Commentary

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Biomarkers in Medicine

Circulating biomarkers for high-grade glioma

Hannah K Loo[1], Peter Mathen[1], Jennifer Lee[1] & Kevin Camphausen*,[1]

[1]Radiation Oncology Branch, National Cancer Institute, 9000 Rockville Pike, Building 10 B3B55, Bethesda, MD 20892, USA

*Author for correspondence: Tel.: +1 301 496 5457; camphauk@mail.nih.gov

"Elevation of panel marker expression in urine was also correlated with increased expression in tumor tissues, and longitudinal follow-up demonstrated a sustained correlation between MMP urinary presence and disease progression. However, the diagnostic ability of this panel was developed for a wide variety of brain tumors, pointing to a need for a urine biomarker specific to GBM."

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Glioblastoma multiforme (GBM) is the most common malignant primary tumor of the brain. Originating from glial cells, GBM is an invasive tumor characterized by extensive angiogenesis and genetic heterogeneity that contributes to treatment failure and disease recurrence. Patients diagnosed with GBM have a median survival of 15 months and an average 5-year survival rate of less than 10% [1]. Given the insidious nature of GBM, biomarkers are needed to aid in diagnosis, prognosis and predicting outcome to treatment. Currently, there are several molecular biomarkers available; however, some have been shown to be limited in prognostic potential, and all require the biopsy of tumor tissue. Furthermore, relying on a tissue sample from a single location has its own limitations, as GBM tends to be a heterogeneous cancer. To this end, the identification of new circulating biomarkers has been of considerable interest in the past decade. Unlike current biomarkers, blood- and serum-based biomarkers represent attractive candidates due to ease of access, relative lack of invasiveness to the patient, and a lower cost. This review highlights advances in the identification of circulating biomarkers for GBM, as well as their limitations and obstacles to translation into clinical care.

Serum proteome

Changes in the serum proteome have previously been shown to be reliable biomarkers of various types of cancer. Serum protein tests for non-small cell lung cancer [2], colorectal cancer [3] and breast cancer have all shown high sensitivity. This is especially true in breast cancer, where serum protein profiles were altered up to 3 years before cancer detection [4], suggesting the possibility of serum protein profiling as an early detection method. A global investigation of serum proteome changes in GBM by Gollapalli *et al.* identified 27 differentially expressed proteins in the serum of GBM patients, five of which were identified as significant due to their known roles in glioma growth [5]. A partial least squares discriminant analysis model developed from a subset of these identified proteins was shown to have 93% accuracy in discriminating between patients with gliomas and a control set of samples.

Several specific serum-based biomarkers have been investigated in patients with GBM. Haptoglobin, an acute phase reactant, was first identified in 2010 by Kumar *et al.* as a potential GBM marker in preoperative samples [6]. Expression levels were shown to be significantly elevated in samples from patients with gliomas in proportion to tumor grade, being highest in GBM samples and lowest in diffuse astrocytomas. Additionally, overexpression of haptoglobin in tumors implanted into mice led to worse survival. Molecular studies identified a role for haptoglobin in both cancer proliferation and motility, which is consistent with the behavior of GBMs. However, work by Van Linde *et al.* has shown no significant changes in serum haptoglobin levels after chemoradiation, noting a decrease only after adjuvant therapy [7]. Furthermore, in samples collected post-op, there was no significant correlation between serum haptoglobin concentration and progression-free survival (PFS) [7].

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Expression of *YKL-40*, an extracellular matrix glycoprotein, was shown by Shostak *et al.* to be differentially upregulated in GBM tumors using a serial analysis of gene expression analysis in GBM and normal brain tissue [8]. Since then, multiple studies have independently associated *YKL-40* expression with worse survival [9], although the strength of association for these studies varies from a hazard ratio of 1.4–2.13 [10,11]. YKL-40 is also associated with the loss of chromosome 10 [12], the most frequent genetic deletion in GBM. As a serum biomarker, increases in YKL-40 serum levels are associated with decreased survival, and have been shown to have prognostic power when taken as a baseline measurement in newly diagnosed GBM [13]. Furthermore, YKL-40 serum concentrations have shown to be elevated in patients who underwent subtotal resections, as compared with those who underwent total resections [14]. The exact reliability of YKL-40 as a serum biomarker is still unclear. While many studies have supported its predictive potential, others have not. Work by Van Linde *et al.* found no association between YKL-40 serum concentration and PFS [7], and another study by Perez-Larraya *et al.* found no association between plasma YKL-40 levels and both survival and tumor volume [15].

Serum concentration of AHSG, a plasma glycoprotein, was found by Petrik *et al.* to be increased in GBM samples by mass spectroscopy [16]. Correlation to patient outcomes showed an independent association with overall survival, which was strengthened in combination with age and Karnofsky performance status (KPS). Supporting this finding, others have shown that serum levels of AHSG also correlate with tumor grade and have suggested that the serum concentration pre-treatment may be a valuable indicator of GBM survival [17]. Like YKL-40, however, work done by Van Linde *et al.* have found no association between AHSG serum concentration and PFS [7]. It is possible the discrepancy in clinical findings is due to the timing of sample collection – work done by Petrik *et al.* looked at samples pre-treatment, and work by van Linde *et al.* examined post-operative samples – however, variability resulting from assays used and sample characteristics should be considered as well.

Platelets

Among blood-based biomarkers, platelets have served as a source of interest for many years. They have been shown to play active roles in cancer growth and metastasis, providing angiogenic factors to support vascularization [18]. Cervi *et al.* demonstrated that concentrations of angiogenesis-regulatory proteins selectively increase in platelets in the presence of a tumor and suggested that analysis of platelet-sequestered protein levels may help physicians detect tumor progression [19]. Indeed, angiogenesis-related proteins can be detected in platelets before tumors have reached detectable sizes [20], suggesting a potential avenue for early detection of cancer. As potential circulating biomarkers for GBM, however, the value of platelets lies in their ability to sequester tumor mRNA. This ability to sequester tumor RNA has been demonstrated in GBM tumors. Research conducted by Nilsson *et al.* showed that platelets can take up RNA-containing membrane vesicles both *in vitro* and *in vivo*, and that platelets in glioma patients can take up vesicles carrying mutant EGFRvIII, a well-established GBM biomarker [21]. In patients with EGFRvIII-mutation positive GBM tumors, 80% also had the mutation found in platelets compared with none in healthy controls [21]. This finding likely extends to other tumor-related RNA, as RNA profiling from glioma and healthy patients led to the identification of a glioma-associated signature [21]. Further investigation into upregulated RNAs may yield a new host of potential circulating biomarkers.

Urine/cerebrospinal fluid

Urine and cerebrospinal fluid (CSF) represent valuable tools for the detection of GBM, urine due to its ease of access and CSF due to its proximity to the brain, which allows for detection of changes in the CNS. A panel of urinary biomarkers was first identified in 2008, which showed significant predictive ability in detecting primary brain tumors, including GBM [22]. Among the panel markers, the majority were members of the matrix metalloproteinase (MMP) family, as well as VEGF, a known angiogenic regulator of GBM. All markers showed significant elevation relative to controls, including MMP-9, which was also shown to be significantly elevated in CSF samples from GBM patients. Elevation of panel marker expression in urine was also correlated with increased expression in tumor tissues, and longitudinal follow-up demonstrated a sustained correlation between MMP urinary presence and disease progression. However, the diagnostic ability of this panel was developed for a wide variety of brain tumors, pointing to a need for a urine biomarker specific to GBM.

Identification of GBM biomarkers in CSF was first demonstrated with the identification of tenascin in 1994 by Yoshida *et al.* [23]. Concentration of tenascin, an extracellular matrix glycoprotein, was shown to be elevated in the CSF of patients with gliomas, with higher levels found in glioblastomas as compared with astrocytomas. Furthermore, the presence of tenascin in the CSF decreased upon remission of astrocytomas, suggesting that

monitoring tenascin might represent a tool for evaluating disease progression. While the use of tenascin as a CSF GBM biomarker has not been explored since, more recent studies have suggested tenascin might be a CSF biomarker for Alzheimer's disease and mild cognitive impairment [24].

Another potential avenue for CSF biomarkers lies in miRNA, discussed in greater detail in the following section. microRNA or miRNA are short stretches of RNA which regulate gene transcription. In recent years, studies have begun to develop methodologies for using CSF miRNA in GBM detection. In 2017, Akers *et al.* developed a CSF miRNA biomarker signature [25]. They found that levels of miRNAs detected in both tumor tissue and CSF correlated with each other, and that a signature consisting of nine miRNAs both correlated with tumor volume and exhibited high sensitivity and specificity of detection. Their work suggests that miRNA profiling in CSF may represent a new avenue for diagnosis.

miRNAs & extracellular vesicles

Extracellular vesicles (EVs) used as biomarkers typically fall into two categories: exosomes, which are generated intracellularly and fuse with the plasma membrane upon release; and microvesicles, which are produced directly from the extracellular membrane via budding. EVs are present in nearly all bodily fluids and, like platelets, are capable of taking up contents from their host cell. Microvesicles, in particular, contain components from the host cell membrane and cytoplasm, along with RNA and other proteins. These contents are subsequently transferred into nearby cells, raising the possibility of malignant transformation by horizontal transmission. Because contents within EVs are protected from their surrounding environment, both exosomes and microvesicles may be valuable sources of information regarding tumor progression and disease state.

Among potential biomarkers contained within EVs, miRNAs are one of the most promising. It has previously been shown that miRNAs comprise approximately a third of all noncoding RNAs in GBM EVs [26]. A number of miRNA have been shown to correlate with GBM survival, particularly miR-21. Yang *et al.* showed that expression of miR-21 in GBM patients was inversely correlated with survival and demonstrated that miR-21 may exert its pro-tumorigenic effects via downregulation of IGFBP3, a GBM tumor suppressor [27]. A meta-analysis performed by Qu *et al.* explored the diagnostic abilities of miRNAs in 11 studies and found that while miRNA panels might improve the accuracy of diagnosis, miR-21 as a single miRNA exhibited high sensitivity and specificity [28]. Another GBM-associated miRNA, miR-451, has also been detected in EVs [29]; however, its role in GBM progression is less clear. While some have shown significant expression of miR-451 in GBM EVs, others have suggested the miRNA is downregulated in comparison to normal brain tissue. This discrepancy may be due to the needs of the tumor, as miR-451 has been shown to play a role in the transition between proliferation and migration of tumor cells [30] and may adjust its expression depending on metabolic needs.

Conclusion

At present, molecular markers for patients with GBM are most commonly assessed via tumor tissue from biopsy or resection. Given the costly and invasive nature of these procedures, circulating biomarkers represent an exciting prospect for diagnosis, treatment and prognosis. Analysis of the CSF, urine, blood and serum from patients with GBM have identified many biomarkers which may have clinical potential; several of which we have discussed here. Despite the promise of recent studies, there remain several challenges. Not all studies are consistent as to the diagnostic and prognostic power of potential biomarkers. This may be dependent on the timing of the sample taken (pre- vs. post-treatment), and points to a need to further analyze the impact of surgery and chemoradiation on biomarker expression. Variation in study conditions and analysis, which may also contribute to conflicting results, also suggests that clinical studies may benefit from standardization of reporting; for example, adhering to guidelines set forth by the REporting recommendations for tumor MARKer prognostic studies (REMARK) [31]. Furthermore, while many of the biomarkers discussed here and in current literature are targeted toward diagnosis of GBM, there remains a great need for prognostic and predictive biomarkers, which may allow for the development of tailored therapies for GBM patients. Nevertheless, the robust and growing number of circulating biomarkers being identified point to the hope that perhaps such biomarkers will be used in conjunction with, or in lieu of, tissue biomarkers in the treatment of GBM.

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