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Research Article

Microvesicles Secreted by Glioblastoma Multiforme DBTRG-05MG Tumor Cell Line Contain Proteins Involved in Tumor Invasion, Stemness and Immunosuppression.

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Abstract

Microvesicles or ectosomes are heterogeneous extracellular vesicles ranging in size from 100-1000 nm in diameter. Research suggests that tumor associated microvesicles have a role in immune suppression, multi-drug resistance, invasion, metastasis and angiogenesis via intercellular communication and re-programming. This research study analyzed microvesicles secreted from serum-starved glioblastoma multiforme, the most common brain tumor, noted for having the worst five-year survival rate of any glioma (5%). Live cell imaging studies have shown that microtumor spheroid formation *in vitro* involves microvesicles that mediate cell-to-cell associations to produce aggregates of cells that coalesce to microtumors, an activity that is consistently observed also in organoid cultures of patient tumors. To study further their role in malignant progression, microvesicles were harvested from the culture medium of serum-starved glioblastoma cell line DBTRG-05MG by ultracentrifugation. Transmission electron microscopy showed that microvesicles comprised the largest fraction of extracellular vesicles, based on measurements of average diameter. Western blot analysis showed that proteins involved in tumor spread, immune system resistance, and early stage neural development were present in microvesicles secreted by the tumor cells. Among these, beta-tubulin III, neuroligin III, and integrin alpha-V were present at levels roughly comparable to glioblastoma whole cell lysates. CD63, CD 47, flotillin-2 and pan-cadherin were also detected in the microvesicle preparations. Notably absent were beta-actin, tsg101, epidermal growth factor receptor and HCAM proteins. A model is proposed, "insertional to mediate tumor invasion and spread.

Key Words: microvesicles, glioblastoma, neuroligin-3, integrin-alpha V, beta-tubulin III, insertional membrane editing

Introduction

According to the World Health Organization (WHO), Astrocytoma Grade IV, otherwise known as Glioblastoma Multiforme (GBM), is the most commonly diagnosed adult malignant primary brain tumor, with a 5-year survival rate of only 5.5% [1] and a median survival time estimated between 12 to 14 months from the time of diagnosis [2]. Gliomas are glial-precursor or glial cell-derived tumors of the brain, comprised of four main types that include astrocytomas (including GBM), oligodendrogliomas, ependymomas and oligoastrocytomas (mixed gliomas), all of which predominantly arise in white matter [3, 4]. Computerized tomography (CT) and magnetic resonance imaging (MRI) are currently the primary diagnostic tools for GBM [5], which is usually detected at advanced stages of disease when surgery, chemotherapy and radiotherapy are unlikely to result in complete disease remission.

Research suggests that tumor-secreted membrane-bound vesicles budded from the plasma membrane, termed extracellular vesicles (EVs) may potentially serve as biomarkers and/or therapeutic vectors for drug delivery [6] to provide a noninvasive approach to both the diagnosis and treatment of GBM that might improve GBM survival rates. Most, if not all, cells shed EVs into the extracellular environment. EVs are typically divided into three categories based on their size, content and biogenesis [7]. These subsets of EVs include exosomes (EXO), ectosomes or microvesicles (MV), and apoptotic blebs or apoptotic bodies (AB). MVs are large, heterogeneous membrane-enclosed EVs ranging in size from 100-1000 nm in diameter that comprise the bulk of intermediate size-range EVs [8]. Their secretion is the result of a regulated redistribution of membrane lipids followed by the outward budding and fission of the plasma membrane. [9].

Each of the three vesicle types has been shown to communicate intercellularly in various physiological and pathological conditions [10, 11]. Of these, exosomes have been the most studied, while MVs the least. EVs are encased by a lipid bilayer membrane that contains distinct internal cargo, specific to each vesicle type. MVs have a larger diameter than exosomes with a heterogeneous size range from 100-1000 nm in diameter [12]. The third major category of EVs is apoptotic bodies that are released during apoptosis or programmed cell death with diameters ranging from 50-4000nm [12].

The ability of secreted EVs to interact with and transport signaling molecules to other cells and regions of the body presents a new paradigm in our understanding of biological processes in health and disease. To this end, research suggests that EVs are an important mechanistic form of intercellular communication [13]. EVs have been shown to shuttle biological molecules including proteins, lipids, RNA, DNA and metabolites to neighboring cells under normal physiological and pathological conditions [14, 15, 16]. Research on tumor associated

microvesicles (TAMVs) suggests that they may play a role in immune suppression, multi-drug resistance, invasion, metastasis, and angiogenesis via intercellular communication and reprogramming [17].

Previous research from our laboratory provided evidence that tumor associated MVs may play an important role in cell-to-cell associations that mediate micro-tumor spheroid formation, an activity that is consistently displayed by tumor cells, both *in vitro* and in organoid cultures of patient tumors [18]. Live cell imaging studies of glioblastoma microtumor formation *in vitro* led us to propose that the process of tumor spheroid formation *in vitro* is characterized by MV mediated cell-to-cell associations leading to spheroid aggregation.
This current study involved the isolation and purification of EVs from serum starved monolayers of the GBM cell line DBTRG-05MG. Electron micrographs of EVs isolated using this production/recovery protocol showed that the isolated the EV fraction is largely comprised of MVs. Western Blot (WB) analysis of MV protein content by immunolabeling demonstrated that MVs produced by this GBM cell line express proteins involved in extracellular matrix (ECM) remodeling, migration, immunosuppression and early stage neurodevelopment. The significance of these observations will be discussed.

Materials and Methods

Cells and culture conditions

The DBTRG-05MG cell line was obtained from the American Type Culture Collection (ATCC) [catalog number CRL-2020] and cultured in (RPMI) 1640 medium (Thermo Fisher Scientific) (1g/L Glucose and 25mM HEPES) supplemented with 5mL 100x Penicillin/Streptomycin (PenStrep) (Fisher Scientific), 5mL 100x Adenine (Sigma), 5mL 100x Hypoxanthine (Sigma), 500uL 1000x Thymidine (Sigma), 5mL 100mM (11mg/ml) sodium pyruvate (Fisher Scientific), 1.25g Glucose (Sigma), 1g sodium bicarbonate and 10% fetalgro® Bovine Growth Serum (BGS) Rocky Mountain Biologicals (RMBIO) at 37^0C in 5% CO_2 . To stimulate EV production, culture medium was replaced by serum-free RPMI medium in confluent cultures for 48-72 hours prior to harvesting (see below).

MV Isolation

DBTRG-05MG cell monolayers were grown to approximately 70-80% confluence in NEST™ 175cm2 cell-culture flasks in standard culture medium (see Cells and Culture Conditions), then washed twice in phosphate buffered saline (PBS) to remove any residual culture medium. Serum-free RPMI 1640 medium, devoid of serum or protein additives (i.e. bovine serum albumin (BSA) or fetal bovine serum (FBS)), was then added to each flask to a volume of 20mL to stimulate EV release. A single EV prep consisted of 10-15 T175 confluent flasks incubated 48-72 hours to yield maximum EV recovery from the serum starved cells. Cell culture medium (containing EVs) was collected into 50mL conical tubes, placed on ice and centrifuged in a Damon IEC Division CRU-5000 centrifuge using a 6/81 fixed angled rotor at 4C at 500g for 20-25 min to pellet cell floaters. The supernatant was transferred into new 50mL conical tubes on ice. A second centrifugation was carried out at 1500g for 20-25min to pellet any remaining cell debris. Following the second spin, the supernatant was centrifuged in a Beckman Coulter Type 45Ti ultracentrifuge fixed rotor/Beckman Coulter L-80 ultracentrifuge at 21,130g or 13,500 RPM at 4C for 4hrs. The supernatant was discarded leaving only 0.5-1ml remaining. The collected microvesicle suspensions were aliquoted into 1.5mL epitubes and centrifuged at 17,000g at 4C in a tabletop accuSpin Micro 17 (Fisher Scientific) microcentrifuge for 90 minutes to pellet the microvesicle suspensions. 500ul of PBS was added to resuspend and wash the pellets which were then spun again at 17,000g for 60 min at 4C. The supernatant was carefully removed and the EV pellet was resuspended in 50-100uL of PBS or 1X RIPA buffer for further analysis. After media containing EVs were harvested, monolayer cultures were processed to assay cell viability by standard trypan blue staining protocol. Viable cell counts were performed using a hemocytometer.

Transmission Electron Microscopy

To assess vesicle size distribution and morphology, a modified version of transmission electron microscopy (TEM) sample preparation was used [19, 20]. EVs were resuspended in PBS, then mixed 1:1 with 4% paraformaldehyde BBP to dilute the solution to 2%. The EV solution was incubated for one hour on ice before further processing. A carbon coated formvar coated 200-mesh grid was placed onto the edge of double-sided tape attached to the bottom section of a petri dish and 5uL of the fixed microvesicle solution was added to the grid for 20 min. 0.8mm thick cellulose filter paper wedges were used to absorb liquid from the grids. 5uL of sterile deionized distilled water was then added for 30-40 seconds then absorbed to wash and prevent drying of grid. Immediately thereafter, 5uL of 2% uranyl acetate was added and allowed to sit for 30-40 seconds and then removed. All images were captured using a Tecnai Spirit G2 transmission electron microscope (FEI company) at 100kV. The (Gatan Spirit) camera was used to obtain close-up images and for wide field images the (Gatan First-light) camera was used.

SDS-PAGE/Immunoblotting

Prior to loading Novex™ Value 10% Tris-Glycine Gel #XV00100PK20 (Invitrogen), proteins were mixed in a 3:1 ratio with 4X protein sample buffer and then incubated at 95C for five minutes following standard protocol. Approximately ~2-5µg of MV lysate and ~23µg of WCL were added to each well and electrophoresed under standard conditions. Western blots were probed with Actin (C4) SC-47778 SCBT 200µg/ml (1: 5000), CD47 (B6H12) SC-12730 SCBT 200µg/ml (1:500), EGFR (A-10) SC-373746 SCBT 200µg/ml (1:500), CD63 (MX-49.129.5) SC-5275 SCBT 200µg/ml (1:500), Flotillin-2 (B-6) SC-28320 SCBT 200µg/ml (1:500), HCAM (F-4) SC-9960 SCBT 200µg/ml (1:500), Pan-Cadherin #4068 CST 20µL (1:1000), tsg101 (C-2) SC-7964 SCBT 200µg/ml (1:500), and Integrin □V (Q20)-R SC-6617-R 200µg/ml (1:600). Secondary antibodies used were Donkey anti-mouse IgG horseradish peroxidase (HRP) NB120-6820 (1:15000), and Donkey anti-rabbit HRP NB7185. SuperSignal® West Fempto Dura kit (Thermo Scientific) was used to visualize the bands using a Bio-Rad CHEMI DOC XRS gel imager using (Quantity One) Version 4.6.6 Basic software.

Statistical Analysis

Measurements were exported to the R statistics generator (BoxPlotR) for analysis of cell viability under varying conditions. Individual experiments were conducted in duplicate, triplicate, or quadruplet when necessary. In the cell viability assessments, three-quadruplicate and three-duplicate experiments were conducted to generate data. Averages, standard deviations, and standard error for each experimental condition were determined using excel software. Particle size distributions were measured in ImageJ and exported to excel for further analysis.

Results

Isolation and purification of extracellular vesicles from serum-starved DBTRG-05MG glioblastoma monolayers

Monolayer cultures of DBTRG-05MG GBM cell line at approximately 75% confluence were incubated in serum-free RPMI for 48-72 hours to stimulate EV production, followed by differential ultracentrifugation to pellet cell fraction enriched with (MVs) (see **Materials and Methods**). Trypan blue viability assays of monolayer cells after cultivation in serum-free medium showed approximately 95% cell viability (**Fig. 1**), indicating that the vesicles produced by this method did not consist of a significant fraction of apoptotic bodies.

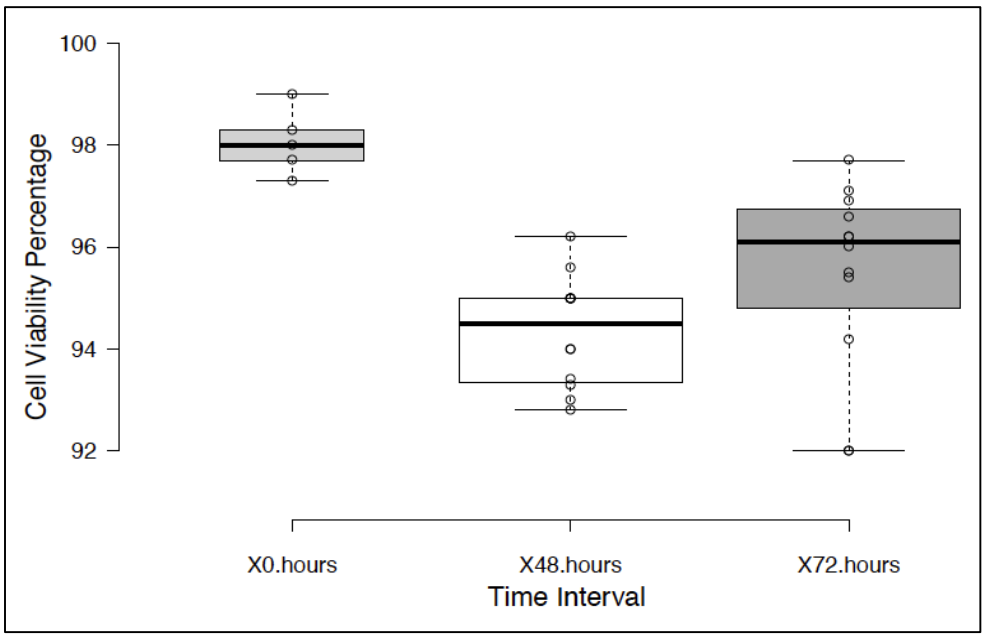


Figure. 1: Average cell viability of Glioblastoma Multiforme DBTRG-05MG cells before and after 48hrs and 72hrs culture in serum free medium. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentile; width of the boxes is proportional to the square root of the sample size; data points are plotted as open circles. Cell viability analyses of (0hrs) (n=5), (48hrs) (n=12), and (72hrs) (n=12). Bars at the 48 and 72 hour time intervals represent the combined percent viability of three experiments consisting of four flasks each, with the bar at the 0 hour time interval representing the control consisting of 5 flasks.

Transmission electron microscopy (TEM) of extracellular vesicles isolated by differential centrifugation

Size data collected from TEM images of EVs prepared from serum-starved cells were used to determine the overall EV morphology and particle distribution in the sample (**Fig. 2**).

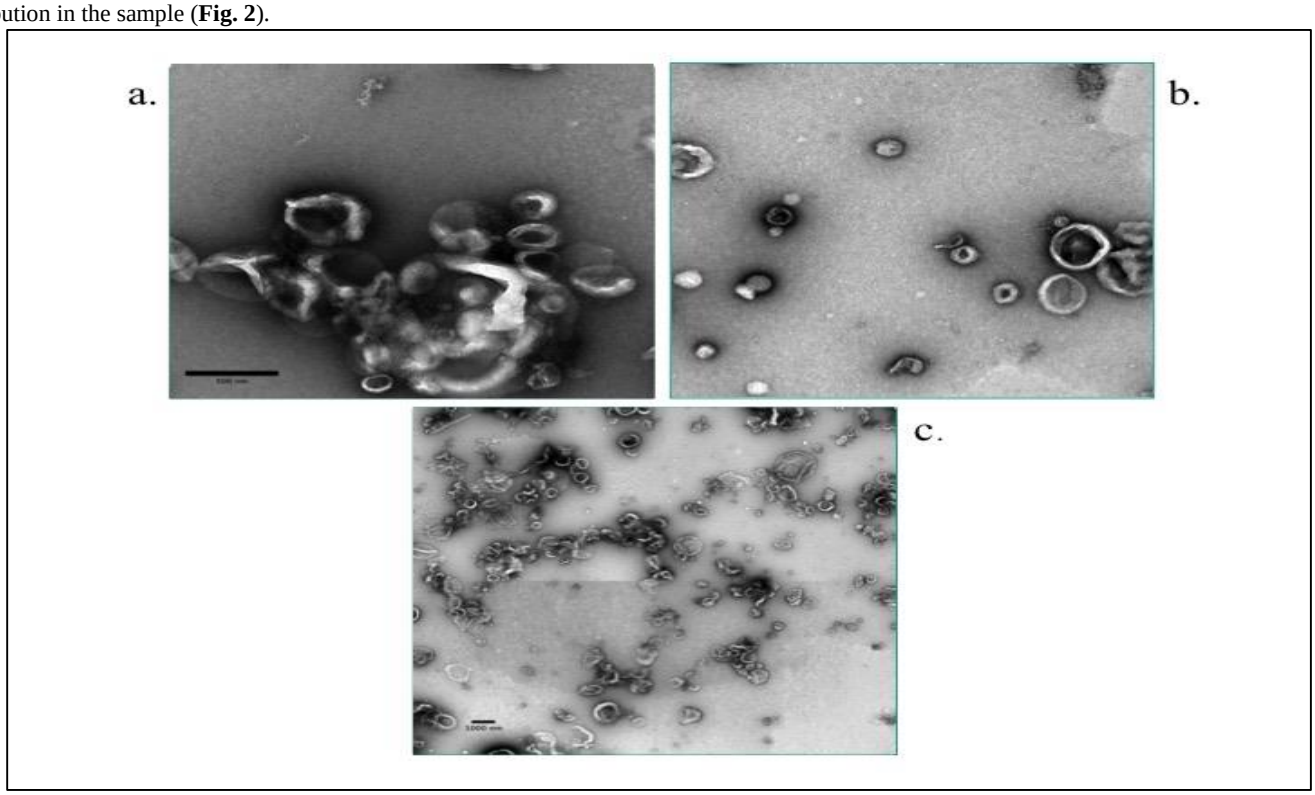


Figure. 2: Transmission electron micrograph of EVs collected from 20,000 g pellet. Images of negatively stained DC20kG microvesicles with cup-shaped morphology due in part by staining (MV, a. & b). (Scale bar 500nm). Image (c) is a wide field via of (a) and (b) locations. (Scale bar 1000 nm). Images (a) and (b) were taken using a Tecnai Spirit G² TEM at 100kV with a Gatan Spirit camera and (c) was taken using a Gatan First-light camera.

TEM particle measurements taken for approximately 130 vesicles showed that the majority (75%) of the vesicles ranged in diameter from 100-500 nm, in the size range characteristic of MVs. Approximately 15% EVs showed a diameter of less than 100 nm, suggesting they may be comprised of exosomes of endosomal origin. The EM data showed that the EV isolation protocol allowed the recovery of EVs predominantly in the size range of MVs.

Western blot analysis of MV protein composition

MV lysates were analyzed against a panel of primary antibody probes and compared to whole cell lysates (WCLs) of DBTRG-05MG cells from which the MVs were isolated.

Primary Antibody	*Source	GBM DBTRG-05MG Microvesicles	GBM DBTRG-05MG Whole Cells Lysates
Actin (C4)	SC-47778 SCBT S	ND	+
CD47 (B6H12)	SC-12730 SCBT	+	+
EGFR (A-10)	SC-373746 SCBT	ND	+
CD63 (MX-49.129.5)	SC-5275 SCBT	+	+
flotillin-2 (B-6)	SC-28320 SCBT	+	+
HCAM (F-4)	SC-9960 SCBT	ND	+
pan-cadherin	#4068 CST	+	+
tsg101 (C-2)	SC-7964 SCBT	ND	+
Integrin αv	(Q20)-R SC-6617-R	++	+
neuroligin-3	(ab186307)	++	+
B tubulin III	(ab18207)	++	+

Table 1. Antibodies used against microvesicles secreted from DBTRG-05MG tumor cells (ND=not detectable).

Each of the proteins tested (**Table 1**) was present at detectable levels in WCLs prepared from DBTGR-05MG monolayer cultures. Among these, pan-cadherin, flotillin-2, integrin alpha V, CD47, CD63, neuroligin-3, and β-tubulin-III were also detected in lysates of GBM MVs (**Fig. 3)**.

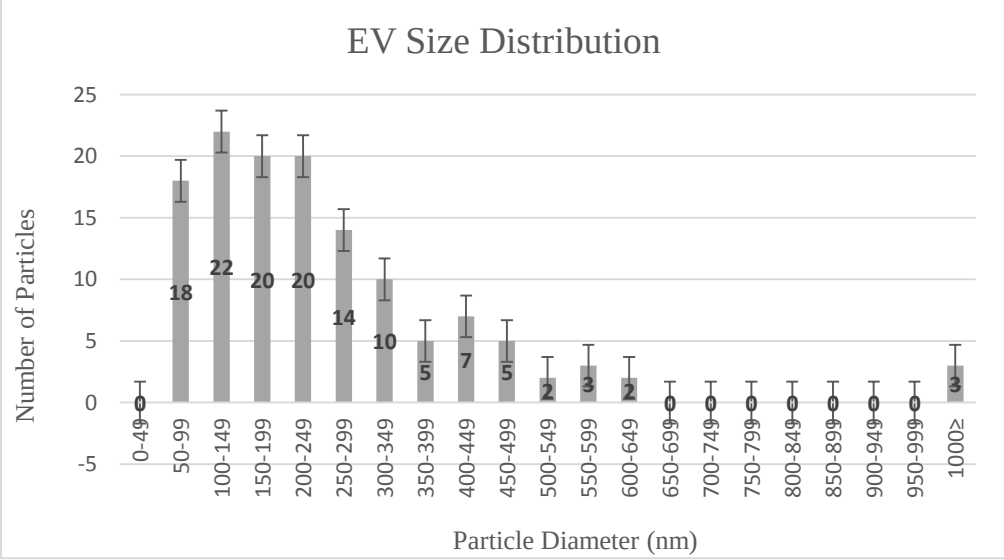
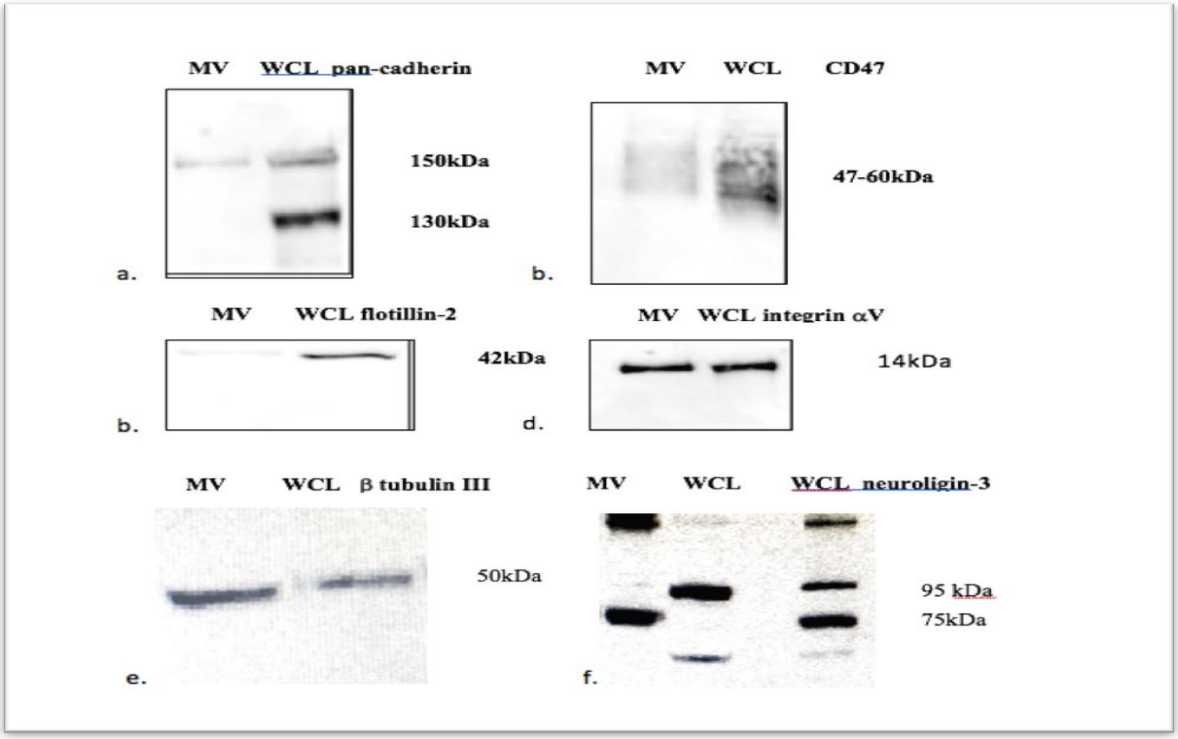


Figure 3: Size distribution of DC20kG MV measured via transmission electron micrographs. Microvesicles (MVs) were measured using ImageJ software and plotted in increments of 50nm. Size data collected was used to determine the overall MV particle distribution among the MV sample used for TEM imaging. Corresponding standard error of the mean (SEM) for each vesicle size group: (0-49) 0; (50-99) 2.9; (100-149) 4.6; (150-199) 3.2; (200-249) 3.5; (250-299) 4.6; (300-349) 6.5; (350-399) 3.0; (400-449) 5.1; (450-499) 16.5; (500-549) 16.0; (550-599) 6.4; (600-649) 17.6; (1000≥) 26.4. Vesicle size groupings from 0 to 49 and 650 to 999 nm were not calculated.

Although the relative amounts of these proteins in MVs as compared to whole cell lysates were difficult to quantitate, OD600 spectrophotometry measurements of MV lysates as compared to whole cell lysates indicated that MV density was approximately one-third that of whole cell lysates used in Western blot analyses (data not shown). The intensity of the bands corresponding to integrin alpha V, neuroligin-3 and β-tubulin III were comparable in MV and WCLs, despite the fact that the total protein content in the WCL was significantly greater. In contrast, the relative levels of CD63, CD47, pan-cadherin and flotillin-2 appeared to be significantly lower in MVs than WCLs, based on staining intensity. Several proteins present at detectable levels in WCLs were not observed in lysates prepared from MVs produced by these cells. Notably absent from MV lysates were B-actin, Tsg101, HCAM and EGFR (**Fig. 4)**.



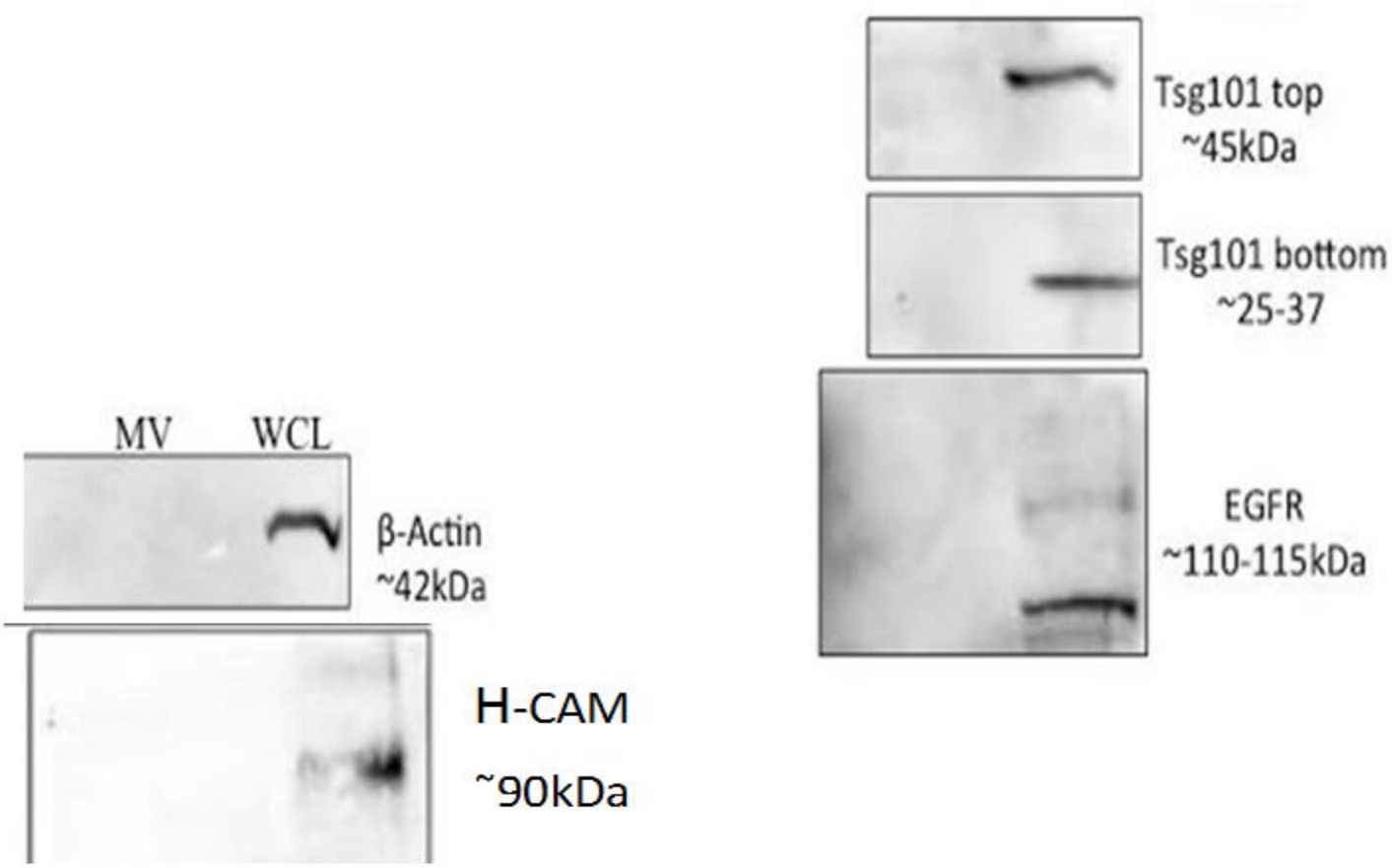


Figure. 4: Western blot characterization of DBTRG-05MG MVs purified from 20,000 g pellet showing proteins identified in both GBM DBTRG-05MG whole cell lysates (WCL) and lysates prepared from microvesicles (MV).

The potential significance of these observations is discussed below.

Discussion

Research suggests that EVs mediate communication within the tumor microenvironment, contributing to cancer progression [21] and metastasis [22, 23]. The research reported in this paper demonstrates that some proteins linked to GBM invasion and spread are also present in tumor-secreted microvesicles (**Table 1**). High levels of integrin αV, a transmembrane ECM adhesion protein, were detected in GBM EVs. Biopsies from five cerebral GBM patients were shown by Gladson and Cheresh [24] to express vitronectin and the αV-β3 integrin receptor, which was not found in low grade astroglial tumors, reactive astrogliosis or normal cortex or cerebral white matter. Moreover, cultured GBM cells were shown to require vitronectin receptor for brain tissue attachment and, therefore, could not attach to normal brain tissue. This mode of adhesion may be a requirement for GBM invasion into normal brain tissue, potentially implicating GBM secreted MVs containing integrin αV in this process. Francovic *et al.* [25] have demonstrated that integrin αV upregulation is correlated with tumor progression from low grade to aggressive gliomas, directly implicating this receptor complex as a biomarker for invasion and metastasis. The study authors suggested that integrin αV may be involved in escape from senescence.

B-tubulin III, a biomarker for tumor aggressiveness and resistance to taxanes, is a poor prognosis indicator whose upregulation is induced by oxidative stress [26]. Its presence in tumor secreted MVs suggests that diagnostic screening of cerebral spinal fluid for MVs containing this biomarker could be a valuable addition to current assessment protocols.

Very high levels of neuroligin-3 and its 75kDa cleaved product were also detected in MVs from GBM tumor cells. Neuroligin-3 activates multiple tumor promoting signal pathways that enhance cell proliferation. An important mechanism that mediate neural regulation of brain cancer is activity-dependent cleavage and secretion of this synaptic adhesion molecule, which promotes glioma proliferation through the PI3K-mTOR pathway [27]. Research has shown that when this signaling pathway is blocked, high grade gliomas cannot proliferate in mice [28]

CD47 blocks phagocytosis of tumor cells by binding to SIRPα receptors on microglial phagocytes; this represents an important component of immune system evasion by tumor cells [29]. Likewise, MVs expressing CD47 secreted by GBM tumor cells may play a direct role in immunosuppression by blocking microglia associated tumor cell phagocytosis. To this end, therapeutic inhibition of CD-47/SIRP-alpha by humanized antibodies has been developed as a treatment for pediatric brain tumors as it activates microglial phagocytosis of brain tumor tissue [30].

CD63 is a tetraspanin that plays an important role in cell adhesion and motility; it is a biomarker for poor prognosis when detected in many types of cancer. [31]. Interestingly, this protein is expressed in undifferentiated neural stem cells, but not in their differentiated counterparts [32]. Its presence in GBM MVs further implicates these vesicles in activities associated with tumor stemness and progression. CD44 is a previously identified biomarker associated with tumor migration and adhesion to the extracellular matrix via binding to hyaluronic acid [33]. Pan-cadherin was also detected in GBM MVs; cadherins are transmembrane epithelial cell-to-cell adhesion proteins regulated via calcium-mediated interactions. Additional research by Kaur et al. [34] showed that cadherin-11 is expressed in human glioma cell lines and is associated with tumor cell migration and growth factor independence.

Surprisingly, B-actin was not detected in MVs secreted from GBM cells. It is possible that MV budding from the plasma membrane may occur in actin-poor regions in the cell membrane where the absence of cytoskeletal components may facilitate membrane extrusion to form extracellular vesicles. In contrast, the presence of flotillin-2 in secreted MVs suggests that cell membrane areas containing this membrane protein may be associated with MV budding and release. The absence of detectable levels of tsg101, a biomarker for exosomes, is consistent with our data showing that the EV preparation used in this study consisted largely of EVs in the size range characteristic of microvesicles (MVs).

Cultivation of monolayer GBM cells in serum-free medium for 48-72 hours enhanced MV production without significant loss of culture viability and associated production of apoptotic bodies. Moreover, the ultracentrifugation protocol used in this study to recover MVs produced by serum-starved GBM cells permitted a high yield isolation of EVs in the particle size range of MVs, based on TEM size range data. A small, but significant (15%), fraction of the recovered EVs were in the size range (50-100nm) of exosomes, and this may have accounted for the cup-shaped morphology observed for some of the vesicles in TEM imaging studies.

An important question raised by this and similar studies is the connection between serum depletion and increased EV production by cultured cells. It is probable that serum starvation affects cell-to-cell and cell-to-substrate adhesion parameters in cultured cells to promote EV production. For example, the major source of fibronectin *in vitro* is serum. As a consequence of serum starvation, decreases in the levels of proteins important in cellular attachment may act as a trigger for MV production; moreover, cell detachment may represent an important trigger for EV formation in both physiological and *in vitro* conditions, consistent with their suggested role in cell movement and migration. It is possible that the composition of secreted MVs may vary depending on the conditions in which their production is induced; therefore, it will be important to characterize the protein composition of MVs induced by physiological factors as well. Previous research in our laboratory has shown that MV release from cultured GBM cell line DBTRG-05MG is stimulated by $CaCl_2$ [18]. Unlike serum starvation, $CaCl_2$ mediated induction of MV production represents a physiologically relevant condition. In cancer patients, tumor induced hypercalcemia, a poor prognostic parameter, may contribute to tumor progression, in part, by contributing to a tumor microenvironment that promotes tumor spread and metastasis associated with and related to elevated MV production. Despite the fact that CaCl2 represents a more physiological trigger of MV production, serum starvation produces a higher, more consistent yield of tumor MVs without inducing significant apoptosis.

That cell membrane biomarkers of GBM tumor invasion and progression are also displayed in tumor secreted MVs supports the role of these vesicles in these processes. The secretion of MVs containing proteins involved in tumor invasion/spread, immunosuppression and "stemness" may represent an important mechanism of glioma progression. In this context, MVs may serve as an intricate intercellular communication platform for initiating invasion and tumorigenesis within the tumor microenvironment, leading to tumor progression and metastasis. Research suggests that the overriding function of EVs is the transport and deposition of cargo from the cell of origin to a cellular recipient and to exert a primary effect on cell biology through this mechanism [35]. MMVs may, however, exert a more comprehensive and fundamental role in tumor biology by modifying the tumor niche to induce stromal malignant transformation and tumor spread by morphogenetic remodeling of the tumor microenvironment. The proposed model by which this process occurs is termed "insertional membrane editing". (**Fig. 5**).

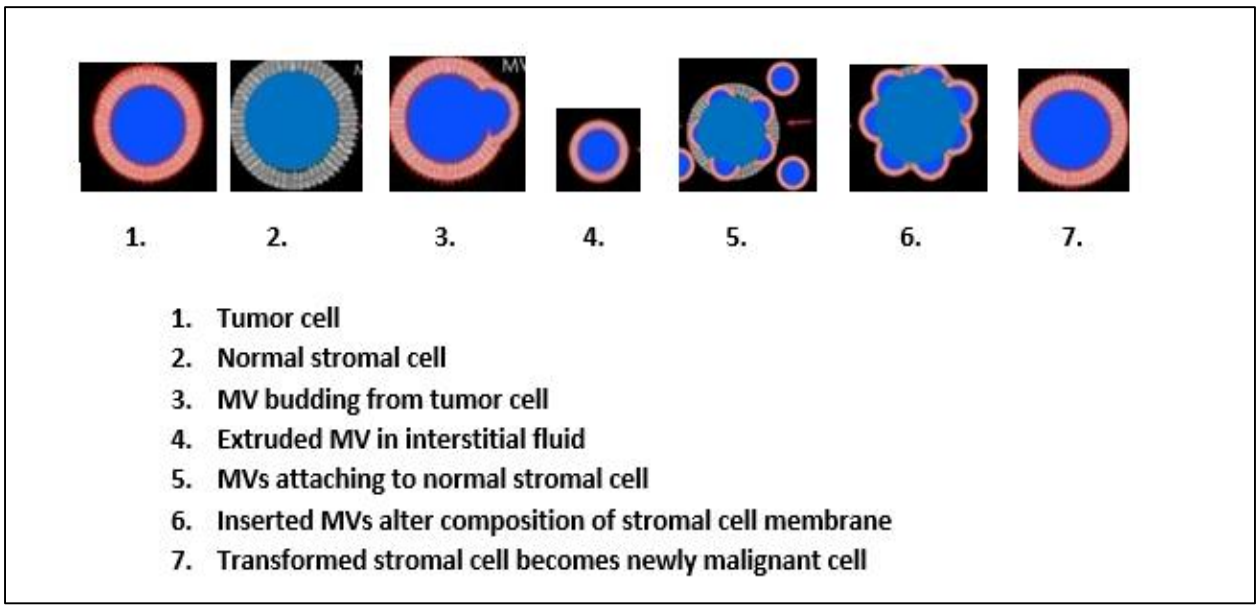


Figure. 5: Proposed steps in stromal cell transformation by tumor secreted MVs. Insertional membrane editing model explains primary role of GBM MVs in primary tumor invasion and spread.

This model suggests that MVs secreted by tumor cells bind to the membranes of stromal cells and, in so doing, insert membrane components from the original tumor cell into the recipient cell membrane that induce stromal tissue transformation. Secreted MVs are mobile elements whose production may be initiated by the disruption of tissue architecture caused by primary tumor hyperplasia. Subsequent movement about the stromal niche lead to interactions with stromal cells to induce tissue morphogenesis by membrane insertion. The result is an "edited" stromal cell membrane that contains proteins that may alter the gene expression profile of the recipient cell via the induction of signal transduction cascades that reprogram the gene expression to elicit changes in the stromal recipient associated with malignant transformation. Tumor invasion into normal regions of the brain may, therefore, be initiated, in part, by the uptake of MVs shed from tumor cells. In the tumor microenvironment, changes in cell-to-cell adhesion parameters, such as those involved in epithelial-to-mesenchymal transitions (EMTs), may further promote the production of EVs to amplify tumor invasion and spread, ultimately remodeling the stromal tissue into a tumor invasion zone, associated with continuously amplifying signals for tumor cell migration, ECM attachment and invasion. In this context, insertional membrane editing may be a fundamental component of tumor progression by which tumor associated MVs educate the stromal niche to express critical parameters of malignant transformation that contribute to advanced, systemic disease. By this mechanism, a regionally localized tumor may dispense migratory carriers of key inducers of malignancy, allowing its effects to extend beyond its primary location to effect local microenvironmental and, ultimately, systemic changes that characterize advanced high-grade malignancy.

References

1. Melin BS, Barnholtz-Sloan JS, Wrensch MR et al (2017) Genome-wide association study of glioma subtypes identifies specific differences in genetic susceptibility to glioblastoma and non-glioblastoma tumors. Nat Genet 49(5):789–794. https:// doi:10.1038/ng.3823.
2. Nizamutdinov D et al (2018) Prognostication of survival outcomes in patients diagnosed with glioblastoma. World Neurosurg 109: e67-e74. https://doi.org/10.1016/j.wneu.2017.09.104
3. Jovčevska I, Kočevar N, Komel R (2013) Glioma and glioblastoma - how much do we (not) know? Mol Clin Oncol 1(6):935-941.
4. Ostrom QT et al (2017) CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2010-2014. Neuro Oncol 19(suppl_5): v1-v88. https://doi: 10.1093/neuonc/nox158.
5. Urbańska K et al (2014) Glioblastoma multiforme - an overview. Contemp Oncol (Pozn).18(5):307-12. https://doi: 10.5114/wo.2014.40559.
6. Sluijter JPG et al (2018) Extracellular vesicles in diagnostics and therapy of the ischaemic heart: Position Paper from the Working Group on Cellular Biology of the Heart of the European Society of Cardiology. Cardiovasc Res 114(1):19-34. https://doi: 10.1093/cvr/cvx211.
7. 7. Kalra H, Drummen GP, Mathivanan S (2016) Focus on Extracellular Vesicles: Introducing the Next Small Big Thing. Int J Mol Sci.17(2):170. https://doi: 10.3390/ijms17020170.
8. Tricarico C, Clancy J, D'Souza-Schorey C (2017) Biology and biogenesis of shed microvesicles. Small GTPases 8(4): 220–232. https://doi: 10.1080/21541248.2016.1215283.
9. Cocucci E, Meldolesi J (2015) Ectosomes and exosomes: shedding the confusion between extracellular vesicles. Trends Cell Biol.25(6):364-72. https://doi: 10.1016/j.tcb.2015.01.004..
10. Hessvik NP, Llorente A (2018) Current knowledge on exosome biogenesis and release. Cell Mol Life Sci.75(2):193-208. https:// doi: 10.1007/s00018-017-2595-9.
11. Kalra H, Drummen GP, Mathivanan S (2016) Focus on Extracellular Vesicles: Introducing the Next Small Big Thing. Int J Mol Sci. 2016;17(2):170. https://doi: 10.3390/ijms17020170.
12. McBride, J, Rodriguez-Menocal L, Badiavas E (2017) Extracellular vesicles as biomarkers and therapeutics in dermatology: A focus on exosomes. Journal of Investigative Dermatology 137(8):1622-1629.

13. D'Asti E (2012) Oncogenic extracellular vesicles in brain tumor progression. Front Physiol. 24;3: 294. https:// doi: 10.3389/fphys.2012.00294.
14. Kanada M1, et al (2015) Differential fates of biomolecules delivered to target cells via extracellular vesicles. Proc Natl Acad Sci U S A. 112(12): E1433-42. https://doi: 10.1073/pnas.1418401112.
15. Raposo G, Stoorvogel W (2013) Extracellular vesicles: exosomes, microvesicles, and friends. J. Cell Biol 200(4):373-83. https://doi: 10.1083/jcb.201211138.
16. Desrochers LM, Antonyak MA, Cerione RA (2016) Extracellular vesicles: satellites of information transfer in cancer and stem cell biology. Developmental Cell 37(4):301-9.
17. Vader P, Breakefield XO, Wood MJ (2014) Extracellular vesicles: emerging targets for cancer therapy. Trends in Molecular Medicine 20(7):385-93.
18. Crawford S, Diamond D, Brustolon L, Penarreta R (2011) Effect of Increased Extracellular Ca++ on Microvesicle Production and Tumor Spheroid Formation. Cancer Microenvironment 4(1):93-103.
19. Théry C, Amigorena S, Raposo G, Clayton A (2006) Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Current Protocols in Cell Biology 1:3-22.
20. 20. Fertig ET, Gherghiceanu M, Popescu LM (2014) Extracellular vesicles release by cardiac telocytes: electron microscopy and electron tomography. J Cellular and Molecular Medicine. 10:1938-43.
21. Muralidharan-Chari V, Clancy JW, Sedgwick A, D'Souza-Schorey C (2010) Microvesicles: mediators of extracellular communication during cancer progression. J Cell Sci 123(10):1603-11.
22. Vehlow A, Cordes N (2013) Invasion as target for therapy of glioblastoma multiforme. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer 1836(2):236-44.
23. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D (2016) Extracellular vesicles in cancer: cell-to-cell mediators of metastasis. Cancer Cell 30(6):836-48.
24. Gladson CL, Cheresh DA (1991) Glioblastoma expression of vitronectin and the alpha v beta 3 integrin. Adhesion mechanism for transformed glial cells. J Clin Invest 88(6):1924-32.
25. Franovic A, Elliott KC, Seguin L, Camargo MF, Weis SM, Cheresh DA(2015) Glioblastomas require integrin αvβ3/PAK4 signaling to escape senescence. Cancer Resb75.; (21) 4466-4473; DOI: 10.1158/0008-5472.CAN-15-0988.
26. Mariani M, Shahabi S, Sieber S, Scambia G, Ferlini C (2011) Class III β-tubulin (TUBB3): more than a biomarker in solid tumors? Current Molecular Medicine 11(9):726-31.
27. Nagano O, Murakami D, Hartmann D, De Strooper B, Saftig P, Iwatsubo T, Nakajima M, Shinohara M, Saya H (2004) Cell–matrix interaction via CD44 is independently regulated by different metalloproteinases activated in response to extracellular Ca2+ influx and PKC activation. J. Cell Biol 165(6):893-902.
28. Venkatesh HS, Tam LT, Woo PJ, Lennon J, Nagaraja S, Gillespie SM, Ni J, Duveau DY, Morris PJ, Zhao JJ, Thomas CJ (2017) Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma. Nature 549(7673):533.
29. Hutter G, Theruvath J, Graef CM, Zhang M, Schoen MK, Manz EM, Bennett ML, Olson A, Azad TD, Sinha R, Chan C. (2019) Microglia are effector cells of CD47-SIRPα antiphagocytic axis disruption against glioblastoma. Proc Natl Acad Sci 116(3):997-1006.
30. Gholamin S, Mitra SS, Feroze AH, Liu J, Kahn SA, Zhang M, Esparza R, Richard C, Ramaswamy V, Remke M, Volkmer AK (2017) Disrupting the CD47-SIRPα anti-phagocytic axis by a humanized anti-CD47 antibody is an efficacious treatment for malignant pediatric brain tumors. Science Translational Medicine 9(381): eaaf2968.
31. Pols MS, Klumperman J (2009) Trafficking and function of the tetraspanin CD63. Exp Cell Research 315(9):1584-92.
32. Rorive S, Lopez XM, Maris C, Trepant AL, Sauvage S, Sadeghi N, Roland I, Decaestecker C, Salmon I (2010) TIMP-4 and CD63: new prognostic biomarkers in human astrocytomas. Modern Pathology 23(10):1418.
33. Nagano O, Saya H (2004) Mechanism and biological significance of CD44 cleavage. Cancer Science 95(12):930-5.
34. Kaur H, Phillips-Mason PJ, Burden-Gulley SM, Kerstetter-Fogle AE, Basilion JP, Sloan AE, Brady-Kalnay SM (2012) Cadherin-11, a marker of the mesenchymal phenotype, regulates glioblastoma cell migration and survival in vivo. Molecular Cancer Research 10(3):293-304.
35. Lee TH, D'Asti E, Magnus N, Al-Nedawi K, Meehan B, Rak J (2011) Microvesicles as mediators of intercellular communication in cancer—the emerging science of cellular 'debris'. In Seminars in Immunopathology (Vol. 33, No. 5, pp. 455-467). Springer-Verlag.

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