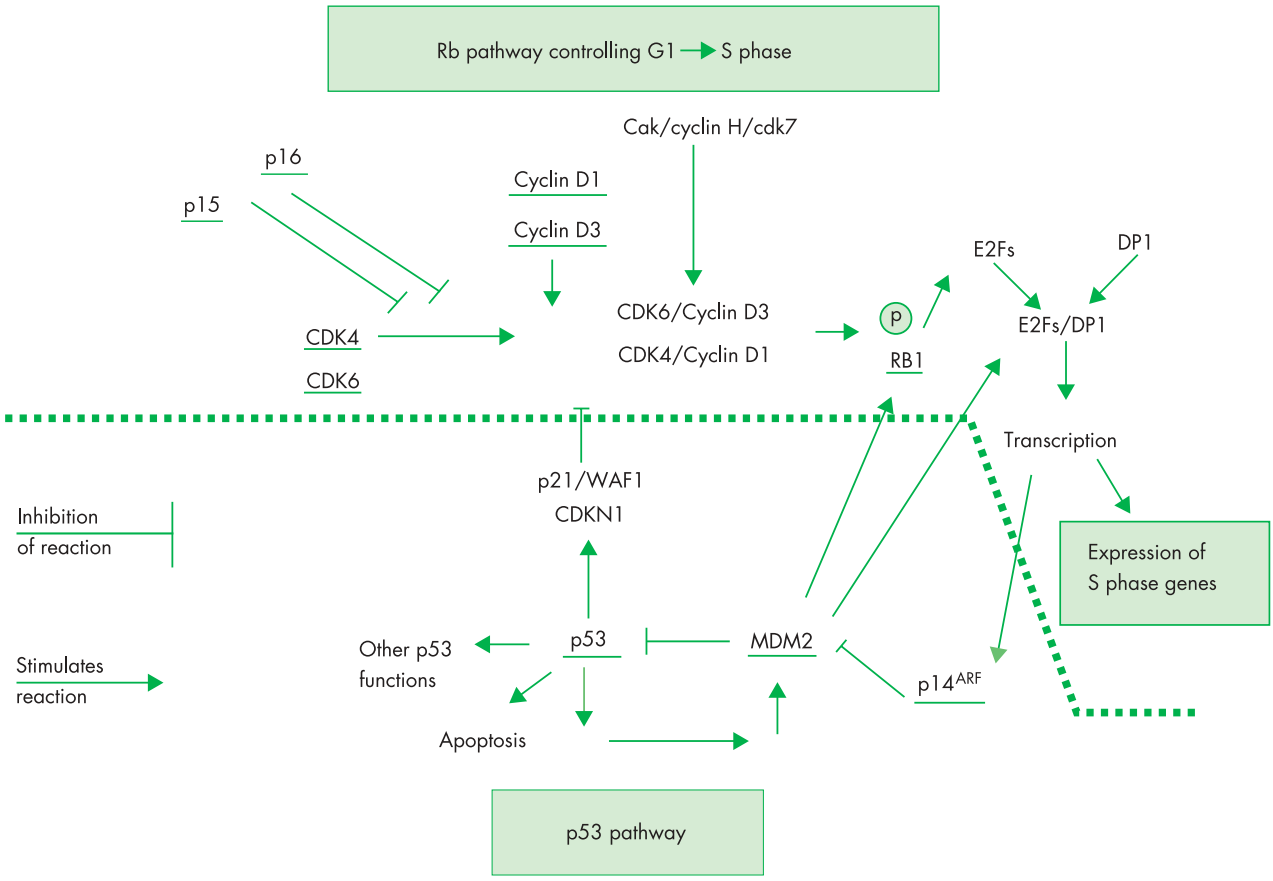

Figure 5 Simplified diagram of interactions between the proteins of the Rb1 pathway (above) and the p53 pathway (below). The genes coding for p16 and p15 proteins are *CDKN2A* and *CDKN2B*, respectively . The genes for all of the proteins underlined have been shown to be abnormal in the astrocytic and some other gliomas as well as many other tumour cell types in other organs. In the vast majority of cases where a pathway is disrupted in a tumour it is due to only **one** of the genes coding for a protein in that pathway being abnormal (loss of both wild type copies in the case of most tumour suppressor genes or amplification and overexpression in the case of proto-oncogenes). Thus it appears that **pathways** are targeted in oncogenesis and progression, and can be disrupted in many ways by losing, mutating, or amplifying the genes coding for the protein components of the pathway.

numbers of genetic abnormalities among the astrocytic tumours and clear patterns of genetic aberrations are emerging. The p53 pathway in glioblastomas is targeted through mutation of the *TP53* gene (approximately 37%[49]), as is seen in astrocytomas and anaplastic astrocytomas, but also by targeting other genes coding for proteins that control cellular p53 levels. The two genes whose products are involved in controlling p53 levels are $p14^{ARF}$ and *MDM2*. $p14^{ARF}$ controls the activity of MDM2,[70] which in its turn controls the breakdown of p53.[71] Loss of both copies of the $p14^{ARF}$ gene or amplification and over-expression of *MDM2* will lead to the rapid breakdown of wild type p53 protein resulting in a cell with little or no wild type p53. The vast majority of glioblastomas (> 70%) have either no wild type p53 or no $p14^{ARF}$ or over express MDM2 as mutually exclusive genetic abnormalities.[49] Methylation of the $p14^{ARF}$ promoter with decreased or non-expression are further mechanisms that have been shown to be involved in some tumours. In glioblastomas additionally the retinoblastoma pathway and the PI3 kinase–Act pathway are also targeted.

In a similar manner one or other of the genes coding for proteins involved in the control of entry into the S phase of the cell cycle (the retinoblastoma pathway) are mutated in glioblastomas (fig 1). Entry into S phase is normally initiated by the release of transcription factors from newly phosphorylated Rb1 at the restriction point in G_1. At the end of the cell cycle Rb1 is unphosphorylated. Unphosphorylated Rb1 normally sequesters the E2F transcription factors.[72] Loss of wild type *RB1* gene resulting in no functional RB1 or inappropriately phosphorylated Rb1 will result in any expressed E2F being free to initiate transcription of the genes necessary for entry into S phase. Inappropriate phosphorylation may be achieved in glioblastomas with wild type Rb1 by either loss of wild type p16 expression or over-expression of CDK4 caused by amplification of its gene. These would make inappropriate phosphorylation of a wild type Rb1 more likely with the release of the E2Fs. p16 normally binds CDK4 and thus inhibits the formation of the CDK4/cyclin D1 heterodimer.[73] In the absence of p16 all expressed CDK4 is available for heterodimer formation. When *CDK4* is overexpressed in the presence of normal levels of p16 there will be excess CDK4 available for heterodimer formation. One or the other of these abnormalities are present in over 90% of glioblastomas and are, with very few exceptions, mutually exclusive.[49] While disruption of the p53 and Rb1 pathways seem essential for glioblastomas, the ways in which the pathways are rendered dysfunctional may confer slightly different biological characteristics on the individual glioblastoma.

In addition to the genetic abnormalities resulting in the disruption of the p53 and Rb1 pathways, over 90% of glioblastomas lose alleles from 10q. The regions consistently lost include the variously named *PTEN/MMAC1/TEP1* tumour suppressor gene at 10q23–24.[74–76] *PTEN* has been shown to be mutated in up to 45% of glioblastomas.[77] The gene is a dual specificity phosphatase (necessary for its ability to function as a tumour suppressor) and has homology to the cytoskeletal protein tensin.[78 79] One of its major substrates is phosphatidylinositol-3, 4, 5-triphosphate (PIP3)[80] and lack of control of PIP3 is likely to have a major effect on the activation of the Akt pathway, affecting among other things apoptosis and HIF-1 activity.[81] Other genes coding for proteins involved in the PI3K/AKT pathway have recently been shown to be mutated, albeit infrequently.[82] This is supported by reports on the affect of Akt activation in an animal model of astrocytoma.[83]

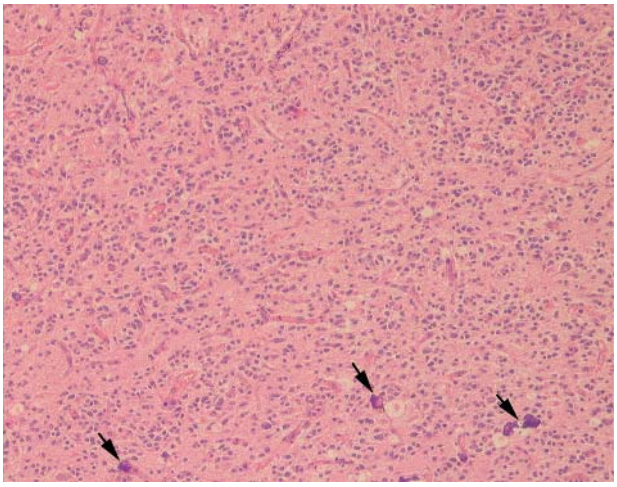

Figure 6 Oligodendroglioma malignancy grade II (H&E) with the typical tumour cell morphology—round nuclei and swollen cytoplasm. Note the microcalcifications (arrows) mainly in the lower right of the field. This is a common feature in oligodendrogliomas but is not unique to this form of glioma.

Amplification of the epidermal growth factor receptor (EGFR) gene (7p11–12) is found in about 35% of glioblastomas. When amplified this gene is always over-expressed but may also be over-expressed in glioblastomas without amplification. Rearrangements of the amplified gene occur in almost half of the tumours with amplification. The most common rearrangement results in a transcript that is aberrantly spliced, remains in frame,[84–86] and codes for a mutated EGFR that has lost 267 amino acids of its extracellular domain and does not bind ligand.[87 88] This mutated EGFR is constitutively activated and attempts are ongoing to target treatment to this aberrant cell surface molecule.[89] Other rearrangements of the amplified EGFR gene occur less frequently and may result in abnormalities of the cytoplasmic domain[90]

Glioblastomas can develop from an astrocytoma or as a de novo glioblastoma. It is tempting to try to sort all these findings into a series of events explaining the development of the two forms of glioblastoma. Both have disrupted the normal p53 and Rb1 pathways, but in different ways. The de novo tumours do this by a single genetic event when amplification of the 12q14 region encompassing the *CDK4* and *MDM2* genes results in their over-expression and the disruption of both pathways. Two genetic events are required to disrupt the two pathways when homozygous deletion of the region on 9p encompassing the genes coding for p16 (CDKN2A), p15 (CDKN2B), and $p14^{ARF}$ ($p14^{AR)}$) occurs (requires loss of both autosomes). Occasionally de novo tumours may also show more complex patterns of mutations with loss of one allele of each of TP53 and RB1, with mutation of the retained alleles, requiring four genetic mutational events. However, in de novo glioblastomas these are in the minority. Secondary glioblastomas generally have no wild type p53 due to loss of one allele and mutation of the retained allele, and lose a functional Rb1 pathway in a similar manner. Other correlations are that EGFR amplification is unusual in cases with no wild type p53, although this does

occur occasionally. In addition to the abnormalities of the genes listed there are likely to be many other genetic changes affecting other regions of the genome that have been found to be manifestly abnormal in these tumours by deletion or amplicon mapping. The genes targeted by these changes have yet to be identified.

Oligodendrogliomas

Oligodendrogliomas occur mainly in the cerebral hemispheres of adults. They are believed to derive from oligodendrocytes. They consist of moderately cellular, monomorphic tumours with round nuclei, often artefactually swollen cytoplasm on paraffin section (fig 6), few or no mitoses, no florid microvascular proliferation or necrosis, and are classified as malignancy grade II according to the WHO. Classically they show a ''chicken wire'' pattern of capillaries. They do not express any antigen characteristic of normal oligodendrocytes and may express GFAP. Grade II oligodendrogliomas are relatively indolent, although they usually recur at the primary site and may display a tendency for subependymal spread with a 5% incidence of cerebrospinal fluid (CSF) seeding. Oligoastrocytomas consist of tumour cells with either astroctytic or oligodendroglial morphological characteristics. Tumour cells with these two phenotypes can be either diffusely mixed or combined as discrete areas in an individual tumour. The morphological borderlines between astrocytomas, oligoastrocytomas, and oligodendrogliomas are difficult and controversial issues.

Increases in nuclear pleomorphism and hyperchromatism, as well as pronounced hypercellularity, brisk mitotic activity, prominent microvascular proliferation, and/or spontaneous necrosis, results in a picture that is histologically classified as anaplastic oligodendroglioma (malignancy grade III). Anaplastic forms of oligoastrocytomas also occur and similar criteria are used to distinguish them from oligoastrocytomas. Since 1990, when combination chemotherapy (procarbazine, lomustin, and vincristine (PCV)) was demonstrated to result in sometimes a dramatic tumour response,[91] the identification of all forms of glioma with oligodendroglial components has become crucial.

Oligodendrogliomas show relatively specific genetic abnormalities that differ from the other gliomas. Loss of genetic information from 1p and 19p was demonstrated in a genomic wide analysis in 1994[92] and this was later linked to a good response to PCV treatment, an association that is currently under intense scrutiny as it provides the first molecular indicator of treatment response in brain tumours.[93 94] The losses on 1p and 19q are most common among the grade II oligodendrogliomas (reports of up to 90%) and are present in over 50% of anaplastic oligodendrogliomas (malignancy grade III). Despite the fact that almost 10 years has elapsed since the identification of these relatively specific losses the genes targeted on these two chromosomes are still unknown. Oligodendrogliomas grade II also show methylation of $p14^{ARF}$, over-expression of EGFR and both ligands and receptors of the platelet derived growth factor (PDGF) system. Malignant progression is associated with additional genetic abnormalities similar to those described above for the astrocytic tumours—that is, disruption of the Rb1 pathway due to homozygous deletions or in some cases hypermethylation of the *CDKN2A*/$p14^{ARF}$ locus, or the *RB1* locus or CDK4 amplification and overexpression as is also seen in the progression of the diffuse astrocytic tumours. Some anaplastic oligodendrogliomas have no wild-type PTEN although this is usually in tumours without 1p and 19q loss. Anaplastic oligodendrogliomas also have abnormalities of many other chromosomal regions including chromosomes 4, 6, 7, 11, 13, 15, 18, and 22.[93]

Oligoastrocytomas and anaplastic oligoastrocytomas tend to have either aberrant genetic patterns similar to the oligodendroglial tumours or the diffuse astrocytic tumours. As yet there are no specific abnormalities associated with these mixed glial tumours.

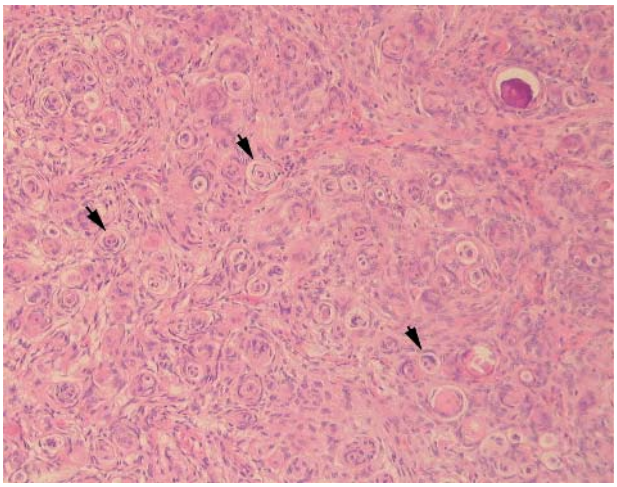

Figure 7 Typical meningioma specimen malignancy grade I showing an example of the common transitional meningioma with multiple whorles (a few marked with arrows).

Meningiomas

Meningiomas are usually solitary lobulated tumours arising in the meninges and attached to the dura. They are believed to develop from meningothelial (arachnoidal) cells, despite the fact that the meningothelial form is far from the most common. Symptomatic meningiomas represent 13–26% of primary intracranial tumours, are most common in middle aged and elderly patients, and show a pronounced female predominance. Small asymptomatic meningiomas are found incidentally in 1.4% of necropsies.[95] Patients with NF2 and members of some other non-NF2 familial syndromes may develop multiple meningiomas, often early in life. Ionising radiation is a well recognised predisposing factor. The cellular morphology, growth pattern, and the presence of extracellular material allow differentiation into the various histological subtypes (fig 7). Meningiomas are graded as malignancy grade I, atypical meningiomas as malignancy grade II, and anaplastic meningiomas as grade III.[96] Meningeal sarcomas are WHO malignancy graded as IV. The vast majority (about 80%) of meningiomas are of malignancy grade I. Atypical meningiomas constitute less than 20% of meningiomas while anaplastic variants are unusual ($< 2\%$). Both atypical and anaplastic meningiomas are more common in men. Meningiomas may progress and therefore should be thoroughly sampled to identify areas with a histology associated with a more aggressive behaviour. The histological criteria indicating a more aggressive behaviour and thus an increase in the malignancy grade include frequent mitoses, regions of hypercellularity, sheet-like growth, high nuclear–cytoplasmic ratio, prominent nucleoli, and spontaneous necrosis. The criteria for the different malignancy grades are strictly defined by WHO.[96] Some

subtypes, characterised by particular tumour cell phenotypes, are associated with more frequent recurrence and they are now classified as malignancy grade II or III. For example, tumours with a papillary growth pattern or areas of rhabdoid cells (rounded tumour cells with an eccentric nucleus with nucleolus and a prominent eosinophilic cytoplasm) are classified as papillary and rhabdoid meningiomas, respectively (malignancy grade III) as they have been documented to behave in a very aggressive fashion.

Meningiomas generally expand and displace but do not invade adjacent brain or spinal chord. Invasion of the dura and skull does occur and has no significance for malignancy grading. Invasion of the skull may elicit an osteoblastic reaction. Brain invasion can occur in meningiomas of all malignancy grades and indicates a greater likelihood of recurrence, but is not considered sufficient criteria alone to increase the malignancy grade.

The higher incidence of meningiomas in women, the apparent manifestation of tumours during pregnancy, and the association of meningiomas with breast and genital cancer have suggested oestrogen and progesterone dependency of the tumours. Meningiomas generally express progesterone receptors and some cases also express oestrogen receptors.

Meningiomas were one of the first solid tumours in humans to be shown to have consistent chromosomal abnormalities, the loss of one copy of chromosome 22.[97] The fact that the second most common tumour in neurofibromatosis type 2 patients was meningioma pointed to the *NF2* gene as a target on chromosome 22. Loss of wild copy NF2 is found in roughly half of sporadic and the majority of NF2 associated meningiomas. In the sporadic cases this is usually by the loss of one copy (monosomy 22) and mutation of the retained copy. The *NF2* gene encodes a protein known variously as merlin[98] or schwannomen.[99] This protein has been shown to be involved in control of cell growth and motility in culture.[100 101] Genetically engineered mice with only one wild-type allele have been shown to develop many tumour forms[102] while tissue specific inactivation in Schwann cells or leptomeningeal cells results in schwannoma or meningioma formation, respectively.[103 104]

As stated above meningiomas may progress from grade I tumours to tumours of higher malignancy grade. This is associated with losses on chromosomal arms 1p, 6q, 9p, 10p and q, 14q, 18q, as well as gains and some amplifications on many other chromosomes.[105–113] The genes targeted by these abnormalities are mostly unknown, but the losses on 9p are associated with loss of both wild-type copies of CDKN2A, p14ARF, and CDKN2B as is commonly seen in the de novo glioblastomas and anaplastic oligodendrogliomas.[113]

CONCLUSIONS

The goal for all histopathology is to provide—on the basis of an analysis of the tissue received—as much useful information as possible to the clinician, informing him/her of all the details of any pathological process present, and forming the basis for the choice of the most appropriate treatment, and a judgement of prognosis. Up until relatively recently this process was mainly based on a morphological analysis. However, as different forms of treatment become more and more based on targeting molecular mechanisms, the analysis of the specimens received must be extended to provide relevant data. This has already occurred—examples include determination of oestrogen receptor, or HER2 expression in breast cancer, which will determine whether the patient will benefit from tamoxifen or herceptin treatment. New technologies currently being introduced will permit a detailed analysis of the genome and transcriptosome of clinical material impossible in the past and the integration of genetic and expression data with the histological information. Eventually we can hope that the molecular information will provide the basis for specific treatments that will only annihilate brain tumour cells, leaving the brain and the rest of the patient unharmed.

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Cover picture

There is a focally haemorrhagic necrotic tumour deep to the insula of the left cerebral hemisphere. The lesion has poorly defined margins and histologically has the features of glioblastoma (WHO grade 4). The tumour and associated swelling have acted as a space occupying lesion with a shift of the midline structures and a supracallosal hernia to the right, narrowing and convex deformity of the third ventricle, again to the right, and compression of the ipsilateral ventricle and enlargement of the contralateral ventricle. Figure courtesy of Professor DI Graham