1500 words (excluding references)

Uncovering the Genetics of Brain Cancer: Recent Advances

And Their Diagnostic and Clinical Significances

Yizhou Wan MB. BS. BSc (Hons). M.R.C.S (Eng)

Introduction

Gliomas constitute the most common form of central nervous system malignancy (Ren et al, 2013; Appin and Brat, 2015). Apart from well circumscribed low-grade gliomas (LGG), most are challenging to treat due to their diffusely infiltrative nature. Complete resection is impossible and prognosis can be dismal, especially for glioblastoma multiforme (GBM).

Current diagnosis is based on morphological criteria defined by the World Health Organisation (WHO) classification system. Histological grading is problematic for several reasons. Firstly, subjective grading is prone to inter and intra-observer variability leading to poor prediction of clinical behaviour and diagnostic difficulties (Guan et al, 2014; TCGA, 2015). Secondly, genetic profiling of tumours identifies markers of immediate relevance to predicting treatment benefit and aids development of novel therapeutics.

1500 words (excluding references)

Recent advances in low-grade glioma genetics

Isocitrate Dehydrogenase 1/2 (IDH) mutations are found in more than 70% of grade II/III gliomas (> 90% are IDH1 mutations) and whilst wild-type IDH catalyses the conversion of isocitrate to alpha-ketoglutarate, mutant IDH1 catalyses the generation of the oncometabolite D-2HG from α-KG (Venniti & Huse, 2015). Introduction of IDH1 mutations into primary human astrocytes induces DNA hypermethylation, similar to the epigenetic changes seen in a subset of LGG known as the CpG-island hypermethylator phenotype (G-CIMP) (Noushmehr et al, 2010, Turcan et al, 2012).

IDH1 mutations are found in both astrocytic and oligodendroglial gliomas and it is thought to be an early event which occurs prior to other lineage-specific mutations has occurred (Appin and Bratt, 2015). Furthermore, IDH1 mutations are strongly associated with both p53 mutations and 1p/19q codeletions. Patients with IDH mutations show longer overall survival (OS) compared to IDH wild-type patients, regardless of grade.

1p/19q codeletion is seen in 70% of oligodendrogliomas and is a favourable prognostic factor (Venniti & Huse, 2015). CIC (homolog of the Drosophila gene capicua) on chromosome 19q and FUBP1 (FUSE-binding protein 1) on chromosome 1p mutations are observed in 60 to 80% of oligodendrogliomas. CIC and FUBP1 serve as negative regulators of RTK signalling and c-Myc transcription respectively (Venniti & Huse, 2015). In three randomised-control trials comparing radiotherapy to radiotherapy and alkylating agents for anaplastic gliomas, patients with 1p/19q codeletion demonstrated a survival advantage regardless of treatment modality (Weller et al, 2012).

1500 words (excluding references)

In IDH-mutant LGG ATRX mutation was mutually exclusive with 1p/19q codeletion and associated with p53 mutation (Liu et al, 2012). The DNA helicase, ATRX (a-thalassemia/mental retardation syndrome X-linked) is described in both adult and paediatric gliomas (Kannan et al, 2012). It is associated with pathological telomere maintenance via the Alternative Lengthening of Telomeres (ALT) phenotype and is found in 33- 67% of grade II astrocytic tumours.

Telomerase reverse transcriptase (TERT) point mutations maintain telomere length and cellular immortality. They have been discovered in 63-78% of oligodendrogliomas and between 0 to 32% of diffuse astrocytomas (Venniti & Huse, 2015). TERT mutations are inversely associated with IDH mutations but are closely associated with 1p/19q codeletion and are mutually exclusive with ATRX mutations (Venniti & Huse, 2015). Whilst the ATRX mutation is common in astrocytomas, it is rare in oliodendrogliomas where instead the TERT mutations play the role of maintaining telomere length.

An early event in gliomaenesis is thought to be methylation of MGMT promoter. It is found in grade II/III gliomas as well as GBM and is associated with p53 mutations in diffuse astrocytomas (Nakamura et al, 2001). The RTOG 0525 study showed that patients with MGMT promoter methylation had longer OS versus patients without (23.2 months vs. 16 months) (Gilbert et al, 2006). The Nordic trial demonstrated that elderly GBM patients with MGMT mutation had an improved OS when treated with temozolamide but not when treated with radiotherapy alone (Weller et al, 2012).

Genome wide analysis of 293 LGG by TCGA network demonstrated that LGG with IDH mutation have either 1p/19q codeletion or p53 mutation in a dichotomous fashion. Patients with IDH wild-type had the worst survival followed by patients with IDH mutation but no codeletion. Patients with both mutations demonstrated the most favourable prognosis (TCGA, 2015). In fact patients with wild-type IDH LGG only had slightly longer survival compared to patients with wild-type GBM. This demonstrates

that genomic analysis may better prognosticate patients and there are common molecular mechanisms which drive both LGG and GBM.

In a recent study, classifying gliomas into IDH, TERT and 1p/19q codeletion demonstrated excellent consistency between molecular groups and clinical behaviour across three data sets (Eckel-Passow et al, 2015). The vast majority of TERT only mutated gliomas were GBM, although 9.4% were grade II/III tumours, these tumours exhibited an aggressive course. Some TERT tumours also harboured ATRX mutations, challenging the mutual exclusivity of these two mutations. In the presence of IDH and 1p/19q codeletion, TERT mutations are favourable. A possible hypothesis is that telomere maintenance is a prerequisite event in gliomagenesis.

Recent advances in High-grade glioma genetics

Data from the TCGA network has demonstrated that the majority of GBMs analysed harboured mutations in the p53, retinoblastoma and receptor tyrosine kinase pathways. This suggests that these pathways are necessary for GBM pathogenesis. Further genomic profiling has classified GBM into four subtypes; proneural, neural, classical and mesenchymal (Verhaak et al, 2010). In murine models exposure to radiation caused a proneural to mesenchymal shift in the glioma expression pattern (Haliday et al, 2014). This may have important implications for cell-differentiation treatment strategies.

The proneural subtype is associated with PDGFR, p53 and IDH mutations. IDH mutations are prevalent in secondary GBM and the proneural class may reflect transformation from a LGG, which may be clinically silent or arise from a common progenitor cell. 75% of lower grade gliomas and most grade

1500 words (excluding references)

III gliomas can be classified into proneural and neural subtypes (Philips et al, 2006). The neural subtype shares the greatest expression similarity with normal brain tissue, suggesting possible transformation from a differentiated cell phenotype. Clinically the proneural substype shows a trend towards longer survival but no benefit from aggressive treatment protocols. The improved survival of proneural tumours both LGG and GBM may be associated with the hypermethylator phenotype seen with IDH mutations. Furthermore, computational models of proneural gliomas suggest that genetic mutations such as p53 and IDH1 are selected for and temporally constrained throughout tumourgenesis (Sonabend et al, 2014).

The classical subtype is defined by the most common genomic abnormalities seen in GBM. EGFR amplifications, chromosome 7 amplifications, chromosome 10 deletions and Ink4a/ARF deletions are seen, however there is a lack of abnormalities in p53, PDGFRA, IDH1 and NF1. Indeed EGFR amplifications may be a key driver mutation in classical GBMs and can be rarely found in grade II gliomas (Guan et al, 2014).

The mesenchymal subtype is characterised by high expression levels of CHI3L1 and MET, as well as a high frequency of NF1 mutations. Mesenchymal GBMs typically have a poorer prognosis compared to the proneural subtype and is resistant to conventional treatment. Interestingly, these tumours show a significantly extended survival when treated with combination adjuvant therapy in a phase I trial of dendritic cell vaccination. Potentially, one hypothesis is that this gene signature correlates with an increased intra-tumoural infiltration of CD3-/CD8+ lymphocytes (Prins et al, 2011).

All of the aforementioned studies are based on samples derived from resected tumour specimens or biopsy specimens. In many cancers such as breast or head and neck cancers, it has been recognised

that intratumour heterogeneity can play an important role in treatment prediction and outcome (Parker and Perou, 2015). Single-cell analysis of GBM has shown that subclone selection and competition changes the spatial characteristics of GBM tumours. For example, high-levels of EGFR amplification subclones repopulate mice xenografts and that secondary transplants form tumours faster than primary xenografts, reflecting clinical reality (Piccirillo et al, 2015).

In chemotherapy naïve patients, treatment resistance may already be present in a small population of subclones (Piccirillo et al, 2015). Treatment is a selection pressure which can alter the clonal dynamics of the tumour. The mutual exclusivity of RTK pathways can be shifted, in the TCGA dataset 7.3% of tumours harboured focal amplifications of 2 or more RTKs (Szerlip et al, 2012). Temozolamide has also be associated with a hypermutation phenotype in recurrent GBM, highlighting the importance of understanding epigenetic changes (Watts, 2015).

Conclusions

It is recognised that histology poorly predicts and prognosticates in both low-grade and grade gliomas. Recent advances in genomic classifications offer improved classification of tumours based on underlying alterations during tumourgenesis. They demonstrate common genomic signatures between different histological grades of glioma. The classification of GBM into four subtypes hints at possible multiple cellular pathways which converge during gliomagenesis.

1500 words (excluding references)

Furthermore, Darwinian selection pressures caused by treatment can spatially and temporally alter subclone dynamics within tumours, potentially affecting clinical outcomes. Monitoring this throught treatment will prove challenging.

Future work needs to understand both inter and intra-tumoural heterogeneity, particularly as current studies focus on tissue samples from the contrast-enhancing surgical site. Depending on the GBM subtype the infiltrative margins of non-enhancing sites differentially express genes, associated with different cellular phenotypes (Gill et al, 2014). This is crucial as disease recurrence commonly depends on residue disease at the tumour margins.

References

Appin C, Brat D. Molecular Pathways in Gliomagenesis and Their Relevance to Neuropathologic Diagnosis. Adv Anat Pathol 2015; 22:50–58.

Capper D, Weissert S, Balss J, et al. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. Brain Pathol. 2010;20:245–254.

Gill BJ, Pisapia DJ, Malone HR, et al. MRI-localized biopsies reveal subtype-specific differences in molecular and cellular composition at the margins of glioblastoma. Proc Natl Acad Sci USA. 2014;111(34):12550–12555.

Guan X, Vengoechea J, Zheng S, Sloan, A.E, Chen Y, Brat, D et al. Molecular Subtypes of Glioblastoma Are Relevant to Lower Grade Glioma. Plos One 2014; 9(3):e91216.

1500 words (excluding references)

Kannan K, Inagaki A, Silber J, et al. Whole-exome sequencing identifies ATRX mutation as a key molecular determinant in lower-grade glioma. Oncotarget. 2012;3:1194–1203.

Liu XY, Gerges N, Korshunov A, et al. Frequent ATRX mutations and loss of expression in adult diffuse astrocytic tumors carrying IDH1/IDH2 and TP53 mutations. Acta Neuropathol. 2012;124:615–625.

Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma groups based on 1p/19q, *IDH*, and *TERT* promoter mutations in tumors. N Engl J Med 2015;372:2499-2508.

Gilbert MR, Wang M, Aldape KD, et al. RTOG 0525: A randomized phase III trial comparing standard adjuvant temozolomide (TMZ) with a dose-dense (dd) schedule in newly diagnosed glioblastoma (GBM). J Clin Oncol 29: 2011 (suppl; abstr 2006).

Guan X, Vengoechea J, Zheng S, et al. Molecular subtypes of glioblastoma are relevant to lower grade glioma. PLoS One 2014;9:e91216-e91216.

Halliday, J., et al., *In vivo* radiation response of proneural glioma characterized by protective p53 transcriptional program and proneural-mesenchymal shift. Proc Natl Acad Sci USA. 2014;111: 5248-53.

Nakamura M, Watanabe T, Yonekawa Y, et al. Promoter methylation of the DNA repair gene MGMT in astrocytomas is frequently associated with G:C –> A:T mutations of the TP53 tumor suppressor gene. Carcinogenesis. 2001;22: 1715–1719.

1500 words (excluding references)

Noushmehr H, Weisenberger DJ, Diefes K, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell. 2010; 17:510–522.

Parker, J.S. Perou, C.M. Tumor Heterogeneity: Focus on the Leaves, the Trees, or the Forest? Cancer Cell. 2015. 28(2); 149-150.

Phillips, H.S., Kharbanda, S., Chen, R., Forrest, W.F., Soriano, R.H., Wu, T.D., Misra, A., Nigro, J.M., Colman, H., Soroceanu, L., et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell. 2006;9:157–173.

Piccirillo SG, Spiteri I, Sottoriva A *et al.* Contributions to drug resistance in glioblastoma derived from malignant cells in the sub-ependymal zone. 2015. Cancer Res. 75(1), 194–202.

Prins RM, Soto H, Konkankit V, Odesa SK, Eskin A, Yong WH, Nelson SF, Liau LM. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. Clin Canc Res. 2011;17:1603–1615.

Sonabend AM, Bansal M, Guarnieri P, Lei L, Amendolara B, Soderquist C, Leung R, Yun J, Kennedy B, Sisti J, Bruce S, Bruce R, Shakya R, Ludwig T, Rosenfeld S, Sims PA, Bruce JN, Califano A, Canoll P (2014) The transcriptional regulatory network of proneural glioma determines the genetic alterations selected during tumor progression. Cancer Res 74:1440–1451.

Szerlip NJ, Pedraza A, Chakravarty D *et al.* Intratumoral heterogeneity of receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma defines subpopulations with distinct growth factor response. Proc. Natl Acad. Sci. USA. 2012;109(8):3041–3046.

1500 words (excluding references)

The Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med 2015;372:2481-2498.

Turcan S, Rohle D, Goenka A, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature. 2012;483:479–483.

Venneti S, Huse JT. The evolving molecular genetics of low-grade glioma. Adv Anat Pathol. 2015;22:94–101.

Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010;17:98-110.

Watts, C. Clonal diversity in glioblastoma: is it clinically relevant? Future Oncol. 2015;11(12):1703-6.

Weller M, Stupp R, Hegi ME, et al. Personalized care in neuro-oncology coming of age: why we need MGMT and 1p/19q testing in malignant glioma patients in clinical practice. Neuro Oncol 2012; 14: 100–08.