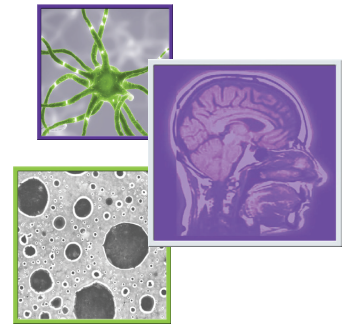
EDITORIAL

CNS Oncology

Genomic landscape of glioblastoma and the potential clinical utility



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Glioblastoma multiforme (GBM) is the most common primary brain cancer with more than 10,000 new cases per year in the USA [1]. Despite steady advances in neurosurgery, radiation and chemotherapy, GBM remains essentially incurable with the survival usually about a year. Complete surgical removal is generally unattainable because of its infiltrative nature. Radiation has consistently shown to improve survival, but the disease inevitably recurs and nearly always within the area targeted with high doses of radiation [2][3]. As for chemotherapy, adding temozolomide to radiation concurrently and adjuvantly modestly improved survival among young patients with good performance status and has become the standard of care [4]. However, the 5-year survival rate was still less than 10%. In short, current treatments are ineffective. What causes such therapeutic resistance in GBM is unclear but novel therapeutic approaches are desperately needed. Understanding the molecular basis for GBM may be the key to overcome treatment resistance and improve clinical outcomes.

In order to elucidate the pathogenesis of GBM, it has been the subject of intense molecular characterization over the last decade. One of the first landmark studies to utilize genome-scale analysis to characterize the molecular heterogeneity of high-grade gliomas was by Phillips and colleagues [5]. Using gene expression microarray data from 76 tumor samples, they described three GBM subtypes, designated proneural, proliferative and mesenchymal. Molecular subtyping was important for prognosis, independent of other established prognostic factors, such as the World Health Organization tumor grade. They further showed that the subtype with favorable prognosis displayed neuronal lineage markers, whereas those with poor prognosis were enriched for markers of neural stem cells, proliferation and angiogenesis. The proposition that there exist distinct GBM subtypes with prognostic implication was corroborated by Verhaak and colleagues [6]. They performed consensus clustering of 200 GBM samples from The Cancer Genome Atlas (TCGA) and identified four subtypes, each of which was characterized by association with a specific set of genomic alterations. For example, EGF receptor (EGFR) amplification was observed in 97% of the 'classical' subtype, whereas the 'proneural' subtype was typified by amplification and overexpression of PDGF receptor-α (PDGFRA) and mutation in IDH1. The potential clinical significance was suggested by the observation that "more intensive treatment" (defined as concurrent chemoradiation or more than

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three subsequent cycles of chemotherapy) was associated with improved survival in all subtypes but not in the 'proneural' type. Although it is tantalizing to envision classifying GBM into various subtypes and to administer specific treatment based on its molecular characteristics, it currently remains experimental and needs clinical validation. What has become more certain though is that, as the name glioblastoma *multiforme* suggests, GBM encompasses a heterogeneous group of diseases that likely have different genetic etiology, treatment response and clinical outcomes.

Using gene expression profiling to obtain prognostic information is feasible and likely more informative than the traditional histology-based approach that relies on morphologic features, such as atypia, mitotic figures and presence of necrosis. In fact, some of the molecular features of GBM are already in clinical use for diagnostic and prognostic purposes, including 1p19q deletion, MGMT promoter methylation and IDH1 mutation status. However, beyond prognosis, what will really impact clinical management of GBM is if genomic analyses can lead to customized treatment and ultimately improved survival. With this as the goal, TCGA was created to provide a comprehensive database that integrates massive genome-scale information from tumor tissues. This database would also contain clinical annotations, so that biological patterns can be correlated with patient, treatment, and cancer characteristics. GBM was the first cancer type to be catalogued by TCGA, and profiles of gene sequences, copy number variations, DNA methylation, mRNA expression, and miRNA expression were collected using various array technologies [7, 8]. The utility of TCGA for GBM was first demonstrated in their publication in 2008 [7], which provided an integrated overview of the complex genomic landscape from over 200 patients samples. It showed three fundamental pathways in gliomagenesis, namely receptor tyrosine kinase (RTK) signaling and the p53 and Rb tumor suppressor pathways, which were altered in 88, 87% and 78%, respectively [7]. In their updated publication with now more than 500 GBM samples [8], a list of GBM signature genes was proposed based on somatic mutation analysis. The list includes well-known cancer genes, such as PTEN, p53, NF1, Rb, IDH1, and EGFR. It also identified new genes, which were not previously implicated in gliomagenesis, such as LZTR1.

The ultimate goal of molecularly characterizing GBM is to develop effective pharmaceutical agents against its core molecules. One successful example is the discovery of IDH1 mutations in GBM. In a large project that sequenced 20,661 coding genes in 22 human GBM samples, 11% harbored an identical mutation in R132 of the IDH1 gene [9]. Subsequent analyses found that IDH1 mutations occurred in more than 70% of grade II/III gliomas [10]. The mutation was found more commonly in younger patients or secondary GBM, and it was associated with better survival. Biochemical characterization showed that the mutant IDH1 converts α-ketoglutarate to 2-hydroxyglutarate (2-HG) instead of isocitrate [11]. 2-HG may be an oncogenic metabolite that can impair histone modification and block neural differentiation [12]. Although the exact role of mutant IDH1 in gliomagenesis remains to be clarified, a high-throughput compound screen was conducted and identified a potent small molecule inhibitor of mutant IDH1 [13]. When tested *in vivo*, the inhibitor induced glial differentiation and delayed tumor growth in IDH1-mutant glioma cells but not IDH1-wildtype cells. Clinical testing of this compound is underway.

Although promising in laboratory studies, many agents targeting core GBM pathways have been disappointing when tested in clinical trials. For example, EGFR is a tyrosine kinase receptor that appears to play a critical role in GBM pathogenesis [7]. There have been several clinical trials investigating the efficacy of EGFR inhibitors in GBM, but the results have been inconclusive at best. When gefitinib, a small molecular inhibitor of EGFR was tested with radiation in newly diagnosed GBM patients, the benefit was negligible with the median survival of 11.5 months, which was not different from the historical control treated with radiotherapy alone [14]. Another trial using erlotinib combined with temozolomide and radiation suggested improved survival (19.3 months) [15], but it had no effect when tested in recurrent GBM [16]. Another approach was to develop a vaccine against EGFRvIII, the most common variant of EGFR mutants found in GBM. In a phase II trial of patients who had surgery and concurrent radiation with temozolomide, intradermal vaccine targeting EGFRvIII was administered. The survival improved to 26 months compared to the historic control (14 months) [17]. Interestingly, among patients who mounted an immune response to the vaccine,

the median survival was dramatically improved to 48 months. A Phase III trial testing the vaccine in newly diagnosed GBM is currently recruiting. As amply demonstrated in many other malignancies, such as non-small cell lung cancer, identifying the subgroup of patients with specific genetic alteration may improve the overall efficacy. It is interesting to note that EGFR is frequently co-activated with other RTK (e.g., c-MET), and simultaneous inhibition of two or more kinases may be necessary before any clinical benefit is observed [18].

In addition to genetic alterations, there are epigenetic mechanisms mediating GBM pathogenesis. Epigenetic alterations refer to biochemical modification to the DNA or other proteins that can alter gene expression without any changes in the DNA sequence. DNA methylation is one such epigenetic modification that has been shown to play an important role in cancer. For example, hypermethylation of the MGMT promoter is strongly associated with improved survival in GBM [19]. Even with similar transcriptional patterns, TCGA showed a subgroup can be further divided into groups with distinct patient/disease characteristics based on their methylation profiles [8, 20]. Within the 'proneural' subgroup in TCGA, the median age of diagnosis was substantially younger in patients with hypermethylation at a large number of loci, compared to those without (41 vs 56 years). The presence of so-called glioma CpG island methylator phenotype (G-CIMP) increases the likelihood of MGMT promoter methylation. Intriguingly, TGCA analysis suggested MGMT promoter methylation was preferentially associated with mismatch repair (MMR) deficiency [7]. The involvement of MMR suggests a possible mechanism of treatment resistance and potential synergistic effect when combined with genotoxic agents.

Recent large-scale studies in GBM have provided an unprecedented amount of genomic and epigenomic information about this devastating disease. Interpretation and organization of this information so that it can be applied clinically will be the next task. Using genomic profiling for prognostication is ready for clinical validation, and it will become the mainstay in the management of GBM in the near future. Development of targeted agents and identification of specific subgroups who will benefit from such agents will be more challenging, but with continued effort in both laboratory and clinical sciences, a therapeutic strategy that is precisely tailored to individual patients with GBM is anticipated.

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