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Morphological and molecular features of diffuse infiltrating astrocytomas

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INTRODUCTION

Diffusely infiltrating astrocytomas (DIAs) are the most common primary brain tumors. The 2000 World Health Organization (WHO) classification of tumors of the nervous system divides them into the following clinicopathologic entities: diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III) and multiforme glioblastoma (WHO grade IV). DIAs have the tendency for malignant progression, with the glioblastoma multiforme (GBM) as the most malignant phenotypic endpoint (1).
It was pointed out that the classification, grading, subtyping and prognosis of DIAs only on the basis of morphological and immunohistochemical analysis may be insufficient. However, the majority of these problems were recently resolved by identification of the most common molecular genetics alterations associated with this group of tumors.

MORPHOLOGICAL FEATURES

The morphological features of DIAs as well as their WHO grade are defined by 2000 WHO criteria. These include: cell types (different forms of astrocytes, multinucleated giant cells, small undifferentiated cells, granular cells, lipidized cells); rate of tumor cell proliferation assessed by counting mitoses and by determining the fraction of Ki-67/MIB-1 positive nuclei; necrosis (not present, band-like with pseudopalisading, large ischaemic); microvascular proliferation (not present, classic with capillary-like microvessels, bizarre with glomeruloid/garland-like vascular formations); thromboses and GFAP and p53 expression levels.
Diffuse astrocytomas (WHO grade II) are predominantly manifested in young adults (mean 34 years). They are characterized by high degree of cellular differentiation, slow growth and diffuse infiltration of neighboring structures. Microvascular proliferation and necrosis are absent while the single mitoses may be present. The tendency for malignant progression to anaplastic astrocytoma and eventually to glioblastoma is evident. Anaplastic astrocytoma (WHO grade III) arise de novo or from less malignant diffuse astrocytoma. Males are frequently affected and the mean age is 41 years. Histopathologically, there is increased cellularity, distinct nuclear atypia and marked mitotic activity. Microvascular proliferation and necrosis are not present. They show tendency for malignant progression to GBM.
Glioblastoma multiforme is the most common type of malignant primary brain tumor in adults. Median patient survival is around 1 year while the 5-years survival rate is <5%. Histopathological features include cellular polymorphism, nuclear atypia, mitotic activity, microvascular proliferation, vascular thrombosis and necrosis. GBM may develop de novo ("primary GBM") or from less malignant precursor lesion ("secondary GBM"). However, the majority of GBM develop de novo with short clinical history usually less than 3 months. They may manifest at any age, but are more common in adults (mean 55 years). The males are more frequently affected. The secondary GBM occur in younger age group (mean 39 years), show a slightly more favorable outcome and develop far less often than primary GBM. The time interval for progression from diffuse low-grade astrocytoma to secondary GBM varies considerably (mean 4-5 years). In regard to histopathological and immunohistochemical features there are no differences between primary and secondary GBM (2).

MOLECULAR GENETIC FEATURES

Molecular genetics of diffuse astrocytoma (WHO grade II) include point mutations in the TP53 tumor suppressor gene (50-80%) (3). Since approximately 25% of them do not contain TP53 mutation, other genetic alterations may be involved. Overexpression of PDGF-A and PDGFR-α is observed in astrocytic tumors of all stages (60%), but gene amplification was only detected in a small subset (<10%) of secondary GBM. Anaplastic astrocytoma (WHO grade III) has a high frequency of TP53 mutations. Additional genetic changes found in some percentage of cases include: p16 and p19 deletion, RB alterations, and LOH on chromosome 19q (50%) (4). Recent studies have identified distinct molecular alterations in GBM, adding a novel set of parameters for evaluation of its subtyping, clinical course and therapeutic responses (5).
The genetic hallmark of primary GBM that typically lack a TP53 mutation is MDM2 amplification/overexpression (50%). Additional genetic changes are EGFR amplification (40% of cases) and/or overexpression (60%), CDKN2-A, CDKN2-B and PTEN mutations (30%), RB alteration and p16 deletion (30-40%) (6). Their genetic characteristic is LOH on the entire chromosome 10 (50-80%). The sequence in which gene alterations are acquired is not known since these neoplasms develop very rapidly, without a clinically or histopathologically identifiable precursor lesion. The TP53 mutations are less common in primary GBM (<10%). Secondary GBM frequently associate with mutations of gene TP53. These mutations in more than 90% cases are already present in the first biopsy of diffuse low grade or anaplastic astrocytoma. Most likely, the TP53 mutation is the initial gatekeeper lesion in astrocytic tumors, which then, through genetic instability undergoes malignant progression. The pathway to secondary glioblastoma is further characterized by LOH on chromosomes 19q and 10q (but not on the entire chromosome 10 as it is seen in primary GBM) (7,8). Recently it was pointed out that genomic alterations of LOH 1p and 19q, which are observed in the majority of oligodendroglioma, may be observed in GBM. However, in contrast to oligodendroglioma, in GBM loss of 19q is more likely to be partial than complete and loss of 1p is uncommon (approx. 10%). It was suggested that combined losses of chromosome arms 1p and 19q may indicate the better prognosis and potential sensitivity to chemotherapy in GBM patients, while isolated loss of either 1p or 19q is of no prognostic significance (9).

PROGNOSTIC PREDICTORS IN GBM

Although survival is short in most patients, a small fraction of long-term survivors is noted. Recent clinicopathologic studies have shown that several

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morphological and genetic features in GBM correlate with overall survival and thus allow prognostic predictors. These features comprise: younger age, lateral tumor localization, macroscopic complete resection, areas of better histopathological differentiation and abundant presence of giant cells (gigantocellular glioblastoma), TP53 mutations and p53 expression levels, rate of tumor cell proliferation, microvascular proliferation and LOH on chromosome 1p and 19q (10).

TP53 mutations. Recent studies indicate that TP53 mutations are a favorable prognostic factor independent of primary or secondary GBM. These mutations show the tendency to occur more frequently in younger than in older GBM patients. This may explain the better survival associated with secondary GBM since these patients frequently combine 2 favorable parameters - younger age and TP53 mutations. The high p53 expression levels (>50% of tumor cells nuclei immunolabeled) assessed by immunohistochemistry indicate the presence of a TP53 mutations and correlate with a more favorable survival (11).

Rate of tumor cell proliferation. Survival analysis disclosed an association between high Ki-67 expression levels (>27% of tumor cells nuclei immunolabeled) and prolonged survival. The possible explanation is that GBM patients with high rate of tumor cell proliferation have the beneficial effect of adjuvant therapy (12).

Microvascular proliferation. The prominent classic vascular pattern and low content of bizarre vascular formations is a factor for longer survival. The even distribution of densely arranged delicate vessels within tumor tissue as it is seen in classic vascular pattern may provide better access of chemotherapeutic drugs to tumor tissue and more effective radiotherapy due to better oxygenation of tumor tissue (13).

LOH on chromosome 1p and 19q. It was noted on a small number of patients that a combined losses of chromosomal arms 1p and 19q may indicate the better prognosis and potential sensitivity to chemotherapy in GBM. If this were to be confirmed, LOH analyses may allow identification of a subgroup of chemosensitive GBM patients that could not be distinguished by morphological investigation.

CONCLUSION

Morphological evaluation should remain the chief support of DIAs diagnosis and classification. Nevertheless, molecular genetic studies play an important part in resolving some diagnostic problems and can be used to subdivide GBM into biologically distinct subtypes. Recent clinicopathologic studies have shown that several morphologic and genetic features in GBM correlate with overall survival and thus allow prognostic predictors. The prognostically favorable features include TP53 mutations, prominent tumor cell proliferation, predominant classic vascular pattern and LOH on chromosomes 1p and 19q.

REFERENCES

1. Kleihues P, Cavenee WK, editors. WHO classification of tumours: Pathology and genetics of tumours of the nervous system. Lyon: WHO/IARC; 2000.

2. Kleihues P, Ohgaki H. Primary and secondary glioblastoma: from concept to clinical diagnosis. Neuro-Oncology 1998;1:44-51.

3. Louis DN. The p53 gene and protein in human brain tumors. J Neuropath Exp Neurol 1994;5:11-21.

4. Louis DN. A molecular genetic model of astrocytoma histopathology. Brain Pathol 1997;7:755-64.

5. Von Deimling A, von Ammon K, Schoenfeld DA, Wiestler OD, Seizinger BR, Louis DN. Subsets of glioblastoma multiforme defined by molecular genetic analyses. Brain Pathol 1993;3:19-26.

6. Hayashi Y, Ueki K, Waha A, Wiestler OD, Louis DN, von Deimling A. Association of EGFR gene amplification and CDKN2 (p16/MTS1) gene deletion in glioblastoma multiforme. Brain Pathol 1997; 7:871-5.

7. Lang FF, Miller DC, Koslow M, Newcomb EW. Pathways leading to glioblastoma multiforme: molecular analyses of genetic alterations in 65 astrocytic tumors. J Neurosurg 1994;81:427-36.

8. Von Deimling A, Fimmers R, Schmidt MC et al. Comprehensive allelotype and genetic analysis of 466 human nervous system tumors. J Neuropath Exp Neurol 2000;59(6):544-58.

9. Smith JS, Perry A, Borell TJ. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas and mixed oligoastrocytomas. J Clin Oncol 2000;18:636-45.

10. Schmidt MC, Antweiler S, Urban N, Mueller W, Kuklik A, Meyer-Puttlitz B et al. Impact of genotype and morphology on the prognosis of glioblastoma. J Neuropath Exp Neurol 2002;61(4):321-8.

11. Birner P, Piribauer M, Fisher I, Gatterbauer B, Marosi C, Ungersbock K et al. Prognostic relevance of p53 protein expression in glioblastoma. Oncol Reports 2002;9(4):703-7.

12. Bredel M, Piribauer M, Marosi C, Birner P, Gaterbauer B, Fisher I et al. High expression of DNA topoisomerase II alpha and Ki-67 antigen is associated with prolonged survival in glioblastoma patients. European J Cancer 2002;38(10):1343-7.

13. Birner P, Piribauer M, Fisher I, Gatterbauer B, Marosi C, Ambros PF et al. Vascular patterns in glioblastoma influence clinical outcome and associate with variable expression of angiogenic proteins: evidence for distinct angiogenic subtypes. Brain Pathol 2003;13(2):133-43.