Brain-derived extracellular vesicles

Brain-derived extracellular vesicles (BEVs) in blood allows for minimally-invasive investigations of central nervous system (CNS) -specific markers of age-related neurodegenerative diseases (NDDs). Polymer-based extracellular vesicle and immunoprecipitation (IP)-based BEV-enrichment protocols from blood have gained popularity.

Polymer-Based EV Enrichment Protocols Principle: Polymer-based methods use water-soluble polymers to precipitate BEVs from blood. These polymers reduce the solubility of BEVs, causing them to aggregate and precipitate.

Commonly Used Polymers:

Polyethylene glycol (PEG) Dextran ExoQuick Protocol:

Sample Preparation:

Collect blood and centrifuge to separate plasma/serum. Filter the plasma/serum to remove cells and debris using a 0.22 µm filter. Polymer Addition:

Add an appropriate amount of polymer solution to the filtered plasma/serum. For PEG: typically, a final concentration of 8-10% is used. For ExoQuick: follow the manufacturer’s instructions. Incubation:

Incubate the mixture at 4°C for several hours (usually overnight) to allow BEVs to precipitate. Centrifugation:

Centrifuge the mixture at high speed (e.g., 10,000 x g) for 30-60 minutes at 4°C to pellet the BEVs. Resuspension:

Carefully remove the supernatant and resuspend the BEV pellet in an appropriate buffer (e.g., PBS). Purification (optional):

Further purify the BEVs using density gradient ultracentrifugation or size exclusion chromatography if higher purity is required. Immunoprecipitation (IP)-Based BEV Enrichment Protocols Principle: IP-based methods use antibodies that specifically bind to surface markers on BEVs. These antibodies are conjugated to beads, allowing for the selective capture and isolation of BEVs.

Commonly Used Surface Markers:

CD9 CD63 CD81 Protocol:

Sample Preparation:

Collect blood and centrifuge to separate plasma/serum. Filter the plasma/serum to remove cells and debris using a 0.22 µm filter. Antibody Conjugation:

Incubate magnetic or agarose beads with antibodies against BEV surface markers (e.g., CD9, CD63, CD81) at the recommended ratio and temperature for a few hours. Binding:

Add the antibody-conjugated beads to the filtered plasma/serum. Incubate the mixture at 4°C with gentle mixing for several hours (typically overnight) to allow the antibodies to bind to the BEVs. Washing:

Use a magnetic separator or centrifuge to collect the beads. Wash the beads multiple times with a washing buffer (e.g., PBS with 0.1% BSA) to remove non-specifically bound material. Elution:

Elute the bound BEVs from the beads using an elution buffer (e.g., low pH glycine buffer or another suitable buffer). Neutralize the eluted BEVs if necessary. Purification (optional):

Further purify the BEVs using additional techniques like ultracentrifugation or size exclusion chromatography if higher purity is required. Comparison of Methods Polymer-Based Methods:

Advantages: Simple, cost-effective, and scalable for large volumes. Disadvantages: Can co-precipitate non-EV proteins and other contaminants, requiring additional purification steps. IP-Based Methods:

Advantages: High specificity due to antibody-antigen interactions, suitable for targeting specific EV subpopulations. Disadvantages: More expensive, requires high-quality antibodies, and the process can be time-consuming. Both methods have their specific applications and can be selected based on the required purity, available resources, and specific research goals. Combining these methods with other purification techniques can enhance the quality and yield of BEVs.

Neural stem cell-derived extracellular vesicle

Neural stem cell-derived extracellular vesicle

Tumor-derived small extracellular vesicles

Small extracellular vesicles (SEVs) are extracellular vesicles containing DNA, RNA, and proteins and are involved in intercellular communication and function, playing an essential role in the growth and metastasis of tumors.

Since the first descriptions of extracellular vesicles (EV) [1) 2)], much interest has arisen regarding these small secreted organelles and their role in human disease.

Many diverse names have been used to refer to these vesicles released by healthy cells including ectosomes, microparticles, and shedding microvesicles, just to name a few. In order to bring harmonization to the field, researchers are now encouraged to use the term extracellular vesicles (EVs) as a generic term for all secreted vesicles. Although confusion on the nomenclature of EVs has spread throughout the literature, EVs may be broadly classified into exosomes, microvesicles (MVs), and apoptotic bodies according to their cellular origin.

Normal and pathologic cells secrete extracellular vesicles (EV), which are defined as 30-2,000 nm spherical organelles.

Neurosurgery

- Brain-derived exosomal hemoglobin transfer contributes to neuronal mitochondrial homeostasis under hypoxia
- Delivery of LOXL1-AS1-siRNAs using targeting peptide-engineered extracellular vesicles with focused ultrasound to suppress medulloblastoma metastasis
- Direct SERS profiling of small extracellular vesicles in cerebrospinal fluid for pediatric medulloblastoma detection and treatment monitoring
- Biomimetic extracellular vesicles derived from chimeric antigen receptor monocytes to treat glioblastoma: An efficient and safe intranasal drug delivery nanoplatform
- Multimodal Imaging of Brain Metastasis-Derived Extracellular Vesicles Using Superparamagnetic Iron Oxide Nanoparticle Labeling
- First-In-Human Application of Human Umbilical Cord-Derived Extracellular Vesicles in Tethered Spinal Cord Release Surgery
- Advanced Therapeutic Approaches Based on Small Extracellular Vehicles (sEVs) For the Regeneration of Spinal Cord Injuries
- Cargo of small extracellular vesicles from neuronal origin shows progression of dementia in individuals with Down syndrome

Importance

Understanding the biology and functions of extracellular vesicles has the potential to revolutionize our knowledge of cell-to-cell communication and open up new avenues for medical research and clinical applications.

Extracellular vesicles (EVs) are highly sought after as a source of biomarkers for disease detection and monitoring. Tumor EV isolation, processing, and evaluation from biofluids is convoluted by EV heterogeneity and biological contaminants and is limited by technical processing efficacy. A study rigorously compares common bulk EV isolation workflows (size exclusion chromatography, SEC; membrane affinity, MA) alongside downstream RNA extraction protocols to investigate molecular analyte recovery. EV integrity and recovery is evaluated using a variety of technologies to quantify total intact EVs, total and surface proteins, and RNA purity and recovery. A comprehensive evaluation of each analyte is performed, with a specific emphasis on maintaining user (n = 2), biological (n = 3), and technical replicates (n≥3) under in vitro conditions. A subsequent study of tumor EV spike-in into healthy donor plasma samples is performed to further validate biofluid-derived EV purity and isolation for clinical application. Results show that EV surface integrity is considerably preserved in eluates from SEC-derived EVs, but RNA recovery and purity, as well as bulk protein isolation, are significantly improved in MA-isolated EVs. EV isolation and RNA extraction pipelines govern recovered analyte integrity, necessitating careful selection of processing modality to enhance recovery of the analyte of interest [3)]

Functions

EV is well recognized as potential contributor to the complex and dynamic pathophysiology of brain tumors [4) 5) 6) 7) 8) 9) 10) 11) 12) 13) 14)].

Numerous studies have demonstrated that EVs not only regulate changes in brain physiology but also regulate synaptic plasticity and neuroregeneration in traumatic brain injury (TBI), which opens a new approach for the treatment of TBI. In view of the fact that most human cells can secrete EVs, and relevant experiments have proved that different doses of EVs have different therapeutic effects on TBI [15)].

It has been found that EV mediate various biological functions including cellular remodeling and export of biomolecules, extracellular communication, immune stimulation and suppression, and modulation of the cellular microenvironment.

To evaluate whether tumour-derived microvesicles (T-MV), originating from the plasma membrane, represent suitable cancer biomarkers, we isolated MV from peripheral blood samples of cancer patients with locally advanced and/or metastatic solid tumours (n = 330, including 79 head & neck cancers, 74 lung cancers, 41 breast cancers, 28 colorectal cancers and 108 with other cancer forms) and controls (n = 103). Whole MV preparations were characterised using flow cytometry. While MV carrying the tumour-associated proteins MUC1, EGFR and EpCAM were found to be enhanced in a tumour-subtype-specific way in patients' blood, expression of the matrix metalloproteinase inducer EMMPRIN was increased independent of tumour type. Higher levels of EMMPRIN+-MV correlated significantly with poor overall survival, whereas the other markers were prognostic only in specific tumour subgroups. By combining all four tumour-associated antigens, cancer patients were separated from healthy controls with an AUC of up to 0.85. Ex vivo, whole MV preparations from cancer patients, in contrast to those of controls, induced a tumour-supporting phenotype in macrophages and increased tumour cell invasion, which was dependent on the highly glycosylated isoform of EMMPRIN. In conclusion, the detection of T-MV in whole blood, even in minor amounts, is feasible with standard techniques, proves functionally relevant and correlates with clinical outcome [16)].

Tumor-derived small extracellular vesicles in glioma

Glioma microvesicles.

International Society for Extracellular Vesicles

Basso M, Gori A, Nardella C, Palviainen M, Holcar M, Sotiropoulos I, Bobis-Wozowicz S, D'Agostino VG, Casarotto E, Ciani Y, Suetsugu S, Gualerzi A, Martin-Jaular L, Boselli D, Kashkanova A, Parisse P, Lippens L, Pagliuca M, Blessing M, Frigerio R, Fourniols T, Meliciano A, Fietta A, Fioretti PV, Soroczyńska K, Picciolini S, Salviano-Silva A, Bergese P, Zocco D, Chiari M, Jenster G, Waldron L, Milosavljevic A, Nolan J, Monopoli MP, Witwer KW, Bussolati B, Di Vizio D, Falcon Perez J, Lenassi M, Cretich M, Demichelis F. International Society for Extracellular Vesicles Workshop. QuantitatEVs: multiscale analyses, from bulk to single extracellular vesicle. J Extracell Biol. 2024 Jan;3(1):e137. doi: 10.1002/jex2.137. Epub 2024 Jan 24. PMID: 38405579; PMCID: PMC10883470.

1)

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;101:942–948.
2)

Harding C, Heuser J, Stahl P: Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J Cell Biol 1983;97: 329–339.
3)

Hsia T, You DG, Politis MG, Batool SM, Ekanayake E, Lee H, Carter BS, Balaj L. Rigorous Comparison of Extracellular Vesicle Processing to Enhance Downstream Analysis for Glioblastoma Characterization. Adv Biol (Weinh). 2023 Sep 5:e2300233. doi: 10.1002/adbi.202300233. Epub ahead of print. PMID: 37670402.
4)

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.
5)

Akers JC, Ramakrishnan V, Kim R, Skog J, Nakano I, Pingle S, Kalinina J, Hua W, Kesari S, Mao Y, Breakefield XO, Hochberg FH, Van Meir EG, Carter BS, Chen CC: MiR-21 in the extracellular vesicles (EVs) of cerebrospinal fluid (CSF): a platform for glioblastoma biomarker development. PLoS One 2013;8(10):e78115.
6)

Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J: Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol 2008;10:619–624.
7)

Chaput N, Thery C: Exosomes: immune properties and potential clinical implementations. Semin Immunopathol 2011;33:419–440.
8)

Chen C, Skog J, Hsu CH, Lessard RT, Balaj L, Wurdinger T, Carter BS, Breakefield XO, Toner M, Irimia D: Microfluidic isolation and transcriptome analysis of serum microvesicles. Lab Chip 2010;10: 505–511.
9)

Chen WW, Balaj L, Liau LM, Samuels ML, Kotsopoulos SK, Maguire CA, Loguidice L, Soto H, Garrett M, Zhu LD, Sivaraman S, Chen C, Wong ET, Carter BS, Hochberg FH, Breakefield XO, Skog J: BEAMing and droplet digital PCR analysis of mutant IDH1 mRNA in glioma patient serum and cerebrospinal fluid extracellular vesicles. Mol Ther Nucleic Acids 2013;2:e109.
10)

Gonda D, Goyal A, Akers J, Chen C, Carter B: Glioblastoma microvesicles transport RNA and proteins, promoting tumor growth; in Hayat MA (ed): Tumors of the Central Vervous System. Dordrecht, Springer, 2014, vol 11, pp 101–112.
11)

Gonda DD, Akers JC, Kim R, Kalkanis SN, Hochberg FH, Chen CC, Carter BS: Neuro-oncologic applications of exosomes, microvesicles, and other nano-sized extracellular particles. Neurosurgery 2013; 72:501–510.
12)

Kucharzewska P, Christianson HC, Welch JE, Svensson KJ, Fredlund E, Ringnér M, Mörgelin M, Bourseau-Guilmain E, Bengzon J, Belting M: Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development. Proc Natl Acad Sci U S A 2013;110:7312–7317.
13)

Raposo G, Stoorvogel W: Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013; 200:373–383.
14)

Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO: Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008;10:1470–1476.

15)
Gao Y, Wang C, Jin F, Han G, Cui C. Therapeutic effect of extracellular vesicles from different cell sources in traumatic brain injury. Tissue Cell. 2022 Mar 4;76:101772. doi: 10.1016/j.tice.2022.101772. Epub ahead of print. PMID: 35272241.
16)
Menck K, Bleckmann A, Wachter A, Hennies B, Ries L, Schulz M, Balkenhol M, Pukrop T, Schatlo B, Rost U, Wenzel D, Klemm F, Binder C. Characterisation of tumour-derived microvesicles in cancer patients' blood and correlation with clinical outcome. J Extracell Vesicles. 2017 Jul 16;6(1):1340745. doi: 10.1080/20013078.2017.1340745. eCollection 2017. PubMed PMID: 28804596; PubMed Central PMCID: PMC5533131.