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Circulating glioma biomarkers

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Validated biomarkers for patients suffering from gliomas are urgently needed for standardizing measurements of the effects of treatment in daily clinical practice and trials. Circulating body fluids offer easily accessible sources for such markers. This review highlights various categories of tumor-associated circulating biomarkers identified in blood and cerebrospinal fluid of glioma patients, including circulating tumor cells, exosomes, nucleic acids, proteins, and oncometabolites. The validation and potential clinical utility of these biomarkers is briefly discussed. Although many candidate circulating protein biomarkers were reported, none of these have reached the required validation to be introduced for clinical practice. Recent developments in tracing circulating tumor cells and their derivatives as exosomes and circulating nuclear acids may become more successful in providing useful biomarkers. It is to be expected that current technical developments will contribute to the finding and validation of circulating biomarkers.

Keywords: biomarker, blood, cerebrospinal fluid, circulating tumor cell, nucleic acid, exosome, glioma, Omics.

Gliomas are the most common type of primary brain tumor and have an invariable fatal outcome and dismal prognosis. Each year, ~200 000 patients are diagnosed with a glioma worldwide.[1] Gliomas are subdivided into astrocytoma, oligodendroglioma, and oligoastrocytoma based on immunophenotypical similarity to a cell of putative origin. The tumors are assigned malignancy grades according to WHO criteria.[2] Gliomas usually recur and tend to increase in malignancy grade over time (Fig. 1). Glioma progression is accompanied by extensive neovascularization, and the newly formed blood vessels are leaky, which is reflected by tumor enhancement on MRI. Glioblastomas (GBMs) represent astrocytomas of the highest malignancy grade (WHO grade IV) and are the most common gliomas and the most aggressive primary brain tumors in adults, with a median survival of only 14.6 months.[3,4] Various molecular aberrations of gliomas (eg, the combined loss of chromosome arms 1p and 19q, the presence of isocitrate dehydrogenase 1(IDH1) mutation, epidermal growth factor receptor (EGFR) amplification, copy number aberrations of chromosomes 7 and 10, and MGMT promoter hypermethylation) harbor diagnostic, prognostic, or predictive information.[5–8] The molecular tests are carried out on tumor biopsies or resection specimens. Mutant IDH1, MGMT promoter methylation, and loss of 1p and 19q can also be detected in serum and cerebrospinal fluid (CSF) of glioma patients, and efforts to trace these aberrations in circulating tumor cells or circulating DNA are ongoing.[9–13]

Therapeutic modalities for gliomas include surgical resection, radiotherapy, and chemotherapy. The gold standard for measuring the effects of treatment in patients with gliomas is the application of the Response Assessment in Neuro-Oncology (RANO) criteria to the radiological appearance of the tumor on MRI.[14,15] There is considerable variation in the radiological presentation of gliomas and their recurrences.[16] A notorious problem in measuring the effects of treatment is so-called pseudoprogression, a treatment-related response of brain tissue to chemotherapy and radiation. Glioma pseudoprogression causes an increase in enhancement and edema on MRI that mimics true tumor progression.[17,18] This condition is probably induced by treatment-related local inflammation, resulting in edema and increased abnormal vessel permeability. There is a need for diagnostic discrimination because combined chemotherapy and radiation (the standard of care for GBM) may induce pseudoprogression in ~30% of cases.[19,20] Unfortunately, there are no reliable radiological techniques to distinguish between pseudoprogression and tumor recurrence or progression.[21,22] The identification of proliferating tumor cells in tissue biopsies taken in situations of pseudoprogression may be troublesome, and the significance of the presence of scattered cells with morphological or molecular characteristics of the original lesion is disputable (Fig. 2).

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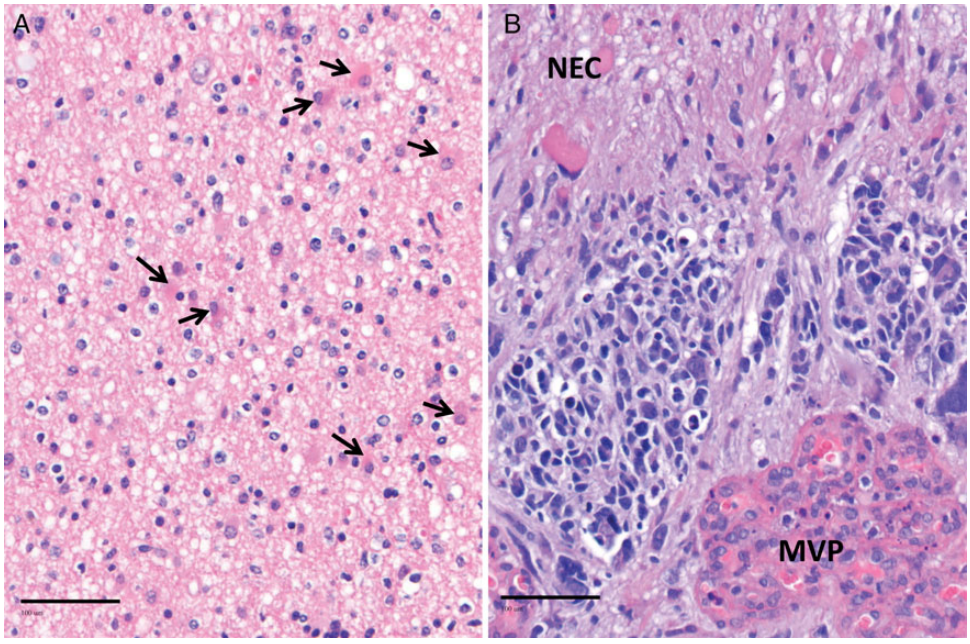

Fig. 1. Astrocytoma in low-grade (A) and high-grade (B) stages. (A) Low-grade astrocytoma (H&E; 200 x; bar = 100 micron). The infiltrating low-grade astrocytoma is composed of genetically altered glial tumor cells that infiltrate into brain tissue and are surrounded by reactive astrocytes (arrows), oligodendrocytes, and microglial cells. The tumor vasculature at this stage is inconspicuous and will be recruited for sprouting angiogenesis. (B) High-grade astrocytoma (grade IV or glioblastoma) (H&E, 200x). The advanced tumor grade is reflected by high cell density, polymorphism of the nuclei of the tumor cells, proliferated blood vessels ("microvascular proliferation"; MVP) and necrosis (NEC). The proliferated blood vessel walls are associated with breakdown of the blood-brain barrier causing leaking vessels, which is represented by enhancement on CT and MRI.

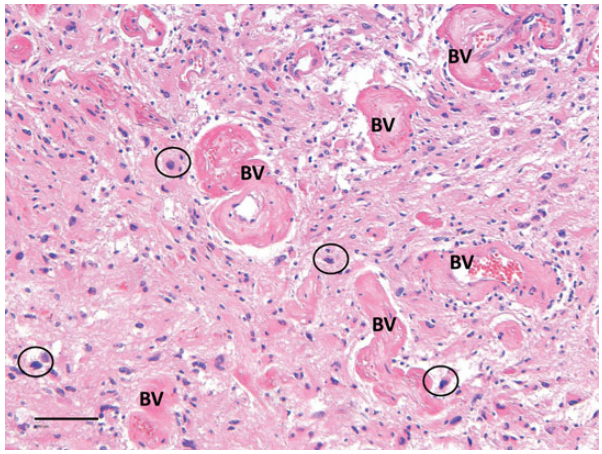

Fig. 2. Histology of a glial tumor with effects of radiation therapy. (H&E; 200 x; bar = 100 micron). The radiation therapy has caused homogenization of vascular walls (BV), edema of the neuropil, and proliferation of reactive astrocytes. The glial tumor cells cannot reliably be distinguished from reactive astrocytes because nuclear polymorphism (circles) may be present in both.

Currently, there are no biomarkers or radiographic or clinical modalities to reliably distinguish glioma recurrence from radiation necrosis or to monitor tumor response to therapy. Objective measurable parameters for the presence of tumor, tumor activity, and response to treatment would be a welcome addition to the currently available diagnostic arsenal.

Recently, advances in "omics" based technologies, including genomics, transcriptomics, proteomics, and metabolomics, have led to an explosion of activity in the field of biomarker research, particularly related to cancer. The general definition of a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[23,24] Cancer biomarkers include a broad range and level of biochemical entities such as nucleic acids, proteins, sugars, lipids, and small metabolites, and cytogenetic and cytokinetic parameters as well as whole tumor cells and exosomes (microvesicles). The advantage of biomarkers present in blood or CSF is their relatively easy accessibility, which facilitates repetitive measurements with obviously better monitoring of disease. In order to evaluate and compare tumor biomarkers, the "Tumor Marker Utility Grading System" has been proposed by the National Comprehensive Cancer Network (NCCN).[25,26] In this system, potential tumor markers are evaluated for their diagnostic, prognostic, or predictive performance as reflected by overall survival, disease-free survival, quality of life, or cost of care.[25–27] In the NCCN system, levels of evidence have been applied to several potential glioma biomarkers including IDH1 mutation, MGMT methylation, loss of 1p/19q, BRAF fusion, and CpG island methylator phenotype (CIMP).[26,27] Among these biomarkers, only 1p/19q testing has been credited for the highest level of evidence because of its ability to improve clinical decision-making and predict patient outcome (IA level).[26,27]

Because of its anatomical proximity to the CNS, CSF is a promising source of biomarker discovery for diseases of the

CNS. CSF samples are used for traditional cytology and have also been recently used for detecting brain metastasis by employing sensitive techniques such as flow cytometry, fluorescence in-situ hybridization (FISH), and PCR/reverse transcription PCR.[28,29] The relative low protein concentration of CSF (100–400 times lower as compared with serum) allows rapid screening, low sample consumption, and accurate identification or profiling by proteomic technologies, which have been facilitated by the recent publication of the normal human CSF proteome.[28,29] Under pathological conditions, one may find altered levels of normal constitutive proteins or proteins that are usually absent from normal CSF. These proteins may have entered the CSF due to disruption of the blood–brain barrier or intrathecal secretion, or shedding by tumor cells of primary brain tumor or metastasis, and/or their microenvironment. By now, various candidate protein biomarkers for gliomas have been found in CSF.[30–51]

In this review, different categories of tumor-associated circulating biomarkers, which have been identified in blood and CSF of glioma patients, are addressed including circulating tumor cells (CTCs), exosomes (microvesicles), circulating nucleic acids (DNA, RNA and miRNA), proteins, and metabolites.

Circulating Tumor Cells

CTCs are detected when diagnosing metastatic disease or tumor recurrence and may be used to monitor disease progression and therapeutic response. CTCs are found in the peripheral blood of patients with advanced stages of solid cancers with or without clinically detectable metastasis.[52–56] It has been shown that the presence of CTCs is related to tumor response, progression-free survival, and overall survival in patients suffering from various tumor types and that the presence of CTCs may hint at the existence of a hitherto undiscovered primary tumor.[57–59] Only one cell per 10^9 cells represents a CTC in the blood of patients with metastatic cancer, and the specificity and sensitivity of CTC detection is a technical challenge.[60] Various detection technologies have been recently developed including microchips, filtration devices, quantitative reverse-transcription PCR assays, automated microscopy systems, and telomerase promoter-based assays.[61–64] CTCs are particularly valuable for tumor characterization in situations in which tissue biopsies are unavailable or the collected tissue is of poor quality and/or insufficient quantity.[63] To what extent CTCs represent the cell population in the solid tumor part remains questionable. Because CTCs reflect the molecular heterogeneity of the tumor, they are important for therapeutic strategies. CTCs are probably not only released from the primary tumor but also from metastatic sites. However, most cancer cells are rapidly destroyed in the circulation, and the metastatic potential of CTCs seems limited.[65–67] Current clinical investigations of CTCs focus on their molecular characterization and the classification of heterogeneous subsets in relation to treatment resistance. CTCs are subjects of investigations in basal processes of epithelial-mesenchymal transition (EMT), collective cell migration and more, with the aim being to better understand the mechanisms of tumorigenesis, invasion, and metastasis.[63,64,68,69]

Until recently, the spread of glial tumor cells outside the brain was considered to be a rare event. However, many isolated cases of metastasizing glial neoplasms have been reported.[70–81] Several cases of transmission of metastatic GBM from donor to organ transplant recipients further supports the notion that appearance of glioma cells in the circulation is not as rare as previously believed and that occurrence rates may match those of other solid tumors.[82–89] Novel sensitive imaging techniques contribute to higher detection rates. So far, data on circulating CTCs associated with brain tumors are limited, and the use of CTCs as biomarkers in glioma patients is just beginning. The identification of CTCs was carried out by using markers for neural lineage in one study,[90] and a telomerase promoter-based assay was used for the detection of these cells in another study (Table 1).[64] There are limitations in the use of lineage markers for the identification of CTCs because of overlap in marker expression of tumor cells and normal cells. Further characterization of CTCs at the DNA, RNA, or protein level will improve the identification of true CTCs and their subfractions from other circulating cells.

Circulating Tumor-derived Exosomes

Exosomes (microvesicles or extracellular vesicles) are 30–100 nm in diameter and are released into the microenvironment of cells or into surrounding body fluids by both normal and cancer cells, where they perform a variety of functions.[91–93] Exosomes can be taken up by particular host cells and thus provide signaling between various cell types including cancer cells.[94–96] Circulating tumor-derived exosomes contain a variable spectrum of molecules representative of the parental cells including proteins, nucleic acids, lipids, metabolites, and other molecules. Cancer cell exosomes carry molecular signatures and effectors of diseases such as mutant oncoproteins, oncogenic transcripts, microRNA, and DNA sequences. Their contents can help identify the cells of origin for the exosomes, thereby offering the opportunity to identify biomarkers or therapeutic targets in body fluids.[91–93,97,98] Circulating exosomes in the body fluids of brain tumor patients may be used to decipher molecular features of the neoplasms or measure their responses to therapy.[96,97] So far, various tumor-related molecules with altered expression patterns have been found in circulating exosomes of glioma patients including EGFRvII,[96,99] EGFR,[99] podoplanin (PDPN),[99] phosphatase and tensin homolog (PTEN),[12] miR-21,[100] and mutant IDH1 mRNA (Table 1).[9,99] Exosomes may carry substantial amounts of bound antibody-recognizing tumor antigens (autoantibodies), which can be used to reveal the presence of tumor antigens; exosome-based immunotherapy is under development.[96,97,101,102] Although exosomes are promising targets of biomarker research, their tracing and quantification in clinical samples remain challenging.[103,104] New technologies, such as ExoScreen, are being developed for eventual clinical use.[103]

Circulating Tumor-associated Nucleic Acids

Circulating nucleic acids (CNAs) have been identified in blood and other bodily fluids of patients with various diseases.[108,118] CNAs are promising targets for development as tumor biomarkers (circulating tumor-associated nucleic acids [ctNAs]) because of the possibility to profile tumors at the genomic and transcriptomic levels. Nucleic acids appear in body fluids

Table 1. Circulating tumor cells, tumor-associated nucleic acids, and exosomes in glioma patients

Reference	Biomarker	Source	Time of Take	Methodology	*n*	Tumor Type	Treatment Response	Survival	Radiology	Median Follow-up
Macarthur et al 2014[64]	CTCs	Blood	Pretreatment	TPBA	5	HGG	NA	NA	NA	NA
Tumilson et al 2014[106]	miR-15b	CSF	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-15b	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-17–5p	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-20a	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-21	CSF	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-21	Plasma	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-23a	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-31	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-106a	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-146b	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-148a	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-150	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-193a	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-197	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-200b	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-200	CSF	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-221	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-222	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-342–3p	Plasma	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
	miR-548b-5p	Serum	NA	qRT-PCR	NA	Glioma NOS	NA	NA	NA	NA
Salkeni et al 2013[109]	EGFRvIII	Plasma	Pre/postoperative	PCR	13	GBM	Yes	NA	NA	NA
Majchrzak-Celinska et al 2013[13]	Methyl. MGMT	Serum	Pretreatment	PCR	17	AII/GBM	NA	NA	NA	NA
	Methyl. RASSF1A	Serum	Pretreatment	PCR	17	AII/GBM	NA	NA	NA	NA
	Methyl. p15INK4B	Serum	Pretreatment	PCR	17	AII/GBM	NA	NA	NA	NA
	Methyl. p14ARF	Serum	Pretreatment	PCR	17	AII/GBM	NA	NA	NA	NA
Chen et al 2013[9]	Mutant IDH1	Serum MVs	Intraoperative	BEAMng/ddPCR	24	AII/GBM	NA	NA	NA	NA
	Mutant IDH1	CSF MVs	Intraoperative	BEAMng/ddPCR	24	AII/GBM	NA	NA	NA	NA
Yang et al 2013[131]	miR-15b	Serum	Preoperative	Sequencing/qRT-PCR	177	AIII	NA	NA	NA	NA
	miR-23a	Serum	Preoperative	Sequencing/qRT-PCR	177	AIII	NA	NA	NA	NA
	miR-133a	Serum	Preoperative	Sequencing/qRT-PCR	177	AIII	NA	NA	NA	NA
	miR-150	Serum	Preoperative	Sequencing/qRT-PCR	177	AIII	NA	NA	NA	NA
	miR-197	Serum	Preoperative	Sequencing/qRT-PCR	177	AIII	NA	NA	NA	NA
	miR-497	Serum	Preoperative	Sequencing/qRT-PCR	177	AIII	NA	NA	NA	NA
	miR-548b-5p	Serum	Preoperative	Sequencing/qRT-PCR	177	AIII	NA	NA	NA	NA
Cohen et al 2012[90]	CTCs	Blood	Pretreatment	Cell lineage markers	11	GBM	NA	NA	NA	NA
Boisselier et al 2012[108]	Mutant IDH1	Plasma	Posttreatment	PCR	80	Astro II-IV	NA	NA	NA	NA
Noerholm et al 2012[116]	Expression patterns	Serum MVs	Pretreatment	Microarray/qRT-PCR	9	GBM	NA	NA	NA	NA
Teplyuk et al 2012[130]	miR-10b	CSF	Intraop./posttreat.	RT-PCR	19	GBM	NA	NA	NA	NA
	miR-21	CSF	Intraop./posttreat.	RT-PCR	19	GBM	NA	NA	NA	NA

Baraniskin et al 2012[132]	miR-15b	CSF	Intraop./postoperative	qRT-PCR	10	AII/GBM	NA	NA	NA	NA
	miR-21	CSF	Intraop./postoperative	qRT-PCR	10	AII/AIII/GBM	NA	NA	NA	NA
Ilhan-Mutlu et al 2012[134]	miR-21	Plasma	Pretreatment	qRT-PCR	10	GBM	NA	NA	NA	NA
Wang et al 2012[135]	miR-21	Plasma	Pre/posttreatment	qRT-PCR	30	GBM	Yes	NA	NA	NA
	miR-128	Plasma	Pre/posttreatment	qRT-PCR	30	GBM	Yes	NA	NA	NA
	miR-342-3p	Plasma	Pre/posttreatment	qRT-PCR	30	GBM	Yes	NA	NA	NA
Balana et al 2011[11]	Methyl. MGMT	Serum	Intraoperative	PCR	37	GBM	NA	Yes	NA	120 weeks
Nilsson et al 2011[118]	EGFRvIII	Platelets	NA	RT-PCR	26	AII/AIII/GBM	NA	NA	NA	NA
Roth et al 2011[136]	miR-128	Blood cell pellets	Postoperative	qRT-PCR	20	GBM	NA	NA	NA	NA
	miR-342-3p	Blood cell pellets	Postoperative	qRT-PCR	20	GBM	NA	NA	NA	NA
Lavon et al 2010[12]	1p LOH	Serum	Posttreatment	PCR	70	AII/OII/OIII	NA	NA	NA	NA
	10q LOH	Serum	Posttreatment	PCR	70	AII/OII/OIII	NA	NA	NA	NA
	19q LOH	Serum	Posttreatment	PCR	70	AII/OII/OIII	NA	NA	NA	NA
	Methyl. MGMT	Serum	Posttreatment	PCR	70	AII/OII/OIII	NA	NA	NA	NA
	Methyl. PTEN	Serum	Posttreatment	PCR	70	AII/OII/OIII	NA	NA	NA	NA
Liu et al 2010[10]	Methyl. MGMT	Serum	Pretreatment	MeDIP/PCR	66	AOA/GBM	NA	Yes	NA	11.3 months
	Methyl. p16	Serum	Pretreatment	MeDIP/PCR	66	AOA/GBM	NA	Yes	NA	11.3 months
	Methyl. TIMP-3	Serum	Pretreatment	MeDIP/PCR	66	AOA/GBM	NA	No	NA	11.3 months
	Methyl. THBS1	Serum	Pretreatment	MeDIP/PCR	66	AOA/GBM	NA	Yes	NA	11.3 months
	Methyl. MGMT	CSF	Pretreatment	MeDIP/PCR	66	AOA/GBM	NA	No	NA	11.3 months
	Methyl. p16	CSF	Pretreatment	MeDIP/PCR	66	AOA/GBM	NA	No	NA	11.3 months
	Methyl. TIMP-3	CSF	Pretreatment	MeDIP/PCR	66	AOA/GBM	NA	No	NA	11.3 months
	Methyl. THBS1	CSF	Pretreatment	MeDIP/PCR	66	AOA/GBM	NA	No	NA	11.3 months
Wakabayashi et al 2009[113]	Methyl. p16	Serum	Pretreatment	PCR	40	Glioma NOS	NA	NA	NA	NA
Skog et al 2008[96]	EGFRvIII	Serum MVs	Intraoperative	RT-PCR	25	GBM	NA	NA	NA	NA
Weaver et al 2006[111]	Methyl. p16	Plasma	Intraoperative	PCR	10	AII/AIII/GBM	NA	NA	NA	NA
	Methyl. MGMT	Plasma	Intraoperative	PCR	10	AII/AIII/GBM	NA	NA	NA	NA
	Methyl. p73	Plasma	Intraoperative	PCR	10	AII/AIII/GBM	NA	NA	NA	NA
	RARbeta	Plasma	Intraoperative	PCR	10	AII/AIII/GBM	NA	NA	NA	NA
Balana et al 2003[110]	Methyl. DAPK	Serum	Pre/Intraoperative	PCR	28	GBM	NA	NA	NA	NA
	Methyl. p16	Serum	Pre/Intraoperative	PCR	28	GBM	NA	NA	NA	NA
	Methyl. MGMT	Serum	Pre/Intraoperative	PCR	28	GBM	Yes	NA	NA	NA
	Methyl. RASSF1A	Serum	Pre/Intraoperative	PCR	28	GBM	NA	NA	NA	NA
Ramirez et al 2003[112]	Methyl. MGMT	Serum	Pre/Intraoperative	PCR	28	GBM	NA	NA	NA	NA
	Methyl. p16	Serum	Pre/Intraoperative	PCR	28	GBM	NA	NA	NA	NA
	Methyl. DAPK	Serum	Pre/Intraoperative	PCR	28	GBM	NA	NA	NA	NA
	Methyl. RASSF1A	Serum	Pre/Intraoperative	PCR	28	GBM	NA	NA	NA	NA

Abbreviations: AII, astrocytoma WHO grade 2; AIII, astrocytoma WHO grade III; AOA, anaplastic oligoastrocytoma; BEAMing, beads, emulsion, amplification, magnetics; CSF, cerebrospinal fluid; CTC, circulating tumor cell; DAPK, death-associated protein kinase 1; ddPCR, droplet digital PCR; EGFRvIII, endothelial growth factor variant 3; GBM, glioblastoma (or astrocytoma WHO grade IV); Glioma NOS, glioma not otherwise specified; HGG, high-grade glioma; IDH1, isocitrate dehydrogenase 1; MeDIP, methylated DNA immunoprecipitation; Methyl, methylation; MGMT, O6-alkylguanine DNA alkyltransferase; miR-, microRNA; NA, not available; OII, oligodendroglioma WHO grade II; OIII, oligodendroglioma WHO grade III; PTEN, phosphatase and tensin homolog; qRT-PCR, real-time reverse-transcription PCR; RARbeta, retinoic acid receptor beta; RASSF1A, Ras association domain-containing protein 1A; THBS1, thrombospondin1; TIMP-3, metalloproteinase inhibitor 3; TPBA, telomerase promoter-Based Assay; intraop, intraoperative.
All studies listed in Table 1 were observational studies.

as a sequel of apoptotic tumor cells or tumor necrosis, but they may also be actively secreted into the circulation.[105] Levels of CNAs are influenced by many factors: the turnover of (tumor) cell populations, cell degradation rates, filtering processes present in the blood or lymphatic circulation, clearance by liver and kidney, infection, age, sex, treatment, stress on epigenetic mechanisms, diet, lifestyle, and more.[105,106] Although nucleic acids are valuable as biomarkers because they can be measured in sensitive high-throughput PCR detection assays, the identification, quantitation, and validity of ctNAs remain challenging. In order to link the presence of ctDNA, circulating tumor-associated RNA (ctRNA), or circulating tumor-associated microRNA (ctmiRNA) in body fluids of cancer patients to tumor-specific molecular events, the preanalytic conditions must be well defined and standardized.[58,105]

Circulating Tumor-associated DNA

ctDNA may harbor the genetic and epigenetic aberrations present in tumors and their metastases including point mutations, rearrangements, amplifications, and aneuploidy. The aberrations may be highly specific for an individual tumor and may also represent its molecular heterogeneity.[107] ctDNAs have been detected in patients with tumors of breast, bladder, colon, liver, lung, ovaries, pancreas, and prostate as well as non-Hodgkin's lymphoma and melanoma. ctDNAs have also been detected in glioma patients, and the aberrations found included IDH1 mutation,[108] loss of heterozygosity for 1p, 10q, 19q[12]; EGFRvIII mutation;[109] as well as abnormal methylation of the promoters of MGMT[11–13,110–112], *p16*,[110–113] *DAPK*,[110,112] *RASSF1A*,[13,110,112] p73,[111] RARbeta,[111] PTEN,[12] p15INK4B,[13] and p14ARF.[13] At this point, the clinical utility has not been validated for any of the candidate ctDNAs as biomarkers for glioma patients. Prospective settings are needed for clinically applicable tests.

Circulating tumor-associated RNA and microRNA

The characterization of ctRNAs has not been explored to the extent of ctDNA, and the tumor specificity of these CNAs has been challenged more vigorously. One reason is that cell-free RNA is prone to degradation by the ubiquitous presence of RNA-degrading enzymes, which are generally elevated in the serum of cancer patients.[114,115] Extracellular RNA is usually present in the exosomes.[116] Aberrant RNA expression has been associated with stage, progression, and spread of various cancer types.[117] In patients with gliomas, exosomes and platelets have been used as sources for detecting tumor-associated RNA profiles, among which are mutant EGFRvIII and mutant IDH1.[9,96,116,118] As with the ctDNAs, the ctRNAs have also not been validated as biomarkers for introduction into clinical practice.

microRNAs (miRNAs) are noncoding, single-stranded RNAs of ~22 nucleotides that constitute a novel class of gene regulators and function as tumor suppressors and oncogenes.[119] Because miRNAs, unlike RNA, are relatively stable and are present in blood and other bodily fluids, they are potential tumor biomarkers and may be more sensitive and specific for detecting tumors than currently available methods for early diagnosis of cancer.[120] The peripheral blood contains large amounts of stable miRNAs derived from various tissues, and alterations in these miRNA have been reported for many tumors including gliomas.[119–129] Deviant miRNA expression patterns in the blood of glioma patients include miR-10b,[130] miR-15b,[106,131,132] miR-17–5p,[106] miR-20a,[106] miR-21,[96,130,132–135] miR-23a,[106,131] miR-31,[106] miR-106,[106] miR-128,[106,135,136] miR-133a,[131] miR-146b,[106] miR-148a,[106] miR-150,[106,131] miR-193a,[106] miR-197,[131] miR-200b,[106] miR-221,[106] miR-222,[106] miR-342-3p,[135,136] miR-497,[131] and miR-548b-5p.[131] Some significant technological pitfalls and limitations need to be addressed before the miRNAs can be introduced as clinically applicable glioma biomarkers.[124,137]

Circulating Tumor-associated Protein Biomarkers

Efforts have been made over the last decades to identify candidate protein biomarkers for gliomas that could be measured in body fluids (eg, urine, serum/plasma, or CSF) for making a diagnosis, detecting recurrence, or monitoring tumor activity following therapy (Table 2).[32,33,36–39,41–43,45,47–51,138–155] Recent advances in proteomics have led to an explosion of activity in the field of biomarker research, particularly that related to cancers. The identification of biomarkers in body fluids such as serum is difficult due to the large dilution factor and the abundance of other constitutive serum proteins. Sample enrichment is necessary to enhance the sensitivity, and extensive validation of the methodology is necessary to ensure the specificity of candidate biomarkers.[156] Several reports on the analysis of the glioma proteome, in which tumor tissue, serum, plasma, CSF or cyst fluids have been implicated.[34,36,157–161] Attempts have also been made to identify biomarkers by using xenografts in animal models.[162]

Growth Factors and Angiogenesis-related Biomarkers

Vascular Endothelial Growth Factor

Gliomas are highly vascularized tumors, and the process of angiogenesis is progressive throughout tumor development. Obviously, the newly formed vessels are attractive targets for antiangiogenic therapy. Vascular endothelial growth factor (VEGF) is a key molecule for triggering the process of angiogenesis in pathological conditions including neoplasms.[163] Tumor hypoxia due to increased cell density triggers the angiogenic switch by upregulating VEGF.[164] Given the importance of VEGF in tumor angiogenesis, several drugs to suppress VEGF signaling have been developed.[165] Bevacizumab is the most well-characterized antiangiogenic drug currently being used for the treatment of human GBM. Bevacizumab is a humanized monoclonal antibody that binds to circulating VEGF and prevents its interaction with the VEGF receptor suppressing VEGF signaling.[166–172] Antiangiogenic strategies are targeted to endothelial cells, although the main sources of VEGF are the glial tumor cells.[165,173] Patients usually become resistant to anti-VEGF therapy after an initial response due to various compensation mechanisms.[165,169,174–176] VEGF has been considered to be a potential protein biomarker in CSF and serum/plasma of glioma patients, and its elevated levels have appeared to correlate with the microvascular density of the tumors.[38,39,177–182] Because VEGF levels of serum are also

Table 2. Potential glioma protein biomarkers in cerebrospinal fluid, serum and plasma

Reference	Biomarker	Study Type	Source	Time of Take	Methodology	*n*	Tumor Type	Treatment Response	Survival	Radiology	Median Follow-up
Yamaguchi et al 2013[30]	OPN	Obser	CSF	Pretreatment	ELISA	63	GBM/metastases	NA	NA	NA	NA
Verschuere et al 2013[258]	Galectin-1	Obser	serum	Intraop./ postoperative	ELISA	125	HGG/recurrent HGG	NA	NA	NA	NA
Chinnaiyan et al 2012[150]	IGFBP-5	Inter Phase I	plasma	Pre/posttreatment	ELISA	10	Recurrent GBM	Yes	Yes	NA	5.7 months
	PDGF	Inter Phase I	plasma	Pre/posttreatment	ELISA	10	Recurrent GBM	Yes	Yes	NA	5.7 months
Li et al 2012[149]	IGFBP-2	Obser	serum	Preoperative	ELISA	145	Glioma NOS	NA	NA	NA	NA
Bemardi et al 2012[235]	YKL-40	Obser	Serum	Postoperative	ELISA	60	GBM	Yes	Yes	NA	12 months
Iwamoto et al 2011[218]	MMP-9	Obser	Serum	Pre/posttreatment	ELISA	343	AII/AIII/GBM	NA	No	No	29 to 52 months
Li et al 2011[149]	IGFBP-2	Obser	CSF	Preoperative	ELISA	94	AGG/carcinomas	NA	Yes	NA	7–24 months
Mittelbronn et al 2011[32]	MIF	Obser	CSF	Pretreatment	ELISA	14	HGG	NA	NA	NA	NA
Schuhmann et al 2010[33]	OPN	Basic	CSF	Preoperative	MALDI-TOF-MS	11	GBM	No	No	NA	NA
	AACT	Basic	CSF	Preoperative	MALDI-TOF-MS	11	GBM	No	No	NA	NA
	TTHY	Basic	CSF	Preoperative	MALDI-TOF-MS	11	GBM	No	No	NA	NA
	ALB	Basic	CSF	Preoperative	MALDI-TOF-MS	11	GBM	No	No	NA	NA
Batchelor et al 2010[194]	VEGF	Inter Phase II	Serum	Pre/posttreatment	ELISA	31	Recurrent GBM	Yes	No	No	227 days
	PlGF	Inter Phase II	Serum	Pre/posttreatment	ELISA	31	Recurrent GBM	Yes	Yes	Yes	227 days
	VEGFR2	Inter Phase II	Serum	Pre/posttreatment	ELISA	31	Recurrent GBM	Yes	No	No	227 days
	FGF-ß	Inter Phase II	Serum	Pre/posttreatment	ELISA	31	Recurrent GBM	Yes	Yes	Yes	227 days
	MMP2	Inter Phase II	Serum	Pre/posttreatment	ELISA	31	Recurrent GBM	Yes	Yes	Yes	227 days
	MMP10	Inter Phase II	Serum	Pre/posttreatment	ELISA	31	Recurrent GBM	Yes	No	No	227 days
	SDF-1a	Inter Phase II	Serum	Pre/post-treatment	ELISA	31	Recurrent GBM	Yes	No	No	227 days
	Tie2	Inter Phase II	Serum	Pre/posttreatment	ELISA	31	Recurrent GBM	Yes	No	No	227 days
	Agn2	Inter Phase II	Serum	Pre/posttreatment	ELISA	31	Recurrent GBM	Yes	No	No	227 days
Sreekanthreddy et al 2010[207]	OPN	Obser	Serum	Preoperative	ELISA+WB	30	GBM	NA	Yes	NA	34 months
Ohnishi et al 2009[259]	Gelsolin	Basic	CSF	Pretreatment	MALDI-TOF-MS	2	AII/GBM	NA	NA	NA	NA
Lin et al 2009[195]	IGFBP-2	Obser	Plasma	Pre/posttreatment	ELISA	196	AGG	NA	Yes	NA	1 year
Ilhan et al 2009[182]	Ang2	Obser	Plasma	Pretreatment	ELISA	78	AGG/metastases	No	Yes	NA	8 months
	VEGF	Obser	Plasma	Pretreatment	ELISA	78	AGG/metastases	No	Yes	NA	8 months
	PDGF	Obser	Plasma	Pretreatment	ELISA	78	AGG/metastases	No	Yes	NA	8 months
Petrik et al 2008[260]	AHSG	Obser	Serum	Preoperative	SELDI-TOF, ELISA	214	AII/AIII/GBM	No	Yes	NA	2 years
Reddy et al 2008[261]	PBEF1	Obser	Serum	Preoperative	ELISA	95	AIII/GBM	NA	Yes	NA	8 months
Iwadate et al 2008[262]	PAI-1	Obser	Serum	Pre/posttreatment	ELISA	57	AGG	NA	Yes	NA	NA
Jung et al 2007[143]	GFAP	Obser	Serum	Pretreatment	ELISA	104	AGG/metastases	No	NA	NA	NA
Brommeland et al 2007[226]	GFAP	Obser	Serum	Preoperative	ELISA	31	HGG/metastases	No	NA	Yes	NA
Todaro et al 2007[263]	NCAM	Obser	Serum	Pretreatment	WB	61	AGG/metastases	Yes	No	NA	1–3 months
Zheng et al 2007[155]	eNOS	Obser	Plasma	Pretreatment	ELISA	115	AGG/metastases	NA	NA	NA	NA
Quaranta et al 2007[264]	EGFR	Obser	Serum	Pre/postoperative	ELISA	65	HGG	NA	Yes	NA	13 months
Khwaja et al 2006[35]	Attractin	Obser	CSF	Pretreatment	2-DE + cICAT	47	Glioma NOS/ metastases	NA	NA	NA	NA
Hormigo et al 2006[219]	YKL-40	Obser	Serum	Pre/postoperative	ELISA	143	HGG	NA	Yes	Yes	4.6 to 22 months
	MMP9	Obser	Serum	Pre/postoperative	ELISA	143	HGG	NA	No	No	4.6 to 22 months
Ilzecka et al 2006[265]	APRIL	Obser	Serum	Preoperative	ELISA	25	GBM	NA	NA	No	NA

Continued

Table 2. *Continued*

Reference	Biomarker	Study Type	Source	Time of Take	Methodology	*n*	Tumor Type	Treatment Response	Survival	Radiology	Median Follow-up
Zheng et al 2005[198]	L-CaD	Obser	Serum	Pretreatment	ELISA	57	AGG	No	NA	NA	NA
Fukuda et al 2005[266]	Cathepsin D	Obser	Serum	Pre/post-operative	ELISA	20	AGG	NA	NA	NA	NA
Peles et al 2004[38]	VEGF	Obser	CSF	Pre/Intraoperative	ELISA	26	AGG	NA	Yes	NA	652 days
	VEGF	Obser	Serum	Pre/Intraoperative	ELISA	26	AGG	NA	No	NA	652 days
	FGF-ß	Obser	CSF	Pre/Intraoperative	ELISA	26	AGG	NA	Yes	NA	652 days
	FGF-ß	Obser	Serum	Pre/Intraoperative	ELISA	26	AGG	NA	No	NA	652 days
Sampath et al 2004[39]	VEGF	Obser	CSF	Pre/Intraoperative	ELISA	27	AII/AIII/ metastases	NA	NA	NA	NA
	Recoverin	Obser	serum	Pre/Intraoperative	ELISA	24	AGG	NA	NA	NA	NA
Zheng et al 2003[40]	L-CaD	Basic	CSF	Intraoperative	2D + MALDI	10	AGG	No	NA	NA	NA
Batabyal et al 2003[37]	CEA	Obser	CSF	Pretreatment	RIA	22	AGG/metastases	No	NA	NA	NA
Ribom et al 2003[41]	PDGF	Obser	CSF	Pre/postoperative	Radioreceptor assay	7	LGG	NA	NA	NA	NA
	VEGF	Obser	CSF	Pre/postoperative	ELISA	7	LGG	NA	NA	NA	NA
	VEGF	Obser	Serum	Pre/postoperative	ELISA	7	LGG	NA	NA	NA	NA
	FGF-ß	Obser	CSF	Pre/postoperative	ELISA	7	LGG	NA	NA	NA	NA
	FGF-ß	Obser	serum	Pre/postoperative	ELISA	7	LGG	NA	NA	NA	NA
Tanwar et al 2002[256]	YKL-40	Obser	Serum	NA	ELISA	65	AGG	NA	NA	NA	NA
Fine et al 2000[138]	FGF-ß	Inter Phase II	Serum	Pre/posttreatment	ELISA	39	HGG	Yes	Yes	Yes	80 weeks
	VEGF	Inter Phase II	Serum	Pre/post-treatment	ELISA	39	HGG	Yes	No	No	80 weeks
Streffer et al 1998[42]	CD95	Obser	CSF	Pretreatment	ELISA	20	GBM	NA	NA	NA	NA
Rudenko et al 1996[230]	CEA	Obser	CSF	Preoperative	SFI	83	Brain tumors NOS	NA	NA	NA	NA
	CEA	Obser	Serum	Preoperative	SFI	83	Brain tumors NOS	NA	NA	NA	NA
	AFP	Obser	CSF	Preoperative	SFI	83	Brain tumors NOS	NA	NA	NA	NA
	AFP	Obser	Serum	Preoperative	SFI	83	Brain tumors NOS	NA	NA	NA	NA
	HCG	Obser	CSF	Preoperative	SFI	83	Brain tumors NOS	NA	NA	NA	NA
	HCG	Obser	Serum	Preoperative	SFI	83	Brain tumors NOS	NA	NA	NA	NA
	CEA	Obser	Serum	Pretreatment	SFI	101	Brain tumors NOS	NA	NA	NA	NA
Rombos et al 1988[141]	CEA	Obser	CSF	Pre/postoperative	SFI	41	AGG/metastases	No	NA	NA	NA
	CEA	Obser	Serum	Pre/postoperative	SFI	41	AGG/metastases	No	NA	NA	NA
	AFP	Obser	CSF	Pre/postoperative	SFI	41	AGG/metastases	No	NA	NA	NA
	AFP	Obser	Serum	Pre/postoperative	SFI	41	AGG/metastases	No	NA	NA	NA
Suzuki et al 1980[50]	CEA	Obser	CSF	Pre/posttreatment	RIA	253	AGG/metastases	Yes	NA	NA	NA
	CEA	Obser	Plasma	Pre/posttreatment	RIA	253	AGG/metastases	Yes	NA	NA	NA
Miyake et al 1979[51]	CEA	Obser	CSF	Pre/posttreatment	RIA	97	AGG/metastases	Yes	NA	NA	NA

Abbreviations: AGG, all grade of gliomas; AII, astrocytoma WHO grade 2; AIII, astrocytoma WHO grade III; AACT, alpha-1-antichymotrypsin; ABL, N-terminal residue of albumin; AHSG, 2-Heremans-Schmid glycoprotein; Ang2, angiopoietin2; APRIL, a proliferation-inducing ligand; CSF, cerebrospinal fluid; EGFR, epidermal growth factor receptor; eNOS, endothelial nitric oxide synthase; FGF-β, basic fibroblast growth factor; GBM, glioblastoma; GFAP, glial fibrillary acidic protein; HGG, high-grade glioma; IGFBP-2, insulin-like growth factor-binding protein 2; IGFBP-5, insulin-like growth factor-binding protein 5; intraop, intraoperative; L-CaD, low molecular; LGG, low-grade glioma; MIF, macrophage migration inhibitory factor; MMP2/9/10, matrix metalloproteinase-2/9/10; NCAM, neural cell adhesion molecule; NOS, not otherwise specified; Obser, observational studies; OPN, osteopontin; PAI-1, plasminogen activator inhibitor 1; PBEF1, pre-B-cell colony enhancing factor 1; PDGF, platelet-derived growth factor; PlGF, placental growth factor; SDF-1α, stromal cell-derived factor 1; Tie2, angiopoietin receptors; TTHY, transthyretin; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2; Wb, Western blot; YKL-40, (tyrosine (Y), lysine (K) and leucine (L) and the apparent molecular weight).

increased in other systemic cancers, including breast cancers,[183–185] lung cancer,[186,187] and colon cancer,[188–190] they are not specific to glial tumors. In several clinical studies, it was demonstrated that the serum VEGF levels of cancer patients, including gliomas, remained significantly high following surgery, radiotherapy, or chemotherapy.[179,191] Moreover, particular inhibitors of the VEGF receptor tyrosine kinases induce increased levels of serum VEGF.[192] Taken together, the value of VEGF as a biomarker has not been established in glioma.

Other Growth Factors and Angiogenesis-associated Molecules

Aside from VEGF, various growth factors and other angiogenesis-associated molecules have been used to monitor the effects of antiangiogenic therapy in gliomas.[38,138,155,168,193,194] Growth factors are potential targets for therapeutic strategies because they are essential for tumor progression. Changes in plasma placental growth factor, basic fibroblast growth factor (FGF-β), soluble VEGF receptor 1, soluble VEGF receptor 2, stromal cell-derived factor-1alpha (SDF-1alpha), and soluble Tek/Tie2 receptor were all used to monitor the effects of cediranib (a pan-VEGF receptor tyrosine kinase inhibitor) in several clinical studies.[168,193,194] All of these molecules reportedly correlated with radiological response and overall survival.[194] FGF-β levels related to tumor progression and overall survival were also evaluated apart from the cediranib trial.[38,138] The factors or molecules evaluated in more than one study include platelet-derived growth factor (PDGF),[41,150,182] insulin-like growth factor binding protein 2 (IGFBP-2),[149,195] and angiopoietin2 (Ang2)[182,194] (Table 1). Endothelial nitric oxide synthase (eNOS), a specific isoform of the nitric oxide-producing enzyme of endothelial cells (ECs), is a well-characterized marker of ECs.[196] This molecule is activated in the process of angiogenesis and vasculogenesis and plays an intimate role in VEGF signaling.[197] The blood level of eNOS largely reflects the activity of cells with endothelial lineage.[196,197] The concentration of plasma eNOS is significantly greater in glioma patients as compared with control groups.[155] The expression of low molecular isoform of caldesmon (*l-CaD*), a cytoskeleton-associated protein, was also increased in CSF[40] and serum[198] in glioma patients. The expression of *l-CaD* in blood vessels was further confirmed in tissue sections of glioma patients.[199–202] A zebrafish *l-CaD* knockdown model further confirmed that this molecule plays a crucial role in vasculogenesis and angiogenesis in vivo.[203] So far, the performance of *l*-CaD and eNOS as biomarkers for glioma has not been tested in additional studies.

Matricellular Proteins

The group of matricellular proteins consists of structurally diverse glycoproteins that are secreted by tumor cells and neighboring stromal cells.[204–206] These proteins are secreted into the extracellular environment, and they interact with cell-surface receptors, proteases, hormones, and structural matrix proteins such as collagens.[205] Matricellular proteins are also involved in various aspects of tumor biology such as EMT, angiogenesis, cell proliferation and survival, motility, and ECM degradation.[204–206] Glial tumor cells need to break down the environmental substances in order to infiltrate diffusely into the surrounding brain tissue. Among the many proteins that serve in this context are thrombosponin-1 and-2 (TSP1, TSP2), tenascin-C (TNC), secreted protein acidic and rich in cysteine (SPARC), osteopontin (OPN), angiopoietin-like protein 4 (ANGPTL4), CCN family members cysteine-rich angiogenic inducer 61 (Cyr61/CCN1) and CCN6, periostin, and more.[204] Some of these proteins have been scrutinized for their value as glioma biomarkers in CSF and serum (eg, OPN[30,33,207] and tenascin).[43] CSF levels of tenascin were reportedly higher in anaplastic gliomas as compared with astrocytomas of lower malignancy.[43] Increased expression of OPN has been associated with the presence of a variety of cancers including breast cancer, ovarian cancer, melanoma, and glioblastoma.[208] The presence of metastases was also found to be associated with high OPN levels.[209,210] CSF and serum levels of OPN appeared to be higher in patients with gliomas as compared with patients with other primary brain tumors or systemic cancers, and the levels were associated with worse outcomes.[30,33,207] However, no correlation between the radiographic properties of the tumor and the OPN level was found. Interestingly, significant differences in OPN levels between patients with gliomas of WHO grades II, III, and IV were found, and the survival times of patients with high serum OPN levels (>20 ng/mL) appeared to be significantly shorter than those of patients with low OPN levels. Postoperative levels of OPN were, however, not measured in these studies.[30,33,207] So far, the value of OPN for monitoring treatment response is unclear. Since OPN levels were reportedly higher in CSF of patients with atypical teratoid/rhabdoid tumors[211] and other tumors,[208] OPN is not specific for glioma and cannot be used as a diagnostic biomarker.

Matrix Metalloproteinase

Matrix metalloproteinases (MMPs) represent a family of degrading enzymes involved in the breakdown of extracellular matrices necessary for invasion of tumor cells.[212] The zinc- and calcium-dependent MMP family also plays a role in various physiological processes such as embryonic development, angiogenesis, wound healing, and more.[212,213] MMPs comprise a relatively large and ever-growing family, and more than 20 enzymes are now known.[212] MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) are the most abundant MMPs in malignant gliomas.[214–216] In glial tumors, MMP-9 in particular enables tumor cells to migrate or infiltrate, and its level is upregulated by the expression of astrocyte elevated gene-1 (AEG-1).[217] MMP-9 has been measured in serum[218,219] and CSF[220,221] in glioma patients. Levels of MMP-9 have been measured in the CSF of patients treated for glioma recurrence, and elevated concentrations were interpreted to be indicative of treatment failure.[220] The presence of activated MMP-9 also correlated with positive CSF cytology.[221] There are, however, conflicting results for the value of MMP-9 levels in serum.[218,219] In a longitudinal study with a larger patient population, no statistically significant association was observed between levels of serum MMP-9 and radiographic disease status, and changes in serum MMP-9 did not appear to be associated with survival in a cohort of patients with anaplastic glioma.[218] In contrast, serum MMP-9 levels were associated with disease status and were inversely correlated with prognosis in 77 GBM and 66 anaplastic glioma patients.[219] A caveat of these studies is the fact

that leukocytes secrete MMP-9, and increased numbers of leukocytes in CSF or serum can cause increased levels of MMP-9.[222–224] Serum or CSF levels of MMP-9 in glioma patients may therefore be influenced by concomitant inflammation. Because MMPs are crucial for angiogenesis, tumor invasion, tumor growth, and metastatic potential, MMPs are promising targets for potential therapies.[225]

Proteins Associated With Cell Lineage

Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein (GFAP) is an intermediate filament-associated protein, and its immunohistochemistry is used for revealing astrocytic lineage of glial cells and glial tumor cells. Serum levels of GFAP have been analyzed in several clinical studies[143,226,227] and were significantly elevated in high-grade gliomas, as compared with those of nonglial tumors, with 100% specificity for the diagnosis of gliomas.[143,226,227] Jung et al prospectively examined 50 patients with GBM, 18 with anaplastic gliomas, 13 with low-grade gliomas, 17 with a single cerebral metastasis, and 50 healthy controls.[143] Serum GFAP levels were measured by ELISA and were detectable in 40 of the 50 GBM patients (median, 0.18 μg/L; range, 0–5.6 μg/L). Only 2 patients with gliomas of low malignancy grade had detectable serum GFAP levels. Serum GFAP levels in patients with metastases and healthy people were below the detection limit (≤0.012 μg/L). The GFAP serum levels correlated with both tumor volumes and estimated volumes of tumor necrosis in the GBM patients. Brommeland et al found GFAP serum levels with a broad range from 30–1210 ng/L (mean, 239 ng/L) and demonstrated a significant association between preoperative serum GFAP levels and tumor volume in 31 high-grade glioma patients by using ELISA.[226] An ELISA has been recently developed for an autoantibody to GFAP, to be used for detection of glioma.[228] The GFAP serum level may well become a useful protein biomarker. At this point, GFAP should be validated in appropriate clinical studies.

Embryonic Antigens

Carcinoembryonal antigen (CEA), human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) are useful markers for the differential diagnosis between primary brain tumors and metastases or germ cell tumors (GCTs). The cell adhesion molecule CEA is an embryonic antigen and is produced in gastrointestinal tissues during fetal development.[229] Levels of CEA were monitored in the serum, CSF, plasma, and tumor cyst fluid[48,50,51,141,230] of patients with primary brain tumors and cerebral metastases. CEA levels in patients with cerebral metastases and leptomeningeal dissemination were consistently higher than those in patients with primary brain tumors.[37,45,48,51,141] In a study with postoperative follow-up, it was found that patients with metastatic brain tumors and leptomeningeal tumor spread showed high levels of CEA in CSF preoperatively (which normalized following surgery).[45] These data support the use of CEA levels in CSF for the differential diagnosis of primary and metastatic brain tumors.[37] The detection of hCG in serum or CSF supports the diagnosis of intracranial GCTs and proves the presence of trophoblastic cells.[231] AFP is an oncofetal glycoprotein that plays an important role during embryo- and fetogenesis. Elevated serum AFP concentrations have been associated with hepatocellular carcinoma and pediatric tumors,[231] and measurement of hCG and AFP in CSF or serum is considered to be of significance in the differential diagnosis of GCTs and other tumors including glioma.[147,148,231]

Miscellaneous Proteins and Circulating Oncometabolites

2-hydroxyglutamate

Cancer-associated IDH mutations produce the metabolite 2-hydroxyglutarate (2HG). Circulating levels of 2HG are significantly elevated in patients with cholangiocarcinoma[232] and acute myeloid leukemia.[233] In a recent study, it was reported that the concentration of the metabolite 2HG in serum from glioma patients did not correlate with the IDH1 mutational status or the size of the tumor.[234] More clinical studies are required to evaluate the clinical utility of 2HG.

YKL-40

YKL-40 (tyrosine (Y), lysine (K) and leucine (L) and the apparent molecular weight) is also known as chitinase-3-like-1 or human cartilage glycoprotein-39.[235,236] There are indications that YKL-40 may promote degradation of the ECM and play a role in cell migration,[237] connective tissue modeling,[238–240] and inflammatory responses.[241,242] Serum levels of YKL-40 are elevated in patients with lymphoma,[243] lung cancer,[244] leukemia,[245] melanoma,[246,247] colon cancer,[248,249] ovarian cancer,[250–252] breast carcinoma,[253,254] and prostate cancer,[255] and raised levels of YKL-40 correlate with shorter survival times. YKL-40 is highly expressed in tissue microarray studies of gliomas.[256] In multivariate analysis, the tissue expression of YKL-40 was identified as an independent predictor of survival after adjusting for patient age, performance status, and extent of resection.[257] The expression of YKL-40 appeared to be associated with loss of chromosome arm 10q.[236] YKL-40 is secreted into the bloodstream by tumor cells and tumor-associated macrophages and can be detected by ELISA.[256] The value of YKL-40 as a serum marker was evaluated during a follow-up period of 27 months after surgery for high-grade glioma.[219] Levels of YKL-40 were significantly correlated with radiographic evidence of disease and survival times in GBM ($n = 76$) and anaplastic glioma ($n = 66$).[219] In a prospective study in which 1740 MRI matched serum samples of 343 anaplastic glioma patients were implicated, the YKL-40 levels appeared to be significantly lower in patients with no radiographic tumor progression as compared with patients with progressive disease.[257] Increases in YKL-40 levels were also associated with worse survival.[257] In various other clinical studies, serum YKL-40 levels of glioma patients were also elevated and correlated with radiographic evidence of disease and worse overall survival.[219,235,256,257] Since serum levels of YKL-40 are also correlated with poor outcome in various cancers,[243–245,249–255] additional validation studies need to be done, focusing on the specificity of YKL-40 and testing of its value as a glioma biomarker in prospective, controlled settings.

Other proteins

Various other proteins, which are not discussed here, are listed in Table 1 and include galectin-1,[258] nerve growth factor (NGF),[31] macrophage migration inhibitory factor (MIF),[32] alpha-1-antichymotrypsin (AACT),[33] transthyretin (TTHY),[33] gelsolin,[259] 2-Heremans-Schmid glycoprotein (AHSG),[260] Pre-B-cell colony enhancing factor 1 (PBEF1),[261] plasminogen activator inhibitor 1 (PAI-1),[262] neural cell adhesion molecule (NCAM),[263] EGFR,[264] attractin,[35] a proliferation-inducing ligand (APRIL),[265] Cathepsin D,[266] recoverin,[39] CD95,[42] G-22,[267] and somatomedins.[47]

Concluding Remarks

Current strategies in the therapy for patients suffering from primary brain tumors necessitate the development of practical and standardized assays for monitoring disease activity and therapy effects. Intracranial tumors are not accessible for frequent sampling, and therefore body fluids such as blood and CSF are preferable sources for biomarkers. A large number of candidate biomarkers have been discovered, but neither circulating tumor cells, nor their exosomes, DNA, RNA, and particular proteins have passed the requirements of the Tumor Marker Utility Grading System Levels of Evidence/NCCN for clinical application or for serving as monitors in trials. The road from the discovery of new candidate biomarkers to their clinical validation is long. Many issues need to be addressed including biological relevance, sensitivity, specificity, and reproducibility of the measurements. Technical standardization is crucial to achieve clinical utility for candidate biomarkers. Collaborating consortia are needed for standardization and validation of sample collection and isolation, and large prospective multicenter studies are needed to reach the level of evidence required for introducing new biomarkers into clinical practice.

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