HSEV Magyar EV kutatók szimpóziuma

21st June 2024, Friday

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Introduction

Dear Participants,

On behalf of the Organizers, we welcome you to the second symposium of the HSEV!

The aim of the symposium is to provide an opportunity for Hungarian EV research workshops to present themselves and to interact. As with EV research, the symposium is highly interdisciplinary: we welcome applications from groups active in basic and applied research in physics, biology, medicine, engineering, and industrial development.

We hope that you find the program inspiring and wish you a successful meeting.

Zoltán Giricz

Hungarian Society for Experimental and Clinical Pharmacology

Edit Buzás

Hungarian Society for Immunology

Hargita Hegyesi

Hungarian Society for Immunology

Csenger Kovácsházi

Hungarian Society for Experimental and Clinical Pharmacology

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Schedule

10:00 – Registration

10:30 – 10:45 Opening

10:45 – 12:15 – Oral Session 1, Biogenesis/release of EVs and their function in signal transmission

12:00 – 13:30 – Lunch break, poster viewing

13:30 – 14:30 – Oral session 2

14:30 – 15:00 – Coffee break, poster viewing

15:00 – 16:30 – Oral session 3

16:15 – 16:30 – Closing remarks, group photo

16:45 – Meeting of senior scientists

Venue

Semmelweis University, NET Building, Tanácsterem.

1098 Budapest, Nagyvárad tér 4.

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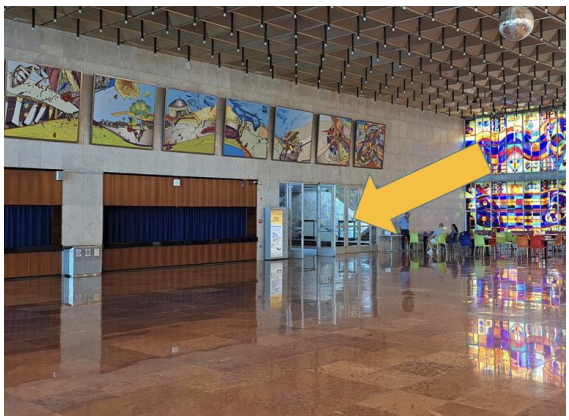

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Scientific program

10:30 - 10:45 Opening

Dr. Zoltán Giricz
Section head, Extracellular Vesicle Section, Hungarian Society for Experimental and Clinical Pharmacology
Semmelweis University, Department of Pharmacology and Pharmacotherapy

Prof. Dr. Edit Buzás
Section head, Extracellular Vesicle Section, Hungarian Society for Immunology
Semmelweis University, Department of Genetics, Cell- and Immunobiology

10:45 - 12:15 – Oral Session 1, Biogenesis/release of EVs and their function in signal transmission

Session chairs:
Ákos M. Lőrincz, Department of Physiology, Semmelweis University
Beáta Szebeni, HUN-REN–SU Pediatrics and Nephrology Research Group, Semmelweis University

10:45: **Andrea Gálisová**
Department of Diagnostic and Interventional Radiology - Computed tomography, magnetic resonance imaging, and clinical and experimental spectroscopy, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
MRI tracking of targeted extracellular vesicles

11:00: **Meir B Kestecher**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Reduced CD63+ extracellular vesicles associate with hypercholesterolaemia in mice and humans

11:15: **Sponsor presentation**
Csaba Bankó, *Kromat Ltd.*
Meet Leprechaun – the sEV specific analytical tool

11:30: **Dorina Lenzinger**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
"Torn bag" release of small extracellular vesicles via limiting membrane rupture of amphiectosomes

11:45: **Krisztina V. Vukman**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Immunomodulatory effect of mast cell-derived extracellular vesicles

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12:00 – 13:30 – Lunch break, poster viewing

Poster 1: **Zsuzsanna Adamecz**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Characterization of human red blood cell-derived extracellular vesicles (RBC-EVs)

Poster 2: **Mirjam Balbisi**
MTA-TTK Lendület (Momentum) Glycan Biomarker Research Group - HUN-REN Research Centre for Natural Sciences, Semmelweis University Doctoral School
Proteomic and glycomic characterization of A549 and BEAS-2B cell line-derived extracellular vesicles

Poster 3: **Bernadett Bodnár**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Isolation and characterisation of extracellular vesicles from lymph nodes

Poster 4: **Edina Bugyik**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Initial steps to follow cancer-induced cardiac atrophy in an in vivo model

Poster 5: **Mátyás Bukva**
Lendület Laboratory of Microscopic Image Analysis and Machine Learning, Biological Research Centre, Szeged
Extracellular vesicles as carriers of tumor-associated protein patterns – a meta-analysis using machine learning methods

Poster 6: **Kelsey Fletcher**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Characterization of mast cell-derived extracellular vesicles during degranulation

Poster 7: **Sayam Ghosal,**
HCEMM-SU Extracellular Vesicles Research Group, Institute of Genetics, Cell and Immunobiology, Semmelweis University
Unraveling the origins and heterogeneity of extracellular vesicles and nanoparticles: Insights from advanced characterization techniques

Poster 8: **Edina Gyukity-Sebestyén,**
Institute of Biochemistry , HUN-REN Biological Research Centre; Department of Immunology University of Szeged
Decoding the Post-COVID syndrome: leveraging molecular signatures of extracellular vesicles for predictive analysis

Poster 9: **Delaram Khamari**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Role of extracted Ginger Extracellular Vesicles-Like Particles

Poster 10: **Júlia Kvasznicza**
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary
In vitro effects of cyclodextrins on extracellular vesicle secretion in melanoma

Poster 11: **Gréta L. Bányai**
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary
Microfluidic-based method to analyze extracellular vesicles in liquid biopsies

Poster 12: **Mátka Nagy**
Department of Physiology, Semmelweis University, Budapest, Hungary
Neutrophil-derived extracellular vesicles modulate the inflammatory response of monocytes and macrophages

Poster 13: **Afrodité Németh,**
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics,Budapest, Hungary
Extracellular vesicles promote migration despite BRAF inhibitor treatment

Poster 14: **Afrodité Németh,**
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary
Surface marker detection of extracellular vesicles isolated from pleural fluids of NSCLC, MPM and pleuritis patients

Poster 15: **Attila Oláh**
Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
Extracellular vesicles and experimental dermatology – An almost empty niche to fill

Poster 16: **Zóra Szilovics,**
Department of Microbiology, University of Szeged, Szeged, Hungary
Studying the interaction between oral pathogenic bacteria and Candida species in an indirect manner: interkingdom communication at the level of extracellular vesicles

Poster 17: **Gábor Valcz**
HUN-REN-SU Translational Extracellular Vesicle Research Group, Budapest, Hungary; Department of Image Analysis; DHISTECH Ltd, Budapest, Hungary
Examination of horizontal gene transfer in colon cancer cell model

Poster 18: **Éva Veres**
Department of Microbiology, University of Szeged, Szeged, Hungary
Oral squamous cell carcinoma-Candida interaction: The role of extracellular vesicles

Poster 19: **Tímea Böröczky**[1,2,3], Mária Harmati[1,2],Gabriella Dobra [1,2,3], Mátyás Bukva[1,2,3],
Edina Gyukity-Sebestyén[1,2], Marie-Anne Debily[4], Clémentine Barry[4], Krisztina Buzás[1,2]
1HUN-REN Biological Research Centre, Szeged, Hungary; 2Department of Immunology, University of Szeged, Szeged, Hungary; 3Doctoral School of Interdisciplinary Medicine, University of Szeged, Szeged, Hungary; 4Vectorologie
Extracellular vesicle based comparative analysis of highly and low invasive diffuse midline glioma H3K27M-mutant

Poster 20: **Ilona Barbara Csordás**[1,2], Tünde Szatmári[1], Eric Andreas Rutten[3], Tamás Visnovitz[4], Katalin Lumniczky[1]
1 Unit of Radiation Medicine, Division of Radiobiology and Radiohygiene, National Centre for Public Health and Pharmacy; 2 Semmelweis University Doctoral School, Patological and Oncological Division; 3Centre for Radiation, Chemical and Environmental Hazards, UK Health Security Agency; 4 Semmelweis University, Faculty of Medicine, Department of Genetics, Cell- and Immunobiology
Identifying Extracellular vesicle-miRNAs with a possible role in ionizing radiation induced leukaemogenesis, and their EV packaging mechanisms in the bone marrow

13:30 – 14:30 – Oral session 2, EV-based biomarkers

Session chairs:

Zoltán Varga, HUN-REN Research Centre for Natural Sciences
Tamás Garay, Faculty of Information Technology and Bionics, Pázmány Péter Catholic University

13:30: **Csenger Kovácsházi**
Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest
Effect of Hypercholesterolemia on circulating and cardiomyocyte-derived extracellular vesicles

13:45: **Anikó Zeöld / Zoltán Wiener**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
MiR-200b categorizes patients into pancreas cystic lesion subgroups with different malignant potential

14:00: **Sponsor presentation**
Árpád Sebestyén, Biomarker Ltd.
Qiagen termékek a vezikula kutatásban

14:15: **Bence Nagy**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Changes in the miRNA cargo of EV-mediated fetal-maternal communication following light treatment

14:30: **Tasvilla Sonallya**
Biomolecular self assembly group,HUN-REN TTK
Comprehensive Study and Categorization of Host Defense and Cell-Penetrating Peptides by Their Affinity for Extracellular Vesicle Interactions

14:30 – 15:00 – Coffee break, poster viewing

15:00 – 16:30 – Oral session 3, EVs in therapy

Session chairs:

Erika Kajdácsi, Research Laboratory of Semmelweis University, Department of Internal Medicine and Hematology
Mária Harmati, HUN-REN Biological Research Centre

15:00: **Mohamed Sobhy Kishta**
Medical Research and Clinical Studies Institute, National Research Centre - Egypt
Exploring the anti-skin cancer potential of copper-tin nanocomposites-loaded exosomes via promotion of apoptosis and cell cycle arrest

15:15: **Luigi Menna**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Anti-inflammatory role of endothelial colony forming cells-derived extracellular vesicles

15:30: **Sponsor Presentation**
Emese Sinkó, Bio-Science Ltd.
Flow Cytometry Solutions for Extracellular Vesicle Research

15:45: **Péter Bokrossy**
Pediatric Center, MTA Center of Excellence, Semmelweis University, Budapest, Hungary
The Effect of Extracellular Vesicles Originated from Mesenchymal Cells of Peritoneal Dialysate on the Mechanism of Fibrosis

16:00: **Ákos M. Lőrincz**
Department of Physiology, Semmelweis University, St George's Hospital
Neutrophil EVs increase the viability of leukocytes and regulate the inflammation

16:15 – 16:30 – Closing remarks, group photo

16:45 – Meeting of senior scientists

HSEV Conference, June 21, 2024, Budapest, Hungary

Characterization of human red blood cell-derived extracellular vesicles (RBC-EVs)

Zsuzsanna Adamecz

Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest

Background: Extracellular vesicles (EVs) are cell-derived membrane particles. Red blood cells (RBCs) are anucleated cells without an extended intramembranous system. RBC-EVs can form under different conditions, such as during eryptosis, a process similar to apoptosis.
Our purpose is to characterise RBC-EVs, induced by different in vitro stimuli.
RBCs were isolated from EDTA-anticoagulated whole peripheral blood of healthy volunteers. Formation of RBC-EVs was induced in vitro by A23187 or cold stress, modulated by the administration of Ca2+, glucose, adenosine or 2-deoxy-D-glucose. RBC-EVs were characterized by flow cytometry, nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM).
Spontaneous formation of RBC-EVs is a time-dependent process and can be enhanced by the administration of A23187 and/or Ca2+. EV formation could be reversed by glucose and adenosine. Glucose treatment is more effective in inhibiting EV formation than adenosine, but the two substances act synergistically. This effect of inhibition of EV formation is not observed by using the glucose analogue 2-deoxy-D-glucose, suggesting that glucose has important role in RBC-EV release.
Released RBC-EVs are annexin-V positive, indicating externalized phosphatidylserine on their surface, and positive for CD235a (glycophorine-A). On the other hand they do not carry any of the tetraspanin markers CD9, CD63 and CD81. In flow cytometry, the particles are sensitive to Triton-X 100 detergent, suggesting their vesicular nature.

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Proteomic and glycomic characterization of A549 and BEAS-2B cell line-derived extracellular vesicles

Mirjam Balbisi *1),2), Tamás Langó 3), Virág Horváth 1), Zoltán Varga 4), Kinga Ilyés 4), Nikolett Nagy 3), Otília Tóth 3), Beáta G. Vértessy 3), Lilla Turiák 1)*

1) MTA-TTK Lendület (Momentum) Glycan Biomarker Research Group, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary
2) Semmelweis University Doctoral School, Budapest, Hungary
3) Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary
4) Biological Nanochemistry Research Group, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

Background: Extracellular vesicles (EVs) are lipid-bound particles released from cells that play essential roles in cell signaling and intercellular communication. Protein glycosylation impacts EV stability, cellular uptake, and interactions with target cells, thereby modulating their roles in intercellular communication. Despite significant advancements in EV research, exploring their post-translational modifications, particularly glycosylation, remains challenging and relatively underexplored.
In this study, we developed a methodology to examine the proteomic, N-glycopeptide, and chondroitin sulfate glycosaminoglycan profiles of small EVs (sEVs) obtained from A549 lung adenocarcinoma and BEAS-2B non-tumorigenic cell lines. A549 cells underwent culturing in non-completed F12 medium, while BEAS-2B cells were cultured in BEGM medium for 72 hours. The supernatant was then collected, and centrifugation and filtration steps were carried out to remove cells, apoptotic bodies and microvesicles. Small EVs were isolated by mini-size exclusion chromatography on in-house prepared columns. Microfluidic resistive pulse sensing and transmission electron microscopy were used to characterize sEV size distribution and shapes. For mass spectrometric analysis, isolated sEVs were subjected to repeated freeze-thaw cycles and digested with trypsin and chondroitinase ABC enzymes to produce peptides and chondroitin sulfate disaccharides, respectively. The peptides obtained were enriched for N-glycopeptides by acetone precipitation, while supernatant contained non-glycosylated peptides. After solid phase extraction purification, the resulting peptides, N-glycopeptides and chondroitin sulfate disaccharides were analyzed by nanoUHPLC-MS(MS).
Proteomic analysis confirmed the presence of about half of the 100 most common vesicular marker proteins, indicating high quality sEV samples. Approximately 100 N-glycopeptides per sample were identified, including glycopeptides of vesicular marker proteins, while chondroitin sulfate analysis successfully identified the most abundant disaccharides. Preliminary results highlight the importance of exploring sEV glycosylation from cancerous and non-cancerous cell lines to increase our understanding of the molecular mechanisms involved in disease progression.

Acknowledgement: Lendület (Momentum) Program of the Hungarian Academy of Sciences,
Semmelweis 250+ Excellence PhD Scholarship, Markus Ralser.

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Isolation and characterisation of extracellular vesicles from lymph nodes

Bernadett Bodnár

Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest

Background: Extracellular vesicles (EVs) are lipid membrane-enclosed small particles that are released into the extracellular environment by all cells in nature. EVs can be obtained from cell conditioned media, biofluids, or from the interstitial space of primary tissues. EV isolation from lymph nodes still remains a challenge, despite the well-described involvement of EVs in immune processes.
Here we carried out isolation and characterisation of EVs from mouse lymph nodes. C57BL/6 male mice were immunised with a stable emulsion of complete Freund's adjuvant alone or in combination with ovalbumin. The inguinal and popliteal lymph nodes were isolated on day 9. EVs were separated from lymph nodes using differential centrifugation combined with size-exclusion chromatography and were further characterised using standard EV methodologies following the MISEV2023 guidelines. Particle concentration was determined with Nanoparticle Tracking Analysis. Total protein concentration of the EV preparations was assessed with BCA assay and the total lipid content was measured with the SPV lipid assay. EV markers were analysed with a CytoFlex S Flow Cytometer. Within the medium- and small-sized EV populations, we detected CD9, CD63 and Annexin V EV surface markers.
Overall, here we present the isolation and characterisation EVs from the solid tissue of lymph nodes and the analysis of the effect immunisation on the isolated vesicles. Also, this work allows for further studies to understand immune processes and provides an opportunity to better understand the relationship between EVs and immune processes.

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The Effect of Extracellular Vesicles Originated from Mesenchymal Cells of Peritoneal Dialysate on the Mechanism of Fibrosis

Péter Bokrossy

Pediatric Center, MTA Center of Excellence, Semmelweis University, Budapest, Hungary

Background: Introduction: The literature is abundant in the topic of benefits of cell therapy in different experimental fibrosis models. Using extracellular vesicles (EVs) as an alternative to cell therapy promises benefits like lower imunnogenicity, a possible crossing of the blood-brain barrier, and not inducing acute immune rejection.
Aims: We investigated the effect of EVs originated from mesenchymal cells (MCs) of peritoneal dialysate (PDE) on the activation of primary MCs and fibroblasts.
Methods: MCs were isolated, characterised and cultured from PDE. From the serum free cultures supernatant EVs were isolated by tangential flow filtration and size exclusion chromatography. After the isolation EVs were characterized based on their particle number, size distribution, morphological features, surface markers, and the composition of cargo proteins. Their effect on fibroblast activation was tested by in vitro experiments on primary peritoneal fibroblasts (pFBs) isolated from peritoneal biopsy collected during removal of the Tenchoff catheter. The effect of EVs on the pFBs were examined by using functional assays such as MTT proliferation assay, Sirius Red assay and Transient Agarose Spot assay.
Results: The mesenchymal cells isolated in the study displayed positive expressions of CK-18, α-SMA, CD73, CD105, and CD90, while lacking the CD34, HLA-DR, CD45, and CD19 markers as indicated by immunofluorescent staining and RT-PCR analysis. The isolated EVs exhibited stem cell and CK18 positivity, implying that their original source cells were MCs that had undergone mesothelial mesenchymal transition. The EVs successfully internalised by pFBs and reduced their PDGF induced proliferation, TGF-β induced collagen accumulation and EGF induced migration as shown by various functionals assays.
Conclusion: Due to the potential antifibrotic properties exhibited by these extracellular vesicles (EVs), they could hold therapeutic promise. Nevertheless, further in vivo testing is required to substantiate this hypothesis.
Funding: NKFIH K-142728, K-131594; 2020-1-1-2-PIACI-KFI_2020-00021, Semmelweis University, TKP2021-EGA-24, TKP2021-EGA-31, RRF-2.3.1-21- 2022-00003; HUN-REN, ELKH-POC-2022-024, Development and Innovation Fund, ÚNKP-23-3-I-SE-36, ÚNKP-23-3-I-SE-42, ÚNKP-23-4-II-SE-29, ÚNKP-23-5-SE-15; Hungarian Academy of Sciences, János Bolyai Research Scholarship.

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Extracellular vesicle based comparative analysis of highly and low invasive diffuse midline glioma H3K27M-mutant

1,2,3Tímea Böröczky, 1,2Mária Harmati, 1,2,3Gabriella Dobra, 1,2,3Mátyás Bukva, 1,2Edina Gyukity-Sebestyén, 4Marie-Anne Debily, 4Clémentine Barry, 1,2Krisztina Buzás

1HUN-REN Biological Research Centre, Szeged, Hungary; 2Department of Immunology, University of Szeged, Szeged, Hungary; 3Doctoral School of Interdisciplinary Medicine, University of Szeged, Szeged, Hungary; 4Vectorologie et Nouvelles Thérapies Anticancéreuses, Gustave Roussy, Université Paris-Saclay, France

Background: Introduction: Diffuse midline gliomas (DMG) are the most frequent and aggressive forms of brain tumor among young individuals, with a very low 12-month survival rate. The success of surgical procedures and radiotherapy is hindered by the tumor's peritumoral infiltrative nature and its tendency to form metastases. Since the latter can vary significantly among different patients and may be linked to the invasive capacity of tumor cells, our goal is to develop an alternative method suitable for estimating invasive capacity, which could facilitate therapeutic decision-making.
Methods: 3D cultures (tumoroids) were created from tumor cells of 5 low and 4 high invasive patients. Cells were allowed to form gliomaspheres and they were embedded in a Matrigel basement membrane matrix. The small extracellular vesicles (sEV) were isolated from the supernatants using size exclusion chromatography. The particle number of the samples was determined using Nanosight NS300, and their molecular fingerprint was analyzed using Raman spectroscopy.
Results: The production of sEVs from low invasive tumoroids was significantly higher compared to the high invasive ones, and the two groups could be well distinguished based on the Raman spectra of sEVs.
Conclusion: Our Raman-based investigative approach, using vesicles isolated from patient-derived tumoroids, proved promising for distinguishing between low and high invasive DMGs. However, further sample addition is necessary to validate this method.
Funding: EUGLOHRIA Research seeding grant 101017572

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Initial steps to follow cancer-induced cardiac atrophy in an in vivo model

Edina Bugyik

Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest

Background: Tumor anorexia-cachexia syndrome is a multifaceted condition characterized with decreased skeletal muscle mass, generalized weakness, diminished quality of life, and poor prognosis. It occurs in 50-80% of cancer patients and is estimated to be responsible for 20-30% of all cancer-related deaths. Although cardiac atrophy is a significant component of this syndrome, early detection of cardiac changes is still an unmet clinical need due to the focus on treating the underlying disease and limitations in diagnostic methods.

The C26 colorectal adenocarcinoma primary tumor model was used to develop tumor anorexia-cachexia syndrome in vivo. The animals' weight, tumor size, heart weight and tibia length were recorded upon termination. Platelet-free plasma (PFP) and extracellular vesicles (EV) were isolated from the blood samples. Wheat Germ Agglutinin (WGA-Alexa-488) labeling was applied for histology. Mass spectrometry measurements were performed from the tumor tissue of diseased animals, from the hearts of healthy and diseased animals, as well as from PFP and EV samples.

The body weight and heart weight of the control and tumor groups showed a significant ($p<0.05$) difference. We confirmed a decrease ($p<0.05$) in the size of cardiomyocytes in the tumor group. A total of 6,969 proteins were detected during the proteomic measurements. We found differentially expressed proteins between the control and tumor groups in the various samples. There were also proteins identified exclusively in the control or tumor group (ON/OFF). The differentially or exclusively identified proteins are mainly involved in the acute phase reaction, immunity (with an excess of neutrophil function), metabolic and cell adhesion processes, apoptosis and cytoskeletal organization.

These findings provide valuable insights into the molecular changes associated with tumor-induced cardiac atrophy. Further exploration of these proteins may lead to the development of specific biomarker panel for early detection and intervention.

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Extracellular vesicles as carriers of tumor-associated protein patterns – a meta-analysis using machine learning methods

Mátyás Bukva

Lendület Laboratory of Microscopic Image Analysis and Machine Learning, Biological Research Centre, Szeged

Background: The omics analysis of extracellular vesicles (EVs) can significantly contribute to the identification of tumor markers and the comprehensive characterization of cancerous conditions. The evolving and increasingly popular machine learning methods and public databases create opportunities to integrate results from independent EV research studies, mapping the role of EVs in tumor processes.
Objective: The objective of this meta-analysis is to investigate the tumor specificity of the proteome transported by EVs using proteomic and cell physiological data from multiple studies.
Methods: During the meta-analysis, we examined the proteome of EVs isolated from the supernatant of the National Cancer Institute 60 (NCI60) cell line panel, as well as the invasive and proliferative capacity of the cell lines. The NCI60 cell line panel includes 60 cell lines across 9 tumor types. The specificity and classification efficiency of the proteome were determined using logistic regression with k-fold validation. The proteins playing the most significant role in differentiation, as well as the protein patterns predicting invasive and proliferative capacity, were selected using the LASSO method. The biological processes and pathways influenced by the proteome were determined using Reactome software.
Results: Based on the 5908 proteins identified in the vesicles isolated from the supernatant of the 60 cell lines, we distinguished samples belonging to the 9 tumor types with an average classification efficiency of 70%. Out of the 5908 proteins, 172 were found to be exceptionally important in the simultaneous differentiation of tumor types. Based on the selected proteins, we achieved a classification accuracy of 97%. The Reactome pathway analysis highlighted tumor-specific biological pathway patterns. The invasive and proliferative capacity was predicted with specific protein patterns with an efficiency of $R2=0.73$ and $R2=0.63$ ($p<0.0001$).
Conclusion: EVs are not only sources of highly specific molecular patterns, but they can also help determine tumor-specific biological pathways and predict invasive and proliferative capacity.

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Identifying Extracellular vesicle-miRNAs with a possible role in ionizing radiation induced leukaemogenesis, and their EV packaging mechanisms in the bone marrow

Ilona Barbara Csordás[1,2], Tünde Szatmári[1], Eric Andreas Rutten[3], Tamás Visnovitz[4], Katalin Lumniczky[1]

1 Unit of Radiation Medicine, Division of Radiobiology and Radiohygiene, National Centre for Public Health and Pharmacy
2 Semmelweis University Doctoral School, Patological and Oncological Division
3Centre for Radiation, Chemical and Environmental Hazards, UK Health Security Agency
4 Semmelweis University, Faculty of Medicine, Department of Genetics, Cell- and Immunobiology,

Hematological malignancies identified as the primary long-term consequences of bone marrow (BM) irradiation, may be initiated by ionizing radiation (IR) induced BM-stem and progenitor cell damage, and altered intercellular signaling. One way of intercellular signaling and transmitting IR-induced damages between cells is through extracellular vesicles (EVs). We have previously shown in vivo that BM-EVs from irradiated mice initiate radiation induced alteration is non-irradiated naïve mice with the contribution of EV-miRNAs. To further understand EV-mediated IR effects on BM microenvironment after irradiation, EV-cargo and it's packaging were analyzed.
RNA-binding proteins (RBP) and miRNAs were analyzed in the BM and BM-derived-EVs of CBA mice isolated 24h after IR (0.1Gy,3Gy). Among the deregulated EV-miRNAs, AML, cellular senescence and inflammation related miRNAs were identified and linked to RBP through sequence motif analysis, interactions were validated with immunoprecipitation and qPCR.
More than half of the EV-miRNAs associated with AML/senescence/inflammation were linked to three RBPs: Anxa2, hnRNpQ and hnNRPA2b1. Among them, hnRNPQ and hnRNPA2b1 expression altered upon IR. HnRNPA2b1 exhibited upregulation in the BM but downregulation in BM-EVs; the miRNAs carrying recognition motifs of hnRNPA2b1 mirrored the quantitative changes of their binding partner in EVs. We established the specific binding of miRNAs to hnRNPA2B1 through their binding motifs, as miRNAs lacking these motifs did not co-precipitate with the protein.
In summary, the miRNA content of BM-EVs following IR exposure results from a selective sorting and packaging mechanism. One observed mechanism through which IR influences this process is by deregulating RBPs. Selectively packaged EV-miRNAs associated with AML or related pathways contribute to AML development by influencing the BM microenvironment.

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Characterization of mast cell-derived extracellular vesicles during degranulation

Kelsey Fletcher

Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest

Background: Characterization of mast cell-derived extracellular vesicles during degranulation

Kelsey Fletcher, Tamás Visnovitz, Péter Lőrincz, Tünde Bárkai, Dorina Lenzinger, Edit I. Buzás, Krisztina V. Vukman.
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Mast cells (MCs) play a crucial role in allergic reactions and are implicated in numerous diseases, where degranulating MCs are involved in inflammatory reactions including atherosclerosis. Beyond the well documented release of cytokines, degranulating MCs also secrete extracellular vesicles (EVs). However, despite extensive research in the area, the differentiation of EVs and granules from one another remains poorly explored. Therefore, this project aims to characterise and differentiate MC-derived EVs from extracellular granules released during degranulation providing a deeper understanding of their role in diseases. This knowledge could spark the development of novel diagnostic assays, therapeutic targets and improved methodologies.
EVs were isolated from bone marrow derived murine MCs from both wild type and GFP-expressing transgenic mice with differential and density gradient centrifugation and were investigated using Flow cytometry, confocal microscopy, TEM and biochemical assays. MCs were stimulated with calcium ionophore or DNP/IgE. Cell activation and recovery was monitored by beta-hexosamainadase, proliferation and viability assays.
We established a model system to monitor real time EV production during degranulation by flow cytometry and confocal microscopy. Our findings indicate that during degranulation MCs secrete a heterogeneous particle population. These particles exhibit variations in size, concentration and detergent sensitivity suggesting a simultaneous production of EVs, extracellular granules and membrane fragments. Furthermore, the composition of the secreted corpuscles depends on the stimuli used to induce degranulation. These results highlight the challenges and critical importance of distinguishing MC-derived EV secretion and degranulation from one another while emphasizing the necessity for precise characterization in future research.

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Unravelling the origins and heterogeneity of extracellular vesicles and nanoparticles: Insights from advanced characterization techniques

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Background: Research on extracellular vesicles (EVs) has revealed their highly heterogeneous nature and significant potential in diagnosing and treating various diseases. Despite extensive studies on EV biogenesis, the origin of small-size EVs (sEVs) remains debated. There is evidence sEVs may derive directly from the plasma membrane (ectosomal origin) or from intraluminal vesicles (ILVs) within multivesicular bodies (MVBs). This study aims to address this important knowledge gap using advanced techniques to differentiate between EV subpopulations and extracellular nanoparticles (EPs). We analyzed 145 articles, selecting 22 with relevant transmission electron microscopy (TEM) data, and found that ILVs averaged 115 ± 25.8 nm in diameter. TEM analysis of three cell lines (EBC-1, Hek293T, HL-1) revealed similar diameters of 125 ± 32 nm, 118 ± 22.1 nm, and 132 ± 28 nm, respectively. Subsequent isolation of lEV subpopulations from Hek293T cells showed significant size differences compared to ILVs, suggesting that lEVs do not originate from ILV. However no significant size difference was observed between sEV and ILVs. Morphology, size distribution, concentrations, biomarkers, and protein to lipid ratio for EVs and EPs were characterised using TEM, nanoparticle tracking analysis (NTA), zeta-potential, single-particle interferometric reflectance imaging sensor (SP-IRIS), total protein assay and lipid assays. Protein-to-lipid ratio distinguished EPs from EVs, with EPs showing significantly higher ratios. Raman spectroscopy further discriminated 100k sEVs from 167k EPs, highlighting differences in protein and lipid composition. Marker analysis by high-resolution flow cytometry and direct stochastic optical reconstruction microscopy (dSTORM) revealed no exclusive markers for EV subpopulations, though distribution patterns provided insights into their origins. Lipidomic analysis identified unique lipid signatures for EPs, particularly the enrichment of Cholesteryl Ester (CE) in 167k EPs.

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Decoding the Post-COVID syndrome: leveraging molecular signatures of extracellular vesicles for predictive analysis

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Background: Introduction: The post-COVID-19 condition (also known as long COVID) refers to long-term symptoms that some people experience more than 12 weeks after recovering from COVID-19. These symptoms may persist from the onset of their initial illness, develop after their recovery, disappear over time and then recur. We hypothesized that the molecular pattern of small extracellular vesicles (sEV) found in the plasma of patients during acute SARS-CoV-2 infection could predict the course of COVID-19 disease, the risk of developing post-COVID syndrome, and its symptoms.
Methods: We conducted a detailed investigation involving 60 volunteers, from whom we isolated - by size exclusion chromatography - small extracellular vesicles (sEVs) from serum samples collected during their acute phase of COVID-19 infection. Based on the symptoms after COVID-19, we perform hierarchical clustering classifying the patients into 3 groups. Subsequent analyses are carried out on the 3 groups: i) a group exhibiting a wide range of symptoms cumulatively, ii) a group manifesting a limited set of symptoms, and iii) a group showing no residual symptoms post-infection. Following the characterization of sEV samples, we employed advanced Raman spectroscopic measurement, liquid chromatography-mass spectrometry (LC-MS) analysis and enzyme-linked immunosorbent assay (ELISA) to examine their properties.
Results: The Raman spectra of the 3 patient groups showed different characteristics allowing us to draw predictions over the potential complications. According to a proteomic analysis of sEVs, more than 25 proteins were significantly enriched in the two patient groups exhibiting post-COVID symptoms compared to the symptom-free control group. These proteins include some members of the complement system, such as C2, C4, C5, and C1 inhibitors.
Conclusion: As the protein composition of sEVs isolated from serum collected during acute SARS-CoV-2 infection shows a significant difference between patients exhibiting post-COVID symptoms and those who are asymptomatic, plasma EV analysis might be suitable for forecasting the post-COVID syndrome and may help the patient care.

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Reduced CD63+ extracellular vesicles associate with hypercholesterolaemia in mice and humans

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Background: The association and co-isolation of low-density lipoproteins (LDL) and extracellular vesicles (EVs) have been shown in blood plasma. Here we study the consequence of this association and of EVs in atherogenesis.
Wild type (WT), PCSK9−/−, and LDLR−/− C57BL/6J mice were used in this study. Eleven week-old male mice were fed high-fat diet (HFD) for 12 weeks or kept on normal diet until old age (22-months). Cardiac function was assessed by ultrasound, cholesterol was quantified with a commercial kit, and circulating EVs were measured by flow cytometry. Plaques were analysed post-mortem using Oil-Red-O staining of the aortic arch. EVs were measured from platelet free blood plasma of patients with normal cholesterol or hypercholesterolaemia. Based on annexin V and CD63 staining, we found a significant increase in EV levels in LDLR−/− and PCSK9−/− mice after HFD, but CD81 showed no significant change in either group. There was no significant change in plaque formation after HFD. PCSK9−/− mice show a favourable cardiac function after HFD. Blood cholesterol levels progressively increased during HFD, with LDLR−/− mice always elevated whereas PCSK9−/− were maintained lowered cholesterol compared to WT animals. In old age mice, similar cholesterol levels were observed to that of 11-week old animals. At old age, ejection fraction was decreased in all groups of mice, as were CD63+ and annexin V+ EVs. Here, LDLR−/− mice showed significantly increased plaques. Patients with hypercholesterolaemia showed significantly lower CD63+ EVs.
This research demonstrates an inverse relationship between circulating EVs and cholesterol, making EVs a potential marker for cardiovascular disease (CVD). HFD causes reduced cardiac function, but atherosclerotic development is slow progressing, even in a hypercholesterolaemic model and only observed with old aged animals. These results also bring further evidence for the benefit of using of PCSK9 inhibitors as therapeutic agents in CVD.

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Role of extracted Ginger Extracellular Vesicles-Like Particles

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Background: Extracellular vesicles (EVs) released universally by cells are of considerable interest because of being potential vectors of the active ingredients in targeted therapies. Some EVs also have a therapeutic effect by themselves without modification. In recent years, plant-derived EVs have been in the center of attention, as they can be produced in a stable, standard and sustainable way. In addition, they are biocompatible and they show a wide range of favourable properties. Ginger root is known to have many beneficial effects. These include among others, anti-inflammatory and anti-carcinogenic effects, and its antioxidant role is also well documented. Several studies attribute a central role to ginger-derived EVs in the effects of ginger.

In the framework of the project, ginger extracellular vesicle-like particles are isolated and characterised by nanoparticle tracking analysis (Zetaview) to determine the size distribution and concentration of these particles. Micro-BCA and SPV Lipid assays are performed to determine the protein and lipid contents, respectively. Also, transmission electron microscopy is carried out to characterize the vesicle-like particles. Ginger root-derived extracellular vesicle-like particles are compared to mammalian counterparts (mammalian extracellular vesicles) in their basic parameters. Finally, experimental systems are set up to investigate the effect of ginger-derived extracellular vesicle-like particles on H9C2 cardiomyocytes.

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Effect of Hypercholesterolemia on circulating and cardiomyocyte-derived extracellular vesicles

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Background: Hypercholesterolemia (HC) induces, propagates and exacerbates cardiovascular diseases via various mechanisms that are yet not properly understood. Extracellular vesicles (EVs) are involved in the pathomechanism of these diseases.
Aim: To assess whether and how HC affects circulating and cardiomyocyte (CM)-secreted EVs and how can these changes affect the myocardium.
Methods: Circulating EVs were isolated with density gradient ultracentrifugation followed by size exclusion chromatography using Vezics system from male Wistar rats fed with high-cholesterol or control chow. Plasma and EV metabolome was analyzed using a Biocrates MxP Quant 500 kit. AC16 human CMs were treated with Remembrane HC supplement and EVs were isolated from cell culture supernatant with ultracentrifugation. Samples were analyzed with nanoparticle tracking analysis, and atomic force microscopy. Monocyte activation was measured in THP1-ASC-GFP cells after treatment with AC16-EVs. AC16-EV proteomics was measured with liquid chromatography-tandem mass spectrometry.
Results: HC diet induced hyperlipidemia in rats and reduced the amount of certain phosphatidylcholines in circulating EVs. Furthermore, plasma EV metabolome showed only minor correlation with that of the plasma. HC treatment significantly increased EV secretion of AC16 CMs but did not affect their elasticity and total phosphatidylcholine concentration. AC16 EVs, regardless of the treatment, did not induce the activation of THP1 monocytes. HC treatment modified AC16 EV metabolome greatly, with 77 enriched proteins and 33 proteins with decreased abundance. No specific enrichment or reduction of any well-defined molecular pathways was identified among the dysregulated proteins, however some these proteins contribute to tissue remodeling.
Conclusions: HC greatly affects metabolome of EVs. Furthermore, it induces secretion and modifies proteome of CM EVs. These changes do not affect monocyte activity and may contribute to HC-induced cardiac remodeling.

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In vitro effects of cyclodextrins on extracellular vesicle secretion in melanoma

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Background: Introduction: Cyclodextrins (CDs) are cyclic oligosaccharides known for their ability to encapsulate lipids and apolar drug molecules due to their toroidal structure. Additionally, CDs interact with membrane lipids and can influence extracellular vesicle (EV) trafficking, thereby affecting intercellular communication in tumor cells. Various CDs, differing in the number of glucose units and substitution patterns, are extensively utilized in research and pharmaceutical applications.
Methods: Ten different CDs were evaluated for their impact on the viability of a syngeneic melanoma cell pair in the presence of EV-containing or EV-depleted FBS using the SRB assay. Furthermore, the effect of CD treatment on EVs release was examined. EVs from CD-treated melanoma cells were collected after 72 hours of incubation and isolated by differential centrifugation. Their characteristics were analyzed using NTA for median particle size and total particle amount, Qubit Protein Kit for protein content, and SPV assay for lipid content.
Results and Conclusion: Consistent with their syngeneic origin, the two examined cell lines exhibited only modest differences in their sensitivity to CD treatment. Different cyclodextrins exhibited varying effects on cell lines, with methylated cyclodextrins demonstrating a more pronounced impact on cell viability. Most of the investigated CDs demonstrated a more significant inhibitory effect on cell proliferation when examined in media containing EV-depleted FBS. The lipid content of the EV fraction was notably reduced upon CD treatment, except for hydroxypropyl-substituted CDs. However, neither the quantity nor the median size of the isolated EVs was considerably affected by CD treatment. These findings suggest that CDs could play a beneficial role in oncological therapies targeting EV-mediated intercellular communication.

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Microfluidic-based method to analyze extracellular vesicles in liquid biopsies

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Background: Introduction: The quantity and composition of extracellular vesicles (EVs) in human blood can vary based on the patient's health status, reflecting physiological and pathological conditions. Microfluidic techniques might offer a precise and cost-effective method for analyzing these vesicles. By exploiting the diffusion characteristics of particles — such as shape, size, and density — a fingerprint of EV-fraction can be yielded using microfluidic methods. Moreover, microfluidic channels enable efficient handling of small sample volumes and high-throughput analysis, making them suitable for clinical applications.
Methods: Plasma was isolated from human blood samples of breast, prostate, and colorectal cancer patients using multi-step centrifugation. The microsomal fraction was separated using SEC columns. The isolated EVs were characterized by DLS and flow cytometry, and the protein and lipid content were quantified. The samples were subsequently stained with Atto-488 NHS ester, a protein dye according to the established protocol. The labeled samples were introduced into a microfluidic channel, and the distribution of the green fluorescent signal across the channel diameter was analyzed using consecutive fluorescence microscopy images.
Conclusion and future perspectives: Our measurements revealed that the intensity curves of samples from patients with varying oncological statuses exhibited distinct changes within the straight microfluidic channel during analysis. This finding indicates that microfluidic channels can detect variations in the size distribution of extracellular vesicle (EV) fractions in blood samples, demonstrating the sufficient resolution of microfluidic techniques for examining these samples. Furthermore, the use of a spiral channel achieved a higher degree of separation. Consequently, our future research will focus on optimizing this measurement technique, testing alternative channel designs to enhance separation efficiency, and improving the AI tools used for data analysis.

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"Torn bag" release of small extracellular vesicles via limiting membrane rupture of amphiectosomes

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Background: Extracellular vesicles (EVs) are mostly spherical membrane-bound particles found in the extracellular environment. They play an important role in intercellular communication, have antigen presenting functions and maintaining cellular homeostasis. Recent studies revealed an unexpected complexity of EV biogenesis.
Our research focused on a recently observed EV secretion pathway, the release of large EVs containing small intraluminal vesicles. We aimed to investigate the origin of these multivesicular large EVs (MV-lEVs), determine whether the release of this EV subtype was a unique or general phenomenon, and identify specific protein markers.
Confocal microscopy, transmission electron microscopy, super-resolution live cell imaging and Western blot analysis were used. The detection of the MV-lEVs was made possible by our de novo in situ fixation method. We characterized the MV-lEVs through classical (CD63, CD81, ALIX, TSG101) and non-conventional (LC3, Rab7, TSPAN4) protein markers, also different treatments have been used to influence the dynamics of their biogenesis.
MV-lEVs were present in all tested experimental systems. The MV-lEV production was sensitive to our treatments that disrupted the cytoskeleton, endo-lysosomal pathways, and autophagy processes. The studied MV-lEVs were different from migrasomes based on their morphology, biogenesis and protein markers.
The spontaneous rupture of the limiting membrane of the secreted MV-lEVs allowed the release of the intraluminal small EVs (sEVs) into the extracellular environment via a "torn bag mechanism." Based on their intracellular origin and release mechanism, we designated them “amphiectosomes”.
Our model suggests that during amphisome formation, the inner LC3-positive membrane of autophagosomes fragments and forms vesicles. These intraluminal sEVs are then secreted into the extracellular space through the ”torn bag mechanism”. This sEV formation is different from the previously described secretion pathways. This new secretion pathway expands our understanding on the EV field and open up new opportunities for research into the roles of EVs in health and disease.

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Neutrophil EVs increase the viability of leukocytes and regulate the inflammation

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Background:
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Introduction: Extracellular vesicles (EVs) are released by every known cell type, representing a novel mode of intercellular communication. In a previous study, we characterized three distinct populations of EVs derived from neutrophils (PMNs): spEVs produced by resting PMNs, apoEVs generated during apoptosis, and ozEVs induced by opsonized zymosan particles. Now we explore the effects of these three EV populations on the viability of leukocytes, cytokine and reactive oxygen species (ROS) production and the phagocytosis of PMNs and monocytes.
Methods: Human PMNs were either stimulated with opsonized zymosan or left unstimulated for 20 minutes. Another set of PMNs was incubated under unstimulated conditions for 24 hours. Medium-sized EV fraction was pelleted through differential centrifugation and filtration. EVs derived from these distinct cell fractions were then co-incubated with monocytes, lymphocytes, and neutrophils. Viability of untreated and EV-treated leukocytes was assessed by measuring annexinV and propidium iodide positivity using flow cytometry and LDH assay. Phagocytosis was measured by flow cytometry, while ROS production was monitored through chemiluminescence measurements. IL-8 and TGF-beta production were quantified using ELISA.
Results: PMN-EVs enhanced the viability of lymphocytes, monocytes and macrophages, with no change in neutrophil viability. EV treatment did not influence the phagocytosis. SpEVs inhibited the ROS production of PMNs and monocytes, whereas ozEVs increased ROS production of PMNs. Additionally, we observed divergent effects on cytokine production. SpEVs notably elevated the anti-inflammatory TGF-beta production, while ozEVs increased the pro-inflammatory IL-8 secretion.
Conclusion: We propose that PMN-derived EVs are tailored to specific conditions, potentially yielding similar or even antagonistic effects.
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Anti-inflammatory role of endothelial colony forming cells-derived extracellular vesicles

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Background: Introduction: Cardiac dysfunction in sepsis is a well-established determinant of heightened mortality. Cardiomyocytes and endothelial cells (ECs) are the predominant cellular constituents within the myocardium, essential for both cardiac remodeling and myocardial regeneration. Endothelial Colony-Forming Cells (ECFCs), characterized by progenitor properties, possess an augmented capacity for neovascularization within ischemic tissues. Recent research has demonstrated that ECFCs secrete extracellular vesicles (EVs), which act as potent intercellular regulators and facilitate neovascularization in ischemic environments. EVs offer several advantages over traditional cell-based therapies in regenerative medicine, including non-tumorigenic potential, the ability to cross biological barriers for targeted delivery, cell-specific bioactive molecule cargo, preservation of their cargo's integrity, and consistent functional performance.
Objectives: This study aimed to characterize ECFCs and their derived EVs. Additionally, we examined the anti-inflammatory effects of ECFC-derived EVs on neonatal cardiomyocytes (NCMCs) pre-treated with lipopolysaccharide (LPS) and assessed the therapeutic benefits of ECFC-EV administration in a sepsis model.
Methods: ECFCs were isolated from CDH5Cre/mTmG mice. The GFP+ ECFCs were obtained from bone marrow and cultured in supplemented EBM-2 medium for several weeks. EVs were harvested from the conditioned medium of ECFC cultures using differential ultracentrifugation (dUC). Flow cytometry was employed to identify both ECFCs and their EVs. In-vitro, ECFC-EV mediated effects on cardiomyocyte were measured in NCMC cells. Expression of Il-1β, IL-6, TNFα, CCL-2, CCL-11 was assessed by qPCR, IL-6 and troponin were detected by ELISA. In the sepsis model, the effect of administration of ECFC-EVs was measured by immunophenotyping peripheral blood cells (PBCs).
Results: ECFCs exhibited endothelial progenitor markers CD31, CD34, and VEGFR2, while ECFC-EVs expressed CD31 and classical vesicular markers such as CD63 and Annexin V within the GFP gate. GFP+ ECFC-EVs were internalized by LPS-treated NCMCs. Treatment with ECFC-EVs for 24 hours significantly reduced LPS-induced Il-1β expression in NCMCs. In vivo, the number of endothelium-derived vesicles increased following LPS injection, and peritoneal administration of ECFC-EVs four hours after LPS injection moderated the reduction in PBCs counts.
Conclusion: ECFCs can be efficiently maintained in primary culture and identified by specific markers and GFP expression, producing EVs at concentrations ranging from 1*10^10-11/mL. The anti-inflammatory properties of ECFC-derived EVs have been validated in both in vitro and in vivo models, highlighting their potential therapeutic role in sepsis-induced cardiac dysfunction.

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Changes in the miRNA cargo of EV-mediated fetal-maternal communication following light treatment

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Background: Introduction: Light exposure of embryos during assisted reproduction affects embryo quality and implantation capacity in a wavelength dependent manner. We investigated the molecular mechanism of light-induced changes through the comparative analysis of gene expression and regulatory miRNA profile of murine embryos cultured in dark environment or exposed to white / red filtered light. miRNA sequencing was used to assess the role of embryo-derived extracellular vesicles in the endometrium-embryo dialogue.
Aims: Analysis of the miRNA patterns of embryo-derived EVs after light exposure.
Methods: In vitro cultured mouse embryos (3.5 dpc) were exposed to white or red filtered light. After 24 hours, the miRNA content of embryo-derived extracellular vesicles were isolated and RNA-sequencing was performed. Differential expression analysis and functional enrichment analysis were used for evaluating the transcriptome results.
Results: Embryo-derived extracellular vesicles wavelength-dependently enclosed unique miRNA cargos the target genes of which play a role in embryo implantation. We have found many significantly changed miRNA within the embryo-derived extracellular vesicles, independent from the embryonic miRNA. Within the white light treated group, miRNA targeting genes in cell signaling and extracellular matrix reorganization were downregulated.
Meanwhile following the treatment with filtered red light, there were significant elevations in levels of miRNA, targeting genes, that play a role in the implantation of embyros. Furthermore there were significant changes in miRNA targeting immunological signaling pathways, namely Fc gamma signaling, DAP12 signaling and inflammatory signalization.
Conclusion: Extracellular vesicles of light-exposed embryos play a role in blastocyst-decidua communication through the horizontal transfer of regulatory miRNAs. Our data prove that light exposure during in vitro fertilization modifies cell function that might affect the outcome of implantation.

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Neutrophil-derived extracellular vesicles modulate the inflammatory response of monocytes and macrophages

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Background: Introduction: Extracellular vesicles (EVs) are released by every known cell type, their ability to transfer bioactive molecules to recipient cells make them crucial mediators of intercellular communication. Our laboratory formerly characterized three different types of neutrophil- derived (PMN) extracellular vesicles: released spontaneously, generated upon opsonized zymosan treatment and produced by apoptotic cells. Our previous experiments showed that these PMN-EVs exert either anti- or proinflammatory effects on neutrophils.
Aims: We investigated the effect of PMN-EVs on the viability and the effector functions of monocytes. The impact of PMN-EVs on monocyte-macrophage differentiation and migration ability was also examined.
Methods: Neutrophils and monocytes were isolated from human blood, macrophages were differentiated via M-CSF. Spontaneously (spEV), upon opsonized particle stimulation (oZ-EV) and during apoptosis (apoEV) generated EVs were isolated by two-step centrifugation and filtration. EVs effect on viability was observed by flow cytometry (FC) and LDH cytotoxicity assay. Monocytes ROS production was monitored by lucigenin assay, cytokine production was measured by ELISA and CD11b expression were determined by FC. The macrophage differentiation was investigated by FC and fluorescent microscopy, the migration was measured by scratch assay.
Results: Monocyte viability was significantly improved by apoEV. SpEV significantly delayed monocytes' ROS production and reduced LPS-induced IL-8 production. A significant CD11b expression level decrease was occurred on spEV-treated monocytes, which can explain the observed effects. SpEV and apoEV significantly increased the number of differentiated macrophages and apoEV treatment led to an increase in migration capacity.
Conclusions: Neutrophils are key players in inflammation. Pro-inflammatory PMN-EVs regulate processes in the early phase of inflammation. During resolution phase, PMNs interact with monocytes and macrophages to promote tissue homeostasis by releasing anti-inflammatory EVs.

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Extracellular vesicles promote migration despite BRAF inhibitor treatment

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Background: Introduction: Extracellular vesicles (EVs) play a critical role in metastasis formation and the development of resistance in cancer. Melanoma, the deadliest form of skin cancer, is characterized by its high metastatic potential and ability to develop resistance to targeted therapies. Approximately half of melanoma patients harbor BRAF mutations, making BRAF-targeted therapies (e.g., vemurafenib, dabrafenib) common in clinical practice. Despite initial positive responses, resistance often develops, frequently due to sustained MEK phosphorylation. Consequently, MEK inhibitors (MEKi) are often used in combination with BRAF inhibitors (BRAFi). In our previous experiments, we investigated the impact of EVs on cancer cell migration, a crucial early step in metastasis formation. This study further examines the effects of BRAFi (vemurafenib, dabrafenib), MEKi (trametinib), and their combination on the migration-promoting effect of EVs.
Methods: For our investigation syngeneic cell line pairs were used, and their vemurafenib, dabrafenib, trametinib, and the combined dabrafenib and trametinib sensitivity were evaluated by SRB cell viability assay. EVs were isolated from each cell line with ultracentrifugation. The isolates were utilized to assess the EVs' capability to transfer resistance and their potential to mitigate the effects of inhibitors on cell migration. The cells' migratory capacity was assessed using mean squared displacement (MSD) and total traveled distance (TTD) parameters obtained from video microscopy and single-cell tracking.
Results and Conclusion: EVs from resistant cell lines more effectively mitigated the inhibition induced by BRAF inhibitors compared to EVs from sensitive cell lines. In contrast, trametinib and the combination of dabrafenib and trametinib successfully neutralized the migration-promoting effect of EVs. However, it should be taken into consideration that the cell lines were isolated from patients treated only with BRAFi and were not acclimatized to MEKi therapy. In summary, our findings enhance the understanding of resistance mechanisms to targeted therapies.

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Surface marker detection of extracellular vesicles isolated from pleural fluids of NSCLC, MPM and pleuritis patients

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Background: Introduction: Extracellular vesicles (EVs) carry the molecular signatures of their cells of origin and are present in all body fluids, including pleural fluid. Which makes them potential prognostic and predictive biomarkers. Malignant pleural mesothelioma patients (MPM) face a poor prognosis, with approximately 5% 5-year survival rate, largely due to late diagnosis and the difficulty in distinguishing between benign and malignant transformations. Consequently, developing reliable diagnostic methods is crucial for improving mesothelioma care.
Methods: EVs were isolated from the pleural fluids of 29 non-small cell lung cancer (NSCLC), 27 MPM and 26 pleuritis patients using SEC and ultrafiltration. TEM was used to visualize the EVs, and the presence of CD63, CD9, and CD81 was confirmed by nFCM. Total particle number, measured by NTA, and total protein amount, analyzed using the Qubit protein assay, were compared across the three patient groups. Surface markers of the isolated EVs were studied using the MACSPlex EV kit with flow cytometry.
Results: Total particle and protein amounts of the isolated EVs did not differ significantly among the patient groups. However, 3 out of the 37 markers included in the MACSPlex assay showed significant differences. The CD326 (EpCam) signal was significantly higher in NSCLC patients compared to those with MPM and pleuritis. Conversely, MCSp was elevated in the MPM isolates compared to NSCLC isolates. Furthermore, CD44 effectively distinguished MPM patients from NSCLC and pleuritis patients.
Conclusion and Future Perspectives: EVs isolated from pleural fluids could serve as novel diagnostic tools for MPM. CD44 is already regarded as a promising marker for MPM, and our study further confirms its significance in this disease. To achieve a more comprehensive evaluation, the measured signals of all biomarkers will be analyzed in conjunction with detailed clinical histories of the patients using AI-based tools.

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Extracellular vesicles and experimental dermatology – An almost empty niche to fill

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Background: Skin is a vital organ, and its diseases are among the most frequent human pathological conditions. By using molecular and cellular physiology approaches, our group mainly focuses on the investigation of the pathophysiology of the pilosebaceous unit (acne, skin dryness, hair growth disorders, etc.) as well as certain inflammatory skin conditions (e.g., atopic dermatitis). Our key goal is to unveil so-far hidden aspects of the physiological regulation of cutaneous homeostasis, with a special emphasis on the role of the complex cannabinoid signaling (i.e., the "cannabinoid" system), mitochondrial biology, as well as extracellular vesicle (EV)-mediated intercellular communication.
In our experiments, we usually follow a stepwise approach, namely, we investigate basic phenomena first in cell lines, and then in more complex model systems, including primary human cells (derived from healthy individuals as well as from patients), reconstructed 3D human skin equivalents, as well as different organ cultures (i.e., hair follicle and full-thickness human skin organ cultures). Among others, changes in viability, proliferation, lipid production, intracellular ion homeostasis, ROS generation, gene expression (Q-PCR, western blot, immunolabeling; fluorescent, confocal and superresolution microscopy followed by quantitative immunohistomorphometry), and mediator release (ELISA) are monitored. We routinely influence gene expression by siRNA-mediated selective gene silencing, while in case of genomic and lipidomic studies, we rely on the expertise of our collaborators.
Although we have several well-established model systems to study key cellular aspects of several skin diseases (e.g., acne), and we are capable of isolating EVs, we currently lack the tools for appropriate characterization of the vesicles, and our EV-yield is rather low. Thus, we hope to build up mutually beneficial collaborations with the members of the HSEV community by investigating the putative cutaneous effects of EVs isolated and characterized by others as well as by providing EVs isolated in our skin-relevant model systems.

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Exploring the anti-skin cancer potential of copper-tin nanocomposites-loaded exosomes via promotion of apoptosis and cell cycle arrest

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Background: This study aims to explore the efficacy of Cu/Sn nanocomposites loaded into exsosomes against skin cancer A431 cell line. CuS/SnS nananocomposites (S1, S2, S3) were synthesized and characterized, then loaded into exosomes (Exo) isolated from bone marrow-derived mesenchymal stem cells (S1-Exo, S2-Exo and S3-Exo) and characterized again. Afterthat, the loaded samples were investigated in vitro against A431 using cytotoxicity, apoptosis, and cell cycle assays. CuS/SnS nanocomposites were indexed to hexagonal CuS structure and orthorhombic α-SnS phase and showed nanorod shape. The exosomes loaded with nanocomposites were regular and rounded within the size of 120 nm, with no signs of broken exosomes or leakage of their contents. The cytotoxicity assay indicated that S1-Exo enhanced the cytotoxic effect versus of the free nano-form S1 on A431. Interestingly, S1-Exo recorded 1.109 times more than DOX in its anti-skin cancer capacity. Moreover, S1-Exo recorded 40.2% for early apoptosis and 22.1% for late apoptosis. Furthermore, it displayed impact in arresting the cancer cell cycle at G0-G1 and G2-M phases. Noteworthy, loaded nanocomposites were safe against HSF skin cells. In conclusion, the loaded CuS/SnS into the exosomes could be of great potential as anti-skin cancer through stimulation of apoptosis and promotion of the cell cycle arrest.

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Comprehensive Study and Categorization of Host Defense and Cell-Penetrating Peptides by Their Affinity for Extracellular Vesicle Interactions

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Background: Introduction: Host defense peptides (HDPs) exhibit significant potential as biomaterials with applications in both antimicrobial and anticancer fields. Their biological functions involve disrupting or lysing cell membranes. These peptides engage in various membrane interaction mechanisms, such as the carpet, toroidal pore, and barrel stave models. Additionally, cell-penetrating peptides play a role in loading cargo and facilitating the uptake of small molecules and nanoparticles. While much study has been done on the mechanics of these peptides' interactions with model membranes, our understanding of their interactions with extracellular vesicles (EVs) remains restricted. There are various aspects where the interplay between EVs and HDPs could be relevant, spanning from their cooperative presence at infection sites to potential functions in EV cargo loading.
Methods: A series of HDPs were selected: Indolicidin, Aurein 1.2., Dermcidin (DCD-1), DHVAR 4, Bactenecin, Protegrin-1, Transportan, Buforin IIb, KLA, Temporin-La, LL37, FK16, Mellitin, Polybia MPI, Histatin 5, PNC-28, CM15, Buforin II, Gramicidin, Arg-1, Macropin I, Lasioglossin LL-III, R8, Penetratin. Polarised light spectroscopy (Linear dichroism), flow cytometry, nanoparticle tracking analysis, zeta potential and freeze-fracture TEM were used to investigate these interactions.
Results: Biophysical investigations have unveiled distinct mechanisms employed by various host defense peptides (HDPs). These mechanisms encompass vesicle penetration, lytic actions, and the removal of protein corona. Biophysical studies categorize host defense peptides (HDPs) into eight groups based on their mechanisms. LL37 and Lassioglossin removes surface proteins effectively. Melittin disrupts membranes strongly, and Octaarginine and Penetratin use a mechanism with lower disruptive affinity on original vesicle composition.
Summary: These insights provide an overview of the surface interactions of Host defense peptides with EVs, allowing us to gain a wide perspective on the molecular level interactions, which may be useful in tailoring the surface of EVs with short HDPs and manipulating for bioengineering.

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Studying the interaction between oral pathogenic bacteria and Candida species in an indirect manner: interkingdom communication at the level of extracelllular vesicles

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Background: The human oral cavity is colonized by more than 700 microbes, such as bacteria, viruses, fungi, known as the oral microbiota. As a result of environmental effects, such as smoking or infections, the microbial composition may change, which can result in dysbiosis that may lead to diseases, such as oral candidiasis. Oral candidiasis is most commonly caused by Candida albicans, which can alter the bacterial diversity.

To examine the nature of such fungal-bacterial interactions, we aim to investigate the interaction between Candida species- and oral pathogenic bacteria at the level of extracellular vesicles (EV).

For our experiments we used the C. albicans SC5314 and C. parapsilosis CLIB214 strains, along with Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aeruginosa as pathogenic bacterial counterparts. We optimized the fungal and bacterial EV isolation protocol from solid media. The characterisation of the EVs by transmission electron microscopy and NanoSight showed round shaped particles with diameters between 50 and 250 nm.

We examined the effects of EVs released by C. parapsilosis and the yeast and hyphae form of C. albicans on the growth and biofilm formation efficiency of S. aureus, P. aeruginosa and E. faecalis and vica versa.

Regarding the effect of bacteria, the bacterial EV treatment reduced the number of CFUs of C. albicans cells. Bacterial EVs also altered the biofilm formation efficiency of the fungal species in a species dependent manner. Staphylococcus aureus derived EV treatment significantly decreased the efficiency of Candida albicans biofilm formation, while it increased it in the case of Candida parapsilosis.

Altogether these results suggest the presence of an active interaction between fungal and bacterial cells at the level of EVs.

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Immunomodulatory effect of mast cell-derived extracellular vesicles

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Background: Immunomodulatory effect of mast cell-derived extracellular vesicles
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Mast cells are important players in the immune system and they secrete a wide range of mediators during bacterial infections. Mast cells are also able to release extracellular vesicles (EVs). The aim of this study was to investigate their role in cell-to-cell communication and the development of Th1 immune responses.

Bone marrow-derived and peritoneal mast cells from GFP-transgenic and wild-type mice were isolated. EVs were separated from the conditioned media of these cells cultured in the presence or absence of lipopolysaccharide (LPS). EVs were characterised according to the MISEV2023 guidelines using flow cytometry, electron and fluorescent microscopy, TRPS, the SPV lipid and the BCA protein assays. Mast cells and splenocytes were cultured with the conditioned media or isolated EVs of stimulated and non-stimulated mast cells. Cell activation was monitored by proliferation assays and cytokine secretion (ELISA). GFP+ mast cells were seeded in diffusion chambers, which were implanted into the peritoneal cavities of mice enabling us to investigate the continuous in vivo release of EVs. Uptake of GFP+ EVs of peritoneal mast cells were tested using flow cytometry and fluorescent microscopy.

Here, we showed that both the conditioned medium of mast cells and the isolated mast cell-derived EVs could activate mast cells and splenocytes in autocrine and paracrine fashions, respectively. This activation was further enhanced after LPS treatment. Moreover, we confirmed that EVs are transmitted to other peritoneal cells in vivo and may spread the pro-inflammatory response.

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Examination of horizontal gene transfer in colon cancer cell model

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Background: One of the biggest challenges in clinical oncology is the adaptation to the intense selection pressure of therapy, ultimately resulting in tumor recurrence. Generally, genes that confer adaptive properties are transferred vertically (from parent to progeny cells), leading to the context-dependent outgrowth of the fittest population. For instance, anti-EGFR treatment is ineffective in the case of the K-ras gene mutation, so the therapy causes the competitive release of mutant clones. However, several new experiments prove that tumor cells can acquire non-vertically inherited genetic properties through horizontal gene transfer (HGT).

Results: We examined two colon cancer cell lines (SW480 and HT-29) with different mutational backgrounds. Our in vitro analysis showed significant migration of cell-free DNA between donor SW480 and recipient HT-29 cell populations. Using the droplet digital PCR method, we found that the K-ras mutation characteristic of the donor cell line also appeared in the K-ras wild-type HT-29 cells after treatment with the SW480-derived total supernatant, small extracellular vesicles (sEVs), and sEV-free supernatant. Furthermore, with whole exome sequencing, we identified 214 SW480 cell line-specific mutations that appeared after the treatment in the recipient cells originally carrying the wild-type allele (e.g. TP53, MUC3A, and CADM1). The mutation spectrum characteristic of SW480 cells was not detectable in the recipient HT-29 cells when were treated with the supernatant of prostate cancer (PC3) cells.

Discussion: The significant number of donor cell-specific mutations in the recipient population strongly suggests that HGT can play a significant role in the development of the host's adaptive properties including therapy resistance.

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Oral squamous cell carcinoma-Candida interaction: The role of extracellular vesicles

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Background: Oral squamous cell carcinoma (OSCC) accounts for 90% of oral cancer episodes worldwide. In the context of OSCC, the normal oral microbiota is often altered, which may predispose to local infections or conditions such as oral candidiasis. A previous study from our laboratory showed that the presence of Candida albicans enhances the progression of OSCC in vitro and in vivo by enhancing the activity of genes and signaling pathways involved in tumor progression, oncometabolite production and matrix metalloproteinase (MMP) activity. However, the main component causing the changes has not yet been identified. Therefore, in our work, we investigate the effect of Candida-derived extracellular vesicles (EV) on the progression of OSCC. As we hypothesize that EVs play a crucial role in cell-cell communication, they may also play an important role in host-pathogen interaction. During our experiments, we isolated vesicles from Candida albicans and Candida parapsilosis. In the case of C. albicans, it was isolated from both yeast and hyphal forms. The uptake and mechanism of Candida-derived EVs were investigated by flow cytometry and confocal microscopy. We also investigated the effect of Candida-derived EV treatment on various processes involved in the epithelial-mesenchymal transformation of the tumor, such as the migration, MMP activity and gene expression profile of the HSC-2 human OSCC cells used. As a result of the experiments, we found that EV treatment affects the migration and morphology of tumor cells. In addition, C. albicans-derived EVs significantly increase the MMP activity of the cells. Gene expression changes were also detected after Candida EV treatment. These results suggest that Candida EVs play a role in promoting OSCC epithelial-mesenchymal transition and thus in tumor progression.

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MiR-200b categorizes patients into pancreas cystic lesion subgroups with different malignant potential

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Background: In our study we investigated into the diagnostic value of extracellular vesicles (EV) and their miR cargo derived from pancreatic cyst fluids. Although pancreatic cysts are heterogeneous, they can be clustered into the larger groups of pseudocysts (PC), and serous and mucinous pancreatic cystic neoplasms (S-PCN and M-PCN, respectively). In contrast to PCs and S-PCNs, M-PCNs may progress to malignant pancreatic cancers. Since current diagnostic tools do not meet the criteria of high sensitivity and specificity, novel methods are urgently needed to differentiate M-PCNs from other cysts. We show that cyst fluid is a rich source of EVs that are positive and negative for the EV markers CD63 and CD81, respectively. Whereas we found no difference in the EV number when comparing M-PCN with other pancreatic cysts, our EV-based biomarker identification showed that EVs from M-PCNs had a higher level of miR-200b. We also prove that not only EV-derived, but also total cyst fluid miR-200b discriminates patients with M-PCN from other pancreatic cysts with a higher sensitivity and specificity compared to other diagnostic methods, providing the possibility for clinical applications. Our results show that measuring miR-200b in cyst fluid-derived EVs or from cyst fluid may be clinically important in categorizing patients.