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REVIEW ARTICLE

Vesiclemia: counting on extracellular vesicles for glioblastoma patients

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Abstract

Although rare, glioblastoma is a devastating tumor of the central nervous system characterized by a poor survival and an extremely dark prognosis, making its diagnosis, treatment, and monitoring highly challenging. Numerous studies have highlighted extracellular vesicles (EVs) as key players of tumor growth, invasiveness, and resistance, as they carry oncogenic material. Moreover, EVs have been shown to communicate locally in a paracrine way but also at remote throughout the organism. Indeed, recent reports demonstrated the presence of brain tumor-derived EVs into body fluids such as plasma and cerebrospinal fluid. Fluid-associated EVs have indeed been suspected to reflect quantitative and qualitative information about the status and fate of the tumor and can potentially act as a resource for noninvasive biomarkers that might assist in diagnosis, treatment, and follow-up of glioblastoma patients. Here, we coined the name vesiclemia to define the concentration of plasmatic EVs, an intuitive term to be directly transposed in the clinical jargon.

Introduction

Glioblastoma is the most common primary malignant brain tumors in adults, accounting for ~12% of the central nervous system tumors, and the most aggressive one, making it a major therapeutic challenge. Occurring in 70% of the cases between 45 and 70 years old patients, prognosis remains extremely poor despite standardized, combative treatment. Indeed, tumor relapse is almost inevitable 7–10 months post therapy, while the median survival is estimated at 14 months and the 5-year survival rate is about 5% [1]. Few risk factors have been identified and they are mainly non modifiable, such as gender and rare congenital disorders, including Li–Fraumeni and Turcot syndromes, neurofibromatosis type 1–2 and Bourneville tuberous sclerosis [2]. Among possible environmental triggers, only ionizing radiations associate with an increased glioma incidence, especially in pediatric tumors. Other modifiers, such as electromagnetic waves, head trauma, pesticides, and nitrosamines are not established risk factors for glioblastoma [3]. This explains the ineffectiveness of lifestyle preventive measures, such as nutritional or physical activity recommendations, and the absence of personalized or public screen campaign in averting glioblastoma. Conversely, atopic manifestations, like asthma and eczema, have been noted as protective parameters against glioma development [4].

The actual first-line reference treatment has been established by Stupp et al. and combines resective surgery (if possible), followed by a 6-week adjuvant radiochemotherapy and a 6-month chemotherapy, both based on standardized doses of temozolomide (TMZ), an alkylating agent [1]. Despite this harsh therapeutic regime, glioblastoma recurrence is almost inevitable. In this context and given the high degree of vasculature proliferation, antiangiogenic therapies have brought hope for relapsing glioblastoma. This is illustrated by the largely spread use of bevacizumab (AVASTIN), a humanized monoclonal antibody targeting VEGF (vascular endothelial growth factor) also administrated in metastatic renal cell carcinoma. Indeed, this medication offers a 6-month progression-free survival in around 45% of recurrent glioblastoma patients, in comparison to 9–16% obtained in historical phase II studies [5]. Thus, bevacizumab monotherapy has been recommended for second-line treatment. Ever since, several phase II clinical trials using bevacizumab monotherapy

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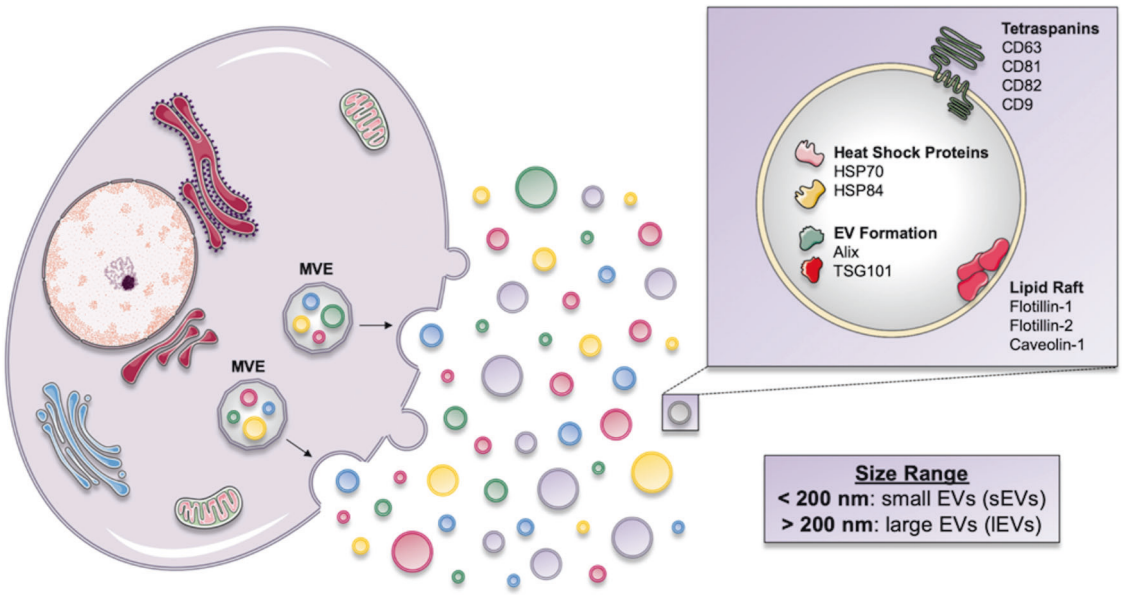

Fig. 1 Biogenesis, nomenclature, and specific markers of extracellular vesicles. Extracellular vesicles (EVs) originate from the budding of the membrane or travel from the intracellular pathway through the MVE (multivesicular endosome). They are heterogeneous in size and qualified as small when < 200 nm and large when > 200 nm. They express markers that are enriched depending on their production routes.

have confirmed its antineoplastic activity in naive recurrent glioblastoma at the expense, nevertheless, of major toxicities such as venous thromboembolism and cerebrovascular accident [5, 6]. Finally, the benefice of its association with other chemotherapeutic agents such as TMZ, lomustine, irinotecan, or etoposide has not been clearly demonstrated [5–8]. In this context of therapeutic impasse, new strategies are currently under evaluation and include chemotherapy sensitization by hyperbaric oxygen, radiotherapy innovations through hypofractionation and brachytherapy, and immunotherapies using immune checkpoint inhibitors, chimeric antigen receptor T cells, and brain tumor vaccines [9].

Glioblastoma progression relies on a harmonious choreography between intrinsic deviated signaling and external cues emanating from the tumor stroma and the stem cell niches [10, 11]. In this context, extracellular vesicles (EVs) had changed the paradigm of intercellular communication, in the immediate ecosystem and at distance.

Extracellular vesicles: linchpin tools for cells communication

Definition and nomenclature

Firstly visualized in 1985 using electron microscopy, EVs are selective fractions of cytosolic content, expelled in the extracellular medium and protected within a double lipid layer [12]. EVs are released virtually by all cells, either through membrane budding or multivesicular endosome (MVE) membrane fusion [13, 14] (Fig. 1). These biologic nanoparticles are highly heterogeneous in size and categorized into two main subtypes: small EVs (sEVs, size < 200 nm) and large EVs (lEVs, size > 200 nm) [15]. Hence, "exosome" and "microvesicle" terms that initially defined EVs according to their biogenesis pathway (i.e., exosomes forming from MVE and microvesicles resulting from membrane budding) have become obsolete and their use is no longer recommended [15]. In addition, if confirmation of EV characteristics cannot be achieved, the term "extracellular particle" is preferable. In agreement, concentration can be expressed as number of particles in a defined volume.

Molecular composition

EVs are nevertheless enriched with proteins reflecting their biogenesis (Fig. 1). These include tetraspanins, a wide family of transmembrane proteins regarded as early endosomes markers [16]. Indeed, CD9, CD63, CD81, and CD82 tetraspanins specifically marked EVs. Likewise, EVs gathered flotillin-1/2 and caveolin-1, both anchored to the inner lipid rafts, and involved in endocytosis and intracellular vesicular trafficking [17]. Cytosolic proteins implicated in MVE formation such as alix and TSG101 are also part of the EV protein signature [18]. Finally, heat shock protein (HSP) families HSP70 and HSP90 accumulated in EVs [18].

A major step toward EV characterization was the demonstration of the presence of both coding mRNAs and

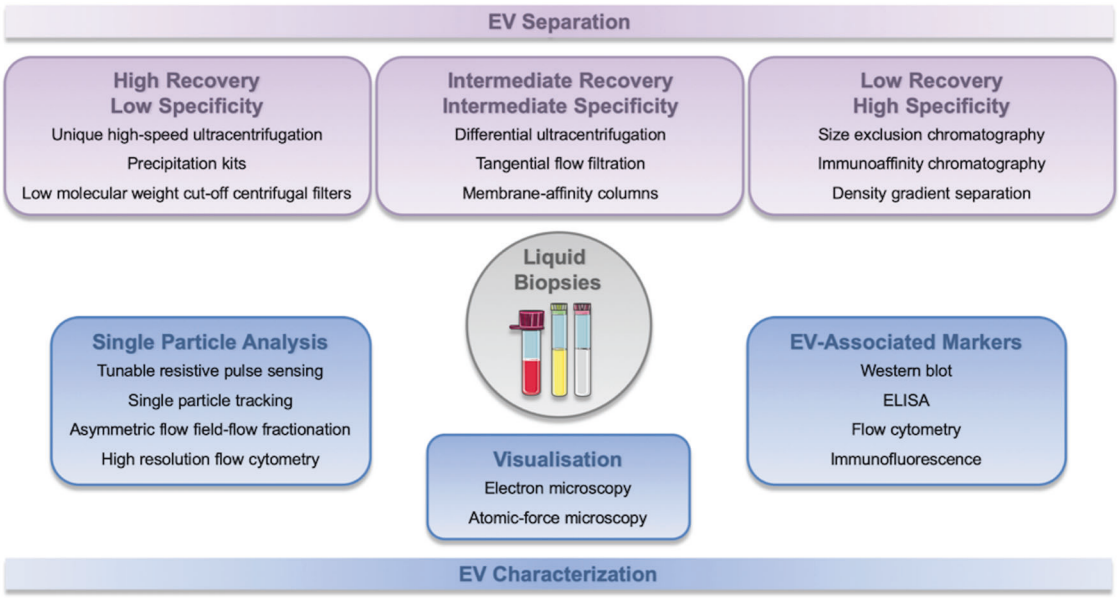

Fig. 2 Separation and characterization of extracellular vesicles. Methods to separate extracellular vesicles (EVs) are classified in three categories that discriminate the yield and purity: high recovery/low specificity; intermediate recovery/intermediate specificity; and low recovery/high specificity. Nanotechnologies are also implemented to characterize EVs based on their size and physical properties.

noncoding RNAs with an experimental potential to deliver their cargo to recipient cells [19, 20]. Indeed, EVs have been shown to incorporate miRNAs, whose profile was similar to the profile of the originating donor cells [19, 20]. Conversely, recent studies established that extracellular mRNAs are actually tethered mostly outside EVs [21–23]. In addition to miRNAs, EVs contain a large range of various noncoding RNAs such as tRNA or vault RNA [24]. On the other hand, lipidomic and glycomic analyses on EVs, especially sEVs, unveiled a selective enrichment in cholesterol, hexosylceramide, and sphingomyelin, while phosphatidylcholine was largely depleted, when compared to originating cell plasma membrane [25, 26]. Glycolipid GM3 and glycerophospholipids containing long, saturated fatty acylchains are equally enriched in EVs [25–27]. Interestingly, glycoconjugates are involved in EV biogenesis, in cellular recognition, and in the efficient uptake of EVs by recipient cells [28]. In this context, particle composition might vary, in quality and quantity, in the course of disease progression and/or in response to therapies and might inform on the tumor status.

Separation and characterization techniques

Because purity cannot be totally guaranteed with biological entities, the term “separation” is recommended over “isolation” and “purification” [15]. In 2015, differential ultracentrifugation was, by far, the most frequently used EV separation procedure [29]. Ever since, the panel of separation methods has become more diversified. This is illustrated by the growing use of various techniques such as size exclusion chromatography, density gradient, precipitation kits or affinity columns. In 2018, the International Society for Extracellular Vesicles has classified these methods according to their yield and degree of purity (Fig. 2). Nevertheless, one has to keep in mind that there is no optimal separation procedure instead chosen methods must satisfy recovery, specificity, and the subsequent use and analysis of the EV fractions. In keeping with this idea, four steps should be specified for each EV preparation and assessed through standardized procedures: preprocessing information, EV abundance, presence of EV-associated markers, and purity control. In an effort to facilitate the interpretation of such EV characterization, a knowledgebase (EV-TRACK) centralizing methodologies and experimental guidelines for EV study has been launched [30]. Therefore, methodologies have to be accurate and reproducible when quantitative and qualitative characterization of EVs ambitions to meet the standard of clinical use.

Extracellular vesicles in glioblastoma

Impact of EVs on glioblastoma progression

Numerous studies have unveiled the central role of EVs as key mediators of intercellular communication in the glioblastoma microenvironment. Indeed, the EV secretory pathway is suspected to be perverted by both tumor and stromal cells, and might distribute oncogenic material and

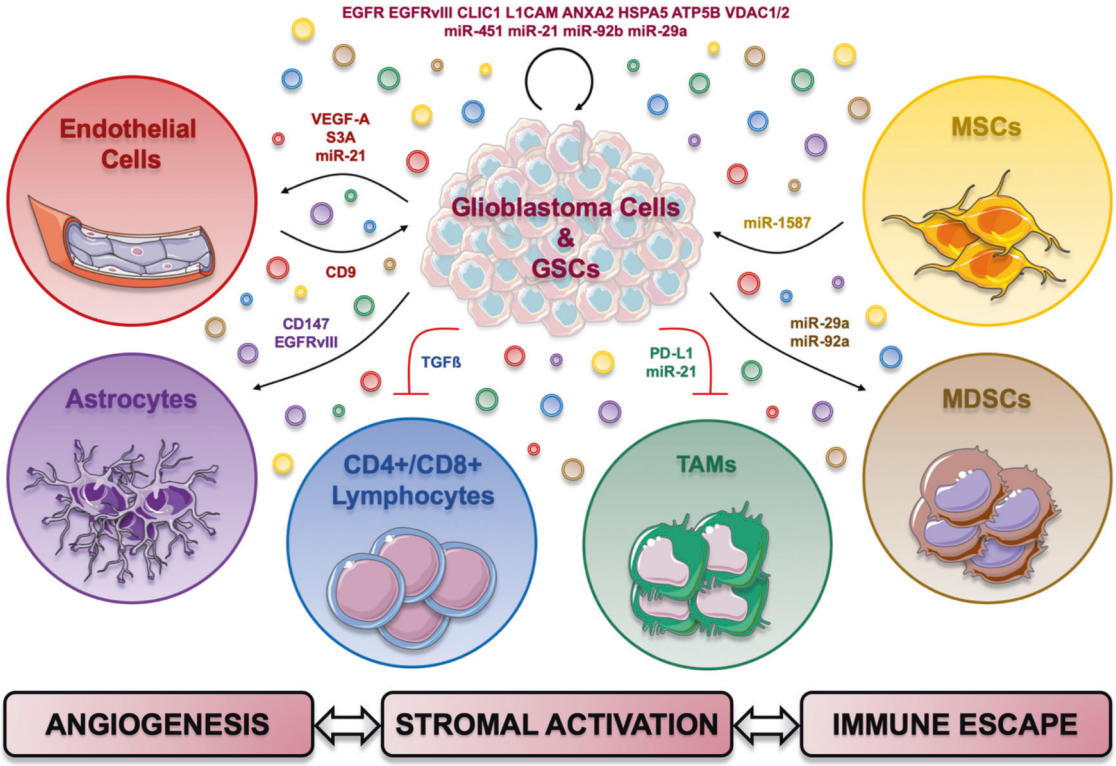

Fig. 3 Possible functions of extracellular vesicles within the glioblastoma microenvironment. Extracellular vesicles (EVs) are key mediators of intercellular communication in the glioblastoma ecosystem. Suspected to be hijacked by both tumor and stromal cells, EVs might carry tumorigenic material from and toward differentiated tumor cells, glioblastoma stem-like cells (GSCs), vascular endothelial cells, supportive and immune cells, thus promoting tumor growth, invasiveness, and survival.

nonphysiological information (Fig. 3). Thereby, EVs have been suggested to serve as major communication tools from and toward stem-like tumor cells, differentiated tumor cells, immune system, and vascular endothelial cells, to support tumor growth, invasiveness, and survival [31].

EVs from glioblastoma cells were shown to transport proto-oncogenes, such as EGFR (epidermal growth factor receptor) and its variant EGFRvIII. They can be further transferred to neighboring tumor cells and therefore sustain proliferation through the activation of intracellular kinases, such as the MAPK and PI3K/Akt pathways [32]. The immunoglobulin superfamily L1 cell adhesion molecule has also been detected in glioblastoma cell-derived EVs and impacts in turn tumor cell migration and invasiveness in vitro [33]. In addition, annexin A2 (ANXA2), a protein sustaining tumor migration, has been highlighted as one of the most abundant proteins in glioblastoma cell-derived EVs [34, 35]. From a metabolic standpoint, large EVs deriving from glioblastoma cells are enriched with HSPA5, a protein from the HSP chaperon family involved in glutamine homeostasis, a well-known mechanism of highly aggressive cancers such as pancreatic adenocarcinoma or melanoma [36]. Likewise, ATP5B, the beta-subunit of F1-ATP synthase accumulated in large EVs, suggesting that glioblastoma cells can transfer and stimulate oxidative pathway elements within recipient cells [37]. Under hypoxic conditions, glioblastoma EVs gather proteins notably involved in actin cytoskeleton and focal adhesion regulation, connoting that hypoxia might support an invasive phenotype in neighboring tumor cells through EV secretion [37].

Glioblastoma malignancy has been further demonstrated to be sustained through intercellular transfer of spliceosomal proteins incorporated in EVs [38]. Transcriptomic analyses have unveiled a wide panel of miRNAs carried by glioblastoma EVs that support proliferation and inhibit apoptosis in neighboring recipient tumor cells [31]. In addition to glioblastoma cells as a source for tumor EVs, endothelial cell-derived EVs sustain tumorigenicity through CD9 activation of the BMX/STAT3 signaling pathway, while proliferation is promoted by EV-harbored miR-1238 secreted by glioma-associated mesenchymal stem cells (MSCs) [39, 40].

One important step remains how host cells tethered and incorporated EVs. For instance, glycosylated EVs released by glioblastoma cells appear to be decorated by CCL18 (C-C motif chemokine ligand 18), promoting their cellular internalization via its cognate receptor CCR8 (C-C motif chemokine receptor 8) [41]. In addition, glioblastoma-derived EV surface-exposed glycans fine-tune receptor-

mediated targeting of dendritic cells, outlining the EV glycocalyx composition as a key factor of the cellular uptake [42].

In summary, EVs turned out to be valuable allies of glioblastoma cells by supporting their proliferation, invasion, and survival, as they broadcast oncogenic proteins and miRNAs. Therefore, EVs emerged as promising therapeutic targets to impair glioblastoma progression, although numerous EV-mediated interactions remain unknown.

Impact of EVs in the glioblastoma microenvironment

EVs emerged as communication mean with tumor-supportive action within the glioblastoma microenvironment (Fig. 3). Tumor EVs have been reported to contribute to tumor-induced angiogenesis by controlling multiple functions of vascular endothelial cells. Indeed, EVs transported pro-angiogenic sustainers including VEGF-A, operating on endothelial migration and sprouting [43–45]. In addition, EGFRvIII containing EVs exacerbate both transcription and release of VEGF by glioblastoma cells [20, 32]. Likewise, under hypoxic conditions, tumor cell-derived EVs are potent inducers of angiogenesis by modulating the phenotype of endothelial cells and pericytes, in human cells and mice models. Indeed, hypoxic EVs mediate paracrine activation of angiogenesis through the stimulation of several pro-angiogenic receptors, including EGFR and VEGFR2, to further promote endothelial cell migration and tube formation [46]. In keeping with this idea, endothelial cells conditioned with hypoxic tumor EVs enhance pericyte migration [46]. Furthermore, EVs isolated from the cerebrospinal fluid (CSF) of glioblastoma patients stimulate endothelial cell proliferation in vitro [46]. In addition, EVs ship molecules involved in vascular permeability increase by acting on the surrounding endothelial cells [43, 44].

Tumor-associated astrocytes also emerged as protagonists of glioblastoma growth notably through EV-mediated interactions with tumor cells. Indeed, EVs secreted by glioblastoma cells remodel normal human astrocytes (NHAs) to acquire an oncogenic phenotype, which might involve myc activation. In addition, EGFRvIII has been demonstrated to convert NHAs into glioblastoma cells in vitro, suggesting that glioblastoma EVs could be at the origin of astrocytes recruitment and oncogenic shift [47]. Moreover, NHAs challenged with glioblastoma EVs display enhanced migratory capacity, alongside with aberrant cytokine secretion, culminating in boosted growth, and invasion in tumor models. EV-treated NHAs also develop tumor-like signaling patterns that may drive astrocytes into tumor-supportive phenotype [48]. Corroborating this, EVs from glioblastoma cells are enriched with CD147, a glycoprotein assisting the release by astrocytes of metalloproteases involved in extracellular matrix remodeling and therefore facilitating tumor invasiveness [49].

In keeping with the idea of impaired immune response in glioblastoma, tumor cell-produced EVs might orchestrate this immunosuppressive atmosphere. Indeed, both tumor-associated macrophages (TAMs) and microglial cells have been shown to adopt an immunosuppressive phenotype when treated with glioblastoma EVs [50, 51]. Likewise, glioblastoma cell-derived EVs are enriched with PD-L1 (programmed death-ligand 1) that may also contribute to the elimination of adaptive immunity in the tumor mass [52]. Moreover, glioblastoma-derived EVs contain TGFß, which acts on cytotoxic lymphocytes to specifically annihilate the expression of granzyme A/B, perforin, as-L, and IFN-γ, while plasmatic EVs from patients with glioblastoma are enriched with cytokines driving pro-tumor T-cell phenotype [37]. In addition, glioblastoma EVs contribute to hinder the adaptive immune response through the activation of tumor-infiltrative myeloid-derived suppressor cells (MDSCs) inhibiting lymphocyte activity [53]. Furthermore, glioblastoma hypoxic EVs stimulate the proliferation of myeloid cells by transferring miR-29a and miR-92a, involved in cell cycle [54].

In summary, the heterogeneity of glioblastoma microenvironment arbitrates growth and survival. In this context, EVs represent essential, reciprocal communication tools between tumor and stromal cells by carrying tumor-supportive material.

EVs in therapeutic resistance

EVs have been reported to contribute to glioblastoma treatment failure by driving cells toward a resistant phenotype and perverting their environment. In this context, glioblastoma stem-like cells promote radiochemoresistance as these cells can cope with therapeutic insults, self-stimulate their proliferation, upregulate the synthesis of efflux transporters, and in turn repopulate the tumor mass [10, 47].

TMZ-resistant cells are suspected to confer drug resistant phenotype to chemosensitive neighboring cells through the secretion of EVs enriched with mRNAs encoding for MGMT (O-6-Methylguanine-DNA-methyltransferase) and APNG (Alkylpurine-DNA-N-glycosylase), two key enzymes of the DNA damage repair (DDR) machinery [55]. Interestingly, MGMT mRNA has also been detected in EVs deriving from tumor-associated astrocytes [56]. In keeping with this idea, tumor EVs have been demonstrated to carry drug efflux pumps, such as P-GP (permeability glycoprotein) and MRP1 (multidrug resistance associated protein 1), altering thereby TMZ efficacy when incorporated in recipient sensitive cells [57]. EVs emanating from resistant glioblastoma cells contain miR-1238 directly targeting

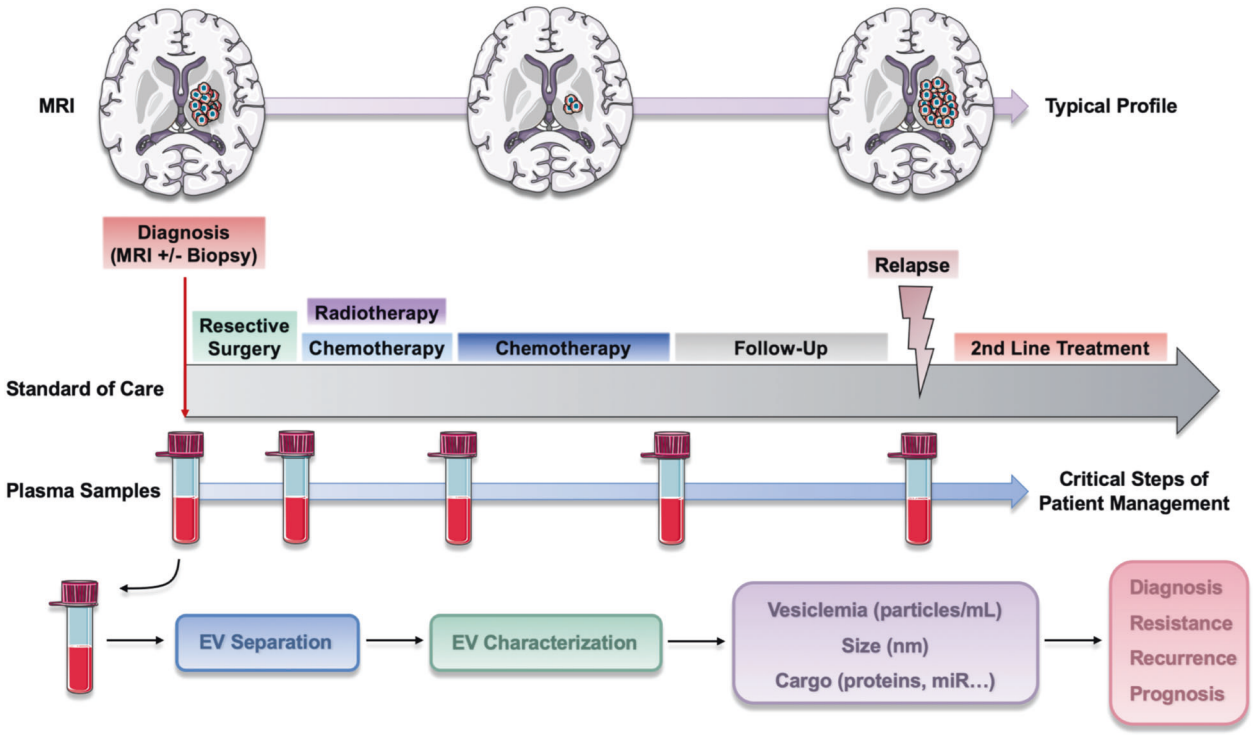

Fig. 4 Use of plasmatic extracellular vesicles in clinics. The standard-of-care for glioblastoma patients is represented in the upper panel, including magnetic resonance imagery (MRI), and therapeutic regime of surgery and radiochemotherapy. Plasma are collected at critical steps of patient management, stored in appropriate conditions and extracellular vesicles (EV) are thus separated, characterized, and qualitatively and quantitatively (vesiclemia) analyzed to serve as predictive biomarkers and/or companion tools for diagnosis, resistance, and recurrence.

caveolin, a tumor suppressor inhibiting EGFR autophosphorylation and its downstream signaling pathways [58, 59]. Likewise, EVs secreted by TMZ-sensitive cells are reported to sustain resistance, through miR-221, which targets in turn the pro-apoptotic protein PUMA (P53 upregulated modulator of apoptosis) [60, 61]. In addition, similar EVs have been demonstrated to contain high concentration of lncSBF2-AS1, a competing endogenous RNA of miR-151-3p implicated in the inhibition of the proto-oncogene XRCC4 (X-ray repair cross-complementing protein 4). Corroborating this observation, elevated levels of lncSBF2-AS1 in plasmatic EVs from glioblastoma patients were associated with a poor response to TMZ treatment [62]. On the other hand, tumor-derived EVs are also implicated in tumor escape to the antiangiogenic antibody bevacizumab. Indeed, bevacizumab can be internalized within the endosomal compartment of glioblastoma cells, further processed and recycled back at the surface of EVs, thereby suggesting that EVs might be served as bevacizumab "shedding tools" [63].

In addition to chemoresistance, EVs have also been reported to contribute to refractoriness to radiations. Hypoxic glioblastoma cells secrete EVs carrying miR-301a promoting radioresistance in sensitive cells by directly targeting TCEAL7 (transcription elongation factor A protein-like 7), an inhibitor of Wnt/ß-catenin signaling pathway highly involved in DDR [64]. Furthermore, tumor EVs are enriched with hypoxia-inducible factor α inducing radioresistance within recipient cells through the regulation of several factors implicated in angiogenesis and endothelial cell migration, such as VEGF.

Conversely, both radiations and TMZ have been highlighted for their major impact on the content of tumor-derived EVs. Indeed, TMZ-treated glioblastoma stem like cells (GSCs) were shown to release EVs enriched with protein cargos dedicated to cell adhesion [65]. Moreover, radiation-derived EVs have been demonstrated to acquire resistant/proliferative profile, as their cargos are enriched with oncogenic proteins involved in major intracellular pathways [66], but also pro-tumor noncoding RNAs such as miR-603 sustaining GSC stemness and upregulating DDR in recipient cells [67]. Thus, while relatively inefficient in killing GSCs in vitro, radiations and TMZ could additionally promote the dissemination of oncogenic information within the tumor microenvironment [65].

Together, these data suggest that EVs may execute radiochemoresistance and disseminate this pernicious phenotype within the tumor microenvironment. The characterization of EV profile throughout treatments could provide a promising mean to monitor therapeutic response in real time.

EVs as biomarkers of glioblastoma

In an effort to improve glioblastoma patient outcomes, several innovative strategies have been developed to optimize diagnosis and monitoring, among which is the analysis of EVs into liquid biopsies including plasma, CSF, and urine. Suspected to reflect unique information about glioblastoma expansion, circulating EVs are thought to constitute an important source of biomarkers that might help to refine diagnosis, treatment, and follow-up (Fig. 4). In this context, the term "vesiclemia" defines the concentration of EVs in the plasma, an intuitive name to be directly transposed to the clinical jargon. This biological parameter must be estimated through standardized procedures while meeting the current recommendations of quality control (*c.f.* 2.3) [15]. Vesiclemia might be considered rather as a relative, comparative, value, for which extracellular particles have been rigorously separated and measured following similar methodologies.

The vesiclemia has been demonstrated to be higher in patients with glioblastoma in comparison to healthy donors, patients with brain metastasis, and extra-axial brain tumors [65, 68]. Moreover, vesiclemia was found to decrease after resective surgery and to bounce back upon recurrence, connoting that EVs concentration may represent a new parameter for glioblastoma diagnosis and monitoring [68]. However, EVs from biological fluids constitute a heterogenous population of particles, suggesting that a more resolutive separation of the different EV subpopulations could ease their characterization and quantification, and further help the identification of specific biomarkers of cancer progression [69, 70]. In addition to fluctuations in vesiclemia, reciprocally, EV cargo may also represent on its own a platform for noninvasive biomarkers. Indeed, proteomic analysis of plasmatic EVs using sequential window acquisition of all theoretical fragment ion spectra mass spectrometry demonstrated that circulating EV protein profiles cluster according to both histological and molecular subtypes, while grouping with aggressiveness markers in patients with recurrent tumors [71]. In addition, plasmatic EVs containing EGFRvIII have been detected in around a third of glioblastoma patients (7/25) but not in any tested healthy donors [20]. Likewise, syndecan-1 (SDC1) has been identified as a plasmatic EV constituent whose expression might discriminate between glioblastoma and low-grade astrocytoma, with high sensitivity and specificity. Interestingly, the levels of SDC1 in plasmatic EVs were also correlated with SDC1 expression in matched patient tumors and decreased postoperatively depending on the extent of the surgery [72]. Furthermore, the levels of oncogenic miRNAs in plasmatic EVs were found upregulated in patients with glioblastoma, as compared to healthy donors, and were significantly reduced after tumor resection [73]. In keeping with this idea, a panel of plasmatic EV miRNAs (namely miR182-5p, miR-328-3p, miR-339-5p, miR-340-5p, miR-485-3p, miR-486-5p, and miR-543) have been proposed for noninvasive glioblastoma diagnosis. Indeed, six iterations of these miRNAs could distinguish glioblastoma patients from healthy subjects with an elevated accuracy rate [74]. Likewise, the amount of long noncoding RNA lncRNA HOTAIR (Hox transcript antisense intergenic RNA) is significantly elevated in plasmatic EVs from glioblastoma patients and correlated with the corresponding tumor HOTAIR levels [75]. In the same manner, oncogenic miRNA signature in CSF-borne EVs has been unmasked in patients with glioblastoma in comparison to the tumor-free group [76, 77]. Moreover, IDH-1 mutant (IDH1-R132H) transcripts have been detected in EVs isolated from the CSF of IDH-1 mutant glioblastoma patients, thereby suggesting that circulating EVs may also assist in the molecular classification.

In addition of tumor diagnosis, fluid-associated EVs are thought to represent prognosis and predictive biomarkers. The presence of EGFRvIII in plasmatic EVs correlated with a lower overall survival (21 months), as compared to patients with no detectable EGFRvIII (28 months) [78]. Moreover, the concentration of plasmatic EV-harbored annexin V increased upon TMZ chemotherapy, and corroborated with early tumor recurrence and poor survival rate [79]. Plasmatic EVs have been further demonstrated enriched with miR-301a upon relapse [80]. Likewise, the pattern of expression of miRNAs in CSF-derived EVs correlated with tumor recurrence and even radiochemotherapy failure [76, 77].

Concluding remarks

Fluid-associated and circulating EVs embody a valuable source of information and biological materials from evolving tumor and may be exploited for diagnosis and theranostic purposes in glioblastoma. However, most of the studies exploring plasmatic EVs rely on rather small number of patients, often missing longitudinal samples, making thus larger cohorts required to strengthen outcomes. In addition, global EV composition of the body fluids may differ due to numerous, external non-tumor factors such as alimentation, physical activity, pathological disorders, and medication. Thereby, standardized protocols and procedure for sampling, storage, and analysis must be developed, with the aim of achieving reproducibility and robustness compatible with clinical routine. The longitudinal analysis of both vesiclemia and plasmatic EV cargo might emerge as promising companion tests for the monitoring of glioblastoma patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352:987–96.
2. Vijapura C, Saad Aldin E, Capizzano AA, Policeni B, Sato Y, Moritani T. Genetic syndromes associated with central nervous system tumors. Radiographics. 2017;37:258–80.
3. Bondy ML, Scheurer ME, Malmer B, Barnholtz-Sloan JS, Davis FG, Il'yasova D, et al. Brain tumor epidemiology: consensus from the brain tumor epidemiology consortium. Cancer. 2008;113:1953–68.
4. Linos E, Raine T, Alonso A, Michaud D. Atopy and risk of brain tumors: a meta-analysis. J Natl Cancer Inst. 2007;99:1544–50.
5. Lu-Emerson C, Duda DG, Emblem KE, Taylor JW, Gerstner ER, Loeffler JS, et al. Lessons from anti-vascular endothelial growth factor and anti-vascular endothelial growth factor receptor trials in patients with glioblastoma. J Clin Oncol. 2015;33:1197–213.
6. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med. 2014;370:709–22.
7. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol. 2009;27:4733–40.
8. Ghiaseddin A, Peters KB. Use of bevacizumab in recurrent glioblastoma. CNS Oncol. 2015;4:157–69.
9. Jain KK. A critical overview of targeted therapies for glioblastoma. Front Oncol. 2018;8:419.
10. Lathia JD, Mack SC, Mulkearns-Hubert EE, Valentim CLL, Rich JN. Cancer stem cells in glioblastoma. Genes Dev. 2015;29:1203–17.
11. Gimple RC, Bhargava S, Dixit D, Rich JN. Glioblastoma stem cells: lessons from the tumor hierarchy in a lethal cancer. Genes Dev. 2019;33:591–609.
12. Pan BT, Teng K, Wu C, Adam M, Johnstone RM. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. J Cell Biol. 1985;101:942–8.
13. Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. J Neurooncol. 2013;113:1–11.
14. Gandham S, Su X, Wood J, Nocera AL, Alli SC, Milane L, et al. Technologies and standardization in research on extracellular vesicles. Trends Biotechnol. 2020. https://doi.org/10.1016/j.tibtech.2020.05.012.
15. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. 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. 2018;7:1535750.
16. Escola J-M, Kleijmeer MJ, Stoorvogel W, Griffith JM, Yoshie O, Geuze HJ. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. J Biol Chem. 1998;273:20121–7.
17. Wubbolts R, Leckie RS, Veenhuizen PTM, Schwarzmann G, Möbius W, Hoernschemeyer J, et al. Proteomic and biochemical analyses of human B cell-derived exosomes: potential implications for their function and multivesicular body formatION. J Biol Chem. 2003;278:10963–72.
18. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018;19:213–28.
19. Nolte-'t Hoen ENM, Buermans HPJ, Waasdorp M, Stoorvogel W, Wauben MHM, 't Hoen PAC. Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of small non-coding RNA biotypes with potential regulatory functions. Nucleic Acids Res. 2012;40:9272–85.
20. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Curry WT, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol. 2008;10:1470–6.
21. Weaver AM, Patton JG. Argonautes in extracellular vesicles: artifact or selected cargo? Cancer Res. 2020;80:379–81.
22. Van Deun J, Mestdagh P, Sormunen R, Cocquyt V, Vermaelen K, Vandesompele J, et al. The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling. J Extracell Vesicles. 2014;3:24858.
23. Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, Zimmerman LJ, et al. Reassessment of exosome composition. Cell. 2019;177:428–45.e18.
24. Bellingham SA, Coleman BM, Hill AF. Small RNA deep sequencing reveals a distinct miRNA signature released in exosomes from prion-infected neuronal cells. Nucleic Acids Res. 2012;40:10937–49.
25. Laulagnier K, Motta C, Hamdi S, Roy S, Fauvelle F, Pageaux J-F, et al. Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. Biochem J. 2004;380:161–71.
26. Subra C, Laulagnier K, Perret B, Record M. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. Biochimie. 2007;89:205–12.
27. Skotland T, Sagini K, Sandvig K, Llorente A. An emerging focus on lipids in extracellular vesicles. Adv Drug Deliv Rev. 2020; S0169-409X:30014–4.
28. Williams C, Royo F, Aizpurua-Olaizola O, Pazos R, Boons G-J, Reichardt N-C, et al. Glycosylation of extracellular vesicles: current knowledge, tools and clinical perspectives. J Extracell Vesicles. 2018;7:1442985.
29. Gardiner C, Vizio DD, Sahoo S, Théry C, Witwer KW, Wauben M, et al. Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey. J Extracell Vesicles. 2016;5:32945.
30. EV-TRACK Consortium, Van Deun J, Mestdagh P, Agostinis P, Akay Ö, Anand S, et al. EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. Nat Methods. 2017;14:228–32.
31. André-Grégoire G, Gavard J. Spitting out the demons: extracellular vesicles in glioblastoma. Cell Adhes Migr. 2017;11:164–72.

32. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, et al. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol. 2008;10:619–24.
33. Pace KR, Dutt R, Galileo DS. Exosomal L1CAM stimulates glioblastoma cell motility, proliferation, and invasiveness. Int J Mol Sci. 2019;20:3982.
34. An JH, Lee SY, Jeon JY, Cho KG, Kim SU, Lee MA. Identification of gliotropic factors that induce human stem cell migration to malignant tumor. J Proteome Res. 2009;8:2873–81.
35. Bronisz A, Wang Y, Nowicki MO, Peruzzi P, Ansari KI, Ogawa D, et al. Extracellular vesicles modulate the glioblastoma microenvironment via a tumor suppression signaling network directed by miR-1. Cancer Res. 2014;74:738–50.
36. Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature. 2013;496:101–5.
37. Yekula A, Minciacchi VR, Morello M, Shao H, Park Y, Zhang X, et al. Large and small extracellular vesicles released by glioma cells in vitro and in vivo. J Extracell Vesicles. 2020;9:1689784.
38. Pavlyukov MS, Yu H, Bastola S, Minata M, Shender VO, Lee Y, et al. Apoptotic cell-derived extracellular vesicles promote malignancy of glioblastoma via intercellular transfer of splicing factors. Cancer Cell. 2018;34:119–35.e10.
39. Figueroa J, Phillips LM, Shahar T, Hossain A, Gumin J, Kim H, et al. Exosomes from glioma-associated mesenchymal stem cells increase the tumorigenicity of glioma stem-like cells via transfer of miR-1587. Cancer Res. 2017;77:5808–19.
40. Li D, Tian Y, Hu Y, Qi Y, Tian N, Li S, et al. Glioma-associated human endothelial cell-derived extracellular vesicles specifically promote the tumourigenicity of glioma stem cells via CD9. Oncogene. 2019;38:6898–912.
41. Berenguer J, Lagerweij T, Zhao XW, Dusoswa S, van der Stoop P, Westerman B, et al. Glycosylated extracellular vesicles released by glioblastoma cells are decorated by CCL18 allowing for cellular uptake via chemokine receptor CCR8. J Extracell Vesicles. 2018;7:1446660.
42. Dusoswa SA, Horrevorts SK, Ambrosini M, Kalay H, Paauw NJ, Nieuwland R, et al. Glycan modification of glioblastoma-derived extracellular vesicles enhances receptor-mediated targeting of dendritic cells. J Extracell Vesicles. 2019;8:1648995.
43. Treps L, Edmond S, Harford-Wright E, Galan-Moya EM, Schmitt A, Azzi S, et al. Extracellular vesicle-transported Semaphorin3A promotes vascular permeability in glioblastoma. Oncogene. 2016;35:2615–23.
44. Treps L, Perret R, Edmond S, Ricard D, Gavard J. Glioblastoma stem-like cells secrete the pro-angiogenic VEGF-A factor in extracellular vesicles. J Extracell Vesicles. 2017;6:1359479.
45. Lucero R, Zappulli V, Sammarco A, Murillo OD, Cheah PS, Srinivasan S, et al. Glioma-derived miRNA-containing extracellular vesicles induce angiogenesis by reprogramming brain endothelial cells. Cell Rep. 2020;30:2065–74.e4.
46. Kucharzewska P, Christianson HC, Welch JE, Svensson KJ, Fredlund E, Ringner M, et al. Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development. Proc Natl Acad Sci USA. 2013;110:7312–7.
47. Hallal S, Mallawaaratchy DM, Wei H, Ebrahimkhani S, Stringer BW, Day BW, et al. Extracellular vesicles released by glioblastoma cells stimulate normal astrocytes to acquire a tumor-supportive phenotype via p53 and MYC signaling pathways. Mol Neurobiol. 2019;56:4566–81.
48. Oushy S, Hellwinkel JE, Wang M, Nguyen GJ, Gunaydin D, Harland TA, et al. Glioblastoma multiforme-derived extracellular vesicles drive normal astrocytes towards a tumour-enhancing phenotype. Philos Trans R Soc B. 2018;373:20160477.
49. Colangelo NW, Azzam EI. Extracellular vesicles originating from glioblastoma cells increase metalloproteinase release by astrocytes: the role of CD147 (EMMPRIN) and ionizing radiation. Cell Commun Signal. 2020;18:21.
50. de Vrij J, Maas SLN, Kwappenberg KMC, Schnoor R, Kleijn A, Dekker L, et al. Glioblastoma-derived extracellular vesicles modify the phenotype of monocytic cells: glioblastoma vesicles affect monocytic cells. Int J Cancer. 2015;137:1630–42.
51. van der Vos KE, Abels ER, Zhang X, Lai C, Carrizosa E, Oakley D, et al. Directly visualized glioblastoma-derived extracellular vesicles transfer RNA to microglia/macrophages in the brain. Neuro Oncol. 2016;18:58–69.
52. Himes BT, Peterson TE, de Mooij T, Garcia LMC, Jung M-Y, Uhm S, et al. The role of extracellular vesicles and PD-L1 in glioblastoma-mediated immunosuppressive monocyte induction. Neuro Oncol. 2020;22:967–78.
53. Domenis R, Cesselli D, Toffoletto B, Bourkoula E, Caponnetto F, Manini I, et al. Systemic T cells immunosuppression of glioma stem cell-derived exosomes is mediated by monocytic myeloid-derived suppressor cells. PLoS ONE. 2017;12:e0169932.
54. Guo X, Qiu W, Wang J, Liu Q, Qian M, Wang S, et al. Glioma exosomes mediate the expansion and function of myeloid-derived suppressor cells through microRNA-29a/*Hbp1* and microRNA-92a/*Prkar1a* pathways. Int J Cancer. 2019;144:3111–26.
55. Shao H, Chung J, Lee K, Balaj L, Min C, Carter BS, et al. Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma. Nat Commun. 2015;6:6999.
56. Yu T, Wang X, Zhi T, Zhang J, Wang Y, Nie E, et al. Delivery of MGMT mRNA to glioma cells by reactive astrocyte-derived exosomes confers a temozolomide resistance phenotype. Cancer Lett. 2018;433:210–20.
57. Bebawy M, Combes V, Lee E, Jaiswal R, Gong J, Bonhoure A, et al. Membrane microparticles mediate transfer of P-glycoprotein to drug sensitive cancer cells. Leukemia. 2009;23:1643–9.
58. Yin J, Zeng A, Zhang Z, Shi Z, Yan W, You Y. Exosomal transfer of miR-1238 contributes to temozolomide-resistance in glioblastoma. EBioMedicine. 2019;42:238–51.
59. Cosset EC, Godet J, Entz-Werlé N, Guérin E, Guenot D, Froelich S, et al. Involvement of the TGFβ pathway in the regulation of α5β1 integrins by caveolin-1 in human glioblastoma. Int J Cancer. 2012;131:601–11.
60. Yang J-K, Yang J-P, Tong J, Jing S-Y, Fan B, Wang F, et al. Exosomal miR-221 targets DNM3 to induce tumor progression and temozolomide resistance in glioma. J Neurooncol. 2017;131:255–65.
61. Zhang C-Z, Zhang J-X, Zhang A-L, Shi Z-D, Han L, Jia Z-F, et al. MiR-221 and miR-222 target PUMA to induce cell survival in glioblastoma. Mol Cancer. 2010;9:229.
62. Zhang Z, Yin J, Lu C, Wei Y, Zeng A, You Y. Exosomal transfer of long non-coding RNA SBF2-AS1 enhances chemoresistance to temozolomide in glioblastoma. J Exp Clin Cancer Res. 2019;38:166.
63. Simon T, Pinioti S, Schellenberger P, Rajeeve V, Wendler F, Cutillas PR, et al. Shedding of bevacizumab in tumour cells-derived extracellular vesicles as a new therapeutic escape mechanism in glioblastoma. Mol Cancer. 2018;17:132.
64. Yue X, Lan F, Xia T. Hypoxic glioma cell-secreted exosomal miR-301a activates Wnt/β-catenin signaling and promotes radiation resistance by targeting TCEAL7. Mol Ther. 2019;27:1939–49.
65. Andre-Gregoire G, Bidere N, Gavard J. Temozolomide affects extracellular vesicles released by glioblastoma cells. Biochimie. 2018;155:11–5.
66. Garnier D, Meehan B, Kislinger T, Daniel P, Sinha A, Abdulkarim B, et al. Divergent evolution of temozolomide resistance in glioblastoma stem cells is reflected in extracellular vesicles and coupled with radiosensitization. Neuro Oncol. 2018;20:236–48.

67. Ramakrishnan V, Xu B, Akers J, Nguyen T, Ma J, Dhawan S, et al. Radiation-induced extracellular vesicle (EV) release of miR-603 promotes IGF1-mediated stem cell state in glioblastomas. EBioMedicine. 2020;55:102736.
68. Osti D, Del Bene M, Rappa G, Santos M, Matafora V, Richichi C, et al. Clinical significance of extracellular vesicles in plasma from glioblastoma patients. Clin Cancer Res. 2019;25:266–76.
69. Zhang H, Lyden D. Asymmetric-flow field-flow fractionation technology for exomere and small extracellular vesicle separation and characterization. Nat Protoc. 2019;14:1027–53.
70. Zhang H, Freitas D, Kim HS, Fabijanic K, Li Z, Chen H, et al. Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation. Nat Cell Biol. 2018;20:332–43.
71. Hallal S, Azimi A, Wei H, Ho N, Lee MYT, Sim H-W. A comprehensive proteomic SWATH-MS workflow for profiling blood extracellular vesicles: a new avenue for glioma tumour surveillance. Int J Mol Sci. 2020;21:4754
72. Indira Chandran V, Welinder C, Månsson A-S, Offer S, Freyhult E, Pernemalm M, et al. Ultrasensitive immunoprofiling of plasma extracellular vesicles identifies Syndecan-1 as a potential tool for minimally invasive diagnosis of glioma. Clin Cancer Res. 2019;25:3115–27.
73. Godlewski J, Ferrer-Luna R, Rooj AK, Mineo M, Ricklefs F, Takeda YS, et al. MicroRNA signatures and molecular subtypes of glioblastoma: the role of extracellular transfer. Stem Cell Rep. 2017;8:1497–505.
74. Ebrahimkhani S, Vafaee F, Hallal S, Wei H, Lee MYT, Young PE, et al. Deep sequencing of circulating exosomal microRNA allows non-invasive glioblastoma diagnosis. npj Precis Oncol. 2018;2:28.
75. Tan SK, Pastori C, Penas C, Komotar RJ, Ivan ME, Wahlestedt C, et al. Serum long noncoding RNA HOTAIR as a novel diagnostic and prognostic biomarker in glioblastoma multiforme. Mol Cancer. 2018;17:74.
76. Figueroa JM, Skog J, Akers J, Li H, Komotar R, Jensen R, et al. Detection of wild-type EGFR amplification and EGFRvIII mutation in CSF-derived extracellular vesicles of glioblastoma patients. Neuro Oncol. 2017;19:1494–502.
77. Saugstad JA, Lusardi TA, Van Keuren-Jensen KR, Phillips JI, Lind B, Harrington CA, et al. Analysis of extracellular RNA in cerebrospinal fluid. J Extracell Vesicles. 2017;6:1317577.
78. Manda SV, Kataria Y, Tatireddy BR, Ramakrishnan B, Ratnam BG, Lath R, et al. Exosomes as a biomarker platform for detecting epidermal growth factor receptor–positive high-grade gliomas. J Neurosurg. 2018;128:1091–101.
79. Evans SM, Putt M, Yang X-Y, Lustig RA, Martinez-Lage M, Williams D, et al. Initial evidence that blood-borne microvesicles are biomarkers for recurrence and survival in newly diagnosed glioblastoma patients. J Neurooncol. 2016;127:391–400.
80. Lan F, Qing Q, Pan Q, Hu M, Yu H, Yue X. Serum exosomal miR-301a as a potential diagnostic and prognostic biomarker for human glioma. Cell Oncol. 2018;41:25–33.