HSEV 3rd Symposium of Hungarian EV researchers

21st November 2025, Friday

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HSEV

HSEV Conference, November 21, 2025, Budapest,

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Introduction

Dear Participants,

On behalf of the Organizers, we welcome you to the third 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
President of HSEV
Hungarian Society for Experimental and Clinical Pharmacology

Edit Buzás
President of HSEV
Hungarian Society for Immunology

Hargita Hegyesi
Secretary of HSEV
Hungarian Society for Immunology

Csenger Kovácsházi
Secretary of HSEV
Hungarian Society for Experimental and Clinical Pharmacology

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Schedule

9:30 – Registration

10:00 – 10:15 Opening

10:15 - 10:55 – Keynote lecture: Ewa Zuba-Surma

10:55 – 11:50 – **Oral Session 1**, Biogenesis/release of EVs and their function in signal transmission

11:50 – 13:15 – Lunch break, poster viewing

13:15 – 14:10 – **Oral session 2**, EV-based biomarkers

14:10 – 14:30 – Coffee break, poster viewing

14:30 – 15:25 – **Oral session 3,** EVs in therapy

15:25 – 15:40 – Closing remarks, awards ceremony, group photo

15:45 –16:15 Meeting of senior scientists

Venue

Semmelweis University, NET Building, Tanácsterem.

1098 Budapest, Nagyvárad tér 4.

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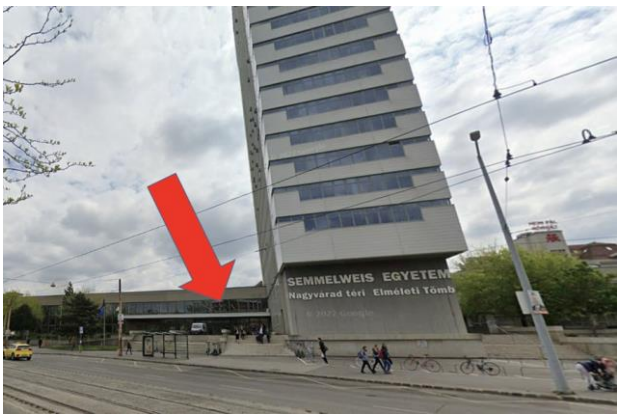

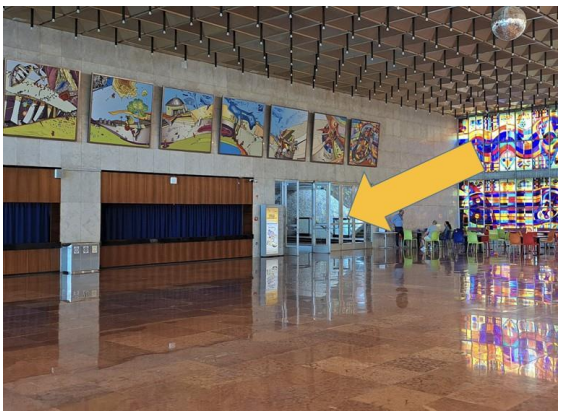

HSEV Conference, June 21, 2024, Budapest, Hungary

Scientific program

Opening:

10:00 - 10:15

Edit Buzás
President, Extracellular Vesicle Section, Hungarian Society for Immunology
Semmelweis University, Department of Genetics, Cell- and Immunobiology

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

Keynote lecture:

10:15: **Ewa Zuba-Surma**
Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology at the Jagiellonian University in Krakow, Poland.
Stem cell- derived extracellular vesicles as a next generation tool for tissue repair

10:55 - 11:50 – Oral Session 1, Biogenesis/release of EVs and their function in signal transmission

Session chairs:
Kitti Garai
Department of Pharmaceutical Biotechnology, University of Pécs

10:50: **Dorina Lenzinger**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Pop, drop and release: novel sEV secretion pathways

11:05: **Lilla Turiak**
MTA-HUN-REN TTK Lendület (Momentum) Glycan Biomarker Research Group, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Glycosylation characteristics of cell-culture derived extracellular vesicles

11:20: **Delaram Khamari**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Characterization of large extracellular vesicles released by apoptotic and pyroptotic cells

11:15: **Sponsor presentation**
Bertrand Damart
Particle Metrix GmbH, Germany
ZetaView: A new edge for Nanoparticle characterization

11:45 – 13:15 – Lunch break, poster viewing

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Poster 1: **Apor Veres-Szekely**
Pediatric Center, Semmelweis University, Budapest, Hungary, HUN-REN – SU Pediatrics and Nephrology Research Group, Budapest, Hungary
Microplate-Based Transient Agarose Spot (TAS) Assay for Quantitative Assessment of Extracellular Vesicle–Mediated Cell Migration

Poster 2: **Bernadett Bodnár**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Isolation and characterisation of extracellular vesicles from lymph nodes

Poster 3: **Edina Bugyik**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
One step further to follow cancer-induced cardiac atrophy in an in vivo model

Poster 4: **Ilona Barbara Csordás**
Unit of Radiation Medicine, Department of Radiobiology and Radiohygiene, National Public Health Centre
Bone marrow (BM) derived EVs activate cytoplasmic DNA sensing and modulate immune phenotypes in BM- MSCs

Poster 5: **Gréta Lilla Bányai**
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary
Microfluidic profiling of extracellular vesicle fractions reveals systemic changes in cancer patients

Poster 6: **István Dudás**
Semmelweis University, Institute of Genetics, Cell- and Immunobiology, Budapest, Hungary
Characterization of Amphiectosome Release in Cardiac Cells

Poster 7: **Judith Mihály**
HUN-REN Research Centre for Natural Sciences, Institute of Materials and Environmental Chemistry
Enhanced Detection and Characterization of Extracellular Vesicles Using Cysteamine-Capped Gold Nanoparticles via Surface-Enhanced Infrared Spectroscopy (SEIRS)

Poster 8: **Lilla Lankovics**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Modulation of Autophagy and Extracellular Vesicle Biogenesis by Novel Drug Candidates

Poster 9: **Luigi Menna**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest, Hungary
Biodistribution and Cellular Uptake of Endothelial Colony-Forming Cell Extracellular Vesicles in Sepsis

Poster 10: **Martina Forgács**
National Centre for Public Health and Pharmacy, Department of Radiobiology and Radiohygiene, Unit of Radiation Medicine, Budapest, Hungary
BONE MARROW-DERIVED EXTRACELLULAR VESICLES FROM IRRADIATED MICE ARE ABLE TO INDUCE SENESCENCE IN THE HEMATOPOIETIC MICROENVIRONMENT

Poster 11: **Mátka Nagy**
Department of Physiology, Semmelweis University, Budapest, Hungary
Impact of storage conditions and cryoprotectants on extracellular vesicle stability

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Poster 12: **Natalia Tokesi**
HUN REN RCNS
Isolation and Characterization of Bovine Milk-Derived Extracellular Vesicles for Potential Therapeutic Applications

Poster 13: **Péter Bokrossy**
Pediatric Center, MTA Center of Excellence, Semmelweis University; Budapest
Investigation of Extracellular Vesicle corona formation with Label-Free Optical Waveguide Lightmode Spectroscopy Method

Poster 14: **Rita Hargitai**
National Center for Public Health and Pharmacy Department of Radiation Biology and Radiation Health Division of Radiation Medicine
Effect of chemical-induced respiratory sensitization on extracellular vesicle production using in vitro and in vivo approaches

Poster 15: **Zoltan Adam**
Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pecs, Hungary
Exercise-Derived Large Extracellular Vesicles Suppress Oncogenic Signaling in Lung Adenocarcinoma Cells

Poster 16: **Zsófia Fodor**
Budapest Universconoity of Technology and Emics, Hungary
Optimization of Extracellular vesicle analysis by flow cytometry

Poster 17: **Dóra Kapui**
Department of Pharmacology and Pharmacotherapy, Semmelweis University
Separation of plasma extracellular vesicles from lipoproteins using density gradient ultracentrifugation and size exclusion chromatography for diagnostic applications

Poster 18: **Szabolcs Hambalkó**
Department of Pharmacology and Pharmacotherapy, Semmelweis University
DIFFERENCES IN THE PROPERTIES OF MSC-EVS TO REDUCE THE CARDIAC SIDE EFFECTS OF ANTI-CANCER DRUGS

Poster 19: **Tasvilla Sonallya**
HUN-REN Research Centre for Natural Sciences
Interaction Pathways of Antimicrobial Peptides with Extracellular Vesicles: Disruption, Penetration, and Corona Removal

13:15 – 14:10 – Oral session 2, EV-based biomarkers

Session chairs:

Csenger Kovácsházi
Semmelweis University, Department of Pharmacology and Pharmacotherapy

13:15: **Zoltán Varga**
HUN-REN Research centre for natural sciences, Budapest
Measurement traceability in the characterization of extracellular vesicles: From principles to reference materials

13:30: **Kelsey Fletcher**
Semmelweis University, Department of Genetics, Cell- and Immunobiology, Hungary
Dissecting Mast Cell-Derived Extracellular Particles: Distinguishing Granules from Vesicles

13:45: **András Försönits**
Semmelweis University, Department of Genetics, Cell- and Immunobiology, Hungary
Stripping the adhered protein corona by high ionic strength may improve immune detection of extracellular vesicles

14:00: **Sponsor presentation**
Zoey Wang
EXODUS BIO LTD.
EXODUS, A High Efficiency System for Automated EVs Isolation

14:10– 14:30 – Coffee break, poster viewing

14:30 – 15:25 – Oral session 3, EVs in therapy

Session chairs:

Osteikoetxea, Xabier
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest, Hungary

14:30: **Rácz Richárd**
Semmelweis Egyetem, Általános Orvostudományi Kar, Farmakológiai és Farmakoterápiás Intézet
Mesenchymal Stem Cell Derived Extracellular Vesicles Functional Study and Their Utilization for the Reduction of Chemotherapeutics Caused Cardiotoxicity

14:45: **Boglárka Nagy**
Department of Genetics Cell-and Immunobiology, Semmelweis University, Budapest
Immunomodulation via engineered embryonic stem cell vesicles

15:00: **Csilla Imola Szíjjártó**
HUN-REN Természettudományi Kutatóközpont
Tuning the protein corona of extracellular vesicles via antimicrobial peptides

15:15: **Sponsor Presentation**
Emese Sinkó
Bio-Science Ltd.
Breaking the boundaries of EV detection

15:25 – 15:400 – Closing remarks, Award ceremony, group photo

16:45 – Meeting of senior scientists

HSEV Conference, June 21, 2024, Budapest, Hungary

Zoltan Adam[1], Kitti Garai[1], Abigel Sebok-Tornai[2,3], Marta Wilhelm[4], Krisztian Kvell[1]

[1] Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pecs, Hungary
[2] Medical School, Department of Laboratory Medicine, University of Pecs, Hungary
[3] Histology and Light Microscopy core facility at the Szentágothai Research Centre, University of Pecs, Hungary
[4] Faculty of Science, Institute of Sport Sciences and Physical Education, University of Pecs, Hungary

Exercise-Derived Large Extracellular Vesicles Suppress Oncogenic Signaling in Lung Adenocarcinoma Cells

Poster 15 – 11.50-13.15

Introduction: Regular physical activity is associated with reduced cancer incidence and improved survival, yet the molecular mechanisms behind its protective effects remain incompletely understood. Extracellular vesicles (EVs) have emerged as key mediators of systemic exercise adaptation, carrying bioactive cargo capable of reprogramming recipient cells. While small EVs have been studied extensively, the role of large EVs (L-EVs) in cancer biology is less clear.

Methods: Here, we investigated whether plasma-derived L-EVs isolated before (Pre) and after (Post) an acute bout of endurance exercise modulate tumor-related pathways in lung adenocarcinoma (PC9) cells. L-EVs were isolated from healthy older adults (61 ± 2 years) with a long history of regular training. Following 24 h incubation with PC9 cells, gene and miRNA expression profiles were assessed using TaqMan array–based RT-qPCR. Differentially expressed genes (DEGs) and miRNAs (DEmiRs) were analyzed using bioinformatics, including Ingenuity Pathway Analysis (IPA), to identify regulatory interactions and predict functional outcomes.

Results: Exercise significantly increased circulating L-EV concentration, confirmed by nanoparticle tracking analysis and transmission electron microscopy. In PC9 cells, Pre- and Post-L-EVs induced distinct molecular signatures. Post-L-EV treatment promoted tumor-suppressive changes, including upregulation of pro-apoptotic and cell cycle inhibitory genes (CASP9, FADD, CDKN2B) and downregulation of oncogenic and immune evasion–associated genes (MAX, FASLG, ELK1). Several DEmiRs, such as miR-21-5p and miR-301b-3p, shifted in the opposite direction to their known oncogenic patterns in NSCLC. IPA predicted enhanced apoptotic signaling and reduced metastatic potential following Post-L-EV exposure.

Conclusion: These results suggest that exercise conditions circulating L-EVs with tumor-suppressive properties, providing a mechanistic link between physical activity and cancer prevention. L-EVs may represent promising biomarkers and mediators of exercise-derived anticancer effects, highlighting their potential relevance for both healthy aging and oncology.

Funding: This work was founded by PTE Research Fund Call for Proposals in Sport Sciences (013_2023_PTE_RK/3) and received partial funding from EKÖP-24-4-II New National Excellence Program (EKÖP-24-4-II-PTE-331).

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Gréta Lilla Bányai[1], Nikolett Kitti Dobos[1], Afrodité Németh[1], András Merényi[2], András Szabó[1], Anikó Gaál[3], Csaba Pongor[1], Tamás Garay[1,4]

[1] Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary

[2] Semmelweis University, Department of Emergency Medicine, Budapest, Hungary

[3] Research Centre for Natural Sciences, Institute of Materials and Environmental Chemistry, Biological Nano-chemistry Research Group, Budapest, Hungary

[4] Semmelweis University, Department of Internal Medicine and Oncology, Division of Oncology, Budapest, Hungary

Microfluidic profiling of extracellular vesicle fractions reveals systemic changes in cancer patients

Poster 5 – 11.50-13.15

Introduction: Microfluidic channels provide a unique platform for analyzing biological particles at the microscale. Owing to their small dimensions, flow remains laminar and diffusion processes dominate, allowing molecular separation to be precisely modeled and interpreted. By introducing plasma-derived extracellular vesicles (EVs) into a sheath flow and monitoring the evolution of fluorescent intensity profiles along the channel, characteristic diffusion patterns can be obtained from minimal sample volumes. Since the readout is based on the combined diffusion properties of the vesicle population, this approach may offer a distinct perspective on EV heterogeneity compared to conventional single-parameter methods.

Methods: Plasma samples were collected from colorectal, prostate, and pancreatic cancer patients, as well as healthy donors. EVs were isolated by size exclusion chromatography, followed by protein and lipid assay and NTA characterization. Samples were fluorescently labeled and introduced into straight microfluidic channels, where fluorescence intensity curves were recorded at twelve positions. Asymmetric sigmoid fitting was applied, and slope changes were analyzed to track diffusion broadening.

Results: Straight-channel microfluidic measurements revealed highly consistent diffusion patterns in prostate and pancreatic cancer patients, while colorectal cancer samples displayed pronounced variability. Interestingly, the control group also showed strong heterogeneity, suggesting that the detected EV diffusion profiles reflect systemic responses of the organism rather than tumor-specific signatures. While certain tumor types (prostate, pancreatic) appear to elicit more uniform responses, colorectal cancer patients exhibit much broader diversity. This contrasts with bulk methods such as protein/lipid assays or NTA, where no discriminative patterns were observed at all. Thus, microfluidic analysis provides a more informative readout of EV populations, offering a new perspective on their role in systemic responses associated with cancer.

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HSEV Conference, June 21, 2024, Budapest, Hungary

Bernadett R. Bodnár[1], Sayam Ghosal[1,2], Brachyahu M Kestecher[1,2,3], András Försönits[1], Nóra Fekete[1], Edina Bugyik[1] , Zsolt I Komlósi[1], Éva Pállinger[1], Edit I Buzás[1,2,3], and Xabier Osteikoetxea[1,2]

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Isolation and characterization of tissue-derived extracellular vesicles from mouse lymph nodes

Poster 2 – 11.50-13.15

Extracellular vesicles (EVs) are lipid bilayer-enclosed particles released by all cells and play key roles in immune regulation. While EVs derived from body fluids are well studied, tissue-derived EVs, especially from lymph nodes (LNs), remain poorly characterized due to technical limitations.

This study aimed to develop a reproducible method for isolating and characterizing large (lEV) and small (sEV) EV subpopulations from murine LNs and to investigate the immunization-induced changes in their properties. Male C57BL/6 mice were immunized with complete Freund's adjuvant (CFA) with or without ovalbumin (OVA). Inguinal and popliteal LNs were excised 9 days later. EVs were isolated using differential centrifugation and size-exclusion chromatography (SEC). EV morphology was evaluated by transmission electron microscopy (TEM), and Particle size distribution and concentration via nanoparticle tracking analysis. Protein and lipid contents were measured by BCA and SPV assays, respectively. EV surface markers were analyzed using bead-based flow cytometry.

OVA+CFA immunization significantly increased LN mass and altered cellular composition. OVA+CFA sEVs consistently showed higher particle counts and protein content compared to lEVs. Protein-to-lipid ratios were higher in sEVs, especially after CFA treatment. TEM confirmed intact vesicles with specific morphological features. Flow cytometry revealed immunization-specific changes in EV markers including CD45, CD146, and MHC class II, highlighting their immunomodulatory potential.

This study establishes a protocol for LN-EV isolation and demonstrates that immunization influences the composition and surface markers of EVs. These findings support the role of tissue-derived EVs in immune regulation and their potential for immunotherapy and vaccine development.

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HSEV Conference, June 21, 2024, Budapest, Hungary

Péter Bokrossy[1], Beáta Szebeni[1,2], Tamás Visnovitz[3,4], Lenzinger Dorina[3], Éva Pállinger[3], Nóra Fekete[3], Zoltán Varga[5, 6, 7], Judith Mihály[5], Domonkos Pap[1,2], Apor Veres-Székely[1,2], Csenge Szász[1], Edit Buzás[3,4,8], Attila J Szabó[1,2], Ádám Vannay[1,2], Nóra Adányi[9]

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[5] Biological Nanochemistry Research Group, Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary
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[7] Semmelweis University, Faculty of Medicine, Department of Biophysics and Radiation Biology - HUN-REN Office for Supported Research Groups, Budapest, Hungary
[8] HCEMM-SU Extracellular Vesicles Research Group, Budapest, Hungary
[9] Insuline BT. Budapest, Hungary

Investigation of Extracellular Vesicle corona formation with Label-Free Optical Waveguide Lightmode Spectroscopy Method

Poster 13 – 11.50-13.15

Introduction: Extracellular vesicles (EVs) are lipid bilayer-delimited particles that are naturally released from almost all types of cells into the extracellular environment. During their biogenesis and release to the extracellular space, EVs adsorb biomolecules, collectively designated as EV corona. Among corona components, proteins like albumin can be particularly important. In this study, we developed a novel method for immobilizing EVs and assessing their capacity to bind human serum albumin (HSA) as a model protein for corona formation.
Methods: The method development for EV-HSA binding was performed using optical waveguide lightmode spectroscopy (OWLS) flow-injection analyser (FIA) system. All experiments were conducted using tris buffer (42 mM, pH 7.4) as flow-through medium, under varying flow rates and flow cell temperatures. To evaluate EV immobilization and surface passivation efficiency for HSA, chip surfaces were treated with poly-L-lysine (PLL) of varying molecular weights, pH, and concentrations. The optimal measuring range of protein concentration was determined using the previously optimised surface treatment and a range of HSA concentrations. Mathematical modelling was used to estimate a theoretical number of HSA can be bound on the EVs surface, then proof measurements were performed to support these calculations.
Results: To conclude the optimisation, the final parameters were found as 10 ng/mL PLL (15-30 kDa) in pH 7.4 buffer solution, at 25 °C; with 100 µl/min flow rate, the isolated EVs were diluted 4.5×1010 particle/mL and the optimal measuring range of HSA can be defined between 1-100 ng/mL. The proof measurements for the mathematical modelling revealed that the results corresponded in magnitude to the theoretical maximum HSA can be bound on the EVs surface, indicating the accuracy of the OWLS measurements.
Conclusions: Our OWLS method is applicable for the examination of real-time EV corona formation.
Funding: K-142728, K-131594, ADVANCED-150077, TKP2021-EGA-24, TKP2021-EGA-23, EKÖP-2025-367; EKÖP-2025-376; VEKOP-2.3.2-162016-00002, VEKOP-2.3.3-15-2017-00016, RRF-2.3.121-2022-00003; NKFIH 150767 Advanced

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HSEV Conference, June 21, 2024, Budapest, Hungary

Edina Bugyik[1], Tamás Visnovitz, Szilvia Bősze, Gábor Valcz, Júlia Opra, Tünde Bárkai, Zsombor Hegedűs, Zoltán Varga, Gertrúd Bajnay, Melinda Rezeli, Sándor Paku, Katalin Dezső, Edit Buzás

[1]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest

One step further to follow cancer-induced cardiac atrophy in an in vivo model

Poster 3 – 11.50-13.15

Tumor anorexia-cachexia syndrome is a complex metabolic condition characterized by progressive weight loss, skeletal muscle wasting, fatigue, and poor prognosis. It affects 50–80% of cancer patients and accounts for up to 30% of cancer-related deaths. While cardiac atrophy is a significant but under-recognized component of this syndrome, early detection remains a clinical challenge.

In this study, we used the C26 colorectal adenocarcinoma model to induce tumor anorexia-cachexia syndrome in male and female mice. Body weight, tumor size, spleen weight, heart weight, and tibia length were measured at the time of sacrifice. The spleen weight was significantly increased in both sexes in the presence of the tumor. Echocardiographic analysis revealed a significant decrease in left ventricular (LV) mass and posterior wall thickness (LVPWd) in female tumor-bearing mice compared to controls, suggesting severe tumor-induced cardiac atrophy. In contrast, male mice showed only a slight, non-significant decrease in these cardiac parameters.

Transmission electron microscopy (TEM) of heart tissue from tumor-bearing animals showed marked ultrastructural alterations, including disrupted actin-myosin filaments, abnormal mitochondrial morphology, and an increased number of vesicle-like structures. Immunohistochemistry revealed the presence of Elane-positive cells in diseased hearts, indicating neutrophil infiltration.

To assess circulating biomarkers, we performed the quantification of two proteins in longitudinal serum samples using the Quanterix Simoa SR-X analyzer. While endoglin (CD105) levels did not change significantly, neurofilament light chain (NfL) levels increased over time with tumor progression, suggesting neuronal or cardiac involvement.

Overall, these findings support the hypothesis that C26 tumors can induce cardiac cachexia, with sex-specific differences in disease manifestation. The observed structural and functional cardiac changes, along with the rise in circulating NfL, offer promising preliminary insights that may contribute to early diagnosis and the development of targeted therapeutic strategies.

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HSEV Conference, June 21, 2024, Budapest, Hungary

Ilona Barbara Csordás[1,2], Tünde Szatmári[1], Katalin Lumniczky[1]
[1] Unit of Radiation Medicine, Department of Radiobiology and Radiohygiene, National Public Health Centre
[2] Doctoral School of Pathological Sciences, Semmelweis University

Bone marrow (BM) derived EVs activate cytoplasmic DNA sensing and modulate immune phenotypes in BM- MSCs

Poster 4 – 11.50-13.15

DNA-damaging agents, such as ionizing radiation (IR), induce cytoplasmic DNA accumulation in targeted cells, activating cytoplasmic DNA-sensing pathways and linking DNA damage to immune activation. Our previous work demonstrated that EVs can transmit IR-induced DNA damage, including double-strand breaks, to non-irradiated cells. In this study, we investigate whether IR and EVs activate DNA-sensing pathways in bone marrow (BM)-derived mesenchymal stem cells (MSCs), aiming to understand how DNA damage impacts BM immune function.

Young C57BL/6 mice received 0 Gy or 2 Gy X-ray irradiation. BM supernatants were collected 24 h later, and EVs were isolated via precipitation. EV markers were confirmed by western blot. OriCell™ C57BL/6 BM-MSCs were cultured to 60% confluence and irradiated with the same doses. MSCs were harvested 24 h post-irradiation for RNA extraction. To assess EV effects, MSCs were co-cultured with EVs from irradiated or control BM for 24 h, followed by RNA isolation. Quantitative PCR measured expression of DNA-sensing and immune-related genes. In 2Gy-irradiated MSCs, we observed increased expression of cytoplasmic DNases, DNA sensors, and inflammatory markers. EVs from both control and irradiated BM similarly induced the expression of cytoplasmic DNases and sensors, along with their downstream effectors. Comparing inflammatory marker expression between irradiated and EV-treated samples suggests that irradiation promotes an immunosuppressive MSC profile, whereas EVs encourage a pro-inflammatory state. Among the cytoplasmic DNA sensing pathways analyzed, STING-related sensors showed the most pronounced alterations.

In summary, both IR and IR-derived EVs activate cytoplasmic DNA sensing in BM-MSCs, with STING-related sensors most prominently involved. EVs from non-irradiated BM also triggered this response but less strongly. The distinct inflammatory profiles after IR or EV treatment suggest different immunomodulatory effects, requiring further investigation at protein levels.

Funding: This research was supported by the European Union's "EURATOM" research and innovation program under the 101061037 grant agreement.

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Bertrand Damart[1]

[1] Particle Metrix GmbH, Germany

ZetaView®: A new edge for Nanoparticle characterization

Oral Presentation – Oral Session 1, Biogenesis/release of EVs and their function in signal transmission- 10.55-11.50

The ZetaView® Evolution is an advanced nanoparticle tracking analyzer (NTA) designed for accurate extracellular vesicle analysis using both scatter and fluorescence detection modes. Its innovative Concentration Scanning Technology enables rapid, calibration-free measurement of up to 20,000 particles in just 30 seconds. With improved optical sensitivity and the user-friendly ZetaSphere software, it delivers one-click, full-sample analysis and automated reporting, fully aligned with the MISEV guidelines for EV research.

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HSEV Conference, June 21, 2024, Budapest, Hungary

István Dudás[1], Lilla Lankovics[1], Tünde Bárkai[1], Kelsey Fletcher[1], Edina Bugyik[1], Krisztina V Vukman[1], Szilvia Bősze[1,2], Edit I Buzás[1,3,4], Dorina Lenzinger[1*], Tamás Visnovitz[1*]

[1] Semmelweis University, Institute of Genetics, Cell- and Immunobiology, Budapest, Hungary

[2] HUN-REN-ELTE Research Group of Peptide Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary

[3] HUN-REN-SU Translational Extracellular Vesicle Research Group, Budapest, Hungary,

[4] HCEMM-SU Extracellular Vesicle Research Group, Budapest, Hungary

*equal contribution

Characterization of Amphiectosome Release in Cardiac Cells

Poster 6 – 11.50-13.15

Extracellular vesicles (EVs) are membrane-enclosed, mainly spherical, non-replicative particles released into the extracellular space. They play an essential role in intercellular communication and the maintenance of cellular homeostasis. Recently, we described a novel mechanism for small EV release, in which large amphiectosomes (500–3000 nm in diameter) bud from the plasma membrane, followed by rupture of their limiting membrane and subsequent release of intraluminal vesicles as exosomes into the extracellular space. We refer to this process as amphiectosome release and the "torn bag mechanism."

The aim of this study was to investigate this EV secretion pathway, previously described by our group in cardiomyoblasts and cardiomyocytes. Specifically, we examined the dynamics of amphiectosome release and characterized them using protein markers.

Experiments were performed using the H9c2 cell line and adult mouse hearts. Cells and tissues were examined by transmission electron microscopy (TEM). Amphiectosome release was monitored by confocal microscopy in H9c2 cells using lactadherin-based fluorescent labelling, classical small EV markers (CD63, CD81, ALIX, TSG101), and the autophagy-specific marker LC3B. The number of secreted amphiectosomes was quantified following treatment with cytochalasin B, colchicine, rapamycin, and bafilomycin A1. Changes in protein expression were analysed by Western blotting.

TEM confirmed the presence of amphiectosomes and the "torn bag mechanism" in both H9c2 cells and mouse heart tissue. In H9c2 cultures, confocal imaging revealed successful modulation of amphiectosome release; however, these findings differed from those previously reported in other cell types, likely reflecting the unique characteristics of cardiomyocytes.

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HSEV Conference, June 21, 2024, Budapest, Hungary

Kelsey Fletcher[1], Tamás Visnovitz[1,2], Péter Lőrincz[3], Tünde Bárkai[1], Dorina Lenzinger[1], Tamara Hermans[1], Csaba Cserép[4], Ádám Dénes[4], Attila Csomós[5], Edit I. Buzás[1,5,6,7], Krisztina V. Vukman[1]

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Dissecting Mast Cell-Derived Extracellular Particles: Distinguishing Granules from Vesicles

Oral Presentation – Oral Session 2 – EV-based biomarkers – 13:15 – 14:10

Mast cells (MCs) play a critical role in allergic and inflammatory disorders, where they release preformed granules through the process of degranulation. Besides tradtional granules, MCs also secrete extracellular vesicles (EVs), yet the distinction between granules and EVs remains unclear. This project's objective is to characterize and distinguish between mast cell-derived EVs and extracellular granules (EGs), thereby enhancing our understanding of MC-derived extracellular particles (EPs).

Bone marrow-derived MCs from both wild-type and GFP+ transgenic mice were stimulated via IgE/DNP or calcium ionophore (A23187), and the resulting EPs were analysed by flow cytometry, confocal microscopy, transmission electron microscopy (TEM), density gradient ultracentrifugation, and biochemical assays.

We developed a real-time system to track EP release and discovered that degranulating MCs release a heterogeneous mix of particles, which differ in size, density, membrane integrity, and detergent sensitivity. Stimulation with A23187 primarily induced a rapid release of large, membrane-bound EGs, whereas DNP stimulation yielded smaller EV-like particles with distinct density and biochemical features. Analysis using detergent lysis and fluorescent staining unveiled stimulus-dependent variations in particle composition, which included differences in membrane and cytoplasmic content. Histamine staining further validated the distinct intracellular cargo among EP subtypes.

Our findings indicate that MC activation leads to a stimulus-specific secretion of structurally and functionally distinct particles, emphasizing the need to discriminate between EVs, EGs, and membrane fragments in both EV and MC research. These insights may alter the interpretations of previous studies and provide a foundation for future therapeutic and diagnostic applications targeting MC-derived EPs.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Optimization of Extracellular vesicle analysis by flow cytometry

Poster 15 – 11.50-13.15

Extracellular vesicles (EVs) have become a focus of biomedical research due to their essential roles in cellular homeostasis and intercellular communication. However, their small size, heterogeneity, and variable concentration creates difficulties for reliable detection and characterization. In flow cytometry-based EV analysis, detergent lysis is a critical step to confirm EV integrity and distinguish EV signals from background noise. As detection technologies evolve, there is a growing need to optimize protocols to accommodate higher sensitivity. Our research aims to develop a standard flow cytometry protocol for EV analysis.

We used EVs derived from HEK293 and HEK-GFP cell lines, isolated from conditioned media using differential centrifugation and filtration. Samples were stained with the lipid dye BioxMLRed, and the effects of dye and sample concentration, buffer composition, washing steps, and instrument settings on EV detection were investigated.

Detergent sensitivity was assessed using Triton X-100, saponin and Tergitol. Our results indicate that instrument settings and EV concentration are critical for reproducible measurements. Among the detergents tested, Triton X-100 effectively disrupted lipid membranes and showed good compatibility with lipid dyes. In contrast, saponin was less effective and gave inconsistent results.

This study contributes to the development of standardized protocols for EV characterization using flow cytometry. These insights will enhance methodological rigor and support a deeper understanding of EV structure-function relationships.

Funding: NVKP_16-1-2016-0004 grant of the Hungarian National Research, Development and Innovation Office (NKFIH), VEKOP-2.3.2-162016-00002, VEKOP-2.3.3-15-2017-00016, the Therapeutic Thematic ProgramTKP2021-EGA-23. RRF-2.3.121-2022-00003 (National Cardiovascular Laboratory Program), 2019-2.1.7-ERA-NET-2021-00015, EU's Horizon 2020 Research and Innovation Program (No. 739593) and János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

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Bone marrow–derived extracellular vesicles from irradiated mice are able to induce senescence in the hematopoietic microenvironment

Poster 10 – 11.50-13.15

Bone marrow (BM) microenvironment is responsible for the normal physiological haematopoiesis. Various cellular stress factors can disrupt the homeostasis of the system, such as ionizing radiation (IR). Extracellular vesicles (EVs) have a key role in these processes via intercellular communication, particularly mediating radiation-induced bystander effects, to modify several biological functions within the BM.

We have set up an in vivo model system to better understand IR-induced processes and the role of EVs potentially involved in the bone marrow pathological alterations. Male 9-12 week-old C57/BL6 and CBA/Ca mice were directly irradiated with 2 or 3 Gy X-rays; or injected with BM-derived EVs isolated from directly irradiated animals. Long bones were isolated 3, 6 and 9 months after treatment, and single cell suspensions were prepared separately from the BM and bone. Cellular senescence was investigated by β-galactosidase staining combined with major phenotypical characterization of the BM microenvironment using flow cytometry. In parallel, miRNA profile changes were analyzed by RT-qPCR, focusing on the biologically relevant miRNAs in the context of cellular senescence.

Cellular senescence showed cell-type- and strain-specific differences after IR and EV treatment. In C57/BL6 mice, increased β-galactosidase activity was observed in lineage-negative haematopoietic stem and progenitor cells, while in CBA/Ca mice, senescence was elevated in microenvironmental cells such as endothelial cells and fibroblasts. In CBA mice, miRNA profiling revealed minimal individual changes, but clustering identified distinct regulatory miRNA groups. Cluster 1 miRNAs were downregulated after IR but upregulated following EV treatment. Notably, miR-21-5p was significantly upregulated in bone tissue after both treatments, suggesting a pro-senescence role.

Overall, IR and EV exposure induced senescence in specific subpopulations, with non-uniform responses between strains and distinct miRNA expression changes in BM and bone in CBA mice.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Stripping the adhered protein corona by high ionic strength may improve immune detection of extracellular vesicles

Oral Presentation – Oral Session 3, EVs in therapy – 14.30-15.25

Our group was among the pioneers to show that extracellular vesicles (EVs) develop a biomolecular corona in biofluids, yet how this layer influences the accessibility of EV surface molecules remains unclear. In this study, we evaluated different corona-removal strategies to improve the immunodetection of EV markers. Washing the EV pellet with high-salt buffer partially stripped the protein corona.

In our experiments, we created an artificial corona by incubating HEK293T-PalmGFP-derived EVs with Cy5-labeled human plasma proteins. EVs were subsequently treated with high-salt washes (NaCl, LiCl, KCl), and corona removal was quantified by measuring the co-localization of plasma proteins with EVs. Corona modifications were further confirmed using annexin-V fluorescent labeling and annexin-V affinity capture of plasma-derived EVs, both with and without salt washing. EVs obtained from THP-1 cells cultured in serum-containing or serum-free media were also analyzed to determine whether growth conditions influenced salt-washing effects. Post-treatment analysis of EV surface markers was performed using the MACSPlex kit.

High-salt washing significantly decreased plasma protein co-localization with EV membranes ($p < 0.0001$, Mann–Whitney test), indicating efficient corona removal. This effect was corroborated by increased annexin-V binding ($p < 0.01$, paired t-test) and annexin-V affinity capture ($p < 0.05$, paired t-test) in both THP-1- and plasma-derived EVs. Enhanced marker detection was observed only in serum-containing conditions, while serum-free cultures showed no change. In plasma-derived EVs, high-salt washing improved immunodetection for 10 of 37 tested surface markers.

These results demonstrate that corona disruption by high ionic strength can improve immune detection of EV markers, providing a strategy to enhance EV identification in biofluids and potentially enabling surface re-engineering for therapeutic applications.

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Differences in the properties of MSC-EVs to reduce the cardiac side effects of anti-cancer drugs

Poster 18 – 11.50-13.15

Anti-cancer drugs can cause several side effects, many of which are serious. Some of the most serious side effects are heart-related, and there is currently no good treatment for these. Extracellular vesicles (EVs) are double-membrane particles that are released by all cells. They help cells communicate with each other by carrying bioactive molecules inside and on their surface. The secretions of EVs by mesenchymal stem cells are known to protect cells and regulate the immune system. Because of this property, they may have a high therapeutic potential and represent an important line of research in cardiovascular therapy. However, EVs from primary cells have been shown to vary widely, which could reduce their effectiveness for medical use.

The objective of this study was to investigate the influence of individual donors on the therapeutic efficacy of MSC-EVs.

EVs were isolated from the conditioned supernatants of MSCs from each donor by ultracentrifugation and characterized according to ISEV guidelines. Finally, AC16 human cardiomyocyte cells were treated with chemotherapeutic agents exhibiting different mechanisms of action, along with EVs derived from MSCs from each donor. The cytoprotective effect was verified by measuring cell viability.

In in vitro cellular assay systems, it has been demonstrated that EVs released by primary MSCs have the capacity to reduce the cardiac side effects of several different anticancer agents.

No differences were found in the physico-chemical characteristics of MSC-EVs isolated from each donor, such as particle number, mean particle size and protein content. However, it was observed that the extent of cytoprotection was significantly influenced by the MSC donor in several different chemotherapeutic agents, with the donor-dependent fraction of EVs exerting a protective effect.

The influence of MSC donors on the cardioprotective effect is significant, and further studies are required to ascertain the genetic and environmental causes of these differences.

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Effect of chemical-induced respiratory sensitization on extracellular vesicle production using in vitro and in vivo approaches

Poster 14 – 11.50-13.15

Chemical-induced respiratory sensitization is a frequent cause of allergic occupational asthma, which poses a growing health concern. However, there are currently no validated in vivo or in vitro tests for the identification of chemicals with sensitizing potency. Production and composition of extracellular vesicles (EVs) was shown to change in asthma patients in the lung, and we assumed that it may be used as a marker of respiratory sensitizer chemical exposure.

Our aim was to develop new approach methodologies for hazard assessment purposes. We used an in vitro model consisting of bronchial epithelial cells (BEAS-2B) and dendritic-like cells (THP-1) in an air-liquid interface (ALI) co-culture model. We collected samples 24 h after chemical exposure from the apical and basolateral compartments, and EVs were isolated applying ExoQuick-TC method. We measured characteristics (concentration, median size, size distribution) of produced EVs using Tunable Resistive Pulse Sensing (Exoid) instrument and validated the EVs by Western blot analysis. In another study, we explored if EVs from sensitized bronchial epithelial cells could induce the activation of THP-1 cells.

An in vivo study was also performed using female BALB/c mice in order to explore the utility of EVs as effect markers of chemical-induced respiratory sensitization. Mice were sensitized to chloramine-T by intranasal exposure and EVs were isolated from the bronchoalveolar lavage fluid.

Our results showed that the concentration and size of EVs of chemical-exposed cells was similar to control cells, and no activation was found in THP-1 cells after EV-treatment. In contrast, sensitized mice released higher amount of EVs in the lung, which was probably due to the higher number of infiltrating immune cells, suggesting the possibility to use this method as an effect marker. The expected outcome of this project is to explore the utility and limitations of an in vitro or in vivo system relevant for respiratory sensitization, and based on these findings, to propose new testing strategies.

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Separation of plasma extracellular vesicles from lipoproteins using density gradient ultracentrifugation and size exclusion chromatography for diagnostic applications

Poster 17 – 11.50-13.15

Background: Biomarkers in circulating extracellular vesicles (EVs) hold great potential for diagnostic applications. However, lipoproteins (LPs) share overlapping physical properties and molecular cargo with EVs, which can obscure EV-related signals. Therefore, efficient separation of EVs and LPs is needed to increase the sensitivity and specificity of EV-based diagnostics.

Aim: Our aim was to develop a method which separates EVs from LPs from human blood samples with optimal purity, yield and time efficiency.

Methods: EVs were isolated from 1 mL human platelet-free plasma by various methods, including iodixanol density gradient ultracentrifugation (DGUC) size exclusion chromatography (SEC) with qEV or Exo-spin columns, Capto Core 400 or 700 bind-elute SEC and iodixanol DGUC followed by SEC using Sepharose CL-2B, CL-4B or 4 Fast Flow (4FF) columns. Isolates were analyzed by nanoparticle tracking analysis, Qubit protein assay and Western blot (WB).

Results: WB analysis of fractions of iodixanol DGUC was compromised by blood-derived contaminants, preventing reliable interpretation. Using qEV, Exo-spin, Capto Core 400 or 700 columns, EV markers were detected by WB, however, substantial LP contamination was observed. DGUC followed by Sepharose CL-2B SEC resulted in isolates positive for CD81 with ApoB below the detection limit. Among Sepharose CL-2B, CL-4B and 4FF SEC columns, the 4FF column with 70 mL volume and 26 mm diameter resulted in the strongest CD81 and TSG101 signals with low ApoA and ApoB signals and good processing time.

Conclusions: Neither iodixanol DGUC nor SEC alone is suitable for separating EVs from LPs from human plasma. Combined DGUC followed by 4FF SEC demonstrated the acceptable EV-LP separation with good processing time, albeit with a trade-off in yield.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Characterization of large extracellular vesicles released by apoptotic and pyroptotic cells

Oral Presentation – Oral Session 1, Biogenesis/release of EVs and their function in signal transmission- 10.55-11.50

Extracellular vesicles (EVs) are emerging key factors maintaining cellular homeostasis, critical mediators of intercellular communication, potential biomarkers, and therapeutic tools. While small EVs have been extensively characterized, the molecular signatures of large EVs (including those generated during regulated cell death pathways) remain poorly defined. Here, we investigated the characteristics of large EVs released during apoptosis and pyroptosis by human monocytic cell lines (THP-1 and U937). Apoptosis was induced by staurosporine and blocked with the pan-caspase inhibitor Q-VD-OPh, whereas pyroptosis was triggered by LPS/nigericin and inhibited with a selective NLRP3 inhibitor. We found that both forms of regulated cell death markedly enhanced the release of large EVs. Both apoptotic and pyroptotic large EVs showed increased Annexin V binding and decreased CD9 expression as compared to those released by healthy living cells. Apoptosis- and pyroptosis-derived large EVs exhibited distinct proteomic profiles. Pyroptotic large EVs were shown to carry interacting protein networks of RNA-binding proteins and chromatin-associated proteins many of which are known as established damage-associated molecular patterns or alarmins. On the other hand, we found that a subpopulation of apoptotic large EVs was characterized by the presence of dsDNA, and active caspase-3/7. Together, our data shed light on the specific protein content of large EVs released by cells undergoing apoptosis and pyroptosis. This study identifies candidate markers of large EVs released by dying cells and may enhance our understanding of the role of EVs in cell death.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Modulation of Autophagy and Extracellular Vesicle Biogenesis by Novel Drug Candidates

Poster 8 – 11.50-13.15

Autophagy is a fundamental cellular process that has been the focus of many recent studies. It includes both degradative and non-degradative intracellular pathways, such as classical lysosomal degradation and amphisome formation through the fusion of autophagosomes with multivesicular bodies. Our recent results show that different cell types can release these amphisomes by ectocytosis as amphiectosomes and later the smaller intraluminal vesicles can be secreted into the extracellular space through a mechanism we refer to as the "torn bag mechanism."

The aim of this study was to investigate the effects of novel drug candidates expected to modulate autophagy. We focused particularly on the number and size distribution of intracellularly formed autophagosomes and amphisomes. In addition, we aimed to understand their mechanisms of action and assess their impact on the release of small extracellular vesicles released by the "torn bag mechanism".

HEK293T-PalmGFP-LC3RFP cells were treated with commonly used autophagy inhibitors, such as chloroquine and bafilomycin A1, as well as novel drug candidates, including salicylanilide and niclosamide. Fixed cells were analysed using a Leica SP8 confocal microscope and evaluated with Leica LAS X software. Gene expression changes were followed by RT-qPCR.

The treatments significantly influenced the number of autophagosomes and amphisomes. Both bafilomycin and chloroquine induced autophagy, but chloroquine had a stronger effect. Salicylanilide caused concentration-dependent increases. RT-qPCR analysis revealed that niclosamide significantly altered the expression of several autophagy-related genes (ATG5, LC3B, MVP, RAB5A, RAB7A, RPTOR).

Our results suggest that the drug candidates affect autophagy through different pathways and modulate extracellular vesicle biogenesis. These findings may support the further development and application of these drugs with novel mechanisms in preclinical and clinical studies.

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Pop, drop and release: novel sEV secretion pathways

Oral presentation – Oral Session 1, Biogenesis/release of EVs and their function in signal transmission – 10.55-11.50

The biogenesis of small extracellular vesicles (sEVs) is still only partially understood due to their diversity. In our recent work, we identified and characterized a novel sEV secretion route, which we termed amphiectosome release and the "torn bag mechanism". In this unconventional biogenesis pathway, amphiectosomes are formed via ectocytosis. Subsequently, their limiting membrane(s) rupture, releasing the intraluminal vesicles into the extracellular space as exosomes. We observed this EV release mechanism in all tested cell lines and mouse organs. Surprisingly, under steady-state conditions, we did not found evidence for the classical exosome secretion through the exocytosis of multivesicular endosomes (MVEs). These findings raise the question of which physiological factors influence the activation of these two distinct EV secretion mechanisms.

The effects of different stress conditions (A23187 Ca^{2+} ionophore treatment and serum depletion) were investigated using transmission electron microscopy, high-resolution flow cytometry, nanoparticle tracking analysis, quantitative confocal microscopy and super-resolution microscopy. Regulation of the two distinct EV secretion pathways was tested by targeted gene silencing.

The transition from the "torn bag mechanism" to exocytosis-based sEV secretion followed the Ca2+ ionophore treatment in a concentration- and time-dependent manner. Silencing ATG5, a key autophagy regulator, inhibited amphiectosome release, whereas RAB27a, a small GTPase that regulates MVE exocytosis, selectively blocked stress-induced exocytosis pathways without affecting amphiectosome release.

Our findings suggest that sEV release via the "torn bag mechanism" represents a general, essential and autophagy-dependent secretion pathway in non-stressed cells, while exocytosis-based exosome release is activated under stress conditions and may play a role in membrane protection.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Biodistribution and Cellular Uptake of Endothelial Colony-Forming Cell Extracellular Vesicles in Sepsis

Poster 9 – 11.50-13.15

Background: Sepsis remains a life-threatening condition characterized by a dysregulated host response to infection, frequently leading to multiorgan failure and high mortality. Up to 60% of patients develop sepsis-induced cardiomyopathy (SICM), a severe myocardial dysfunction that markedly worsens prognosis. Despite advances in critical care, the molecular pathways driving SICM are poorly understood, underscoring the urgent need for innovative therapeutic strategies.

Methods: Endothelial Colony-Forming Cells (ECFCs), highly proliferative endothelial progenitors with strong angiogenic capacity, have emerged as promising candidates for vascular repair in cardiovascular disorders. Their extracellular vesicles (EVs) carry proteins, lipids, and nucleic acids that support immune modulation, endothelial protection, and myocardial repair, thereby offering the regenerative potential of cell-based therapies while circumventing the risks of direct cell transplantation.

In this study, primary ECFCs were isolated from bone marrow of bi-transgenic mice in which only endothelial progenitors and their progeny expressed GFP. Cells were characterized using flow cytometry and fluorescence microscopy. EVs were harvested by differential ultracentrifugation and analyzed through flow cytometry, transmission electron microscopy, and nanoparticle tracking analysis.

Results: To assess biodistribution and uptake, GFP-labelled EVs were injected intraperitoneally into mice. Flow cytometry and confocal microscopy confirmed their efficient internalization by peritoneal cells at multiple time points, indicating effective in vivo intercellular communication. Functional evaluation included Kaplan–Meier survival analyses under different treatment conditions (LPS alone versus LPS combined with small or large EVs). EV administration was well tolerated, and uptake by target cells was consistently demonstrated.

Conclusions: These results provide novel insights into the biodistribution and cellular uptake of ECFC-derived EVs in vivo. Our findings support the therapeutic potential of ECFC-EVs to mitigate cardiac dysfunction in sepsis, establishing a foundation for future translational research aimed at developing EV-based interventions for SICM.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Enhanced Detection and Characterization of Extracellular Vesicles Using Cysteamine-Capped Gold Nanoparticles via Surface-Enhanced Infrared Spectroscopy (SEIRS)

Poster 7, 11.50-13.15

In-house developed cysteamine-capped gold nanoparticles (cysAuNPs) were utilized with infrared (IR) spectroscopy to detect and characterize extracellular vesicles (EVs) through surface-enhanced infrared spectroscopy (SEIRS). Crucially, minimal nanoparticle clustering is required to form electromagnetic hot spots that produce the SEIRS enhancement. In this approach, the cysAuNPs, functionalized with positively charged cysteamine molecules, electrostatically interact with the negatively charged components of EVs. This interaction brings the EVs into close proximity with the nanoparticle clusters, leading to significant amplification of their IR vibrational signals. As a result, SEIRS provides a more intense and distinct IR spectroscopic signature for EVs, facilitating improved detection and compositional analysis. Moreover, cysteamine contributes minimally to the IR spectrum, ensuring that the spectral enhancement originates primarily from the EVs themselves.

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Immunomodulation via engineered embryonic stem cell vesicles

Oral Presentation – Oral Session 3, EVs in therapy – 14.30-15.25

Introduction: Extracellular vesicles (EVs) from embryonic stem cells (ESCs) are emerging as therapeutic tools, transferring proteins, lipids, and RNAs while avoiding the risks and ethical concerns of direct stem cell use. Genetic engineering further enhances their potential by enabling EVs with tailored immunomodulatory properties.

Aims: This study explored the immunoregulatory properties of EVs released by a murine ESC line stably engineered to express multiple immunomodulatory genes. We hypothesized that these vesicles would transfer functional immune checkpoint and regulatory molecules to recipient immune cells, thereby attenuating immune activation.

Methods: A modified ESC line (NT2) was created to express eight immunomodulatory proteins (PD-L1, FasL, CD47, CD200, SerpinB9, MFGE8, CCL21, H2-M3) together with a suicide safety switch (HSV-TK). EVs were purified from conditioned medium using differential ultracentrifugation, characterized by nanoparticle tracking analysis, and examined by flow cytometry for classical EV markers and the introduced proteins. Functional assays tested NT2-EVs in co-culture with peripheral blood mononuclear cells, monitoring effects on CD4+ and CD8+ T cells, B cells, and monocytes. In vivo transplantation experiments in mice were followed by immunophenotyping of blood leukocytes.

Results: NT2-derived EVs carried canonical exosomal markers alongside immunomodulatory proteins such as PD-L1, CD47, and CD200, which were absent from wild-type ESC-EVs. Functionally, both NT2 cells and their EVs significantly reduced activation of CD4+ and CD8+ T cells in vitro. In vivo, NT2 teratomas were associated with decreased circulating CD4+ T cells, consistent with systemic immunosuppression.

Conclusion: Genetically engineered ESC-derived EVs can deliver functional immune checkpoint and regulatory proteins, effectively dampening immune responses. These findings highlight their promise as next-generation, cell-free immunotherapies for transplantation tolerance, autoimmune disease, and regenerative medicine.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Impact of storage conditions and cryoprotectants on extracellular vesicle stability

Poster 11 – 11.50-13.15

Background: The preservation and storage of extracellular vesicles (EVs) poses a critical challenge due to their sensitivity to environmental conditions and the potential impact on integrity, content and function. Their nanoscale properties make EVs particularly vulnerable to damage, necessitating optimized storage protocols to ensure reproducible research outcomes and successful clinical applications.

Aim: This study investigated how different storage conditions affect the physicochemical properties of extracellular vesicles.

Methods: Neutrophils were isolated from human blood, and extracellular vesicles were generated either spontaneously (spEV) or upon stimulation with opsonized particles (ozEV). EVs were isolated by two-step centrifugation and filtration, then stored either at −80 °C or lyophilized and kept for one month. Carbohydrates and albumin were used as cryoprotectants during storage. Particle counts were determined by flow cytometry, while membrane integrity was assessed by LDH release assay.

Results: Particle counts decreased in all stored samples compared to fresh controls, both after storage at −80 °C and following lyophilization. In lyophilized samples the addition of cryoprotectants mitigated this loss, as indicated by significantly higher event counts. This cryoprotective effect was particularly evident when carbohydrate-based cryoprotectants were applied, resulting not only in increased vesicle number but also in better preservation of membrane integrity. LDH release was consistently lower, suggesting reduced membrane disruption.

Conclusion: This study provides a comparative evaluation of EV storage conditions, highlighting the beneficial role of cryoprotectants in maintaining vesicle stability. Our findings support the use of carbohydrate-based cryoprotectants as effective approaches for preserving both EV quantity and integrity. Future studies will explore the extent to which EV functionality is retained during storage.

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Mesenchymal Stem Cell Derived Extracellular Vesicles Functional Study and Their Utilization for the Reduction of Chemotherapeutics Caused Cardiotoxicity

Oral Presentation – Oral Session 3, EVs in therapy – 14.30-15.25

Chemotherapeutic agents, such as some anthracyclines and others, are widely used in the treatment of various cancers. However, their cardiotoxic side effects limit their clinical efficacy and compromise patient health. These drugs induce oxidative stress, inflammation, and cellular apoptosis in cardiac tissues, leading to cardiomyopathy, arrhythmias, and heart failure. As a result, strategies to mitigate these adverse effects are urgently needed to improve the quality of life and survival rates of cancer patients.

Mesenchymal stem cells (MSCs) have gained significant attention for their regenerative potential, partly attributed to their secretion of extracellular vesicles (EVs). MSC-derived EVs carry bioactive molecules, such as proteins, lipids, and RNAs, that can modulate cellular behaviour, promote tissue repair, and reduce inflammation. Recent studies have demonstrated the cardioprotective properties of MSC EVs, suggesting their potential to alleviate the cardiotoxic effects of a wide range of chemotherapeutics.

We aim to validate and understand the therapeutic potential of MSC EVs in counteracting the cardiotoxicity induced by chemotherapeutics. Specifically, we explore the molecular mechanisms by which MSC EVs exert their protective effects, including modulation of oxidative stress, inhibition of apoptosis, changes in mitophagy and autophagy, and promotion of cardiac tissue regeneration.

We work in vitro with AC16 and iUC-MSC cell lines, while utilizing many different methodologies for end-point measurements such as: Viability, cytotoxicity assays, Western blots, PCR/RT-PCR, flowcytometry.

We have already succeeded in reducing the cardiotoxic effect of doxorubicin and others using UC-MSC EVs. Now we are looking further into the biological changes within the cells (autophagy, mitophagy pathways).

In conclusion, MSC-derived EVs represent a promising therapeutic strategy for reducing the cardiotoxic side effects of chemotherapeutics. By harnessing the natural regenerative properties of MSC EVs, it may be possible to enhance the safety and efficacy of cancer treatments, providing a vital improvement in patient outcomes.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Breaking the boundaries of EV detection

Oral Presentation – Oral Session 3, EVs in therapy – 14.30-15.25

EVs play a vital role throughout the human body, and their precise, sensitive, and reproducible analysis is key to better understanding and characterizing diseases, as well as developing innovative therapies. Many techniques can be used to analyze EVs: from single-parameter characterization using ELISA to multi-measurement platforms such as DLS and SRM. However, most existing technologies require researchers to make trade-offs—whether in throughput, accuracy, or resolution.

Nanoscale flow cytometry is a cutting-edge technique that combines the principles of flow cytometry with nanotechnology. It enables the analysis of particles at the nanoscale, providing valuable information about their size, composition, and surface properties. Nanoscale flow cytometry has numerous applications across various fields, including biology, medicine, and materials science. It allows researchers to analyze and characterize nanoparticles, extracellular vesicles (EVs), and other small particles with great precision and sensitivity. In addition to its research applications, nanoscale flow cytometry also holds promise for the development of diagnostic tools and targeted drug-delivery systems. The ability to analyze nanoparticles based on their characteristics opens new possibilities for personalized medicine and nanomedicine.

We are introducing Beckman Coulter's CytoFLEX Nano Flow Cytometer A pivotal development in flow cytometry, the CytoFLEX Nano analyzer enables the analysis of EVs at sizes as small as 40 nm with ease, while simultaneously offering up to six separate fluorescent channels of detection to deliver full characterization. Its high sensitivity and resolution for small particles, combined with automated cleaning, an extensive QC process, and a Fluorescence Sensitivity Monitor, will undoubtedly propel the field of research forward. The capabilities and features of this instrument will enable researchers to explore previously uncharted territories and obtain more comprehensive and accurate data. With this cutting-edge tool, we anticipate significant advancements in research findings and a deeper understanding of extracellular vesicles and their applications.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Tuning the protein corona of extracellular vesicles via antimicrobial peptides

Oral Presentation – Oral Session 3, EVs in therapy – 14.30-15.25

Identifying the spontaneously formed protein corona on the surface of nanoscale materials is of critical importance for the therapeutic and diagnostic development of nanoparticles. In order to explore the potential role in manipulating the surface of nanoparticles and also to progress towards surface engineering, cationic peptides with well-characterized action mechanism were selected. Red blood cell-derived extracellular vesicles (RBCEVs) were isolated, as they are easily produced in high concentrations, and have several unique advantages, such as the lack of DNA, prevention of immune clearance and extended circulation time. The effective peptide concentration was determined using spectroscopy, flow cytometry and microfluidic resistive pulse sensing techniques. Size exclusion chromatography measurements were performed to separate intact vesicles from protein rich soluble fractions after peptide treatment. Nanoscale liquid chromatography coupled to tandem mass spectrometry was performed for characterizing protein corona composition.

Proteomic analysis revealed 125 proteins in the control sample, including membrane-bound and cytosolic proteins as well as metabolic enzymes, of which seventeen were classified as components of the protein corona. The protein profiles of control and peptide-treated samples show a high degree of overlap, supporting the effectiveness of peptide selection strategy. Interestingly, some of the applied peptides also induced the appearance of several proteins that were not detected in the control sample. These proteins may originate from electrostatic interactions between the partially negatively charged EV surfaces and the cationic peptides, which act as membrane anchors facilitating their attachment to the EV surface.

Our results envisage the applicability of cationic peptides in modulating protein corona content, as a possible tool in nanoparticles surface engineering.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Interaction Pathways of Antimicrobial Peptides with Extracellular Vesicles: Disruption, Penetration, and Corona Removal

Poster 11 – 11.50-13.15

Antimicrobial peptides (AMPs) and extracellular vesicles (EVs) are integral to immune defense and regulation. EVs can act as AMP carriers, and certain AMPs can remove the protein corona (PC) from EVs, enabling new bioengineering and proteomics approaches.

To clarify how AMPs and EVs interact, we examined twenty-six AMPs, including cell-penetrating peptides (CPPs), with red blood cell-derived vesicles (REVs). Using spectroscopy, flow cytometry, nanoparticle tracking, electron microscopy, and zeta-potential analyses, we identified distinct interaction patterns. Peptides such as LL-37 and lasioglossin-III efficiently removed the PC with minimal membrane disruption, while AMPs like KLA, bradykinin, histatin-5, and most CPPs (e.g., octa-arginine, penetratin, buforin II) showed cell-penetrating behavior with little vesicle damage.

Our findings outline major AMP–EV interaction pathways and suggest strategies for tuning EV properties for therapeutic applications.

HSEV Conference, June 21, 2024, Budapest, Hungary

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Isolation and Characterization of Bovine Milk-Derived Extracellular Vesicles for Potential Therapeutic Applications

Poster 12 – 11.50-13.15

Milk-derived extracellular vesicles (mEVs) are nanosized, lipid-bound particles rich in regulatory RNAs, proteins, and bioactive lipids. Recent studies show that mEVs can resist digestion, cross intestinal barriers, and influence immune and inflammatory responses. They also help maintain gut integrity, support microbiome balance, and promote tissue repair. Preclinical research indicates beneficial effects in models of intestinal inflammation, metabolic and cardiovascular disorders, and nervous system injury, highlighting their potential as natural therapeutics and oral drug-delivery carriers with low immunogenicity. In our research group, we have established a published protocol for isolating bovine milk EVs with high purity, reproducibility and consistent cargo profiles. This poster presents this isolation workflow and preliminary physicochemical and molecular characterization of mEVs, which will serve as the basis for our upcoming in vivo studies in mouse models of different human diseases.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Measurement traceability in the characterization of extracellular vesicles: From principles to reference materials

Oral Presentation – Oral Session 2 – EV-based biomarkers – 13:15 – 14:10

Metrology—the science of measurement—provides the foundation for reliable, comparable, and traceable data across scientific disciplines. Extracellular vesicles (EVs) exhibit high heterogeneity, and the diversity of measurement technologies further complicates their characterization; thus, metrological principles are essential to ensure reproducibility and inter-laboratory comparability. Techniques like atomic force microscopy (AFM) and small-angle X-ray scattering (SAXS) exemplify how metrological traceability links experimental data to SI units through unbroken chains of comparisons with stated uncertainties. Reference materials (RMs) are essential for calibration, validation, and quality control, ensuring measurement comparability across analytical platforms.

Hollow organosilica beads (HOBs) were developed and evaluated as potential RMs for setting EV size gates in flow cytometry (FCM), addressing the limitations of polystyrene beads arising from refractive index mismatches. Prepared via a hard-template sol–gel method, HOBs exhibit uniform size, tunable shell thickness, and light scattering behavior that closely matches EVs. Their traceable characterization by SAXS, AFM, and single-particle ICP-MS provides reliable size and concentration values. Moreover, fluorescently labeled HOBs synthesized through peptide-assisted conjugation exhibit dim, EV-like fluorescence, making them suitable for fluorescence calibration.

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HSEV Conference, June 21, 2024, Budapest, Hungary

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Microplate-Based Transient Agarose Spot (TAS) Assay for Quantitative Assessment of Extracellular Vesicle–Mediated Cell Migration

Poster 1 – 11.50-13.15

Introduction: Collective cell migration plays a vital role in various biological processes, such as tumor progression and metastasis. The conventional scratch assay (wound healing assay) is widely used to study this phenomenon, but it has significant limitations in terms of throughput, reproducibility, and data analysis. In parallel, mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) have gained attention as potential modulators of tumor cell migration.

Aims: To address the shortcomings of traditional migration assays, we aimed to further improve our previously developed Transient Agarose Spot (TAS) assay by adapting it to a high-throughput, microplate-based format suitable for automated analysis. As a proof-of-concept, we specifically investigated the modulatory effects of EVs on cancer cells.

Methods: We established a 96-well plate version of the TAS assay and integrated it with microplate reader-based automated data acquisition. Hoechst staining was used to label viable cells, allowing for reliable, non-toxic kinetic measurements. Fluorophore-expressing cancer cells were employed to validate the method. MSC-derived EVs were employed to quantify concentration-dependent effects on cell migration.

Results: The microplate-based TAS assay enabled accurate, high-throughput detection of cell migration. It effectively measured the dose-dependent influence of fetal bovine serum (FBS) on migration, as well as the anti-migratory effects of kinase inhibitors and MSC-EVs in lung cancer cells. The assay provided quantifiable, concentration-dependent results, demonstrating both inhibitory and modulatory effects on cell migration.

Conclusion: This improved TAS assay offers a robust, scalable, and cost-effective alternative to traditional microscopy-based methods. Its compatibility with high-throughput screening makes it a valuable tool for studying cell migration and for drug discovery applications.

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EXODUS, A High Efficiency System for Automated EVs Isolation

Oral Presentation – Oral Session 2 – EV-based biomarkers – 13:15 – 14:10

Extracellular vesicles (EVs) play a critical role in intercellular communication and are emerging as biomarkers for disease diagnostics and therapeutic delivery. Traditional methods like UC and TFF often yield impure samples with high levels of protein contamination. The EXODUS system overcomes these limitations by offering a rapid and scalable method to isolate exosomes and viruses with high purity and yield.