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REVIEW ARTICLE

The emerging clinical potential of circulating extracellular vesicles for non-invasive glioma diagnosis and disease monitoring

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Abstract

Diffuse gliomas (grades II–IV) are amongst the most frequent and devastating primary brain tumours of adults. Currently, patients are monitored by clinical examination and radiographic imaging, which can be challenging to interpret and insensitive to early signs of treatment failure and tumour relapse. While brain biopsy and histologic analysis can evaluate disease progression, serial biopsies are invasive and impractical given the cumulative surgical risk, and may not capture the complete molecular landscape of an evolving tumour. The availability of a minimally invasive 'liquid biopsy' that could assess tumour activity and molecular phenotype in situ has the potential to greatly enhance patient care. Circulating extracellular vesicles (EVs) hold significant promise as robust disease-specific biomarkers accessible in the blood of patients with glioblastoma and other diffuse gliomas. EVs are membrane-bound nanoparticles shed from most if not all cells of the body, and carry DNA, RNA, protein, and lipids that reflect the identity and molecular state of their cell-of-origin. EVs can cross the blood–brain barrier and their release is upregulated in neoplasia. In this review, we describe the current knowledge of EV biology, the role of EVs in glioma biology and the current experience and challenges in profiling glioma-EVs from the circulation.

Keywords Biomarker · Extracellular vesicles · Glioblastoma · Glioma · Liquid biopsy

Introduction

Diffuse gliomas (grades II–IV) are among the most frequent and devastating primary brain tumours of adults and are characterised by diffuse infiltrative growth into the brain parenchyma [1]. Glioblastoma (GBM) is the most common and most lethal of these tumours, with a median survival of approximately 15 months [2]. Four distinct molecular types of glioblastoma have been described [3] and molecular heterogeneity within a given tumour is well recognised [4]. Key molecular features of diffuse gliomas include isocitrate dehydrogenase (IDH) mutational status, 1p/19q codeletion, *EGFR* gene amplification, and mutations in *TP53* and/or *ATRX* [1]. In GBM, promoter hypermethylation of the DNA repair gene, *O*-6-methylguanine-DNA-methyltransferase (*MGMT*) is a key predictive and prognostic marker [5, 6]. While surgical resection remains a mainstay of treatment, the diffusely infiltrative nature of GBM makes curative surgery impossible [7, 8]. Adjuvant treatment options are limited, and despite maximal efforts, tumour will almost invariably recur. In lower grade gliomas, progression of malignant features is common upon recurrence, and there is

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often evolution of a 'hypermutation phenotype' after temozolomide treatment [9]. In recurrences of GBM, emergence of subclonal heterogeneity and treatment resistance is the rule, which is partly dependent on *TP53* pathway mutation [10].

Liquid biopsies

Liquid biopsies sample tumour-derived materials released into biofluids, i.e., blood, urine, cerebrospinal fluid (CSF), and saliva, in either free-form (soluble protein [11, 12]; circulating tumour nucleic acids [13, 14]), circulating tumour cells [15], or within membrane-bound vesicles [16, 17]. Unlike traditional tissue biopsies, liquid biopsies can be repeated regularly with minimal risk and serve as a powerful approach to assess dynamic, real-time molecular information from tumours (see Fig. 1). Furthermore, liquid biopsies offer the potential to assess a tumour's molecular diversity in toto, which is not possible in small-biopsy specimens. Liquid biopsy assay development is garnering immense attention for multiple cancer types [18–20] with efforts underway to develop unique panels of descriptive cancer biomarkers. There are three key considerations for the selection of suitable candidate biomarkers for glioma liquid biopsy: ease of access, stability, and detection sensitivity. This is especially relevant to GBM, where the highly dynamic nature of the tumour can lead to clinically relevant changes within weeks–months [21]. The ability to monitor tumour changes, ideally before they are clinically or radiologically apparent, would significantly improve the clinical management of glioma patients as well as provide accurate surrogate endpoints for the new generation of adaptive clinical trials such as GBM-AGILE [22].

Blood-based glioblastoma biomarkers

While gliomas are generally confined to the CNS [23], an abundance of tumour-derived material has been identified in the circulation, including circulating tumour cells (CTCs) [15, 24–26], soluble proteins [27, 28], circulating tumour nucleic acids (DNA [13, 14, 29], miRNA [30, 31]) and extracellular vesicles (EVs) [17, 32]. Protein biomarkers

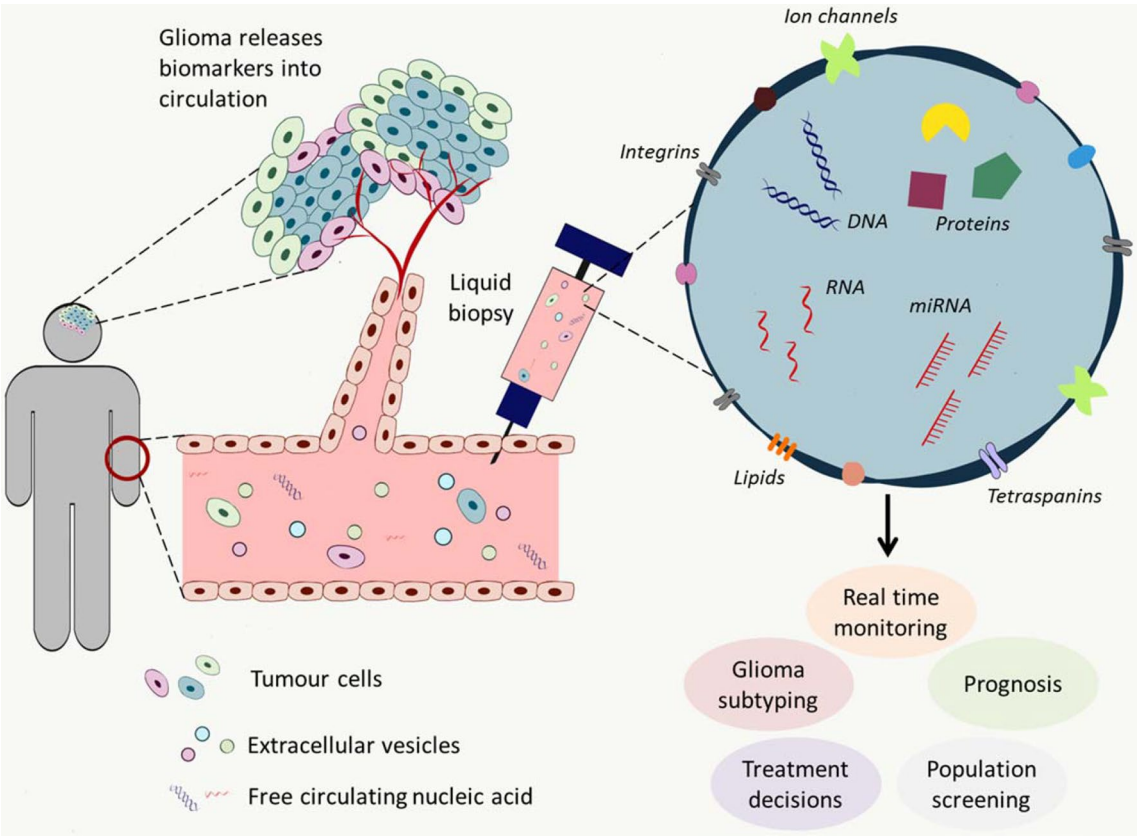

Fig. 1 Potential clinical applications of circulating glioma extracellular vesicles. Glioma biomarkers, such as extracellular vesicles (EVs), circulating tumour cells (CTCs), and circulating DNA and RNA are released into the circulation and can be accessed by a minimally invasive blood test. Glioma-EVs carry molecules (e.g., nucleic acids, proteins, and lipids) that may be useful as diagnostic, prognostic, and predictive biomarkers to assist numerous clinical challenges in glioma patient management, including real-time monitoring, tumour subtyping/stratification, prognostication, therapy selection, and population screening

include factors that are important for GBM pathophysiology, such as cytokines (IL-1β, IL-2, IL-6, IL-8, IL-10, TNF-α), angiogenic proteins (VEGF, FGF-2, TSP-1) [33, 34] and glial fibrillary acidic protein (GFAP) [35, 36]. Matrix metalloproteinases (MMPs) [37] have also been shown to have prognostic value, distinguishing high from low-grade gliomas [38] and predicting treatment response [39, 40]. Plasma 2-hydroxyglutarate (2-HG), the neometabolite of mutant IDH, has also gained interest as a circulating biomarker [41]; however, it has shown limited correlation with IDH mutational status [42]. Free circulating nucleic acids identified in the peripheral circulation include tumour-derived DNA with tumour-specific genetic or epigenetic marks [43–45] and miRNA [46]. Circulating tumour DNA was found in the plasma of 10% glioma patients in one study [47], whilst it was detectable in 55% of patients' sera using methylation assays in another [45]. Free circulating EGFRvIII DNA [14] as well as *IDH1* mutant DNA [13] both show promise as candidate biomarkers in glioma.

Extracellular vesicles (EVs)

EVs are 30–1000 nm membrane-bound nanoparticles that are secreted by most if not all cell types and carry an abundant array of lipids, DNA, RNA and protein that reflect the identity and molecular state of their cell-of-origin [48]. EVs encompass three main classes of secreted vesicles, endosome-derived 'exosomes' (30–100 nm), plasma membrane-derived 'microparticles' (100–1000 nm) and apoptotic cell-derived 'apoptotic bodies' (1000–5000 nm) all varying in size, content and biogenesis. Microparticles form by outward budding of the plasma membrane, while exosomes form through intraluminal invagination of the membrane of late endosomes to form multivesicular bodies (MVB), that then fuse with the plasma membrane to secrete the intraluminal vesicles (ILV) as exosomes [49]. While normal cells secrete microparticles and exosomes, apoptotic bodies are only formed during programmed cell death, which, like in many tumours, plays an important role in GBM pathophysiology [50].

EV biogenesis appears to be a highly regulated, energy-dependent and complex process of membrane trafficking. The ESCRT (endosomal complex required for transport) pathway has overlapping functions for both exosome and microparticle formation [51]. Exosome formation and release involves multiple (~20) ESCRT proteins assembled into four complexes (ESCRT-0, -I, -II and -III) [52] responsible for sorting ubiquitinated proteins into the ILV [53], stimulating inward blebbing of the endosomal membrane [54, 55] and enacting membrane scission, in conjunction with Vps4 ATPase, to complete the budding process [55, 56]. RAB GTPases are involved in docking of the MVB to the cell membrane and SNARE (soluble *N*-ethylmaleimide-sensitive component attachment protein receptor) membrane fusion machinery mediates exosome secretion [54]. Alternative ESCRT-mediated [57] and ESCRT-independent [58] exosome biogenesis pathways have also been described. For microparticles, the ESCRT machinery is involved in the production of vesicles enriched in plasma membrane proteins [51, 59]. In contrast to exosomes and microparticles, less is known of apoptotic body biogenesis. The process of apoptosis involves nuclear chromatin condensation, followed by membrane blebbing and the disintegration of cellular contents into the membrane-enclosed apoptotic bodies [49] with evidence suggesting that membrane blebbing is mediated by actin–myosin interactions [60–62].

There are currently no clear physical or biochemical characteristics that can distinguish between EVs from endosomal and plasma membrane origins [63]. Given this, the International Society for Extracellular Vesicles recommends that the term "Extracellular Vesicle" be used in the place of 'exosome' or 'microvesicle', with operational terms for EV subtypes that refer to physical characteristics of EVs, such as size, density or biochemical composition (MISEV2018) [63]. The term 'EV' is used in this review to encompass small (< 200 nm) and medium/large (> 200 nm) nanosized extracellular membrane-bound particles, without reference to their biogenesis pathway, with the caveat that most studies cited refer to vesicles of smaller size.

EVs were first discovered in the early 1980s from maturing blood reticulocytes [64]. They were initially considered as a conduit for cellular waste, carrying apoptotic material out of the cell [65], but are now recognised as important biological effectors in health and disease [48]. EVs are utilised by cells as unique packaging systems that facilitate the protection and delivery of cellular information through the extracellular milieu. They play integral roles in cancer biology [66, 67], contributing to the preparation of pre-metastatic niches [68, 69], stimulating cell proliferation, angiogenesis [70] and responding to environmental stimuli, such as hypoxia [71].

Glioma-EVs were first identified 3 decades ago from a rat-glioma cell line [72]. Little was known of their physiological importance at the time; however, increasingly, studies show that EVs are integral to glioma-cell signalling and are capable of cultivating a tumour-supportive microenvironment within the brain [73]. GBM-EVs transfer oncogenic material to less malignant GBM cells to induce transformation and therapy resistance [74], influence endothelial cells to promote angiogenesis [70, 75], and maintain intratumoural heterogeneity [71, 76–80]. Recently, GBM-EVs were shown to induce normal astrocytes to acquire a pro-inflammatory, tumour-promoting, senescence-associated secretory phenotype [81]. GBM-derived EVs are taken up by resident microglial cells [82] and skew the activity of classical M1 monocytes/macrophages to an immunosuppressed

M2 phenotype to mediate immune evasion [82–84]. Exosomal transfer of miR-21 is known to downregulate the expression of M1 markers in tumour-associated macrophages and to promote their M2 polarization in other tumours [85], and miR-21 is a key microRNA significantly enriched in glioblastoma EVs [75]. Another cancer-related microRNA that shows a relationship to microglia is miR-504, which negatively regulates the tumour suppressor p53 [86]. The miR-504/miR145/CTGF and miR-504/Grb10/Egr1 pathways were recently identified as important regulators of the mesenchymal transformation of glioblastoma, and miR-504 overexpression in glioma stem cells inhibits their oncogenic potential and the crosstalk with microglia via exosomal delivery [87]. Overexpressed miRNA-21 and miRNA-451 can both be transferred to microglial cells via EVs [88]. Importantly, glioblastoma-derived EVs may modify the phenotype not only of resident microglia but also of invading monocytic cells [84] and even seem to have a predilection for the latter [89].

In addition to their significant roles in intercellular signalling, EVs are also being recognised as promising candidates for liquid biopsy development. The physical and biochemical properties of EVs make them exploitable as stable biomarker reservoirs. Tumour-derived EVs carry constellations of molecules (RNA, DNA, protein, and lipids), enclosed within a lipid bilayer and protected from enzymatic degradation [70, 75, 90]. Moreover, tumour cells secrete EVs in significantly greater quantities than normal cells [91], allowing tumour-derived molecules to be detected above the levels of potential 'noise' from EVs released from other sources. EVs can also cross the blood–brain barrier (BBB) [92, 93], and are significantly increased in the plasma of GBM patients [94], making them excellent candidates for monitoring brain tumours in situ. EVs are relatively stable within the blood, with reports of a half-life of 30–60 min in mice [95] and up to 5 h in patients with thrombocytopenia [96].

The molecular composition of glioma-EVs

Profiling the molecular constituents of EVs derived from primary and established GBM cells [97, 98] and biofluids [99] has identified multiple RNA, DNA and protein species with tumour-supportive ontologies (Table 1). Some molecules are enriched in EVs compared to their cell of origin, indicating that there is selective packaging of some molecules for transport in EVs. The mechanisms driving the selective packaging of molecules into EVs are not well understood. Proteins are directed to MVBs by ubiquitination and ESCRT machinery [100]. miRNAs found within EVs are enriched for species with uradine at their 3′ ends [101] indicating that this post-transcriptional modification is involved in miRNA sorting into exosomes, and may represent a common mechanism for many types of small RNAs such as Y RNAs. Overexpression of neural sphingomyelinase 2 (nSMase2) increases miRNAs within exosomes [102], and some heterogeneous nuclear ribonucleoproteins (hnRNPs) are able to bind to the 3′ end of miRNAs and direct them to the exosomal compartment [103]. Ceramide-rich lipid rafts in the endosomal membrane are thought to be sites of RNA loading into exosomes, influenced by nSMase2, and enhanced affinity for these domains may be influenced by specific sequence motifs [104].

Skog et al. [70] performed the first comprehensive study to profile the molecular contents of glioma EVs. They identified 27,000 enriched transcripts that were characteristic of glioma, including transcripts encoding EGFRvIII and GFAP, within EVs derived from GBM cells in vitro. Selective packaging of a wide variety of nucleic acids occurs, including retrotransposon elements [105], mRNA (including abundant mRNA for the enigmatic vault protein associated with drug-resistance) [70, 75], small non-coding RNAs including miRNAs, tRNAs, vtRNAs and Y RNAs, as well as numerous unannotated small RNAs from intergenic regions of unknown function [70, 75, 106].

Recently, attempts have been made to determine proteomic signatures for GBM-EVs that are reflective of GBM invasiveness and aggression. Proteomic analysis of EVs released by six established GBM cell lines identified a common set of 145 proteins, with diverse biological and molecular annotations and significant functional associations to cell proliferation, cell fate and intercellular signaling [97]. Protein specifically enriched in EVs from primary GBM cultures are involved in extracellular matrix interactions, leukocyte migration [84] and angiogenesis, notably angiogenin, IL-6, IL-8, TIMP-1, VEGF and TIMP-2 [70]. Proteomic profiling of EVs captured from neurosurgical aspirates also shows distinct signatures able to distinguish GBM from grades II to III gliomas [16], including GBM-EV proteins previously associated with tumour invasiveness and reduced survival [97]. One such candidate, chaperonin-containing TCP1 subunit 6A (CCT6A), is a subunit of the key molecular chaperone T-complex protein 1 ring complex (TRiC). Interestingly, CCT6A is co-localised with EGFR at 7p11.2, with a strong tendency for co-amplification and EV-associated CCT6A levels are proposed as a proxy for *EGFR* testing in tumours [16]. Studies addressing the molecular composition of EVs both in vitro and in vivo are summarised in Table 1; an up-to-date compendium of EV-associated molecules from all cell types is curated in Vesiclepedia (http://microvesicles.org) [107].

EVs as glioma biomarkers

An important consideration for any peripheral biomarker of central nervous system disease is their ability to cross the BBB into the circulation. GBM–EVs have been shown to

Table 1 Timeline of publications reporting potential glioma–EV biomarkers

Publication year	Main glioma-EV biomarker findings	Molecule type	EV source	References
2008	EGFRvIII and GFAP transcripts let-7a, miR-15b, miR-16, miR-19b, miR-21, miR-26a, miR-27a, miR-92, miR-93, miR-320, miR-20 Angiogenic proteins: angiogenin, IL-6, IL-8, TIMP-1, VEGF, TIMP-2, FGF-α	mRNA miRNA Protein	Serum and primary GBM cells	[70]
2008–2009	EGFRvIII	Protein	GBM cell lines	[74, 108]
2009	EGFR wild-type, EGFRvIII, TGFβ1	Protein	Glioma cell line	[109]
2012	Downregulation of ribosomal genes	mRNA	Serum	[110]
2013	*Selectively packaged*: miR-451a, -4301, -5096, -4454, -3676-5p, -1303, -1273a, -619, -448, -1246, -4792, -5095, -1273, -4256, -4255, -5100, -1285-1, -1269b, -4500, -1273d, -4443, AC068946.1 Vault RNAs *Highly abundant*: miR-21, -99a, -23a, -30a, -30d, -30b, -22, -125a, -25, -221, -92b, -135b, -29a, -222, -100, -451-a, -4301, -27b, -15b, -23b, -5096, -3676, -30e, -374b, -339, -191, -4454, let-7b	Coding and non-coding RNA Protein	Glioma cell line	[75]
2013	IDH1	mRNA	CSF and serum	[111]
2013	miR-21	miRNA	CSF	[99]
2014	RNU61, miR-320, miR-574-3p, miR-483-5p, miR-197, miR-484, miR-146a, miR-223	Small nuclear RNA miRNA	Serum	[106]
2015	MGMT, APNG	mRNA	GBM cell lines and serum	[21]
2015	miR-21, miR-103, miR-24, miR-125	miRNA	CSF, plasma and primary GBM cells	[112]
2015	miR-21	miRNA	CSF and GBM cell lines	[113]
2016	TrkB	Protein	GBM cell line and Plasma	[114]
2016	ANXA1, ACTR3, ITGβ1, IGF2R, PDCD6IP, CALR, IPO5, MVP, PSMD2	Protein	GBM cell lines and neurosurgical aspirates	[97]
2016	Ras	Protein	GBM cell lines	[115]
2017	miR-221	miRNA	GBM cell line	[116]
2017	IDH1 wild-type and mutant AKT1, AKT3, ASCL1, CDK4, EGFR, ERBB2, IDH2, MDM2, MDM4, MGMT, PIK3CA, RB1	DNA	Xenograft mouse plasma Primary GBM cells Plasma	[32]
2017	miR-21, miR-218, miR-193b, miR-331, miR-374a, miR-548c, miR-520f, miR-27b, miR-130b	miRNA	CSF	[117]
2017	Wild-type EGFR, EGFRvIII	mRNA	CSF	[118]
2017	miR-1587	miRNA	Primary GBM cells	[119]
2018	Upregulation of miR-301a	miRNA	Serum	[120]
2018	PD-L1	DNA	Serum and plasma	[121]
2018	HOTAIR	Long non-coding RNA	Serum	[122]
2018	PTRF	Protein	Serum	[123]
2018	EGFRvIII	mRNA	Serum	[124]

Table 1 (continued)

Publication year	Main glioma-EV biomarker findings	Molecule type	EV source	References
2018	*Markers for IDH wild-type GBM*: miR-182-5p, miR-328-3p, miR-339-5p, miR-340-5p, miR-485-3p, miR-486-5p and miR-543 *Markers for IDH mutant Glioma*: miR-7d-3p, miR-98-5p, miR-106b-3p, 130b-5p and 185-5p	miRNA	GBM, glioma and healthy control serum	[17]
2019	*Markers for IDH wild-type GBM*: CCT6A, S100A10, S100A11, LCP1, CHI3L1, CLIC1, EIF2S3, CD163, MRC2, APOL2, STAB1, RPS27, PYGL, FCGR2A, CAPG, SLC16A3 Markers for IDH-mutant Glioma: CERS4, CBSL, NUBPL, SLC9A6	Protein	GBM and glioma neurosurgical aspirates	[16]
2019	FTL, vWF, AZGP1, Serpin 3, C3, APOE	Protein	Plasma	[94]
2019	SDC1	Protein	Plasma	[125]

traverse the BBB in several mouse models [32, 126]. In an orthotopic xenotransplant mouse model of human glioma with an intact blood-brain barrier, tumour-derived DNA associated with a variety of vesicle types was detected in the peripheral blood [28]. In another elegant experiment, EVs from dendritic cells transfected with a neural surface protein were electroporated with siRNA against GAPDH and injected into the tail vein of mice [126]. After 3 days there was a significant reduction in GAPDH mRNA levels in sampled brain regions but not in peripheral organs, indicating that the EVs were able to cross the BBB in the reverse direction, and could be targeted to specific cell types to deliver an effective molecular cargo.

A variety of studies have examined EVs in patient groups to assess their usefulness as clinical biomarkers, starting with Skog et al. [70]. To date, most studies have used relatively small numbers of patients, but findings from many studies have demonstrated that larger longitudinal studies are well justified. Several studies have utilised CSF as the EV source, as CSF bathes the brain and theoretically should be a good source of brain-derived materials. In an early study using digital droplet PCR, mutant *IDH1* transcripts were detected in CSF–EVs collected during surgery, showing good concordance to tumour IDH mutational status [111] In another CSF study, miR-21 levels in EVs captured from GBM patients were found to be around tenfold higher than in CSF–EVs from non-tumour patients [93]. This finding was further substantiated in a larger cohort with high sensitivity (87%) and specificity (93%), flagging CSF EV miR-21 levels as a potential biomarker with clinical utility [99]. An extended study of 70 glioma patients also demonstrated significantly elevated CSF EV miR-21 levels (with trauma-matched controls), while serum EV levels of miR-21 were unaltered [126]. EGFRvIII mRNA as well as elevated levels of EGFR mRNA have been assessed in CSF-derived EVs. While approximately 60% of EGFRvIII tissue-positive GBM patients had detectable EGFRvIII in CSF-EVs, only one of 48 EGFRvIII tissue-negative patients had detectable EGFRvIII in their CSF-EVs [118].

Serum and plasma EV studies in patients with glioma have also been performed (Table 1). In general these have interrogated pre-defined groups of molecules by RT-PCR, TaqMan Arrays or microarrays. In 2012, serum EV RNA from patients with primary GBM ($n=9$) and healthy controls ($n=7$) were analysed by RNA microarrays, showing profiles with discriminatory power between the two cohorts [106]. Several studies also included serum-derived EVs, where miR-21 was reported as significantly upregulated [112]. Another study found serum exosomal miR-320, mir-547-3p, and RNU6-1 were significantly associated with GBM diagnosis, as well as outcome (RNU6-1) [106] in a group of 25 newly diagnosed GBM patients and matched controls, with validation of the findings in an additional 50 GBM patients. Levels of serum EV-associated miR-301 have also been found to be elevated in glioma patients by quantitative real-time PCR, and that levels fall after surgery and are predictive of overall survival [127]. Microfluidic chip-based analysis of MGMT and APNG levels in blood-derived EVs has demonstrated that the levels of these two drug-resistance enzymes in EVs are correlated with intratumoural levels and may represent a method to predict drug-resistance [21]. EGFRvIII has also been detected in peripheral blood EVs [109, 124]. Using unbiased next-generation sequencing, partially overlapping, yet distinct miRNA signatures for IDH wild-type GBM and IDH mutant grades II–III gliomas were described in serum-derived EVs, and could distinguish preoperative GBM patients from healthy controls with high accuracy [17]. Sampling EVs from the glioma patient blood

with subsequent molecular profiling may serve as a non-invasive alternative to tissue biopsies and allow tumours to be assessed in real-time. Under the 2016 WHO guidelines, diffuse glioma diagnoses now require molecular testing, including IDH-mutational and 1p/19q codeletion states [128]. If circulating EV molecular profiles mirror the mutational status of the respective tumours, glioma subtyping and/or stratification may also be possible via liquid biopsies.

Challenges in developing glioma EV-based liquid biopsies

Despite the suitability of glioma–EVs as biomarker reservoirs, multiple obstacles have hindered their application to the clinic. One of the barriers to blood-derived EV biomarker discovery is the complexity and heterogeneity of the blood. Glioma-EVs are a minor population of the total EVs within the blood; EVs are secreted by all cells into the systemic circulation with a significant proportion in the blood being platelet-derived [129]. Analysis of single vesicles in patient sera suggests that GBM-derived EVs constitute less than 10% of the total EV pool [130]. However, significant associations between specific GBM molecular pathways and altered EV miRNA profiles from GBM patient blood can be observed [17], suggesting that while mixed vesicle populations are present in crude EV preparations, there is a disease-specific signal in the periphery sufficient for biomarker studies. Enrichment strategies for GBM-derived EVs are being investigated, with antibody-based purification of the GBM-specific peptide EGFRvIII as well as podoplanin being used successfully [131].

Primary or immortalised glioma cell cultures have been useful for EV profiling studies (refer to Table 1), however in vitro models are rarely accurate reflections of in vivo disease. With increased passage number, cultured cells lose features that were present in the original tumour. Recently, neurosurgical aspirates from glioma patients were shown to be rich sources of tumour-released EVs and are valuable for determining glioma-EV molecular profiles as a first-pass for the discovery of peripheral EV biomarkers, including markers associated with tumour invasion and patient survival [16, 97]. Moreover, surgical aspirates are collected at a critical window for biomarker discovery; surgery is the only time when the tumour microenvironment is directly accessible, and represents a key standardised time point in a patient's clinical management to which several parameters are measured.

The effective translation of EV-based liquid biopsies to clinic assays requires rigorous standardisation of pre-analytical variables. EV isolation methods include differential ultracentrifugation, polymer-based isolation, affinity methods and filtration systems, each method capturing subtly different sub-populations of EV species [132]. EV isolation procedures are often time-consuming, require large quantities of starting material, and currently cannot separate tumour-derived EVs from other EV populations present. The isolation method is usually selected in accordance with several factors, including the nature and quantity of the starting sample, the degree of required purity and final downstream applications. Some of the variability across existing studies is likely due to differences in EV isolation methods. Other pre-analytical variables, including sampling methods, processing time, preservative agents, storage conditions and freeze/thaw cycles can all impact the yields, purity and subpopulations of EVs recovered, further impacting downstream results and confounding cross-assay comparisons [133]. Methods of nucleic acid extraction also impact upon the profile of RNA or DNA identified in EV preparations [134]. Methods that offer targeted capture of glioma–EVs from patient blood hold major promise for the future of EV-based liquid biopsies. Recently developed microfluidic immunoaffinity-based devices or alternating current electrokinetic (ACE) chips capture tumour-derived EVs directly from the blood of patients without any previous purification or manipulation, offering several advantages for clinical translation, including low cost and, small sample volumes [135–137]. The recent rapid expansion of the EV research field has driven the need for improved standardisation, and minimal requirements for reporting EV isolation, characterisation and analytical approaches have been refined by the International Society for Extracellular Vesicles (MISEV2018) [63]. With this framework in place and access to rich, well annotated clinical sources of tumour-derived EVs, a universal set of surface glioma-EV markers may be described that allows for more targeted biomarker discovery analyses.

Summary

Critical to the clinical management of glioma is the ability to monitor tumour progression and treatment response accurately, non-invasively and in a timely manner. Glioma-associated EVs present in the blood have significant potential to be used for such purposes. The application of multi-omics to glioma-EVs will yield more complete molecular profiles and allow the identification of diagnostic, prognostic and predictive biomarkers signatures with clinical applicability as liquid biopsies.

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References

1. Louis DN, Perry A, Reifenberger G et al (2016) The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol 131(6):803–820
2. Delgado-Lopez PD, Corrales-Garcia EM (2016) Survival in glioblastoma: a review on the impact of treatment modalities. Clin Transl Oncol 18(11):1062–1071
3. Verhaak RG, Hoadley KA, Purdom E et al (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17(1):98–110
4. Patel AP, Tirosh I, Trombetta JJ et al (2014) Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344(6190):1396–1401
5. Rivera AL, Pelloski CE, Gilbert MR et al (2010) MGMT promoter methylation is predictive of response to radiotherapy and prognostic in the absence of adjuvant alkylating chemotherapy for glioblastoma. Neuro Oncol 12(2):116–121
6. Dunn J, Baborie A, Alam F et al (2009) Extent of MGMT promoter methylation correlates with outcome in glioblastomas given temozolomide and radiotherapy. Br J Cancer 101(1):124–131
7. Holland EC, Multiforme G (2000) The terminator. Proc Natl Acad Sci USA 97(12):6242–6244
8. Stupp R, Mason WP, van den Bent MJ et al (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352(10):987–996
9. Hunter C, Smith R, Cahill DP et al (2006) A hypermutation phenotype and somatic MSH6 mutations in recurrent human malignant gliomas after alkylator chemotherapy. Cancer Res 66(8):3987–3991
10. Kim H, Zheng S, Amini SS et al (2015) Whole-genome and multisector exome sequencing of primary and post-treatment glioblastoma reveals patterns of tumor evolution. Genome Res 25(3):316–327
11. Brommeland T, Rosengren L, Fridlund S et al (2007) Serum levels of glial fibrillary acidic protein correlate to tumour volume of high-grade gliomas. Acta Neurol Scand 116(6):380–384
12. Peles E, Lidar Z, Simon AJ et al (2004) Angiogenic factors in the cerebrospinal fluid of patients with astrocytic brain tumors. Neurosurgery 55(3):562–567 **(discussion 567–8)**
13. Boisselier B, Perez-Larraya JG, Rossetto M et al (2012) Detection of IDH1 mutation in the plasma of patients with glioma. Neurology 79(16):1693–1698
14. Salkeni MA, Zarzour A, Ansay TY et al (2013) Detection of EGFRvIII mutant DNA in the peripheral blood of brain tumor patients. J Neurooncol 115(1):27–35
15. Gao F, Cui Y, Jiang H et al (2016) Circulating tumor cell is a common property of brain glioma and promotes the monitoring system. Oncotarget 7(44):71330–71340
16. Hallal S, Russell BP, Wei H et al (2018) Extracellular vesicles from neurosurgical aspirates identifies chaperonin containing TCP1 subunit 6A (CCT6A) as a potential glioblastoma biomarker with prognostic significance. Proteomics 19(1–2):e1800157
17. Ebrahimkhani S, Vafaee F, Hallal S et al (2018) Deep sequencing of circulating exosomal microRNA allows non-invasive glioblastoma diagnosis. NPJ Precis Oncol 2:28
18. Morrison GJ, Goldkorn A (2018) Development and application of liquid biopsies in metastatic prostate cancer. Curr Oncol Rep 20(4):35
19. Best MG, Sol N, Zijl S et al (2015) Liquid biopsies in patients with diffuse glioma. Acta Neuropathol 129(6):849–865
20. Giannopoulou L, Kasimir-Bauer S, Lianidou ES (2018) Liquid biopsy in ovarian cancer: recent advances on circulating tumor cells and circulating tumor DNA. Clin Chem Lab Med 56(2):186–197
21. Shao H, Chung J, Lee K et al (2015) Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma. Nat Commun 6:6999
22. Alexander BM, Ba S, Berger MS et al (2018) Adaptive global innovative learning environment for glioblastoma: GBM AGILE. Clin Cancer Res 24(4):737–743
23. Hamilton JD, Rapp M, Schneiderhan T et al (2014) Glioblastoma multiforme metastasis outside the CNS: three case reports and possible mechanisms of escape. J Clin Oncol 32(22):e80–e84
24. Macarthur KM, Kao GD, Chandrasekaran S, Alonso-Basanta M et al (2014) Detection of brain tumor cells in the peripheral blood by a telomerase promoter-based assay. Cancer Res 74(8):2152–2159
25. Sullivan JP, Nahed BV, Madden MW et al (2014) Brain tumor cells in circulation are enriched for mesenchymal gene expression. Cancer Discov 4(1):1299–1309
26. Liu T, Xu H, Huang M et al (2018) Circulating glioma cells exhibit stem cell-like properties. Cancer Res 78(23):6632
27. Ilhan A, Gartner W, Neziri D, Czech T et al (2009) Angiogenic factors in plasma of brain tumour patients. Anticancer Res 29(2):731–736
28. Quaranta M, Divella R, Daniele A, Di Tardo S et al (2007) Epidermal growth factor receptor serum levels and prognostic value in malignant gliomas. Tumori J 93(3):275–280
29. Balana C, Carrato C, Ramirez JL, Cardona AF et al (2011) Tumour and serum MGMT promoter methylation and protein expression in glioblastoma patients. Clin Transl Oncol 13(9):677–685
30. Yang C, Wang C, Chen X, Chen S et al (2013) Identification of seven serum microRNAs from a genome-wide serum microRNA expression profile as potential noninvasive biomarkers for malignant astrocytomas. Int J Cancer 132(1):116–127
31. Ma C, Nguyen HPT, Luwor RB et al (2018) A comprehensive meta-analysis of circulation miRNAs in glioma as potential diagnostic biomarker. PLoS One 13(2):e0189452–e0189452
32. Garcia-Romero N, Carrion-Navarro J, Esteban-Rubio S et al (2017) DNA sequences within glioma-derived extracellular vesicles can cross the intact blood–brain barrier and be detected in peripheral blood of patients. Oncotarget 8(1):1416–1428
33. Albulescu R, Codrici E, Popescu ID, Mihai S et al (2013) Cytokine patterns in brain tumour progression. Mediat Inflamm 2013:979748. https://doi.org/10.1155/2013/979748
34. Reynes G, Vila V, Martin M, Martin M, Parada A et al (2011) Circulating markers of angiogenesis, inflammation, and coagulation in patients with glioblastoma. J Neurooncol 102(1):35–41
35. Husain H, Savage W, Grossman SA, Ye X et al (2012) Pre- and post-operative plasma glial fibrillary acidic protein levels in patients with newly diagnosed gliomas. J Neurooncol 109(1):123–127
36. Ilhan-Mutlu A, Wagner L, Widhalm G, Wohrer A et al (2013) Exploratory investigation of eight circulating plasma markers in brain tumor patients. Neurosurg Rev 36(1):45–55
37. Lin Y, Wang JF, Gao G-Z, Gao GF, Zhang G-Z et al (2013) Plasma levels of tissue inhibitor of matrix metalloproteinase-1 correlate with diagnosis and prognosis of glioma patients. Chin Med J 126(22):4295–4300
38. Crocker M, Ashley S, Giddings I, Petrik V et al (2011) Serum angiogenic profile of patients with glioblastoma identifies distinct tumor subtypes and shows that TIMP-1 is a prognostic factor. Neuro Oncol 13(1):99–108
39. Eoli M, Di Stefano AL, Aquino D et al (2013) Tumor perfusion during bevacizumab and irinotecan in recurrent glioblastoma: a multimodal approach. J Clin Oncol 31(15_suppl):2074–2074

40. Tabouret E, Boudouresque F, Barrie M, Matta M et al (2014) Association of matrix metalloproteinase 2 plasma level with response and survival in patients treated with bevacizumab for recurrent high-grade glioma. Neuro Oncol 16(3):392–399
41. Lombardi G, Corona G, Bellu L et al (2015) Diagnostic value of plasma and urinary 2-hydroxyglutarate to identify patients with isocitrate dehydrogenase-mutated glioma. Oncologist 20(5):562–567
42. Capper D, Simon M, Langhans C-D, Okun JG et al (2012) 2-Hydroxyglutarate concentration in serum from patients with gliomas does not correlate with IDH1/2 mutation status or tumor size. Int J Cancer 131(3):766–768
43. Wakabayashi T, Natsume A, Hatano H, Fujii M et al (2009) p16 promoter methylation in the serum as a basis for the molecular diagnosis of gliomas. Neurosurgery 64(3):455–461
44. Majchrzak-Celinska A, Paluszczak J, Kleszcz R, Magiera M et al (2013) Detection of MGMT, RASSF1A, p15INK4B, and p14ARF promoter methylation in circulating tumor-derived DNA of central nervous system cancer patients. J Appl Genet 54(3):335–344
45. Lavon I, Refael M, Zelikovitch B, Shalom E et al (2010) Serum DNA can define tumor-specific genetic and epigenetic markers in gliomas of various grades. Neuro Oncol 12(2):173–180
46. Moller HG, Rasmussen AP, Andersen HH, Johnsen KB et al (2013) A systematic review of microRNA in glioblastoma multiforme: micro-modulators in the mesenchymal mode of migration and invasion. Mol Neurobiol 47(1):131–144
47. Bettegowda C, Sausen M, Leary RJ, Kinde I et al (2014) Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 9(224):224ra24
48. Yanez-Mo M, Siljander PR, Andreu Z et al (2015) Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 4:27066
49. Akers JC, Gonda D, Kim R et al (2013) Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. J Neurooncol 113(1):1–11
50. Pavlyukov MS, Yu H, Bastola S et al (2018) Apoptotic cell-derived extracellular vesicles promote malignancy of glioblastoma via intercellular transfer of splicing factors. Cancer Cell 34(1):119–135
51. Bebelman MP, Smit MJ, Pegtel DM et al (2018) Biogenesis and function of extracellular vesicles in cancer. Pharmacol Ther 188:1–11
52. Henne WM, Buchkovich NJ, Emr SD (2011) The ESCRT pathway. Dev Cell 21(1):77–91
53. Katzmann DJ, Babst M, Emr SD (2001) Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. Cell 106(2):145–155
54. Babst M, Katzmann DJ, Snyder WB et al (2002) Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body. Dev Cell 3(2):283–289
55. Babst M, Katzmann DJ, Estepa-Sabal EJ et al (2002) Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting. Dev Cell 3(2):271–282
56. Christ L, Raiborg C, Wenzel EM et al (2017) Cellular functions and molecular mechanisms of the ESCRT membrane-scission machinery. Trends Biochem Sci 42(1):42–56
57. Baietti MF, Zhang Z, Mortier E et al (2012) Syndecan–syntenin–ALIX regulates the biogenesis of exosomes. Nat Cell Biol 14(7):677–685
58. Stuffers S, Sem Wegner C, Stenmark H et al (2009) Multivesicular endosome biogenesis in the absence of ESCRTs. Traffic 10(7):925–937
59. Nabhan JF, Hu R, Oh RS et al (2012) Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at plasma membrane by recruitment of TSG101 protein. Proc Natl Acad Sci USA 109(11):4146–4151
60. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26(4):239–257
61. Sebbagh M, Renvoize C, Hamelin J et al (2001) Caspase-3-mediated cleavage of ROCK I induces MLC phosphorylation and apoptotic membrane blebbing. Nat Cell Biol 3(4):346–352
62. Coleman ML, Sahai EA, Yeo M et al (2001) Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. Nat Cell Biol 3(4):339–345
63. Théry C, Witwer KW, Aikawa E et al (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7(1):1535750
64. Harding C, Stahl P (1983) Transferrin recycling in reticulocytes: pH and iron are important determinants of ligand binding and processing. Biochem Biophys Res Commun 113(2):650–658
65. Johnstone RM, Bianchini A, Teng K (1989) Reticulocyte maturation and exosome release: transferrin receptor containing exosomes shows multiple plasma membrane functions. Blood 74(5):1844–1851
66. Nakano I, Garnier D, Minata M et al (2015) Extracellular vesicles in the biology of brain tumour stem cells–Implications for intercellular communication, therapy and biomarker development. Semin Cell Dev Biol 40:17–26
67. Chistiakov DA, Chekhonin VP (2014) Extracellular vesicles shed by glioma cells: pathogenic role and clinical value. Tumour Biol 35(9):8425–8438
68. Hoshino A, Costa-Silva B, Shen T-L et al (2015) Tumour exosome integrins determine organotropic metastasis. Nature 527(7578):329–335
69. Kaplan RN, Riba RD, Zacharoulis S et al (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 438(7069):820–827
70. Skog J, Wurdinger T, van Rijn S et al (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 10(12):1470–1476
71. Kucharzewska P, Christianson HC, Welch JE et al (2013) Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development. Proc Natl Acad Sci USA 110(18):7312–7317
72. Trams EG, Lauter CJ, Norman S Jr et al (1981) Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. BBA Biomembr 645(1):63–70
73. Broekman ML, Maas SLN, Abels ER et al (2018) Multidimensional communication in the microenvirons of glioblastoma. Nat Rev Neurol 14(8):482–495
74. Al-Nedawi K, Meehan B, Micallef J et al (2008) Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol 10(5):619–624
75. Li CC, Eaton SA, Young PE et al (2013) Glioma microvesicles carry selectively packaged coding and non-coding RNAs which alter gene expression in recipient cells. RNA Biol 10(8):1333–1344
76. Svensson KJ, Kucharzewska P, Christianson HC et al (2011) Hypoxia triggers a proangiogenic pathway involving cancer cell microvesicles and PAR-2-mediated heparin-binding EGF signaling in endothelial cells. Proc Natl Acad Sci USA 108(32):13147–13152
77. Platet N, Liu SY, Atifi ME et al (2007) Influence of oxygen tension on CD133 phenotype in human glioma cell cultures. Cancer Lett 258(2):286–290

78. Li Z, Bao S, Wu Q et al (2009) Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cancer Cell 15(6):501–513
79. Kolenda J, Jensen SS, Aaberg-Jessen C et al (2011) Effects of hypoxia on expression of a panel of stem cell and chemoresistance markers in glioblastoma-derived spheroids. J Neurooncol 103(1):43–58
80. Joseph JV, Conroy S, Pavlov K et al (2015) Hypoxia enhances migration and invasion in glioblastoma by promoting a mesenchymal shift mediated by the HIF1α–ZEB1 axis. Cancer Lett 359(1):107–116
81. Hallal S, Mallawaaratchy DM, Wei H et al (2018) Extracellular vesicles released by glioblastoma cells stimulate normal astrocytes to acquire a tumor-supportive phenotype via p53 and MYC signaling pathways. Mol Neurobiol. https://doi.org/10.1007/s12035-018-1385-1
82. van der Vos KE, Abels ER, Zhang X et al (2015) Directly visualized glioblastoma-derived extracellular vesicles transfer RNA to microglia/macrophages in the brain. Neuro Oncol 18(1):58–69
83. Systemic T (2017) Cells immunosuppression of glioma stem cell-derived exosomes is mediated by monocytic myeloid-derived suppressor cells. PLoS One 12(1):e0169932
84. de Vrij J, Maas SLN, Kwappenberg KMC et al (2015) Glioblastoma-derived extracellular vesicles modify the phenotype of monocytic cells. Int J Cancer 137(7):1630–1642
85. Hsieh CH, Tai SK, Yang MH (2018) Snail-overexpressing cancer cells promote M2-like polarization of tumor-associated macrophages by delivering MiR-21-abundant exosomes. Neoplasia 20(8):775–788
86. Hu W, Chan CS, Wu R et al (2010) Negative regulation of tumor suppressor p53 by microRNA miR-504. Mol Cell 38(5):689–699
87. Rand D, Cazacu S, Hong X et al (2018) Overexpression of miR-504 in glioma stem cells inhibits the oncogenic potential and the crosstalk of these cells with microglia via exosomal delivery. J Extracell Vesicles 7(sup1):1461450
88. Matias D, Balca-Silva J, da Graca GC et al (2018) Microglia/astrocytes-glioblastoma crosstalk: crucial molecular mechanisms and microenvironmental factors. Front Cell Neurosci 12:235
89. Gabrusiewicz K, Li X, Wei J et al (2018) Glioblastoma stem cell-derived exosomes induce M2 macrophages and PD-L1 expression on human monocytes. Oncoimmunology 7(4):e1412909
90. Thakur BK, Zhang H, Becker A et al (2014) Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res 24(6):766–769
91. Eldh M, Olofsson Bagge R, Lasser C et al (2014) MicroRNA in exosomes isolated directly from the liver circulation in patients with metastatic uveal melanoma. BMC Cancer 14:962
92. Yang T, Martin P, Fogarty B et al (2015) Exosome delivered anticancer drugs across the blood–brain barrier for brain cancer therapy in Danio rerio. Pharm Res 32(6):2003–2014
93. Wood MJ, O'Loughlin AJ, Samira L (2011) Exosomes and the blood–brain barrier: implications for neurological diseases. Ther Deliv 2(9):1095–1099
94. Osti D, Del Bene M, Rappa G et al (2019) Clinical significance of extracellular vesicles in plasma from glioblastoma patients. Clin Cancer Res 25(1):266–276
95. Lai CP, Mardini O, Ericsson M et al (2014) Dynamic biodistribution of extracellular vesicles in vivo using a multimodal imaging reporter. ACS Nano 8(1):483–494
96. Rank A, Nieuwland R, Crispin A, Grutzner S et al (2011) Clearance of platelet microparticles in vivo. Platelets 22(2):111–116
97. Mallawaaratchy DM, Hallal S, Russell B et al (2016) Comprehensive proteome profiling of glioblastoma-derived extracellular vesicles identifies markers for more aggressive disease. J Neuro Oncol 131(2):233–244
98. Ricklefs F, Mineo M, Rooj AK et al (2016) Extracellular vesicles from high-grade glioma exchange diverse pro-oncogenic signals that maintain intratumoral heterogeneity. Cancer Res 76(10):2876–2881
99. Akers JC, Ramakrishnan V, Kim R, Skog J et al (2013) MiR-21 in the extracellular vesicles (EVs) of cerebrospinal fluid (CSF): a platform for glioblastoma biomarker development. PLoS One 8(10):e78115
100. Raiborg C, Stenmark H (2009) The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. Nature 458(7237):445–452
101. Koppers-Lalic D, Hackenberg M, Bijnsdorp IV et al (2014) Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. Cell Rep 8(6):1649–1658
102. Kosaka N, Iguchi H, Hagiwara K et al (2013) Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis. J Biol Chem 288(15):10849–10859
103. Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F et al (2013) Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun 4:2980
104. Janas T, Janas T (2011) The selection of aptamers specific for membrane molecular targets. Cell Mol Biol Lett 16(1):25–39
105. Balaj L, Lessard R, Dai L et al (2011) Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. Nat Commun 2:180
106. Manterola L, Guruceaga E, Perez-Larraya J, Gonzalez-Huarriz M et al (2014) A small noncoding RNA signature found in exosomes of GBM patient serum as a diagnostic tool. Neuro Oncol 16(4):520–527
107. Kalra H, Simpson RJ, Ji H et al (2012) Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. PLoS Biol 10(12):e1001450
108. Al-Nedawi K, Meehan B, Kerbel RS et al (2009) Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. Proc Natl Acad Sci USA 106(10):3794–3799
109. Graner MW, Alzate O, Dechkovskaia AM et al (2009) Proteomic and immunologic analyses of brain tumor exosomes. FASEB J Off Publ Feder Am Soc Exp Biol 23(5):1541–1557
110. Noerholm M, Balaj L, Limperg T et al (2012) RNA expression patterns in serum microvesicles from patients with glioblastoma multiforme and controls. BMC Cancer 12:22
111. Chen WW, Balaj L, Liau LM et al (2013) BEAMing and droplet digital PCR analysis of mutant IDH1 mRNA in glioma patient serum and cerebrospinal fluid extracellular vesicles. Mol Ther Nucleic Acids 2:e109
112. Akers JC, Ramakrishnan V, Kim R et al (2015) miRNA contents of cerebrospinal fluid extracellular vesicles in glioblastoma patients. J Neuro Oncol 123(2):205–216
113. Shi R, Wang PY, Li XY et al (2015) Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients. Oncotarget 6(29):26971–26981
114. Pinet S, Bessette B, Vedrenne N et al (2016) TrkB-containing exosomes promote the transfer of glioblastoma aggressiveness to YKL-40-inactivated glioblastoma cells. Oncotarget 7(31):50349–50364
115. Luhtala N, Aslanian A, Yates JR 3rd et al (2017) Secreted glioblastoma nanovesicles contain intracellular signaling proteins and active ras incorporated in a farnesylation-dependent manner. J Biol Chem 292(2):611–628
116. Yang JK, Yang JP, Tong J et al (2017) Exosomal miR-221 targets DNM3 to induce tumor progression and temozolomide resistance in glioma. J Neurooncol 131(2):255–265

117. Akers JC, Hua W, Li H et al (2017) A cerebrospinal fluid microRNA signature as biomarker for glioblastoma. Oncotarget 8(40):68769–68779
118. Figueroa JM, Skog J, Akers J et al (2017) Detection of wild-type EGFR amplification and EGFRvIII mutation in CSF-derived extracellular vesicles of glioblastoma patients. Neuro Oncol 19(11):1494–1502
119. Figueroa J, Phillips LM, Shahar T et al (2017) Exosomes from glioma-associated mesenchymal stem cells increase the tumorigenicity of glioma stem-like cells via transfer of miR-1587. Cancer Res 77(21):5808–5819
120. Lan F, Qing Q, Pan Q et al (2018) Serum exosomal miR-301a as a potential diagnostic and prognostic biomarker for human glioma. Cell Oncol (Dordr) 41(1):25–33
121. Ricklefs FL, Alayo Q, Krenzlin H et al (2018) Immune evasion mediated by PD-L1 on glioblastoma-derived extracellular vesicles. Sci Adv 4(3):eaar2766
122. Tan SK, Pastori C, Penas C et al (2018) Serum long noncoding RNA HOTAIR as a novel diagnostic and prognostic biomarker in glioblastoma multiforme. Mol Cancer 17(1):74
123. Huang K, Fang C, Yi K et al (2018) The role of PTRF/Cavin1 as a biomarker in both glioma and serum exosomes. Theranostics 8(6):1540–1557
124. Manda SV, Kataria Y, Tatireddy BR et al (2018) Exosomes as a biomarker platform for detecting epidermal growth factor receptor-positive high-grade gliomas. J Neurosurg 128(4):1091–1101
125. Indira Chandran V, Welinder C, Mansson AS et al (2019) Ultrasensitive immunoprofiling of plasma extracellular vesicles identifies syndecan-1 as a potential tool for minimally invasive diagnosis of glioma. Clin Cancer Res. https://doi.org/10.1158/1078-0432.CCR-18-2946
126. Alvarez-Erviti L, Seow Y, Yin H et al (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 29(4):341–345
127. Lan F, Qing Q, Pan Q et al (2018) Serum exosomal miR-301a as a potential diagnostic and prognostic biomarker for human glioma. Cell Oncol 41(1):25–33
128. Komori T (2017) The 2016 WHO classification of tumours of the central nervous system: the major points of revision. Neurol Med Chir 57(7):301–311
129. Melki I, Tessandier N, Zufferey A et al (2017) Platelet microvesicles in health and disease. Platelets 28(3):214–221
130. Fraser K, Jo A, Giedt J et al (2018) Characterization of single microvesicles in plasma from glioblastoma patients. Neuro Oncol. https://doi.org/10.1093/neuonc/noy187
131. Roy S, Small J, Lansbury E et al (2018) Plasma-based detection of gliomas. J Extracell Vesicles 7:175–175
132. Konoshenko MY, Lekchnov EA, Vlassov AV et al (2018) Isolation of extracellular vesicles: general methodologies and latest trends. Biomed Res Int 2018:8545347
133. Yuana Y, Boing AN, Grootemaat AE et al (2015) Handling and storage of human body fluids for analysis of extracellular vesicles. J Extracell Vesicles 4:29260
134. Srinivasan S, Cheah P, Danielson K et al (2018) Systematic study of exRNA isolation reveals presence of distinct exRNA carriers. J Extracell Vesicles 7:122–123
135. Ibsen SD, Wright J, Lewis JM et al (2017) Rapid isolation and detection of exosomes and associated biomarkers from plasma. ACS Nano 11(7):6641–6651
136. Liang LG, Sheng YF, Zhou S et al (2017) An integrated double-filtration microfluidic device for detection of extracellular vesicles from urine for bladder cancer diagnosis. Methods Mol Biol 1660:355–364
137. Reategui E, van der Vos KE, Lai CP et al (2018) Engineered nanointerfaces for microfluidic isolation and molecular profiling of tumor-specific extracellular vesicles. Nat Commun 9(1):175