Perspective

Glioblastoma biomarkers: finding a needle in a haystack

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Abstract: Glioblastoma is one of the most aggressive brain cancers. Although this tumor is considered relatively rare, it has considerable clinical economic and social impacts due to the limited success that has been achieved so far for its prevention and treatment. The current drawbacks in early diagnostics are mostly attributable to cancer cells heterogeneity and cerebral localization, which both contribute to reduce the potential diagnostic options, thus making the search for candidate biomarkers more or less like finding a needle in a haystack. However, interesting data emerged from recently published studies attest that the assessment of some epigenetic biomarkers (namely methylguanine-DNA-methyltransferase promoter methylation status, combined measurement of some putative micro RNAs), proteomic analysis and cell-free circulating tumour DNA of circulating cancer cells may pave the way to a paradigm shift in glioblastoma diagnostics. It is hence conceivable that combining these different strategies may yield better diagnostic performance that using one single approach alone. Moreover, the potential identification of specific glioblastoma signatures in cerebrospinal fluid, genetic, epigenetic or even biochemical, will perhaps enable identifying localized cancers much earlier than these biomarkers will appear and be measurable in the bloodstream. Further studies will be needed to explore these promising strategies in the future.

Keywords: Glioblastoma; miRNAs; bio-markers

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Introduction

Glioblastoma, also known as glioblastoma multiforme, is one of the most aggressive and lethal brain cancers. Although this malignancy is considered relative rare (the overall prevalence is approximately 3–10:100,000 persons, accounting for ~2.5% of all cancer deaths), life expectancy is extremely poor, usually comprised between 3–5% at five years, with the vast majority of patients dying within 15 months from initial diagnosis (1). The diagnostic approach of patients with glioblastoma is essentially based on imaging techniques, namely magnetic resonance imaging (MRI) using either traditional or advanced technologies or, less frequently, computed tomography (CT), along with (stereotactic needle) tissue biopsy or tumor resection, followed by pathologic analysis (1).

Although many different therapeutic options have been attempted over the past decades (i.e., surgical ablation, radiation, chemotherapy, immunotherapy, anti-angiogenic drugs and epigenetic modulators) (2-4), most of these have been largely unsuccessful to substantially reverse disease progression, so that the prognosis of this type of cancer remains dramatically poor. Therefore, an early diagnosis of glioblastoma seems now the most effective strategy for improving the outcome or, at least, for prolonging progression-free survival of affected patients.

Glioblastoma biomarkers

Although it is now unquestionable that laboratory diagnostics strongly contributes to the screening and diagnosis of many human conditions, laboratory tests in cancer diagnostics are prevalently useful for therapeutic management and early detection of recurrence.

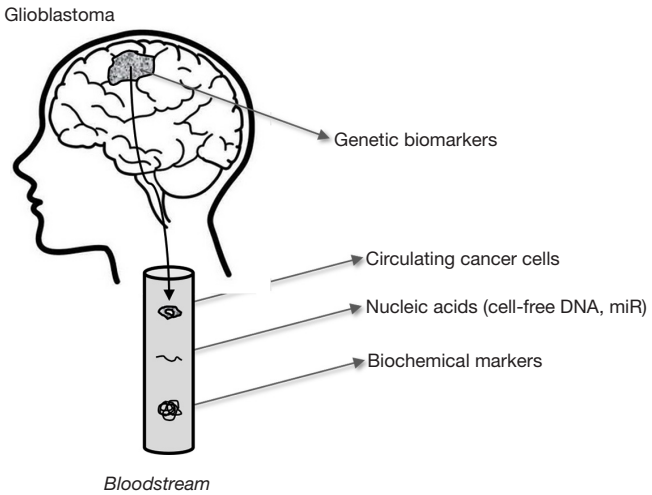

Figure 1 Innovative approaches for glioblastoma diagnostics.

Glioblastoma makes no exception to this rule, but represents an even harder challenge due to many aspects including cancer cell heterogeneity and the fact that the blood-brain barrier further reduces the likelihood that some potentially useful biomarkers, actively released by cancer cells, will early reach the bloodstream where they can then be efficiently assessed. As for many other types of cancers, the putative biomarkers for early detection/diagnosis/monitoring of glioblastoma can be divided in four main categories, encompassing genetic, epigenetic and biochemical biomarkers, as well as circulating tumor cells or DNA (*Figure 1*, *Table 1*).

Regarding the former class, the most studied genetic alterations include mutations or amplifications of genes encoding for growth proteins and cell signaling, namely those belonging to the receptor tyrosine kinase (RTK) signaling [i.e., epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor alpha (PDGFRA), basic fibroblast growth factor receptor 1 (FGFR-1) and insulin-like growth factor receptor (IGFR-1)], *TP53* tumor suppressor gene, retinoblastoma protein (RB1) protein pathway [especially those of cyclin dependent kinase 2a/p16 (CDKN2A/p16) and cyclin-dependent kinase 4 (CDK4)], isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2), phosphatase and tensin homolog (PTEN), loss of heterozygosity (LOH) on chromosome 10q, 1p/19q co-deletion, interleukin-13 (IL-13) receptor α1 and α2, alpha-thalassemia/mental retardation X-linked gene (ATRX) and telomerase reverse transcriptase-encoding gene (TERT) (5-8) (*Table 1*). Albeit the study of these molecular abnormalities is indeed promising not only for glioblastoma diagnostics, but also for developing targeted (personalized) therapies against the involved genetic mutations, their clinical usefulness has been limited so far by the fact that they can be mainly used as prognostic or predictive biomarkers in cancer tissues, whilst their use as early diagnostic biomarkers (i.e., liquid biopsy) has been by far less explored.

The most studied glioblastoma epigenetic biomarkers include the methylguanine-DNA-methyltransferase (MGMT) promoter methylation status, some micro RNAs (miRs) such as miR-21, miR-10b, miR-454-3p, miR-222 and miR-124-3p, among others (5,9,10) (*Table 1*). Notably, a recent epigenetic study carried out in our laboratory allowed to identify a serum exosome signature (i.e., miR-21, miR-222 and miR-124-3p) from patients with glioma, which was characterized by 87% diagnostic accuracy for discriminating cancer patients from healthy controls, as well 83% diagnostic accuracy for discriminating patients with low-grade from those with high-grade cancer (10). This study clearly highlights that the investigation of some exosome-associated miRNAs should strengthen the diagnostic specificity of these biomarkers. Unlike somatic mutations, miRNA have the important advantage that can now be easily, rapidly and economically measured in blood samples, thus representing a less invasive and cumbersome approach compared to other conventional strategies.

Regarding circulating biochemical biomarkers, several studies showed increased concentrations of several proteins, cytokine and hormones, including immunosuppressive acidic protein, alpha-1 acidic glycoprotein, alpha-1 antitrypsin, fibronectin, endothelial cell-derived thrombomodulin-1, vascular endothelial growth factor (VEGF), placental growth factor (PlGF), platelet-derived growth factor (PDGF), specific neuronal protein [i.e., glial fibrillary acidic protein (GFAP), brain-derived neurotrophic factor (BDNF), protein S100 B (S100B), neural cell adhesion molecule (NCAM)], of 2-hydroxyglutarate (2-HG), chitinase-3-like protein 1 (CHI3L1), interleukin-2 (IL-2), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), matrix metalloproteinases (MMPs) or tissue inhibitors of metalloproteinases (TIMPs) such as MMP-2, MMP-9, MMP-10 and TIMP-1, as well as that of other more traditional cancer biomarkers (*Table 1*) (9). Unfortunately, the diagnostic efficiency of all these biomarkers remains relatively low in glioblastoma diagnostics, especially those which are reportedly not tumor-specific (i.e., VEGF, cytokines, MMPs, traditional

Table 1 Putative biomarkers for glioblastoma diagnostics

Genetic markers
Receptor tyrosine kinase (RTK)
Epidermal growth factor receptor (EGFR)
Platelet-derived growth factor receptor alpha (PDGFRA)
Basic fibroblast growth factor receptor 1 (FGFR-1)
Insulin-like growth factor receptor (IGFR-1)
TP53 tumor suppressor gene
Retinoblastoma protein (RB1) protein pathway
Cyclin dependent kinase 2a/p16 (CDKN2A/p16)
Cyclin-dependent kinase 4 (CDK4)
Isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2)
Phosphatase and tensin homolog (PTEN)
Loss of heterozygosity (LOH) on chromosome 10q
1p/19q co-deletion
Interleukin-13 (IL-13) receptor $\alpha 1$ and $\alpha 2$
Alpha-thalassemia/mental retardation X-linked gene (ATRX)
Telomerase reverse transcriptase-encoding gene (TERT)
Epigenetic biomarkers
Methylguanine-DNA-methyltransferase (MGMT) promoter methylation
Micro RNAs
miR-21
miR-10b
miR-454-3p
miR-222
miR-124-3p
Biochemical biomarkers
Immunosuppressive acidic protein
Alpha-1 acidic glycoprotein,
Alpha-1 antitrypsin
Fibronectin
Endothelial cell-derived thrombomodulin-1
Vascular endothelial growth factor (VEGF)
Placental growth factor (PlGF)
Platelet-derived growth factor (PDGF)
Specific neural proteins
Glial fibrillary acidic protein (GFAP)
Brain-derived neurotrophic factor (BDNF)
Protein S100 B (S100B)
Neural cell adhesion molecule (NCAM)
2-hydroxyglutarate (2-HG)
Chitinase-3-like protein 1 (CHI3L1),
Interleukin-2 (IL-2)
Transforming growth factor-β (TGF-β)
Tumor necrosis factor-α (TNF-α)
Complement component C9 (C9)
C-reactive protein (CRP)
Leucine-rich alpha-2-glycoprotein (LRG1)
Gelsolin
Apolipoprotein A-IV (APOA4)
Ig alpha-1 chain C region (IGHA1)
Matrix metalloproteinases (MMPs)
MMP-2
MMP-9
MMP-10
Tissue inhibitors of metalloproteinases (TIMPs)
TIMP-1
Traditional cancer biomarkers
Carcinoembryonal antigen (CEA)
Human chorionic gonadotropin (hCG)
Alpha-fetoprotein (AFP)

cancer biomarkers). Interestingly, in a recent study based on a proteomic approach, eight putative biomarkers for glioblastoma could be identified. Six of these proteins, namely over-expression of complement component C9 (C9), C-reactive protein (CRP), leucine-rich alpha-2-glycoprotein (LRG1) and under-expression of gelsolin, apolipoprotein A-IV (APOA4) and Ig alpha-1 chain C region (IGHA1), yielded a diagnostic efficiency higher than 80%, whilst the concentration of three of these (i.e., C9, CRP and LRG1) was also significantly correlated with tumor size (11).

The assessment of circulating tumor cells is indeed a valuable perspective for diagnostics of many types of cancers. Albeit their early detection in patients with glioblastoma

would be plagued by the same drawbacks as those of other biomarkers (e.g., the presence of the brain-blood barriers, which retards the entrance of tumor material into the bloodstream), the use of fluorescence immunocytochemistry has recently allowed to distinguishing advanced (i.e., metastatic) from localized cancer. Some ongoing studies, using fluorescent probes recognizing specific glioma cells markers (i.e., GFAP, EGFR, Sox2, Tubulin beta-3, A2B5 and c-Met), are now investigating the potential clinical usefulness of this approach for early detection of metastatic cancer (9). Another interesting option is the possibility of detecting cell-free circulating tumour DNA. Although is has not been validated for clinical use so far, promising evidence has been provided that circulating DNA bearing suggestive genetic aberrations (e.g., those involving IDH1, MGMT, EGFR, etc.) may be seen as a valuable perspective for identifying primary aggressive or invasive tumors and their metastases (12), since a consistent number of primary patients with glioblastoma may display detectable cell-free circulating tumor DNA even at an early stage (13).

Conclusions and future perspectives

Despite being currently considered a relatively rare cancer, glioblastoma has a considerable clinical economic and social impact due to the limited success that has been achieved so far for its prevention and treatment. Most of the current drawbacks in early diagnostics are attributable to cancer cells heterogeneity and cerebral localization of the tumor (i.e., unfeasible access for easy and frequent sampling, delayed appearance of tumor material in the bloodstream), which both contribute to diminish the potential diagnostic options (12), and make the search for candidate biomarkers more or less like finding a needle in a haystack. However, interesting evidence emerged from recently published studies attests that the assessment of some epigenetic biomarkers (namely MGMT promoter methylation status and combined measurement of miR-21, miR-222 and miR-124-3p), proteomic analysis and identification of cell-free circulating tumor DNA may pave the way to a paradigm shift in glioblastoma diagnostics. It is also conceivable that combining these different strategies, an approach which has not been validated so far, may yield better diagnostic performance that using one single approach alone. Albeit we would all agree that the use of cerebrospinal fluid (CSF) for biomarkers assessment is invasive and carries the risk of important side effects or complications, it is undeniable that the identification of glioblastoma signatures in CSF, either molecular or biochemical (14-16), will perhaps enable to identify localized cancers much earlier than the appearance and detectability of these biomarkers in the bloodstream. Further studies will hence be needed to explore the value of using these promising strategies in the future, as well as their potential impact on personalized treatment of glioblastoma.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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