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Overview

Pathology of Gliomas and Developments in Molecular Testing

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Abstract

Advances in technology are allowing a molecular characterisation of human brain tumours that is providing a wealth of new information. In this short overview, a summary of the histopathology of the common gliomas is integrated with some molecular data. In some instances, the data are proving clinically relevant with conventional therapies. Some single histological entities are being found to contain a number of molecular subtypes, whereas in others, different histological entities are found to be molecularly similar. The introduction of targeted therapies will necessitate a complete reassessment of the way we characterise tumours, and particularly the adequacy of our pathology reports in a new clinical environment.

Key words: Brain neoplasms; immunocytochemistry; methylation; morphology; oncogenes; tumour suppressor genes

Statement of Search Strategies Used and Sources of Information

This paper reflects expert opinion and current literature accessed by the authors; no formal search strategy has been defined.

Introduction

Gliomas have been classified according to histomorphological criteria for over a century. In the 1920s the classification of primary central nervous system (CNS) tumours by Bailey and Cushing [1] was pivotal, with the later development of criteria for malignancy grades in the late 1940s [2,3]. Initially there were local variations in classification and malignancy grading. The first World Health Organization (WHO) classification was published in 1979 [4] and aimed at being an international standard. Since then there have been three revisions with new editions in 1993, 2000 and 2007, with widespread acceptance [5–7]. The latter two editions have included known molecular data about each tumour type. The next edition will have the task of integrating the enormous advances made in the molecular analysis of the common glial tumours over the last few years. So far only a few single markers have been shown to have diagnostic, prognostic or predictive value with current therapeutic regimens and these have begun to be used in clinical decision-making. They include the point mutations of *IDH1* and *IDH2*, the most common of which is the R132H mutation in IDH1 that can be identified using an excellent monoclonal antibody [8], 1p and 19q co-deletion and *MGMT* promoter methylation. Although the documentation of the less common *IDH1* and *IDH2* mutations simply requires sequencing, the identification of loss on 1p and 19q requires care (see [9–11]). The issue of the appropriate methods for *MGMT* methylation testing are more complex and have been extensively discussed elsewhere [12,13]. Their significance is described below under the relevant tumour types, together with a brief overview of other genetic changes the significance of which remains to be established. In the near future, next generation sequencing combined with automated high-throughput analysis will probably provide, in a clinically relevant timeframe, patterns of genetic, epigenetic and expression abnormalities defining new molecularly homogeneous tumour categories, targets for therapy as well as prognostic and predictive indicators.

The latest WHO classification lists over 120 histological entities [7]. In this short overview only the common

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gliomas will be discussed. Brain tumours are morphologically heterogeneous and are known to become more malignant (progress) with time [14]. Thus, for both reasons, adequate sampling of a tumour is essential for correct classification and grading. Morphological classification of gliomas is dependent on the recognition of areas with the characteristic histology for a particular tumour type, often assisted by immunocytochemistry. The histological criteria for malignancy grading are not uniform for all tumour types and thus classification must precede the assigning of a WHO malignancy grade. The most malignant part of the tumour determines the malignancy grade (often called WHO grade). The WHO system recognises four grades of malignancy and these provide an assessment of how biologically aggressive the untreated tumour may be. The spectrum stretches from grade I tumours, the least aggressive that may be cured by surgery alone (e.g. pilocytic astrocytoma) through grade II tumours where patients survive for a median of about 7 years to grade III tumours where the median survival is around 3 years and the highly aggressive grade IV tumours that grow rapidly, infiltrate surrounding brain and are usually rapidly fatal on average within a year (e.g. glioblastoma). Successful therapy may modify these survival times. General criteria for determining WHO malignancy grade include cellularity, degree of polymorphism and atypia, the incidence of mitoses, the presence of spontaneous necrosis and the degree of angiogenesis induced by the tumour (microvascular proliferation). The criteria for each tumour type have been empirically derived and apply only to untreated tumours, as therapy may alter tumour morphology. There are no premalignant lesions recognised. There are, however, a number of familial cancer syndromes associated with an increased risk of brain tumours, these include neurofibromatosis types 1 and 2 (NF1, -2), Li–Fraumeni syndrome, tuberous sclerosis, Turcot syndrome B, Gorlin's syndrome, Cowden disease and melanoma-astrocytoma syndrome [15]. Recent genome wide association studies have identified genetic variants at several loci that are associated with an increased risk of gliomas [16].

Astrocytomas

The astrocytomas include pilocytic astrocytomas WHO grade I (most common CNS tumour in children) with excellent 10 year survival, diffuse astrocytoma WHO grade II, anaplastic astrocytoma WHO grade III (the latter two most common in middle life) and glioblastoma WHO grade IV (common in later life) with little 2 year survival despite recent therapeutic advances.

Pilocytic Astrocytoma WHO Grade I

Pilocytic astrocytomas occur mainly in the posterior fossa, but can arise anywhere in the CNS. Classically they are of low to moderate cellularity, consisting of both compact bipolar (hair-like) cells with Rosenthal fibres and loosely textured multipolar cells with microcysts. Cells with pleomorphic nuclei, mitoses, microvascular proliferation and infarct-like necrosis are all compatible with a diagnosis of pilocytic astrocytomas, sometimes making the distinction from other gliomas difficult when examining small biopsies. Although macroscopically relatively well defined, microscopically varying degrees of invasion into the adjacent brain are observed. Recently, a variant named pilomyxoid astrocytoma that occurs most commonly in the hypothalamus/chiasmic region has been described and provisionally given a WHO malignancy grade of II. Pilocytic astrocytomas have been found to have mutations of genes coding for components of the MAP-kinase pathway – from cell surface receptor genes (e.g. *FGFR1* or *NTRK2*) to *BRAF*, with fusions between the uncharacterised gene *KIAA1549* and *BRAF* the commonest aberration (about 70%) [17,18]. A further five genes have been identified as fusion partners for *BRAF* and a fusion between *SRGAP3* and *RAF1* has also been found [19,20]. All fusion proteins retain the kinase domain of BRAF or RAF, losing their regulatory domains with consequent constitutive activation of the MAP kinase pathway. Pilocytic astrocytomas are the most common glioma associated with NF1, the mutated *NF1* gene product, neurofibromin being unable to control RAS activity. Identification of the *KIAA1549–BRAF* fusion has been used as a diagnostic marker for pilocytic astrocytomas, although there are some reports that this fusion may also occur rarely in other tumour types [21]. These findings have resulted in clinical trials of inhibitors of BRAF or targets further down the MAP kinase pathway.

Diffuse Astrocytomas WHO Grade II

Although these tumours were previously histologically considered a single entity, molecular analysis indicates that there may be several histologically indistinguishable types, the main differences currently recognised being between the paediatric and adult forms. The tumours are characterised by relatively well-differentiated, astrocyte-like tumour cells with a moderate increase of cellularity compared with normal brain (Figure 1a). Mitotic activity is limited or absent. They have a peak incidence between 20 and 45 years, but can occur at any age and may be located anywhere in the CNS. The adult form is generally characterised by point mutations of one copy of *IDH1* or *IDH2* in about 80% of cases, resulting in substitutions at amino acid 132 in *IDH1* (about 90% are R132H, other substitutions include R132C/S/L/G/V) or substitutions in *IDH2* at amino acid 172 (R172K/M/W/S/G). The second copy of the gene is always wild-type [22,23]. Mutations of either gene are believed to result in similar metabolic changes in the tumour cells, disrupting mechanisms controlling both DNA and histone methylation (recently reviewed in [24]). These mutations are thought to be the earliest in the development of these tumours and occur together with mutations of the DNA-binding domain of *TP53* (>60%) and mutations of *ATRX* (about 70%). The latter is a helicase involved in assisting the deposition of the histone protein H3.3 into chromatin, particularly in telomeric regions, probably ensuring telomere maintenance [25,26]. Other related mutations include

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