1st EV4

EXTRACELLULAR VESICLES IN VISEGRAD COLLABORATION CONFERENCE

PROGRAMME



21-22 OCTOBER 2026

Semmelweis University, Budapest, Hungary

EV4 EXTRACELLULAR VESICLES IN VISEGRAD COLLABORATION CONFERENCE

HUPHAR Hungarian Society for Experimental and Clinical Pharmacology

MAGYAR IMMUNOLÓGIAI TÁRSASÁG

WELCOME

The organizing committee welcomes you at the first EV4 – Extracellular Vesicles in Visegrad Collaboration conference, a joint event of extracellular vesicle researchers of the Czech Republic, Poland, Slovakia, Hungary and beyond, held in Budapest, Hungary between 21-22 October 2026.

The meeting brings together researchers from all career stages, with a special focus on young researchers to meet and share their knowledge and expertise, offering a unique opportunity for regional cross-border scientific exchange and cooperation. One of the key aims of the event is to establish the basis of a strong international cooperation in extracellular vesicle research throughout the V4 countries and beyond. Through keynote talks of outstanding EV researchers, lectures, poster sessions and social events, the conference will support the initiation of joint research projects, cross-border initiatives and long-term scientific collaborations.

Main topics of the conference include:

- Biology of extracellular vesicles
- Extracellular vesicles from non-mammalian sources
- Novel technologies in extracellular vesicle research and development
- Extracellular vesicle-based therapies and diagnostics



ORGANIZERS OF THE CONFERENCE:

Czech Republic, Czech Society for Extracellular Vesicles:

Vendula Hlaváčková Pospíchalová
Masaryk University

Andrea Gálisová
Charles University

Jan Balvan
Masaryk University

Hungary, Hungarian Section for Extracellular Vesicles:

Zoltán Giricz
Semmelweis University

Edit Buzás
Semmelweis University

Csenger Kovácsházi
Semmelweis University

Poland:

Sabina Górska
Hirszfeld Institute, Polish Academy of Sciences

Agnieszka Razim
Hirszfeld Institute, Polish Academy of Sciences

Slovakia, Slovak Society for Extracellular Vesicles:

Zuzana Vince Kážmérová
Biomedical Research Center of the Slovak Academy of Sciences

Kristína Lichá
Comenius University

Barbora Tamášová
Comenius University

STRATEGIC SPONSORS

ANAMET
ANALYTICAL & MEASURING & TESTING

PARTICLE METRIX

ACCELA

IZON

EXODUS

Secretech Biosciences

PREMIUM SPONSORS

BIO-SCIENCE
www.bio-science.hu

BECKMAN COULTER

AURO-SCIENCE CONSULTING

KROMAT

ZEISS

BIO-RAD

RWD

greiner BIO-ONE

ANIMALAB DISCOVERY PARTNER®

everest biolabs

SPONSORS

HSEV Hungarian Section for Extracellular Vesicles

WACŁAW FELCZAK ALAPÍTVÁNY

CzeSEV

SEMMELWEIS UNIVERSITY 1769

SlovSEV SLOVAK SOCIETY FOR EXTRACELLULAR VESICLES

TABLE OF CONTENTS

Welcome 2

Organizers of the conference 3

General informations 6

Programme 8

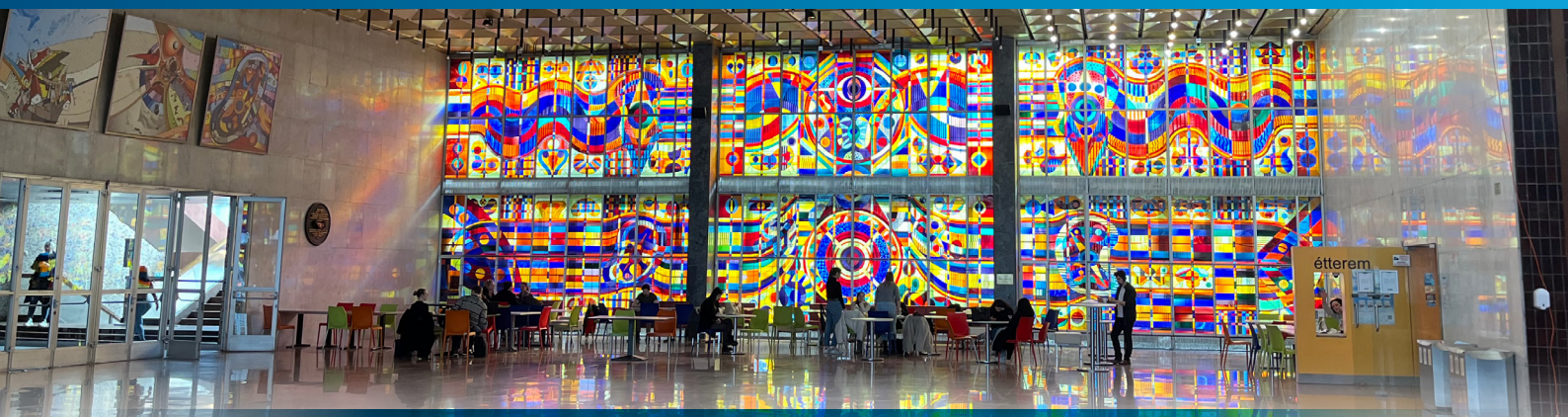
Oral abstracts 19

- W2 - Session 1 - Cell Biology of Extracellular Vesicles – 1 20
- W3 - Session 2 - Cell Biology of Extracellular Vesicles - 2 24
- W4 - Session 3 - Extracellular Vesicles from Bacterial Sources 29
- W5 - Session 4 - Technologies in Extracellular Vesicle Analysis and Therapy 34
- T2 - Session 5 - Extracellular Vesicle-Based Diagnostics 39
- T3 - Session 6 - Extracellular Vesicle-Based Therapeutics 45

Poster abstracts 51

- Cell biology of extracellular vesicle release, uptake and signal transduction 52
- EV-based diagnostic applications 61
- EVs as therapeutics, including efficacy, safety and EVs as delivery systems 67
- EVs in specific disease conditions, including cancer, immune- and cardiovascular diseases 80
- Extracellular vesicles originating from non-mammalian and non-animal sources 94
- New technologies related to extracellular vesicles, including isolation, characterisation, engineering, and synthetic EV-mimetics 100
- Other topics related to extracellular vesicles 116

Sponsors 127



GENERAL INFORMATION

Venue:

Address: 1089 Budapest, Nagyvárad tér 4.

The conference is held at the Semmelweis University Theoretical Building at Nagyvárad tér. The entrance is on the left side of the tower building. Take Exit C from the Metro station.

You can find the exact location of the event venue HERE.

The venue is accessible between 07:00 and 20:00 every day.

Wi-Fi is available at the venue:

- network name: konferencia
- password: Semmelweis

Cloakroom will be operated at the venue during conference hours: October 21, 08:00-19:00, October 22, 08:00-14:30



On-site registration and hospitality desk will be operated at the venue in the following hours:

October 20 - 16:30 - 18:30

October 21 - 8:00 - 17:00

October 22 - 8:00 - 11:00

Access:

The building has excellent public transport connections, take M3 metro to Nagyvárad tér station. Tram lines 23 and 24 also serves Nagyvárad tér. The building is just a 1 minute walk away.
We recommend the 72-hour Budapest-travelcard, that can be bought at major public transport hubs or in the Budapest Go app. Several licenced taxi companies operate in Budapest, look for yellow cabs. Public bicycles are also available citywide in the MOL Bubi app.
From Liszt Ferenc International Airport take airport shuttle 200E to Kálvin tér, then transfer to

M3 metro to Nagyvárad tér (Kőbánya-Kispest direction). Travelling with shuttle bus 200E requires a separate ticket that can be purchased at the airport from vending machines at the bus stop located at the Arrival level of Terminal 2B or in the Budapest Go app.
From Nyugati railway station take M3 metro to Nagyvárad tér (Kőbánya-Kispest direction).
From Kelenföld railway station or Keleti railway station take M4 metro to Kálvin tér and transfer to M3 metro to Nagyvárad tér (Kőbánya-Kispest direction).

Networking event:

Venue: Symbol Budapest
Address: Budapest, Bécsi út 56, 1036
Date: Wednesday, 21 October

Programme:
The programme will start at 20:00.
The evening will offer a relaxed setting to meet fellow participants, continue conversations from the conference, and enjoy Budapest together. The programme will include dinner, drinks, DJ and dancing.

Getting there:
From the NET Building at Nagyvárad tér, take M3 metro to Deák Ferenc tér. From there, you can either take M2 metro towards Déli pályaudvar to Batthyány tér, followed by tram 19 or 41 to Kolosy tér, or take bus 9 from Deák Ferenc tér to Kolosy tér. Symbol Budapest is within a short walking distance from both stops.
You can find the exact location of the event venue HERE.

Please note that access to the Networking Event is reserved for registered participants. **Please wear the name badge** received during conference registration, which will serve as your admission pass for the evening.

Conference Secretariat:

Altagra Business Services and Travel Agency Ltd.
Email: EV42026@altagra.hu
Web: EV42026

1st EV4 PROGRAMME



Tuesday, 20. October, 2026

9:00 – 13:00		**EV4 workshop with Exodus** Morning session
9:00 – 13:00		**EV4 workshop with Bio-Science Kft. & Beckman Coulter** Morning session
13:00 – 17:00		**EV4 workshop with Exodus** Afternoon session
13:00 – 17:00		**EV4 workshop with Bio-Science Kft. & Beckman Coulter** Afternoon session
16:30 – 18:30		**Registration**
17:00 – 22:00		**Young Scientists Social Event**

Wednesday, 21. October, 2026

8:00 – 17:00		**Registration**
9:00 – 9:30		**Opening ceremony**
9:30 – 10:00	W1	**PLENARY LECTURE 1** **PARADIGMS AND OUTSTANDING QUESTIONS IN EV RESEARCH AND DEVELOPMENT (20'+5')** Kenneth Witwer Johns Hopkins School of Medicine Faculty
10:00 – 11:00	W2	**SESSION 1 - CELL BIOLOGY OF EXTRACELLULAR VESICLES - 1** Chair: Andrea Gálisová, Kristína Lichá
10:00 – 10:20	W2-1	**ENGINEERING RBC-DERIVED EXTRACELLULAR VESICLE SURFACES THROUGH PEPTIDE-MEDIATED PROTEIN CORONA REMODELING** (15'+5') [1]Imola Cs. Szigyártó, [1]Tasvilla Sonallya, [1]Tünde Juhász, [1]Priyanka Singh, [2]Anikó Gaál, [2]Judith Mihály, [3]Lilla Túriák, [3]László Drahos, [2]Zoltán Varga, [1]Tamás Beke-Somfai

[1]Biomolecular Self-Assembly research group, [2]Biological Nanochemistry research group, [3]Glycan Biomarker research group, HUN-REN Research Centre for Natural Sciences, Hungary

10:20 – 10:35 W2-2 **WHEN CELLS ARE STRESSED, EXOSOMES TAKE OVER: SWITCHING THE ROUTE OF SMALL EXTRACELLULAR VESICLE RELEASE** (10'+5')

[1]Dorina Lenzinger, [1]Lilla Lankovics, [1]Tünde Bárkai, [2]Mirjam Balbisi, [1]Krisztina V. Vukman, [1]Kelsey Fletcher, [1]Edina Bugyik, [3]Csaba Cserép, [3]Ádám Dénes, [1,4]Szilvia Bősze, [2]Lilla Turiák, [5,6]Gábor Mórotz, [7]Márton Sághi, [7]Attila Fintha, [1,8]Edit I. Buzás, [1,9]Tamás Visnovitz

[1]Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Hungary, [2]MTA-HUN-REN TTK Lendület (Momentum) Glycan Biomarker Research Group, HUN-REN Research Centre for Natural Sciences, Hungary, [3]Laboratory of Neuroimmunology, HUN-REN Institute of Experimental Medicine, Hungary, [4]HUN-REN-ELTE Research Group of Peptide Chemistry, ELTE Eötvös Loránd University, Hungary, [5]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [6]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary, [7]Department of Pathology and Experimental Cancer Research, Semmelweis University, Hungary, [8]HUN-REN-SU Translational Extracellular Vesicle Research Group, Hungary, [9]Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Hungary

10:35 – 10:50 W2-3 **CELLULAR ABUNDANCE SHAPES THE ARCHITECTURE OF EXTRACELLULAR VESICLE MIRNA CARGO MORE STRONGLY THAN CANONICAL SORTING MOTIFS** (10'+5')

[1,2] Vojtěch Šprincl, [1] Nataliya Romanyuk

[1]Institute of Experimental Medicine, Czech Academy of Science, Czech Republic, [2]Second Medicine Faculty, Charles University, Czech Republic

10:50 – 11:00 W2-4 **SPONSORS TALK – IZON**

11:00 – 11:30 **Coffee break**

11:30 – 12:45 W3 **SESSION 2 - CELL BIOLOGY OF EXTRACELLULAR VESICLES - 2**

Chair: Zuzana Vince Kážmérová, Csenger Kovácsházi

11:30 – 11:50 W3-1 **SOURCE-DEPENDENT EFFECTS OF MSC-DERIVED EXTRACELLULAR VESICLES IN TWO IN VITRO MODELS OF OSTEOARTHRITIS** (15'+5')

[1]Denisa Harvanova, [2]Alexander Otahal, [1]Slavomira Gulova, [2]Markus Rothammer, [2]Bernadette Kern, [2]Karina Kramer, [1]Livia Kolesar Fecskeova, [1]Jana Matejova, [1]Hedviga Kosuthova, [1]Jana Bzdilova, [1]Lucia Slovinska, [2]Stefan Nehrer, [2]Andrea De Luna

[1]Associated Tissue Bank, Faculty of Medicine, P.J. Safarik University and L. Pasteur University Hospital in Kosice, Slovakia, [2]Center for Regenerative Medicine, University for Continuing Education Krems, Austria

11:50 – 12:05 W3-2 **PHOSPHATIDYLSERINE-POSITIVE EXTRACELLULAR VESICLES FROM ACIVATED FIBROBLASTS: THEIR PATHOLOGICAL ROLE IN ONCOGENIC SIGNALING IN HEAD AND NECK CANCERS** (10'+5')

[1,2]Jan Balvan, [1]Klara Hanelova, [1]Jiri Navratil, [1]Monika Kratochvilova, [1]Kveta Pilarova, [1,2]Martina Raudenska
[1]Department of Pathophysiology, Faculty of Medicine, Masaryk University, Czech Republik, [2]Department of Physiology, Faculty of Medicine, Masaryk University, Czech Republik

12:05 – 12:20 W3-3 **CHARACTERISATION OF EV-DNA IN JUVENILE IDIOPATHIC ARTHRITIS** (10'+5')

[1,4]Kristína Truchanová, [2,4]Barbora Tamášová, [2]Jakub Janko, [3]Tomáš Dallos, [3]Barbora Balážiová, [2]Michal Pastorek
[1] Institute of Medical Physics and Biophysics, Faculty of Medicine, Comenius University, [2] Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, [3] Department of Pediatrics, Comenius University Medical Faculty in Bratislava and National Institute of Children's Diseases, Bratislava, Slovakia, [4]MicroPoll s. r. o., Bratislava, Slovakia

12:20 – 12:35 W3-4 **MAST CELL SECRETOME AND ITS FUNCTIONAL IMPACT ON CARDIOMYOCYTES** (10'+5')

[1]Kelsey Fletcher, [1]Zsófia Fodor, [1]Zsófia Szász, [1]Dorina Lenzinger, [1]Tamara Hermans, [1]Bence Nagy, [1]Tünde Bárkai, [2,3]Péter Lőrincz, [1,4]Tamás Visnovitz, [1,5]Edit I. Buzás, [1]Krisztina V. Vukman
[1]Semmelweis University, Department of Genetics, Cell- and Immunobiology, Hungary, [2]ELTE Eötvös Loránd University, Department of Anatomy, Cell and Developmental Biology, Hungary, [3]Intracellular Transport & Cytoskeleton Research Group, HUN-REN Biological Research Centre, Hungary, [4]ELTE Eötvös Loránd University, Department of Plant Physiology and Molecular Plant Biology, Hungary, [5]HUN-REN–SU Translational Extracellular Vesicle Research Group, Hungary

12:35 – 12:45 W3-5 SPONSORS TALK – PARTICLE METRIX
ZETAVIEW® EVOLUTION : THE IMPORTANCE OF FLUORESCENCE FOR EVS CHARACTERIZATION USING NTA TECHNOLOGY (8'+2')

Bertrand Damart
Particle Metrix GmbH

12:45 – 13:15 **Lunch break**

13:15 – 14:00 **POSTER SESSION**

14:00 – 15:15 W4 **SESSION 3 - EXTRACELLULAR VESICLES FROM BACTERIAL SOURCES**
Chair: Agnieszka Razim, Tamás Beke-Somfai

14:00 – 14:20 W4-1 **VESICLES FROM PROBIOTIC BACTERIA: IMMUNE MODULATION, ALLERGY PREVENTION AND ENVIRONMENTAL SENSING** (15'+5')
[1]Irma Schabussova
[1]Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria

14:20 – 14:35 W4-2 **BIFIDOBACTERIUM ADOLESCENTIS-DERIVED EXTRACELLULAR VESICLES SHAPE MUCOSAL IMMUNE RESPONSES IN ALLERGIC AIRWAY INFLAMMATION** (10'+5')
[1]Dominika Kozakiewicz, [2]Dagmar Srutkova, [2]Martin Schwarzer, [1]Dominika Jakubczyk, [1]Agnieszka Razim, [1]Sabina Górska
[1]Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland, [2] Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Novy Hradek, Czech Republic

14:35 – 14:50 W4-3 **BIDIRECTIONAL EV-RELATED CROSSTALK BETWEEN MELANOMA CELLS AND GRAM-POSITIVE SKIN BACTERIA** (10'+5')
[1]Richárd Máté, [1]Djenana Vejzovic, [1]Giorgia Magnarini, [1]Silvia Schauer, [1]Simone Klinger, [1]Sophie Steinkogler, [2]Anna Marin, [2]Jasmina Tuzlak, [2]Altea Topicu, [2]Nermina Malanovic, [1,3] Beate Rinner
[1]Diagnostic and Research Institute of Pathology, Medical University of Graz, Austria, [2]Institute of Molecular Biosciences, University of Graz, Austria, [3]BiotechMed, Austria

14:50 – 15:05 W4-4 **CHEMICALLY DEFINED MEDIUM ENHANCES DETECTION OF IRON-DEPENDENT CHANGES IN BACTERIAL EXTRACELLULAR VESICLES** (10'+5')
Subhasree Venkatasubramaniyan, Norbert Łodej, Sabina Górska, Agnieszka Razim
Hirszfeld Institute of Immunology and Experimental Therapy PAS, Wrocław, Poland

15:05 – 15:15 W4-5 SPONSORS TALK – SECRETECH
DISCOVER HIDDEN BIOLOGY WITH SINGLE EV PROTEOMICS (8'+2')
Rudi Schläfli
Business Development Manager Europe, Secretech Biosciences

15:15 – 15:45 **Coffee break**

15:45 – 17:00 W5 **SESSION 4 - TECHNOLOGIES IN EXTRACELLULAR VESICLE ANALYSIS AND THERAPY**
Chair: Vendula Hlaváčková Pospíchalová, Edit Buzás

15:45 – 16:05 W5-1 **PROGRAMMING EXTRACELLULAR VESICLES FOR IMAGE-GUIDED TARGETED DELIVERY** (15'+5')
[1,2]Andrea Galisova, [1]Aida de la Cruz-Gambra, [1]Jiri Zahradnik, [2]Dominik Havlicek, [3]Josef Uskoba, [4]Ziv Porat, [2]Daniel Jirak
[1]BIOCEV, First Faculty of Medicine, Charles University, Czech Republic, [2]Department of Imaging Methods, Institute for Clinical and Experimental Medicine, Czech Republic, [3]Biotech a.s., Czech Republic, [4]Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel

16:05 – 16:20 W5-2 **LIPID REMODELING AND iNOS CARGO IN BACTERIALLY REPROGRAMMED MACROPHAGE EVs** (10'+5')
[1,2]Miriam Dutkova, [1,2]Julia Orlovska, [1,3]Kristyna Turkova, [1]Eva Vojtkova, [1,2]Marek Oslacky, [1,2]Lucia Calekova, [1,2]Veronika Mlcekova, [4]Stepan Strnad, [4]Vladimir Vrkoslav, [5]Vratislav Berka, [6]Josef Chudacek, [6]Stefan Kolcun, [6]Eva Kriegova, [1,2,3]Lukas Kubala, [1]Gabriela Ambrozova
[1]Department of Biophysics of Immune System, Institute of Biophysics of the Czech Academy of Sciences, Czech Republic, [2]Department of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic, [3]International Clinical Research Center, St. Anne's University Hospital Brno, Czech Republic, [4]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic, [5]Contipro a.s., Czech Republic, [6]Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Czech Republic

16:20 – 16:35 W5-3 **WHEN EV DOSE IS NOT WHAT IT SEEMS: MISMATCH BETWEEN PARTICLE NUMBER- AND PROTEIN-BASED DOSING** (10'+5')
Tímea Böröczky[1,2,3], Mária Harmati[1], Edina Gyukity-Sebestyén[1], Tamás F. Polgár[4,5], Csenge Feinek[2], Emma Balog[1], Antal Martinecz[6], Krisztina Buzás[1,2], Gabriella Dobra[1,2]*, Mátyás Bukva[1,2]
[1]Institute of Biochemistry, HUN-REN Biological Research Centre, Szeged, Hungary, [2]Department of Immunology, University of Szeged, Szeged, Hungary, [3]Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged, Hungary, [4]Core Facilities - Transmission Electron Microscope Laboratory, HUN-REN Biological Research Centre, Szeged, Hungary, [5]HUN-REN-

SZTE Neuroscience Research Group, Hungarian Research Network, University of Szeged, Hungary, [6]Certara, Radnor, Pennsylvania, USA

16:35 – 16:50 W5-4 **HIGH-PURITY EXTRACELLULAR VESICLE ISOLATION UNRAVELS DISTINCT BUT OVERLAPPING PROTEIN COMPOSITION WITH LIPOPROTEINS IN ACUTE MYOCARDIAL INFARCTION PATIENTS** (10'+5')

[1,2]Dóra Kapui, [1,2]Szabolcs Hambalkó, [1,2]Yavuz Artuner, [1,2]Orsolya Szalai, [1,2]Richárd Rácz, [3]David Matallanas, [3]Kieran Wynne, [1,2,4]Péter Ferdinandy, [1,2,4]Zoltán Giricz, [1,2]Csenger Kovácsházi

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary, [3]Systems Biology Ireland, University College Dublin, Ireland [4]Pharmahungary Group, Hungary

16:50 – 17:00 W5-5 SPONSORS TALK – EXODUS

NEW TECHNOLOGY HIGHLIGHT- EVS ISOLATION USING THE EXODUS TECHNOLOGY

Yuri D'Alessandra

Exodus Bio

17:00 – 17:15 **Break**

17:15 – 18:15 **General assemblies of CzeSEV, SlovSEV and HSEV**

20:00 – 0:00 **Networking event - Registration is needed**

Venue: Symbol Budapest

Thursday, 22. October, 2026

8:00 – 11:00 **Registration**

9:00 – 9:30 T1 **PLENARY LECTURE 2**
EXTRACELLULAR VESICLES IN THE VASCULATURE: NOT ONLY BLOOD BUT ALSO LYMPH (20'+5')
Wolfgang Holnthoner
Ludwig Boltzmann Institute for Traumatology

9:30 – 11:00 T2 **SESSION 5 - EXTRACELLULAR VESICLE-BASED DIAGNOSTICS**
Chair: Jan Balvan, Barbora Tamášová

9:30 – 9:50 T2-1 **EXTRACELLULAR VESICLES IN THE OVARIAN CANCER MICROENVIRONMENT: FROM ASCITES BIOLOGY TO LIQUID BIOPSY AND PRECISION MEDICINE** (15'+5')
[1]Stanislava Sladeček, [1]Šárka Hrachovinová, [2]Dita Münzová, [2]Michal Felsinger, [3]Markéta Bednaříková, [4]Jitka Hausnerová, [5]Václav Pustka, [5]Zbyněk Zdráhal, [2]Vít Weinberger, [1]Vendula Pospíchalová
[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic, [2]Department of Obstetrics, Gynecology and Neonatology, University Hospital Brno and Medical Faculty, Masaryk University, Czech Republic, [3]Department of Internal Medicine - Hematology and Oncology, University Hospital Brno and Medical Faculty, Masaryk University, Czech Republic, [4]Department of Pathology, University Hospital Brno and Medical Faculty, Masaryk University, Czech Republic, [5]Proteomics Core Facility, Central European Institute of Technology, Masaryk University, Czech Republic

9:50 – 10:05 T2-2 **BEYOND BULK EV ANALYSIS: AN INTEGRATED WORKFLOW FOR SUBPOPULATION-SPECIFIC EXTRACELLULAR VESICLES PROFILING** (10'+5')
[1-2]Elisabetta Pingitore, [2]Selena Mimmi, [2]Valentina Crapella, [2]Anna Maria Zimbo, [2]Annarita Scardamaglia, [2]Costanza Maria Cristiani, [2]Luana Scaramuzzino, [2]Andrea Quattrone, [2]Aldo Quattrone, [1-2]Enrico Iaccino
[1]Department of Experimental and Clinical Medicine, Catanzaro, Italy, [2]Laboratory of Neuroimmunology, Institute of Neurology, Catanzaro, Italy

10:05 – 10:20 T2-3 **NEUROFILAMENT LIGHT CHAIN IN NEURONALLY DERIVED EXTRACELLULAR VESICLES: DIFFERENTIATING PARKINSON'S DISEASE FROM ESSENTIAL TREMOR WITH RESTING TREMOR** (10'+5')
[1,3]Valentina Crapella, [1,3]Selena Mimmi, [2]Elisabetta Pingitore, [1,3]Annarita Scardamaglia, [1,3]Anna Maria Zimbo, [1,3]Andrea Quattrone, [1,3]Aldo Quattrone, [2]Enrico Iaccino
[1]Laboratory of Neuroimmunology, Institute of Neurology, Department of Medical and Surgical Sciences, University Magna Graecia of Catanzaro, Catanzaro, Italy; [2] Department of Experimental and Clinical Medicine, University „Magna Graecia" of Catanzaro, Catanzaro, Italy; [3] Neuroscienze

Research Center, Magna Graecia University, Catanzaro, Italy

10:20 – 10:35 T2-4 **EXTRACELLULAR VESICLE-BASED DIAGNOSTICS AND PROGNOSTICS: CURRENTLY APPLIED TECHNOLOGIES AND UNMET NEEDS – INSIGHTS FROM A SCOPING REVIEW ON HEART FAILURE** (10'+5')

[1,2]Csenger Kovácsházi, [1,2]Dóra Kapui, [1,2]Yavuz Artuner, [1,2]Szabolcs Hambalkó, [1,2]Richárd Rácz [1,2]Teresa Hung, [1,2]Bennet Y Weber, [1,2]Dániel Dobák, [1,2]Tamás G Gergely, [3]Kenan Aloss, [4,5]Gemma Chiva-Blanch, [6,7]Joost P. G. Sluijter, [8,9,10]Edit I Buzás, [1,2,11]Péter Ferdinandy, [1,2,10]Zoltán Giricz

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Budapest, Hungary, [3]Faculty of Medicine, Université de Montréal, Montréal, QC H3C 3T5, Canada, [4]Health Sciences Faculty, Universitat Oberta de Catalunya (UOC), Barcelona, Spain, [5]Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain, [6]Department of Cardiology, Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht, the Netherlands, [7]UMC Utrecht Regenerative Medicine Center, Circulatory Health Research Center, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands, [8]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, [9]HCEMM-SU Extracellular Vesicle Research Group, Budapest, Hungary, [10]HUN-REN-SU Translational Extracellular Vesicle Research Group, Budapest, Hungary, [11]Pharmahungary Group, Szeged, Hungary

10:35 – 10:50 T2-5 **SNEV: A GLOBAL COMMUNITY FOR EARLY-CAREER RESEARCHERS IN EXTRACELLULAR VESICLES** (10'+5')

[1]Reza Abouali, on behalf of the Student Network on Extracellular Vesicles (SNEV) Executive Committee

[1]Laboratory of Experimental and Translational Immunology, University of Gdansk and Medical University of Gdansk, Intercollegiate Faculty of Biotechnology, Gdansk, Poland

10:50 – 11:00 T2-6 **SPONSORS TALK – ACCELA**

11:00 – 11:30 **Coffee break**

11:30 – 13:00 T3 **SESSION 6 - EXTRACELLULAR VESICLE-BASED THERAPEUTICS**

Chair: Zoltán Giricz, Sabina Górska

11:30 – 11:50 T3-1 **CYTOKINE PRIMING ENHANCES THE IMMUNOMODULATORY CARGO AND EFFICACY OF MSC-DERIVED EVS IN T2-LOW AIRWAY INFLAMMATION** (15'+5')

Andrzej Eljaszewicz, Marlena Tynecka, Dominik Cysewski, Magdalena Niemira,

Agnieszka Tarasik

11:50 – 12:05 T3-2 **METHOD MATTERS: COMPARATIVE EVALUATION OF TISSUE-DERIVED EXTRACELLULAR VESICLE ISOLATION METHODS IN NON-SMALL CELL LUNG CANCER** (10'+5')

[1]Anna Biernacka, [1]Jakub Faktor, [1]Martyna Siewiera, [1]Ines Papak, [1]Artur Piróg, [1]Zuzanna Urban-Wójciuk, [2]Magdalena Sitkiewicz, [2]Magdalena Szczepanowska, [2]Tomasz Marjański, [2]Witold Rzyman, [3,4]Aneta Zebrowska, [3,5]Monika Pietrowska, [6]Danuta Gutowska-Owsiak, [1]Sachin Kote, [1,7]Natalia M. Marek-Trzonkowska

[1]International Centre for Cancer Vaccine Science, University of Gdańsk, Poland, [2]Department of Thoracic Surgery, Medical University of Gdansk, Poland, [3]Maria Sklodowska-Curie National Research Institute of Oncology, Poland, [4]Department of Histology and Cell Pathology, Medical University of Silesia, Poland, [5]Department of Biotechnology and Nutrigenomics, Institute of Genetic and Animal Biotechnology of the Polish Academy of Sciences, Poland, [6]Laboratory of Experimental and Translational Immunology, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk, Poland, [7]Laboratory of Immunoregulation and Cellular Therapies, Medical University of Gdańsk, Poland

12:05 – 12:20 T3-3 **EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS DERIVED FROM THE UMBILICAL CORD REDUCE CARDIOTOXICITY OF VARIOUS ANTI-TUMOUR AGENTS** (10'+5')

[1, 2]Szabolcs Hambalkó, Richárd [1, 2]Rácz, [1, 2]Teresa Hung, [1, 2]Nóra Wagner, [3]Tamás Visnovitz, [1,2]Bence Ágg, [1, 2]Dóra Bihary, [1, 2]Csenger Kovácsházi, [1, 2]Dóra Kapui, [1, 2]Yavuz Artuner, [5]László Cervenak, [5]Erika Kajdácsi, [1, 2, 4]Péter Ferdinandy, [1, 2, 4]Zoltán Giricz

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary, [3]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Hungary, [4]Pharmahungary Group, Hungary, [5]Department of Internal Medicine and Hematology, Semmelweis University, Hungary

12:20 – 12:35 T3-4 **THERAPEUTIC APPLICATION OF EV-AMP DELIVERY SYSTEMS IN A MURINE MODEL OF SEPSIS** (10'+5')

[1,2]Tasvilla Sonallya, [3,4]Zoltan Varga, [1,2]Kamal el Battoui, [5]Kata Horvati, *[6,7]Yumi Kumagai and *[1]Tamas Beke-Somfai

[1]Institute of Materials and Environmental Chemistry, Biomolecular Self-assembly Research Group, Research Centre for Natural Sciences, Budapest, Hungary, [3]Institute of Materials and Environmental Chemistry, Biological Nanochemistry Research Group, Research Centre for Natural Sciences, Budapest, Hungary, [4]Department of Physical Chemistry and Materials Science, Budapest University of Technology and Economics, Hungary, [5]MTA-HUN-REN TTK "Momentum" Peptide-Based Vaccines Research Group, Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary, [6]Department of Biochemistry and Systems Biomedicine, Juntendo University Graduate School of Medicine, Tokyo, Japan, [7]Biomedical Research Core Facilities, Juntendo University Graduate School of

Medicine, Tokyo, Japan

12:35 – 12:50	T3-5	**COMPARTMENT-SPECIFIC IMMUNOMODULATORY EFFECTS OF ENDOTHELIAL COLONY-FORMING CELL-DERIVED EVS IN A MURINE MODEL OF SYSTEMIC INFLAMMATION** (10'+5') [1]Luigi Menna, [1]Nóra Fekete, [1]Boglárka Nagy, [1]Kristóf Csont, [1]Sayam Ghosal, [1]Tünde Bárkai, [1]Angéla Takács, [1]Xabier Osteikoetxea, [1,2]Edit I. Buzás, [1]Hargita Hegyesi *[1]Institute of Genetics, Cell and Immunobiology, Semmelweis University, Hungary, [2]HUN-REN SU, Hungary*
12:50 – 13:00	T3-6	**SPONSORS TALK – ANAMET**
13:00 – 13:30		**Lunch break**
13:30 – 14:00		**POSTER SESSION**
14:00 – 14:30		**Closing ceremony & awards**

1st EV4

ORAL ABSTRACTS



W2 - SESSION 1 CELL BIOLOGY OF EXTRACELLULAR VESICLES - 1

W2-1 ENGINEERING RBC-DERIVED EXTRACELLULAR VESICLE SURFACES THROUGH PEPTIDE-MEDIATED PROTEIN CORONA REMODELING

[1]Imola Cs. Szigyártó, [1]Tasvilla Sonallya, [1]Tünde Juhász, [1]Priyanka Singh, [2]Anikó Gaál, [2]Judith Mihály, [3]Lilla Túriák, [3]László Drahos, [2]Zoltán Varga, [1]Tamás Beke-Somfai
[1]Biomolecular Self-Assembly research group, [2]Biological Nanochemistry research group, [3]Glycan Biomarker research group, HUN-REN Research Centre for Natural Sciences, Hungary

Identifying the spontaneously formed protein corona on the surface of nanoscale materials is of critical importance for the development of nanoparticle-based therapeutic and diagnostic applications. To explore the potential of peptides to modulate the surface of nanoparticles and also to progress towards surface engineering, we selected cationic peptides with well-characterized membrane-interacting properties. Red blood cell-derived extracellular vesicles (RBC-EVs) were used as they can easily be produced at high concentrations and possess several unique advantages, such as the lack of DNA, prevention of immune clearance and extended circulation time. The effective peptide concentrations were determined using spectroscopic methods, flow cytometry, and microfluidic resistive pulse sensing techniques. Size exclusion chromatography was performed to separate intact vesicles from soluble protein-rich fractions. The protein composition was characterized by nanoscale liquid chromatography coupled to tandem mass spectrometry.

Proteomic analysis identified 125 proteins in the control RBC-EV sample, including membrane-associated and cytosolic proteins as well as metabolic enzymes. Among these, seventeen proteins were classified as putative components of the protein corona. The overall protein profiles of the control and peptide-treated samples showed a high degree of overlap, indicating that peptide treatment did not cause extensive alteration of the EV-associated proteins. Nevertheless, treatment with selected peptides resulted in changes in the detectable protein corona, including the appearance of several proteins that were not identified in the untreated control. These changes suggest that cationic peptides can remodel the protein composition associated with the EV surface. One possible mechanism involves electrostatic interactions between the cationic peptides and the partially negatively charged regions of the EV membrane, allowing membrane-bound peptides to modify the physicochemical properties of the EV surface and thereby alter the retention or removal of corona proteins.

Overall, our results demonstrate the potential of cationic peptides to modulate the protein corona of EVs and suggest that peptide-mediated corona remodelling could provide a versatile strategy for engineering vesicle surfaces.

Acknowledgements

This work was funded by the National Research, Development and Innovation Office, Hungary (TKP2021-EGA-31, 2020--1.1.2-PIACI-KFI-2020-00021, KKP_22 (144180).

W2-2 WHEN CELLS ARE STRESSED, EXOSOMES TAKE OVER: SWITCHING THE ROUTE OF SMALL EXTRACELLULAR VESICLE RELEASE

[1]Dorina Lenzinger, [1]Lilla Lankovics, [1]Tünde Bárkai, [2]Mirjam Balbisi, [1]Krisztina V. Vukman, [1]Kelsey Fletcher, [1]Edina Bugyik, [3]Csaba Cserép, [3]Ádám Dénes, [1,4]Szilvia Bősze, [2]Lilla Turiák, [5,6]Gábor Mórotz, [7]Márton Sághi, [7]Attila Fintha, [1,8]Edit I. Buzás, [1,9]Tamás Visnovitz

[1]Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Hungary, [2]MTA-HUN-REN TTK Lendület (Momentum) Glycan Biomarker Research Group, HUN-REN Research Centre for Natural Sciences, Hungary, [3]Laboratory of Neuroimmunology, HUN-REN Institute of Experimental Medicine, Hungary, [4]HUN-REN-ELTE Research Group of Peptide Chemistry, ELTE Eötvös Loránd University, Hungary, [5]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [6]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary, [7]Department of Pathology and Experimental Cancer Research, Semmelweis University, Hungary, [8]HUN-REN-SU Translational Extracellular Vesicle Research Group, Hungary, [9]Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Hungary

Our recent findings provide evidence that amphiectosome release through the “torn bag mechanism” represents a novel pathway for small extracellular vesicle (sEV) secretion. We demonstrated in HEK293 cells that stress conditions activate multivesicular endosome (MVE) exocytosis, whereas this pathway is absent under steady-state conditions. The intraluminal vesicle composition and protein profile of amphiectosomes appears to differ from that of exosomes generated through MVE exocytosis.

Here, we also investigated these two different EV release mechanisms in hearth failure models. Transmission electron microscopy was performed on patient-derived samples and on rat models. Our observations suggest that the predominant sEV secretion mechanism differs according to the progression and nature of cardiac pathology. In slowly progressing conditions, such as cardiomyopathy, endothelial cells predominantly release large multivesicular extracellular vesicles (MV-lEVs) through the “torn bag mechanism”. In contrast, during acute heart failure such as ischemic heart disease, endothelial cells primarily release sEVs through MVE exocytosis. These findings were further supported in rats with surgically induced myocardial infarction. Additionally in a previous studies, we demonstrated that cardiomyocytes are also capable of releasing MV-lEVs, most likely representing amphiectosomes. Pathological changes in these secretion pathways may influence the composition of circulating EVs. The characterization of the pathway-specific markers may provide a novel approach for detecting acute pathological changes in heart.

Funding: NKKP ADVANCED_25 152112, NKFIH FK146163, Therapeutic Thematic Programme (TKP2021-EGA-23), RRF-2.3.1-21-2022-00003 and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (C.C., G.M.M. and T.V.).

W2-3 CELLULAR ABUNDANCE SHAPES THE ARCHITECTURE OF EXTRACELLULAR VESICLE MIRNA CARGO MORE STRONGLY THAN CANONICAL SORTING MOTIFS

[1,2] Vojtěch Šprincl, [1] Nataliya Romanyuk
[1]Institute of Experimental Medicine, Czech Academy of Science, Czech Republic, [2]Second Medicine Faculty, Charles University, Czech Republic

Extracellular vesicles (EVs) are produced by all living cells and are involved in extracellular communication. A key component of their cargo is microRNAs (miRNAs), which are thought to carry context-dependent information between cells. It has previously been hypothesized, and to some extent demonstrated, that some miRNAs contain nucleotide motifs that enable their preferential sorting into EVs. This suggests that miRNA cargo may be actively controlled and that cells can selectively influence the information they communicate. However, this model has increasingly been questioned due to a lack of reproducibility across studies. Whether sorting motifs are a major determinant of miRNA content in EVs therefore remains unclear. In this project, we analyzed publicly available RNA sequencing datasets containing matched cellular and EV samples from diverse cell types, including neural stem cells, mesenchymal stem cells, keratinocytes, HEK cells, and cancer cells.

EV miRNA repertoires were highly concentrated, with a small fraction of miRNAs accounting for a substantial proportion of the total EV-associated signal. Across EV datasets, miRNA profiles closely reflected cellular miRNA abundance. Most of the variation in EV miRNA abundance could be statistically explained by cellular miRNA abundance rather than by the tested sorting motifs, and this relationship was also reflected in miRNA rank. The ten most abundant miRNAs accounted for approximately 70% of all EV miRNA reads, and a shared core was dominated by members of the miR-21, miR-10, and let-7 families. We also applied different operational definitions of EV-enriched cargo, including fold-change and EV membership, to evaluate the robustness of motif associations. Importantly, the conclusions were consistent across these definitions, with no evidence for a strong repertoire-wide contribution of the tested sorting motifs. Despite the dominant contribution of cellular abundance, producer-specific patterns remained detectable within the EV miRNA repertoire, including a coherent signal in the MSC datasets.

Our findings support an abundance-constrained model of EV miRNA composition, in which cellular miRNA abundance is the major determinant of the observed EV miRNA repertoire, while sequence-dependent mechanisms may contribute to selected subsets rather than determining the repertoire as a whole.

Funding: GAUK 350225, OP JAK CZ.02.01.01/00/22_008/0004562

W3 - SESSION 2 CELL BIOLOGY OF EXTRACELLULAR VESICLES - 2

W3-1 SOURCE-DEPENDENT EFFECTS OF MSC-DERIVED EXTRACELLULAR VESICLES IN TWO IN VITRO MODELS OF OSTEOARTHRITIS

[1]Denisa Harvanova, [2]Alexander Otahal, [1]Slavomira Gulova, [2]Markus Rothammer, [2]Bernadette Kern, [2]Karina Kramer, [1]Livia Kolesar Fecskeova, [1]Jana Matejova, [1]Hedviga Kosuthova, [1]Jana Bzdilova, [1]Lucia Slovinska, [2]Stefan Nehrer, [2]Andrea De Luna
[1]Associated Tissue Bank, Faculty of Medicine, P.J. Safarik University and L. Pasteur University Hospital in Kosice, Slovakia, [2]Center for Regenerative Medicine, University for Continuing Education Krems, Austria

Osteoarthritis (OA) is a chronic joint disease characterized by progressive cartilage loss and persistent proinflammatory crosstalk between chondrocytes and synovial cells. Although mesenchymal stromal cells (MSC)-based therapies are being investigated for the treatment of joint diseases, their therapeutic effects are thought to be mediated predominantly by paracrine mechanisms, including extracellular vesicles (EVs) activity. However, the mechanisms underlying MSC-EVs activity are not fully understood. Furthermore, the choice of in vitro OA model creates a specific experimental context that can significantly influence the assessment of EV biological efficacy.

MSC were isolated from chorionic tissue (Cho-MSC) and Hoffa's fat pad (HFP-MSC). ChoMSC were expanded and cultured in conventional two-dimensional culture flasks and HFP-MSCs in three-dimensional vertical-wheel bioreactors. EVs were isolated from MSC conditioned medium using ultrafiltration and/or size-exclusion chromatography and characterised by nanoparticle tracking analysis, Western blotting, and multiplex bead-based flow cytometry. Two in vitro models of OA were established, a model using chondrocytes stimulated with conditioned medium derived from inflamed synovial membrane (CMSM), and a co-culture model consisting of chondrocytes and M1-polarised macrophages. Both models were treated with Evs from both tissue sources at fixed particle-to-cell ratios. Gene expression and cytokine release were evaluated by RT-qPCR and multiplex bead-based flow cytometry.

Assessment of EV potency revealed model-dependent differences in the regulation of inflammatory, catabolic, and chondrogenic markers. In the CMSM model, Cho-MSC-EVs reduced the gene expression of *iNOS*, *MMP13*, and *COL2A1*. In the M1 macrophage co-culture model, HFP-MSC-EVs increased *COL2A1* expression and decreased *ADAMTS5* expression compared with the corresponding inflammatory condition. Cytokine release, including IL-8, MCP-1, and MIP-3α, was increased following treatment with both chorion and Hoffa's fat pad MSC-derived EVs in the CMSM model. In contrast, both EV preparations reduced cytokine release in the M1 macrophage co-culture model.

These findings demonstrate that the biological potency of MSC-derived EVs is strongly dependent on the *in vitro* OA model used for evaluation. Careful selection of a model that is responsive to the biomarkers under investigation is therefore essential for the reliable assessment and comparison of EV activity.

The work was cooperatively supported by the Slovak Research and Development Agency (SRDA) and the Austrian Agency for Education and Internationalisation (OeAD) under the contract SK-AT-23-0005 and by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic (VEGA 1/0103/25).

W3-2 PHOSPHATIDYLSERINE-POSITIVE EXTRACELLULAR VESICLES FROM ACIVATED FIBROBLASTS: THEIR PATHOLOGICAL ROLE IN ONCOGENIC SIGNALING IN HEAD AND NECK CANCERS

[1,2]Jan Balvan, [1]Klara Hanelova, [1]Jiri Navratil, [1]Monika Kratochvilova, [1]Kveta Pilarova, [1,2]Martina Raudenska
[1]Department of Pathophysiology, Faculty of Medicine, Masaryk University, Czech Republik, [2]Department of Physiology, Faculty of Medicine, Masaryk University, Czech Republik

Cancer-associated fibroblasts (CAFs), a subtype of activated fibroblasts, facilitate cancer cell progression and immune evasion by secreting immunomodulatory molecules, physically interacting with immune cells, and remodeling the extracellular matrix. Comprehensive CAF phenotyping is necessary for the development of more effective stromal-targeted therapies and for improving our understanding of how CAFs support cancer progression.

In this study, activated fibroblasts isolated from tumor biopsies of head and neck squamous cell carcinoma patients exhibited nuclear localization of transcription regulator YAP1, enhanced formation of micronuclei, distinct distribution of phosphatidylserine in the nuclear envelope, and elevated expression of FAP, a marker of activated fibroblasts. This activated phenotype was also reflected in the secreted extracellular vesicles.

Extracellular vesicles isolated from activated fibroblasts significantly impacted the FaDu tumor cell line, enhancing nuclear translocation of YAP1. This nuclear enrichment was associated with increased expression of the the YAP-target genes. Additionally, phosphatidylserine-positive extracellular vesicles induced changes in mechanical properties and increased the proliferation of a model tumor cell line.

Overall, our findings suggest a new hypothetical mechanism for signal transduction between activated fibroblasts and surrounding cells, with potential implications for pathological conditions beyond the TME.

W3-3 CHARACTERISATION OF EV-DNA IN JUVENILE IDIOPATHIC ARTHRITIS

[1,4]Kristína Truchanová, [2,4]Barbora Tamášová, [2]Jakub Janko, [3]Tomáš Dallos, [3]Barbora Balážiová, [2]Michal Pastorek
[1]Institute of Medical Physics and Biophysics, Faculty of Medicine, Comenius University, [2]Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, [3]Department of Pediatrics, Comenius University Medical Faculty in Bratislava and National Institute of Children's Diseases, Bratislava, Slovakia, [4]MicroPoll s. r. o., Bratislava, Slovakia

Introduction: Small extracellular vesicles (sEVs) are one of the forms of extracellular DNA (EV-DNA) protecting it from degradation in circulation by nucleases. Our hypothesis is that EV-DNA and sEVs can act as damage associated molecular patterns (DAMPs) in various inflammatory diseases including autoimmune diseases such as nonsystemic juvenile idiopathic arthritis (JIA).

Methods: sEVs were isolated from plasma and synovial fluid (SF) obtained from controls (CTRL) and JIA patients by size exclusion chromatography. sEVs were characterized by transmission electron microscopy, nanoparticle tracking analysis and western blot. DNA was extracted using a commercial kit and further analysed.

Results: No significant differences were observed between DNA concentration of CTRL and JIA plasma- or SF-derived sEVs. Plasma-derived sEVs from both CTRL and JIA patients contain approximately 30 – 45 % of DNA located on the surface and 55 – 60% protected inside vesicles. SF-derived sEVs of JIA patients exhibited a higher proportion of DNA associated with the vesicles surface (90%), which may contribute to increased vesicle size and a propensity for aggregate formation. These samples have only 10% of DNA protected within the vesicle lumen. In contrast, CTRL SF-derived sEVs contain about 55% of surface-associated DNA and 45% intravesicular DNA. Nuclear and mitochondrial DNA was detected in both JIA and CTRL sEVs, with a higher copy number of mitochondrial DNA protected against DNase I degradation.

Conclusion: This study demonstrates that DNA, including mtDNA, is present in a protected form within plasma-derived and SF-derived sEVs from both CTRL and JIA patients. Depending on its localization, EV-mtDNA may play different roles: when exposed on the vesicle surface, it could function as a DAMP and amplify inflammatory responses in JIA, whereas intravesicular mtDNA may be protected from pattern recognition receptors, thereby limiting immune activation.

Source(s) of research support: Grant of the Slovak Research and Development Agency APVV-21-0378 and APVV-25-0499.

W3-4 MAST CELL SECRETOME AND ITS FUNCTIONAL IMPACT ON CARDIOMYOCYTES

[1]Kelsey Fletcher, [1]Zsófia Fodor, [1]Zsófia Szász, [1]Dorina Lenzinger, [1]Tamara Hermans, [1]Bence Nagy, [1]Tünde Bárkai, [2,3]Péter Lőrincz, [1,4]Tamás Visnovitz, [1,5]Edit I. Buzás, [1]Krisztina V. Vukman
[1]Semmelweis University, Department of Genetics, Cell- and Immunobiology, Hungary, [2]ELTE Eötvös Loránd University, Department of Anatomy, Cell and Developmental Biology, Hungary, [3]Intracellular Transport & Cytoskeleton Research Group, HUN-REN Biological Research Centre, Hungary, [4]ELTE Eötvös Loránd University, Department of Plant Physiology and Molecular Plant Biology, Hungary, [5]HUN-REN–SU Translational Extracellular Vesicle Research Group, Hungary

Mast cells (MCs) regulate inflammatory responses and are implicated in cardiovascular diseases. Upon activation, MCs release a diverse secretome including extracellular vesicles (EVs) and extracellular granules (EGs). EVs mediate intercellular communication and tissue remodeling, while EGs contain preformed mediators released during classical degranulation. However, similar size and composition of these extracellular particle (EP) populations complicate interpretation of MC-derived secretome profiles and limit understanding of how activation pathways shape EP output and influence cardiomyocytes.

This study aimed to characterize MC-derived EP heterogeneity, determine how activation stimuli influence EP composition, identify features distinguishing EG-like and EV-like particles, and assess their effects on cardiomyocytes. Bone marrow-derived murine MCs were cultured with A23187, DNP/IgE, LPS, or under control conditions. EPs were isolated by differential centrifugation and characterized by flow cytometry, confocal microscopy, transmission electron microscopy, and biochemical assays. HL-1 cardiomyocytes were cultured with separated EPs, and EP uptake, viability, metabolic activity, morphology, and senescence were evaluated.

Activated MCs released heterogeneous EP populations with distinct, stimulus-dependent profiles. DNP/IgE activation primarily generated EG-like, detergent-sensitive, histamine-rich particles well tolerated by HL-1 cells. In contrast, LPS stimulation favored EV-like, detergent-resistant particles with lower histamine content, inducing senescence, reduced viability, decreased metabolic activity, and altered cell density in HL-1 cells. A23187 stimulation produced a mixed EG/EV output.

These findings reveal that MC activation yields multiple EP subtypes and that the EG/EV balance is strongly shaped by the activating stimulus. Discrimination of these populations is vital for accurate interpretation of MC secretion in immunology and EV research. Stimulus-dependent EP composition critically influences cardiomyocyte responses and may support targeted therapies.

Funding: STARTING_25_No.152115, ADVANCED_No.152112, ADVANCED_No.150767, VEKOP-2.3.2-16-2016-00002, VEKOP-2.3.3-15-2017-00016, TKP2021-EGA-23, RRF-2.3.1-21-2022-00003, János Bolyai Research Scholarship

W4 - SESSION 3
EXTRACELLULAR VESICLES FROM BACTERIAL SOURCES

W4-1 VESICLES FROM PROBIOTIC BACTERIA: IMMUNE MODULATION, ALLERGY PREVENTION AND ENVIRONMENTAL SENSING

[1]Irma Schabussova
[1]Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria

Extracellular vesicles are released across all domains of life, but those of bacterial origin (bEVs) hold a distinct position: they are the only vesicle type with an established record in human medicine. A vaccine against serogroup B meningococcal disease, built on bEVs, proved protective in a trial in 1991, and licensed bEV-containing meningococcal vaccines have been in routine clinical use for more than a decade. This is an unusual starting point, in which proof of efficacy and safety in people preceded rather than followed the mechanistic work.

In this talk I will summarise our research on vesicles from Gram-negative and Gram-positive probiotics. OMVs of *Escherichia coli O83* (Colinfant Newborn) are spherical particles of about 110 nm carrying lipopolysaccharide and a defined protein cargo, of which 136 proteins are enriched relative to the parent bacterium, with flagellar proteins predominating. They engage TLR2, TLR4, TLR5, NOD1 and NOD2, induce both pro- and anti-inflammatory cytokines in splenocytes and dendritic cells, and, given intranasally, suppress airway hyperresponsiveness, eosinophilia, Th2 cytokine production and mucus secretion in a mouse model of allergic airway inflammation. In a subsequent study we followed their fate in the host: these vesicles alter oxidative stress and inflammatory protein expression in human nasal epithelial cells, and in mouse models are internalised by airway macrophages, recruit neutrophils and γδ T cells to the lung, and act through NF-kB signalling and nitric oxide production. They thus reproduce key benefits of a live probiotic without administering a live organism.

Turning to Gram-positive bacteria, vesicles of *Lactiplantibacillus plantarum* NCIMB 8826 purified by ultracentrifugation with size-exclusion chromatography are largely free of co-purifying protein and peptidoglycan, carry surface lipid profiles and zeta potentials distinct from the parent cell, and remain stable across a range of pH, ionic strength and mild detergent conditions. Bile exposure induces the release of larger vesicles enriched in bile-metabolism proteins, and infrared spectroscopy shows that these changes differ from those in whole cells. Vesicle composition therefore reports on the environment encountered by the producing bacterium.

This work was carried out with partners in Poland and the Czech Republic, in the Visegrad spirit of this meeting. I will close by arguing that bEVs are non-replicating, stable, inexpensively produced and genetically tractable, with intrinsically adjuvant cargo, making them attractive for mucosal vaccination, allergy prophylaxis and microbiota-derived biomarkers, and that the wider EV field cannot afford to overlook them.

W4-2 BIFIDOBACTERIUM ADOLESCENTIS-DERIVED EXTRACELLULAR VESICLES SHAPE MUCOSAL IMMUNE RESPONSES IN ALLERGIC AIRWAY INFLAMMATION

[1]Dominika Kozakiewicz, [2]Dagmar Srutkova, [2]Martin Schwarzer, [1]Dominika Jakubczyk, [1]Agnieszka Razim, [1]Sabina Górska
[1]Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland, [2] Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Novy Hradek, Czech Republic

The rising incidence of allergic airway diseases is driving demand for safe, effective approaches to immune modulation. Although probiotic bacteria may support immune homeostasis, introducing live microorganisms may entail certain risks, particularly in immunocompromised individuals. Bacterial extracellular vesicles (BEVs) represent a promising cell-free alternative because they preserve biologically active bacterial components without requiring the administration of viable bacteria. Nevertheless, their role in allergic airway inflammation remains insufficiently understood. This study aimed to characterize BEVs produced by *Bifidobacterium adolescentis* CCDM 368 and CCDM 373 and evaluate their immunomodulatory activity in a murine model of ovalbumin (OVA)-induced airway allergy.

BEVs were isolated from bacterial culture supernatants using differential ultracentrifugation followed by chromatographic purification. Their protein and lipid composition was examined by SDS-PAGE, lipidomic and proteomic profiling, and spectroscopic analyses. Recognition by innate immune receptors was investigated using Toll-like receptor (TLR) reporter systems. The immunostimulatory properties of BEVs were further assessed *ex vivo* in bone marrow-derived dendritic cells (BMDCs) and splenocytes, while their effects on macrophage polarization were determined by flow cytometry. *In vivo* activity was examined following BEV administration to mice with OVA-induced airway allergy. Treatment effects were evaluated based on bronchoalveolar lavage (BAL) cell composition, lung histopathology, total and antigen-specific IgA and IgG concentrations in BAL and serum, and cytokine production following tissue restimulation.

The isolated BEVs exhibited characteristic protein and lipid compositions. They selectively stimulated TLR2-dependent signaling without activating lipopolysaccharide-responsive pathways. BMDCs and splenocytes showed different cytokine responses after EV stimulation, with the extent and nature of the effects varying by bacterial strain and vesicle fraction. BEVs also influenced macrophage differentiation towards both M1- and M2-associated phenotypes *in vitro*. In OVA-sensitized mice, BEV treatment modified local pulmonary immune responses, as demonstrated by changes in BAL leukocyte composition, cytokine production, lung histopathology, and antigen-specific IgA and IgG levels.

BEVs demonstrate substantial immunomodulatory activity across cellular, *ex vivo*, and *in vivo* models of allergic airway inflammation. Their effects include regulating inflammatory signaling, pulmonary immune cell populations, cytokine responses, and antigen-specific antibody production. These findings support further investigation of *B. adolescentis*-derived BEVs as cell-free modulators of mucosal immunity and potential candidates for interventions targeting allergic airway diseases.

This study was supported by the National Science Center, Poland (NCN), grant no. 2021/43/I/NZ7/00693.

W4-3 BIDIRECTIONAL EV-RELATED CROSSTALK BETWEEN MELANOMA CELLS AND GRAM-POSITIVE SKIN BACTERIA

[1]Richárd Máté, [1]Djenana Vejzovic, [1]Giorgia Magnarini, [1]Silvia Schauer, [1]Simone Klinger, [1]Sophie Steinkogler, [2]Anna Marin, [2]Jasmina Tuzlak, [2]Altea Topicu, [2]Nermina Malanovic, [1,3] Beate Rinner
[1]Diagnostic and Research Institute of Pathology, Medical University of Graz, Austria, [2]Institute of Molecular Biosciences, University of Graz, Austria, [3]BiotechMed, Austria

Tumor–microbiome research often focuses on Gram-negative outer membrane vesicles (OMVs) and LPS-driven inflammatory signaling. In contrast, cutaneous melanoma develops within a skin ecosystem rich in Gram-positive bacteria, including *Staphylococcus epidermidis* and *Staphylococcus aureus.* These bacteria release cytoplasmic membrane vesicles (CMVs) rather than classical OMVs and also produce cell wall-associated molecules such as lipoteichoic acid (LTA). LTA is particularly relevant because it can occur as a soluble or vesicle-associated bacterial factor and may form micelle-like or nanoscale aggregates at higher concentrations. In this study, we investigated melanoma–skin microbiome crosstalk from two connected angles: melanoma-derived EVs acting on skin bacteria, and Gram-positive bacterial CMVs, soluble factors and LTA acting on melanoma cells.

Melanoma-derived EVs (mEVs) were isolated by ultracentrifugation or tangential flow filtration (TFF) and characterized by nanoparticle tracking analysis, tetraspanin profiling and Western blotting. Their effects on *S. epidermidis* proliferation were assessed by Bioscreen growth-curve analysis. CMV-enriched preparations from *S. epidermidis* and *S. aureus* were obtained by TFF and characterized by nanoparticle analysis, protein quantification and zeta potential measurements. Their effects on melanoma cell viability were evaluated alongside bacterial supernatants, soluble fractions, and *S. aureus*-derived LTA. mEVs altered *S. epidermidis* growth kinetics, reducing growth rate, increasing doubling time and lowering final biomass without a clearly bactericidal effect. This suggests that mEVs can remodel bacterial growth behavior rather than simply inhibit bacterial survival. In the reverse direction, Staphylococcus CMV-enriched preparations caused only modest, cell line-dependent changes in melanoma viability, whereas bacterial supernatants, soluble fractions and LTA induced stronger and more heterogeneous responses. LTA showed concentration-dependent, model-specific effects, with divergent viability responses across melanoma models at higher concentrations The strongest changes overlapped with the concentration range in which LTA may form micelle-like or nanoscale aggregates, suggesting that the physical state of this Gram-positive cell wall molecule may contribute to melanoma responses.

Together, these findings suggest that melanoma–skin staphylococcal crosstalk cannot be reduced to a one-directional tumor EV or bacterial vesicle effect. mEVs can modulate *S. epidermidis* growth, whereas melanoma responses to Gram-positive bacterial products appear to depend on CMV-associated cargo, soluble factors, LTA concentration/aggregation state and tumor cell context. This work highlights a Gram-positive melanoma–microbiome EV-related interaction that differs from classical OMV/LPS-based models and supports future studies on growth-phase-dependent CMV cargo composition and LTA–TLR2-related signaling mechanisms.

W4-4 CHEMICALLY DEFINED MEDIUM ENHANCES DETECTION OF IRON-DEPENDENT CHANGES IN BACTERIAL EXTRACELLULAR VESICLES

Subhasree Venkatasubramaniyan, Norbert Łodej, Sabina Górska, Agnieszka Razim
Hirszfeld Institute of Immunology and Experimental Therapy PAS, Wrocław, Poland

Keywords: Bacterial extracellular vesicles, Undernutrition, stress EVs, Iron deficiency, Chemically defined medium.

Undernutrition represents a significant global health challenge, impacting diverse populations. Its influence on the gut microbiome is complex, causing both quantitative and qualitative shifts that may alter inter-bacterial communication and host interactions. One of the impacted messengers are bacterial extracellular vesicles (bEVs), whose production rate, function and cargo are dependent on the parent bacterial physiological state.

This project aims to elucidate the role of undernutrition-induced stress, i.e. iron depletion, on bEVs production. To achieve this, we are developing a universal chemically-defined medium (CDM) optimized for the reproducible culture of representative commensal strains under stressed and standard conditions. The CDM allows modification of its composition, facilitating the study of nutrient stress under controlled conditions. Furthermore, this medium is devoid of animal-derived components, which can co-purify with bEVs, influencing result interpretation. We analysed commensal Escherichia coli growth and fitness in CDM and a standard medium, and characterized the bEVs produced in both media and in iron-free CDM. We analysed size and concentration (DLS), morphology (TEM), RNA and protein content (Western blot and proteomics).

Comparative analysis of bacteria and the EVs they produce indicates that CDM is suitable for their repeatable production. Furthermore, our results indicate specific changes in the composition of bEVs produced with and without iron. Our research will enable the creation of a universal platform for studying the effects of various stressors on bEV production. The insights obtained may pave the way for novel biomarkers or therapeutic strategies targeting microbiome-mediated pathways in undernutrition and related digestive pathologies.

W5 - SESSION 4 TECHNOLOGIES IN EXTRACELLULAR VESICLE ANALYSIS AND THERAPY

W5-1 PROGRAMMING EXTRACELLULAR VESICLES FOR IMAGE-GUIDED TARGETED DELIVERY

[1,2]Andrea Galisova, [1]Aida de la Cruz-Gambra, [1]Jiri Zahradnik, [2]Dominik Havlicek, [3]Josef Uskoba, [4]Ziv Porat, [2]Daniel Jirak
[1]BIOCEV, First Faculty of Medicine, Charles University, Czech Republic, [2]Department of Imaging Methods, Institute for Clinical and Experimental Medicine, Czech Republic, [3]Biotech a.s., Czech Republic, [4]Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel

Genetic engineering of extracellular vesicles (EVs) represents an emerging strategy for targeted drug delivery, but limited tissue specificity and lack of visualization methods restrict their translation. Current engineering approaches commonly rely on genetic fusion of a single targeting protein to EV scaffolds, which is labor-intensive process with limited modularity. To solve this issue, we introduce the Nanobody-Tag-Ligand (NaTaLi) system, in which EVs display engineered nanobodies that bind a specific protein tag with high affinity. This enables post-isolation functionalization of EVs with any tagged protein, which can be easily produced in bacterial systems. Moreover, engineered EVs can be modified with imaging cargoes allowing in vivo visualization in tissues.

In this study, NaTaLi EVs were functionalized with cancer-targeting proteins (RGD and LinTT1) and characterized according to the MISEV2023 criteria (NTA, cryo-EM, protein content). Binding of tagged proteins to EV surface resulted in a strong and persistent bond, which was confirmed by orthogonal analyses including the Flow Induced Dispersion Analysis (FIDA), Ligand Tracer and surface plasmon resonance (SPR). ImageStream analysis confirmed a possibility of ratiometric functionalization by multiple proteins simultaneously. NaTaLi EVs administered intravenously in mice showed a significantly higher accumulation of cancer-targeted EVs in tumors compared to non-functionalized controls. Doxorubicin-loaded EVs exhibited a chemotherapeutic potential in breast cancer cell line (4T1). EVs loaded with MRI-active polymers and fluorescence dyes were successfully tracked in tumors in vivo by both fluorescence and magnetic resonance imaging (MRI).

Finally, NaTaLi EVs represent a scalable, plug-and-play approach for versatile functionalization of EVs. Together with state-of-the-art imaging methods, this programmable EV platform provides new avenues for the next generation of multiplexed and image-guided therapeutics.

W5-2 LIPID REMODELING AND iNOS CARGO IN BACTERIALLY REPROGRAMMED MACROPHAGE EVs

[1,2]Miriam Dutkova, [1,2]Julia Orlovska, [1,3]Kristyna Turkova, [1]Eva Vojtkova, [1,2]Marek Oslacky, [1,2]Lucia Calekova, [1,2]Veronika Mlcekova, [4]Stepan Strnad, [4]Vladimir Vrkoslav, [5]Vratislav Berka, [6]Josef Chudacek, [6]Stefan Kolcun, [6]Eva Kriegova, [1,2,3]Lukas Kubala, [1]Gabriela Ambrozova

[1]Department of Biophysics of Immune System, Institute of Biophysics of the Czech Academy of Sciences, Czech Republic, [2]Department of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic, [3]International Clinical Research Center, St. Anne's University Hospital Brno, Czech Republic, [4]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic, [5]Contipro a.s., Czech Republic, [6]Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Czech Republic

Macrophages (Mfs) are key players in innate immunity, contributing to host defense, inflammation, and tissue repair. They are also significant producers of extracellular vesicles (Mf-EVs), whose immunological roles remain incompletely understood. We hypothesized that bacterial stimulation modulates the production, composition, and pro-inflammatory function of Mf-EVs.

Mf-EVs were isolated from untreated or bacteria-stimulated RAW 264.7 macrophages by differential ultracentrifugation followed by sucrose cushion purification. EVs were characterized according to MISEV 2023 guidelines using Cryo-EM, nanoparticle tracking analysis, and Western blotting. Lipidomic profiling (LC-MS), iNOS expression and activity, and functional assays in RAW 264.7 and MS-1 cells were performed. EVs from peritoneal lavage of inflamed C57BL/6J mice and peritoneal exudates of patients with acute peritonitis were analyzed to confirm *in vivo* and clinical relevance.

Isolated Mf-EVs were within the expected size range for small EVs, displayed bilayered morphology, expressed EV markers (TSG101, CD63, CD81), and lacked ApoE. Bacterial stimulation increased iNOS incorporation into Mf-EVs, and importantly, EV-associated iNOS retained enzymatic activity. In parallel, bacterial stimulation induced extensive lipid remodeling, generating distinct EV lipidomic signatures enriched in saturated fatty acids and ceramides, which correlated with enhanced uptake by target cells. Together, these changes were associated with enhanced pro-inflammatory activity of Mf-EVs, including increased production of pro-inflammatory mediators by innate immune and endothelial cells.

These findings reveal that bacterial cues significantly reshape Mf-EV cargo and function. We identified enzymatically active EV-associated iNOS as a previously unrecognized inflammatory cargo and demonstrated its presence in murine models of peritoneal inflammation and in patients with acute peritonitis. By protecting functional cargo from degradation and enabling the transport of pro-inflammatory mediators to sites distant from their cellular origin, Mf-EVs may contribute to the propagation of inflammatory responses, highlighting them as promising biomarkers and therapeutic targets.

W5-3 WHEN EV DOSE IS NOT WHAT IT SEEMS: MISMATCH BETWEEN PARTICLE NUMBER- AND PROTEIN-BASED DOSING

Tímea Böröczky[1,2,3], Mária Harmati[1], Edina Gyukity-Sebestyén[1], Tamás F. Polgár[4,5], Csenge Feinek[2], Emma Balog[1], Antal Martinecz[6], Krisztina Buzás[1,2], Gabriella Dobra[1,2]*, Mátyás Bukva[1,2]*
[1]Institute of Biochemistry, HUN-REN Biological Research Centre, Szeged, Hungary, [2]Department of Immunology, University of Szeged, Szeged, Hungary, [3]Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged, Hungary, [4]Core Facilities - Transmission Electron Microscope Laboratory, HUN-REN Biological Research Centre, Szeged, Hungary, [5]HUN-REN-SZTE Neuroscience Research Group, Hungarian Research Network, University of Szeged, Hungary, [6]Certara, Radnor, Pennsylvania, USA

* These authors contributed equally

Background: Extracellular vesicles (EVs) are widely studied as mediators of intercellular communication, yet their quantitative characterization remains inconsistent. In this study, we investigated how cellular proliferation, estimated vesicle release rate, and cancer origin jointly influence EV-associated protein production.

Methods: Using a panel of 19 cancer- and non-cancer cell lines cultured under standardized conditions, we isolated small EV–enriched fractions and quantified particle number, protein content, cell number, and proliferation rate. Then we integrated these parameters into three complementary metrics describing vesicle release characteristics (VRC; particle/cell/24 h), protein content per particle (PP, pg/particle), and total protein release characteristics (PRC, pg/cell/24 h).

Results: Cancer cell lines showed higher estimated EV release (~3.6-fold higher VRC), while their EV-enriched isolates exhibited a substantially lower protein-to-particle ratio (~4.3-fold lower PP) and lower per-cell protein recovery (~2.1-fold lower PRC) than non-cancer cell lines. The lower PP was independently associated with cancer origin and proliferation rate and could not be explained by particle size. This difference has important implications for EV dosing: when normalized for the same protein amount, cancer-derived EV-enriched isolates contained approximately 4.37-fold more particles, whereas particle-based normalization resulted in approximately 4.37-fold more protein in non-cancer derived isolates. Thus, particle number and protein amount should not be considered interchangeable measures for normalizing EV-enriched preparations.

Conclusion: Our results highlight the limitations of single-metric normalization strategies and underscore the need for integrated, multidimensional reporting to enable biologically meaningful comparisons of EV preparations.

Fundings: OTKA-K143255, TKP-2021-EGA-09, EKOP-24-3-SZTE-318

W5-4 HIGH-PURITY EXTRACELLULAR VESICLE ISOLATION UNRAVELS DISTINCT BUT OVERLAPPING PROTEIN COMPOSITION WITH LIPOPROTEINS IN ACUTE MYOCARDIAL INFARCTION PATIENTS

[1,2]Dóra Kapui, [1,2]Szabolcs Hambalkó, [1,2]Yavuz Artuner, [1,2]Orsolya Szalai, [1,2]Richárd Rácz, [3]David Matallanas, [3]Kieran Wynne, [1,2,4]Péter Ferdinandy, [1,2,4]Zoltán Giricz, [1,2]Csenger Kovácsházi
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary, [3]Systems Biology Ireland, University College Dublin, Ireland, [4]Pharmahungary Group, Hungary

Introduction: Lipoproteins (LPs) share overlapping physical properties and molecular cargo with extracellular vesicles (EVs), which can obscure EV-related analytical signals, suggesting that separation of EVs and LPs facilitates distinguishing patient populations. However, single-step isolation methods are unable to separate EVs from LPs. Here we applied two-step EV enrichment to compare EV and LP proteome in patients with acute myocardial infarction (AMI).

Methods: Platelet-free Plasma samples (1 mL) were collected from 17 patients with AMI and from 23 non-AMI patients with acute angina (ethical approval: SE RKEB 67/2022). Samples were processed with 20 hours, 10-50% iodixanol density gradient ultracentrifugation (DGUC). LP and EV-rich fractions were further purified with size exclusion chromatography (SEC) using Sepharose 4 Fast Flow (4FF) columns. Isolates were characterized by Western blot (WB) and nanoparticle tracking analysis (NTA). Proteomics was performed using liquid chromatography coupled with tandem mass spectrometry.

Results: After 4FF SEC of the EV-rich fractions, WB analysis revealed the presence of EV markers, while ApoA1 was also present. LP-rich isolates were positive for ApoA1 and ApoB-100 LP markers, with EV markers below detection limit. Application of the total workflow needed two experimental days for 6 samples. EV samples had significantly lower protein and particle content and larger particle sizes compared to LPs. Proteomics revealed 113 unique EV and 46 unique LP proteins, while 120 proteins were shared between the sample types. AMI did not affect protein and particle amount in the samples. Between AMI and non-AMI patients, 72 proteins were differentially enriched in EVs and 10 in LPs. Two of these were shared between the sample types. Proteins that are associated with AMI were only found in EV isolates.

Conclusion: Combined DGUC followed by 4FF SEC demonstrated acceptable EV-LP separation and processing time for plasma EV isolation. Results demonstrate that two-step isolation methods are needed for unbiased, exploratory-phase experiments, especially for omics, while for targeted analyses, more heterogeneous samples may be adequate, as certain proteins are carried only by EVs.

T2 - SESSION 5 EXTRACELLULAR VESICLE-BASED DIAGNOSTICS

T2-1 EXTRACELLULAR VESICLES IN THE OVARIAN CANCER MICROENVIRONMENT: FROM ASCITES BIOLOGY TO LIQUID BIOPSY AND PRECISION MEDICINE

[1]Stanislava Sladeček, [1]Šárka Hrachovinová, [2]Dita Münzová, [2]Michal Felsinger, [3]Markéta Bednaříková, [4]Jitka Hausnerová, [5]Václav Pustka, [5]Zbyněk Zdráhal, [2]Vít Weinberger, [1]Vendula Pospíchalová
[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic, [2]Department of Obstetrics, Gynecology and Neonatology, University Hospital Brno and Medical Faculty, Masaryk University, Czech Republic, [3]Department of Internal Medicine - Hematology and Oncology, University Hospital Brno and Medical Faculty, Masaryk University, Czech Republic, [4]Department of Pathology, University Hospital Brno and Medical Faculty, Masaryk University, Czech Republic, [5]Proteomics Core Facility, Central European Institute of Technology, Masaryk University, Czech Republic

High-grade serous carcinoma (HGSC) of the ovary, fallopian tube, and peritoneum is the most common and most lethal form of ovarian cancer and is characterized by extensive transcoelomic dissemination and the frequent accumulation of malignant ascites. Rather than representing a passive consequence of advanced disease, ascites constitutes a complex and dynamic tumor microenvironment containing tumor spheroids, immune and stromal cells, soluble factors, and abundant extracellular vesicles (EVs). We hypothesized that ascites-derived EVs integrate molecular information from multiple cellular components of this environment and may therefore provide a particularly informative source of clinically relevant biomarkers.

Using complementary EV isolation and characterization approaches combined with quantitative proteomics, we investigated the molecular composition and cellular origin of EVs isolated from ascites of patients with HGSC. Proteomic profiling revealed an EV-associated molecular signature distinct from corresponding ascitic cells. Importantly, a substantial proportion of EV proteins could be attributed to stromal and immune components of the tumor microenvironment, particularly macrophages and fibroblasts, demonstrating that ascitic EVs capture information extending beyond the tumor cells themselves. Specific EV-associated proteins and their inferred cellular origins were further linked to clinical outcome, supporting the potential of EVs as reporters of disease biology and prognosis.

We are now extending this concept from prognostic characterization towards prediction of treatment response. Our current studies combine EV proteomics with fluorescence-based analysis of EV membrane properties and single-EV characterization to identify signatures associated with platinum sensitivity. In parallel, patient-derived ascitic tumor spheroids are used to establish three-dimensional models for functional drug testing, called ascitoids, providing a complementary approach to interrogate residual disease.

Together, these findings position malignant ascites as an accessible liquid tumor microenvironment and ascites-derived EVs as integrative reporters of its cellular and molecular complexity. Combining EV-based molecular signatures with functional models may provide a path towards prospective prediction of treatment response and more individualized therapeutic decision-making in ovarian cancer.

Acknowledgements: Supported by the Ministry of Health of the Czech Republic in cooperation with the Czech Health Research Council under project No. NW26-03-00442.

T2-2 BEYOND BULK EV ANALYSIS: AN INTEGRATED WORKFLOW FOR SUBPOPULATION-SPECIFIC EXTRACELLULAR VESICLES PROFILING

[1-2]Elisabetta Pingitore, [2]Selena Mimmi, [2]Valentina Crapella, [2]Anna Maria Zimbo, [2]Annarita Scardamaglia, [2]Costanza Maria Cristiani, [2]Luana Scaramuzzino, [2]Andrea Quattrone, [2]Aldo Quattrone, [1-2]Enrico Iaccino
[1]Department of Experimental and Clinical Medicine, Catanzaro, Italy, [2]Laboratory of Neuroimmunology, Institute of Neurology, Catanzaro, Italy

Extracellular vesicles (EVs) circulate as highly heterogeneous populations, meaning that their clinical utility as biomarkers for liquid biopsy depends strongly on our ability to distinguish biologically diverse subpopulations rather than relying solely on bulk measurements. However, one of the main obstacles to clinical translation remains the lack of standardized pre-analytical workflows capable of isolating and preserving specific vesicle fractions with high reproducibility across analyses.

To address this limitation, we established an integrated workflow combining automated EV isolation via EXODUS with downstream molecular profiling using nanoparticle tracking analysis (NTA) and the Leprechaun platform for the assessment of EV concentration, size, surface markers, and molecular cargo, in accordance with IFCC quality indicators and MISEV2023 recommendations. The clinical applicability of the workflow was evaluated in a cohort of patients with neurodegenerative parkinsonian disorders. Neuron-derived extracellular vesicles (NDEVs) were successfully enriched from peripheral blood through L1CAM-based immunomagnetic capture, enabling subsequent detection of disease-associated molecular signatures by automated capillary electrophoresis (JESS, Simple Western). This approach allowed the quantification of the 4R/3R tau isoform ratio from very low amounts of protein lysate in neurodegenerative diseases characterized by overlapping clinical features and challenging differential diagnosis.

Implementation of the automated workflow ensured highly reproducible and operator-independent EV isolation while preserving vesicular structure and macromolecular cargo. Importantly, its clinical application highlighted the value of targeting specific EV subpopulations. While analysis of total circulating EVs did not reveal disease-specific alterations, selective enrichment of NDEVs uncovered a distinct pathological signature. Specifically, the workflow detected a significant increase in the NDEV-associated 4R/3R tau ratio in patients with progressive supranuclear palsy (PSP), enabling molecular discrimination from both Parkinson's disease (PD) patients and healthy controls.

The integration of automated pre-analytical processing with subpopulation-specific phenotyping enables the detection of disease-relevant EV signals that may remain undetectable within heterogeneous bulk populations. This integrated platform provides a structured and scalable framework linking pre-analytical standardization with biomarker characterization and supports the potential application of EV subpopulation profiling in liquid biopsy.

T2-3 NEUROFILAMENT LIGHT CHAIN IN NEURONALLY DERIVED EXTRACELLULAR VESICLES: DIFFERENTIATING PARKINSON'S DISEASE FROM ESSENTIAL TREMOR WITH RESTING TREMOR

[1,3]Valentina Crapella, [1,3]Selena Mimmi, [2]Elisabetta Pingitore, [1,3]Annarita Scardamaglia, [1,3]Anna Maria Zimbo, [1,3]Andrea Quattrone, [1,3]Aldo Quattrone, [2]Enrico Iaccino
[1]Laboratory of Neuroimmunology, Institute of Neurology, Department of Medical and Surgical Sciences, University Magna Graecia of Catanzaro, Catanzaro, Italy; [2]Department of Experimental and Clinical Medicine, University „Magna Graecia" of Catanzaro, Catanzaro, Italy; [3] Neuroscienze Research Center, Magna Graecia University, Catanzaro, Italy.

Parkinson's disease (PD) and resting-tremor essential tremor (rET) often present overlapping clinical features, complicating differential diagnosis. This study investigated whether neurofilament light chain (Nf-L) contained in neuron-derived extracellular vesicles (NDEVs) could support discrimination between PD and rET.

Thirty-three PD patients, thirteen rET patients, and thirty healthy controls were enrolled. Extracellular vesicles (EVs) were isolated from serum samples by size exclusion chromatography and characterized using tunable resistive pulse sensing (Exoid) and transmission electron microscopy to confirm size distribution and morphology. NDEVs were subsequently enriched through L1CAM-based immunocapture. Nf-L levels were quantified using an automated ELISA platform. Group differences, discriminative ability, and associations with demographic or clinical variables were evaluated with standard statistical approaches.

NDEV Nf-L concentrations were significantly higher in PD than in rET and healthy controls, while rET patients showed values comparable to controls. NDEV Nf-L clearly distinguished PD from the other groups, demonstrating strong overall diagnostic potential. No significant associations with clinical measures were found, except for a sex-related effect observed only in the rET group.

Nf-L measured within neuron-derived extracellular vesicles (NDEVs) represents a promising and accessible blood-based biomarker to assist in differentiating PD from rET. These preliminary findings highlight the potential value of NDEVs for improving diagnostic accuracy in tremor disorders. Validation in larger and independent cohorts will be essential to support clinical translation.

T2-4 EXTRACELLULAR VESICLE-BASED DIAGNOSTICS AND PROGNOSTICS: CURRENTLY APPLIED TECHNOLOGIES AND UNMET NEEDS – INSIGHTS FROM A SCOPING REVIEW ON HEART FAILURE

[1,2]Csenger Kovácsházi, [1,2]Dóra Kapui, [1,2]Yavuz Artuner, [1,2]Szabolcs Hambalkó, [1,2]Richárd Rácz [1,2]Teresa Hung, [1,2]Bennet Y Weber, [1,2]Dániel Dobák, [1,2]Tamás G Gergely, [3]Kenan Aloss, [4,5]Gemma Chiva-Blanch, [6,7]Joost P. G. Sluijter, [8,9,10]Edit I Buzás, [1,2,11]Péter Ferdinandy, [1,2,10]Zoltán Giricz
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Budapest, Hungary, [3]Faculty of Medicine, Université de Montréal, Montréal, QC H3C 3T5, Canada, [4]Health Sciences Faculty, Universitat Oberta de Catalunya (UOC), Barcelona, Spain, [5]Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain, [6]Department of Cardiology, Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht, the Netherlands, [7]UMC Utrecht Regenerative Medicine Center, Circulatory Health Research Center, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands, [8]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, [9]HCEMM-SU Extracellular Vesicle Research Group, Budapest, Hungary, [10]HUN-REN-SU Translational Extracellular Vesicle Research Group, Budapest, Hungary, [11]Pharmahungary Group, Szeged, Hungary

Extracellular vesicles (EVs) are intensively studied as biomarkers in various diseases, however, the wide variety of applied methodologies, and reporting rigor hinders the interpretation and clinical application of the results. Heart failure (HF) is one of the leading causes of death worldwide, despite improving therapeutic and diagnostic options, which highlight the needs for novel diagnostic and prognostic applications.

To identify current evidence and limitations, applied technologies and reporting rigor about potential EV-based analytics, a scoping review was applied collecting studies on circulating EVs in HF patients.

Records presenting data on blood-derived EVs from HF patients were collected from PubMed, Embase, Scopus, and Web of Science databases. Applied EV-related technologies, methodological and reporting rigor, EV-related outcomes, and their relation to established cardiac analytes were collected.

Sixty-six reports were included in the final data collection, of which 52 cross-sectional, 16 interventional, 3 case-control, and 6 cohort studies were identified. Marked methodological heterogeneity was observed, with flow cytometry, with or without EV enrichment, being the most frequent applied approach. Collected studies did not apply two-step EV enrichment strategies and most of them did not follow MISEV-related guidelines. Results suggest differences between HF and non-HF populations, however, no superiority over established HF measures has been demonstrated.

In conclusion, the identified reports are mainly exploratory, cross-sectional studies with wide heterogeneity in the applied technologies and measured endpoints, with limited adherence to guidelines. Observed differences between HF and healthy patients suggest that EVs have the potential to be used in future analytics in HF, however, further studies designed to provide strong clinical evidence, including rigorous application of EV-related technologies are needed.

T2-5 SNEV: A GLOBAL COMMUNITY FOR EARLY-CAREER RESEARCHERS IN EXTRACELLULAR VESICLES

[1]Reza Abouali, on behalf of the Student Network on Extracellular Vesicles (SNEV) Executive Committee
[1]Laboratory of Experimental and Translational Immunology, University of Gdansk and Medical University of Gdansk, Intercollegiate Faculty of Biotechnology, Gdansk, Poland

Early-career researchers (ECRs) contribute to the development of the extracellular vesicle (EV) field, while also facing the need to establish professional connections and find support beyond their own laboratories. The Student Network on Extracellular Vesicles (SNEV) was established in 2020 to connect students postdoctoral and ECRs working on EVs across different disciplines and countries. Following a formal partnership with the International Society for Extracellular Vesicles (ISEV) in 2021, SNEV became ISEV's official ECR arm in 2024. Through its activities, SNEV supports scientific exchange and professional development, creating opportunities for ECRs to participate in the wider EV community.

SNEV is coordinated by an international team of volunteers working across events, media, design, newsletters and podcasts. Its activities combine scientific exchange with career support, providing opportunities for ECRs to present their work and learn from researchers at different career stages. Since its establishment, SNEV has organised over 110 online events and two international virtual conferences. Its newsletter has reached 61 issues, sharing community updates, interviews and career opportunities, while podcasts provide another route to hear about the experiences of researchers in the field. Together with social media platforms reaching a combined audience of more than 8,000 followers, these activities maintain regular contact within the community between scientific meetings.

Mentorship and direct interaction are also central to SNEV's activities. The ISEV–SNEV Mentorship Program has completed two editions, connecting ECRs with researchers from different career stages. At ISEV annual meetings, Meet the Experts sessions, satellite events and informal networking activities provide further opportunities to exchange experiences and build professional connections. Initiatives such as the EV Graphical Abstract Competition encourage ECRs to communicate their research and participate in major EV meetings.

Through the SNEV Interconnect Program, 29 ambassadors representing national and regional EV societies connect ECR communities worldwide. This collaboration supports communication between societies and joint activities such as SNEV Ambassadors' Day. Together, these initiatives illustrate how a virtual, volunteer-led network can support scientific and professional development alongside established EV societies. With free registration and opportunities to contribute to its activities, SNEV offers an accessible way for ECRs to become involved in an international community and help shape the support available to their peers.

T3 - SESSION 6

EXTRACELLULAR VESICLE-BASED THERAPEUTICS

T3-1 CYTOKINE PRIMING ENHANCES THE IMMUNOMODULATORY CARGO AND EFFICACY OF MSC-DERIVED EVS IN T2-LOW AIRWAY INFLAMMATION

Andrzej Eljaszewicz, Marlena Tynecka, Dominik Cysewski, Magdalena Niemira, Agnieszka Tarasik

Immunosuppressive properties of mesenchymal stem cells (MSCs) have been demonstrated in numerous preclinical models of chronic inflammatory diseases, including asthma. Despite these promising findings, to date, only one MSC-based therapy has been approved. Given the potential safety risks and logistical challenges of cell-based therapies, attention has increasingly shifted toward cell-free approaches, particularly the MSC secretome. Extracellular vesicles (EVs) released by MSCs are thought to retain many of the biological properties of their parent cells while minimizing the risks associated with whole-cell transplantation. Here we aimed to investigate how proinflammatory signaling in MSCs affects the qualitative composition and efficacy of EVs in regulating T2-low lung inflammation.

EVs were isolated from conditioned media of MSCs cultured under standard conditions or following priming with the pro-inflammatory cytokines, namely IL-1β, TNF-α, and IFN-γ. We standardized EV concentration to total protein content. First, to assess cargo content, we performed untargeted proteomic analysis to identify differences in EV protein cargo. Next, to assess therapeutic potential, we performed in vivo experiments in house dust mite extract (HDM)-induced T2-low lung inflammation. Briefly, C57BL6 mice were challenged with 100μg of HDM. We administered MSCs or EVs (primed or unprimed) intranasally on the 13th day of the experiment. Moreover, the HDM stimulation was prolonged to analyze the long-term effects of EV treatment. Finally, we collected lung tissues for histochemistry, flow cytometry analysis, and transcriptomic profiling.

Differential proteomic analysis revealed a substantial enrichment in protein abundance in primed EVs compared to naïve EVs. Among others, we found increased expression of immunomodulatory proteins, namely IDO1, IL4I1, CD274, ARG1, and HMOX2, when compared to their unstimulated counterparts. Moreover, we confirmed that both MSCs and EVs substantially limited HDM-induced airway inflammation. We observed a decrease in IL-4-producing T cells and a trend toward decreased IFN-producing T cells after MSC treatment, while EV administration significantly reduced IFNγ-producing T cells. The analysis of canonical and noncanonical pathways revealed downregulation in arachidonic acid metabolism and lipid metabolism via LXR, NF-Y, SEBP, and PPAR in the MSC-treated group. Similarly, preconditioned EVs administration caused upregulation in fatty acid degradation and downregulation in linoleic acid metabolism.

In summary, we confirmed that EV administration reflects the beneficial effects of MSCs in regulating T2-high airway inflammation and demonstrates advantages over the originating cells.

T3-2 METHOD MATTERS: COMPARATIVE EVALUATION OF TISSUE-DERIVED EXTRACELLULAR VESICLE ISOLATION METHODS IN NON-SMALL CELL LUNG CANCER

[1]Anna Biernacka, [1]Jakub Faktor, [1]Martyna Siewiera, [1]Ines Papak, [1]Artur Piróg, [1]Zuzanna Urban-Wójciuk, [2]Magdalena Sitkiewicz, [2]Magdalena Szczepanowska, [2]Tomasz Marjański, [2]Witold Rzyman, [3,4]Aneta Zebrowska, [3,5]Monika Pietrowska, [6]Danuta Gutowska-Owsiak, [1]Sachin Kote, [1,7]Natalia M. Marek-Trzonkowska
[1]International Centre for Cancer Vaccine Science, University of Gdańsk, Poland, [2]Department of Thoracic Surgery, Medical University of Gdansk, Poland, [3]Maria Sklodowska-Curie National Research Institute of Oncology, Poland, [4]Department of Histology and Cell Pathology, Medical University of Silesia, Poland, [5]Department of Biotechnology and Nutrigenomics, Institute of Genetic and Animal Biotechnology of the Polish Academy of Sciences, Poland, [6]Laboratory of Experimental and Translational Immunology, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk, Poland, [7]Laboratory of Immunoregulation and Cellular Therapies, Medical University of Gdańsk, Poland

Tumor-derived small extracellular vesicles (sEVs), including exosomes, affect immune cell function and contribute to the establishment of an immunosuppressive tumor microenvironment. Most studies on tumor-derived sEVs focus on vesicles isolated from cancer cell-conditioned media, but these may differ substantially from those present in the native tumor microenvironment. Direct isolation of sEVs from tumor tissue offers a more biologically relevant approach, but isolated material is often contaminated with with intracellular material, protein aggregates, and blood-derived components, making the choice of isolation method critical for reliable downstream analyses.

In this study, we compared six sEV isolation methods for non-small cell lung cancer tissue: ultracentrifugation, iodixanol-cushioned ultracentrifugation, and four commercial kits based on vesicle precipitation, membrane affinity, immunoaffinity, and phosphatidylserine binding. Using transmission electron microscopy, CD9-targeted fluorescence nanoparticle tracking analysis, and quantitative LC-MS/MS proteomics, we assessed the yield, morphology, purity, and molecular composition of the isolated sEV preparations. Ultracentrifugation and iodixanol-cushioned ultracentrifugation yielded samples with the highest sEV enrichment, as evidenced by particle morphology, the abundance of CD9-positive particles, and the enrichment of sEV-associated proteins. The phosphatidylserine-binding approach resulted in moderate sEV enrichment, whereas the other tested approaches predominantly isolated non-vesicular contaminants. While iodixanol-cushioned ultracentrifugation achieved the highest purity, ultracentrifugation provided comparable sEV enrichment with considerably higher yield.

Overall, ultracentrifugation offers the best balance between purity and recovery, making it a practical choice for tissue-derived EV studies requiring high-quality sEV preparations for downstream functional and molecular analyses in solid tumors.

T3-3 EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS DERIVED FROM THE UMBILICAL CORD REDUCE CARDIOTOXICITY OF VARIOUS ANTI-TUMOUR AGENTS

[1, 2]Szabolcs Hambalkó, Richárd [1, 2]Rácz, [1, 2]Teresa Hung, [1, 2]Nóra Wagner, [3]Tamás Visnovitz, [1,2]Bence Ágg, [1, 2]Dóra Bihary, [1, 2]Csenger Kovácsházi, [1, 2]Dóra Kapui, [1, 2]Yavuz Artuner, [5]László Cervenak, [5]Erika Kajdácsi, [1, 2, 4]Péter Ferdinandy, [1, 2, 4]Zoltán Giricz

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary, [3]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Hungary, [4]Pharmahungary Group, Hungary, [5]Department of Internal Medicine and Hematology, Semmelweis University, Hungary.

Cardiotoxicity is a common side effect of several groups of anticancer drugs, prevention or causative treatment of which are unmet clinical needs. The effect of mesenchymal stem cells and their EVs in reducing cardiotoxicity of doxorubicin has been shown, but their benefit against other cancer therapeutics is unknown.

EVs were isolated from human primary (pri-MSC) and immortalized umbilical cord-derived MSCs (imm-MSC) by ultracentrifugation. AC16 human cardiomyocytes were treated with various anti-cancer drugs acting via different molecular mechanisms at doses that induced 80% cell death, as well as with EVs at different concentrations. The effect of EVs on cardiotoxicity was assessed 48h later by CellTiter-Glo kit. Composition of EVs was assessed by Western blots and miRNA transcriptomics. Molecular mechanism of cardioprotection was assessed by investigating apoptosis, autophagy, and proteomic composition of the treated AC16 cells.

Small extracellular vesicles (sEVs) derived from imm-MSCs significantly prevented the cardiac cell death induced by one of the anti-tumour compounds (Compound 1), whereas sEVs derived from pri-MSCs proved ineffective. By contrast, when tested against another agent (Compound 2) having a different mechanism of action, maximum effect size (Emax) values of sEVs from imm-MSCs and pri-MSCs were similar, while maximum viability after treatment with pri-sEVs was significantly higher. Large EVs from imm-MSCs showed significantly higher Emax and maximum viability against Compound 1, while both EVs showed similar protection against Compound 2. Small EVs of immortalized MSCs applied after Compound 1 treatment reduced cardiomyocyte apoptosis and restored autophagy, which may be mediated by microRNAs in EVs.

Our results evidence that MSC-EVs alleviate cardiotoxicity caused by various anti-cancer drugs, via modulating apoptosis and autophagy. Here we also evidence high batch-to-batch variability in efficacy of MSC-EVs and that source and subtype influence efficacy profile of MSC-EVs substantially, the reason of which needs further investigation.

T3-4 THERAPEUTIC APPLICATION OF EV-AMP DELIVERY SYSTEMS IN A MURINE MODEL OF SEPSIS

[1,2]Tasvilla Sonallya, [3,4]Zoltan Varga, [1,2]Kamal el Battoui, [5]Kata Horvati, *[6,7]Yumi Kumagai and *[1]Tamas Beke-Somfai
[1]Institute of Materials and Environmental Chemistry, Biomolecular Self-assembly Research Group, Research Centre for Natural Sciences, Budapest, Hungary, [3]Institute of Materials and Environmental Chemistry, Biological Nanochemistry Research Group, Research Centre for Natural Sciences, Budapest, Hungary, [4]Department of Physical Chemistry and Materials Science, Budapest University of Technology and Economics, Műegyetem rkp. 3, Budapest 1111, Hungary, [5]MTA-HUN-REN TTK "Momentum" Peptide-Based Vaccines Research Group, Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary, [6]Department of Biochemistry and Systems Biomedicine, Juntendo University Graduate School of Medicine, Tokyo, Japan, [7]Biomedical Research Core Facilities, Juntendo University Graduate School of Medicine, Tokyo, Japan

Sepsis is a life-threatening condition marked by dysregulated inflammation and multi-organ failure, for which effective, biocompatible antimicrobial therapies remain an unmet need. Extracellular vesicles (EVs) are promising natural carriers that enhance the stability and delivery of antimicrobial peptides (AMPs), improving outcomes in systemic infection. Building on mechanistic insights into AMP-EV interactions, we investigated blood-derived EVs as carriers for the antimicrobial peptide DHVAR4 and evaluated their therapeutic potential in a cecal ligation and puncture (CLP) model of sepsis.

DHVAR4 loading did not compromise EV integrity: size distribution remained stable before and after loading, and glycophorin A expression was retained after loading and washing. Efficient DHVAR4 loading was confirmed across EV subtypes, and peptide-EV association was validated by CD63-AlexaFluor and CD81-PerCP-Cy5.5-positive fluorescence. Against Escherichia coli, EVs alone showed no inhibitory effect, whereas DHVAR4-loaded EVs achieved potent inhibition, with red blood cell-derived EVs producing complete inhibition at a low bactericidal dose and platelet-derived EVs (PEVs) producing strong inhibition, confirming EV-associated DHVAR4 retains activity after washing.

In the CLP-sepsis model, blood-derived EV-DHVAR4 formulations markedly improved survival versus controls. PEV-DHVAR4 was most effective, improving survival to 75%, versus 50% for red blood cell-derived EV-DHVAR4. These findings show blood-derived EVs preserve structural integrity upon AMP loading and enhance DHVAR4's stability and potency, with PEVs emerging as a highly effective carrier platform, supporting blood-derived EVs, and PEVs specifically, as biocompatible carriers for AMP-based therapeutics in sepsis and other severe infections.

T3-5 COMPARTMENT-SPECIFIC IMMUNOMODULATORY EFFECTS OF ENDOTHELIAL COLONY-FORMING CELL-DERIVED EVS IN A MURINE MODEL OF SYSTEMIC INFLAMMATION

[1]Luigi Menna, [1]Nóra Fekete, [1]Boglárka Nagy, [1]Kristóf Csont, [1]Sayam Ghosal, [1]Tünde Bárkai, [1]Angéla Takács, [1]Xabier Osteikoetxea, [1,2]Edit I. Buzás, [1]Hargita Hegyesi
[1]Institute of Genetics, Cell and Immunobiology, Semmelweis University, Hungary, [2]HUN-REN SU, Hungary

Introduction: Sepsis is a severe systemic condition associated with profound dysregulation of the immune response, underscoring the need for novel therapeutic strategies capable of restoring immune homeostasis. Murine models of LPS-induced systemic inflammation are widely used to investigate these processes. Extracellular vesicles (EVs) derived from endothelial colony-forming cells (ECFCs) have gained attention as potential immunomodulatory agents owing to their anti-inflammatory and regenerative properties. This study examines whether intraperitoneally administered small EVs from ECFCs (ECFC-sEVs) modulate local and systemic immune parameters in a murine model of LPS-induced systemic inflammation.

Methods: ECFCs were isolated from the bone marrow of bi-transgenic (Cdh5-Cre/mTmG) mice and expanded to confluence. Large and small EVs (lEVs, sEVs) were isolated from conditioned medium by differential ultracentrifugation and characterized by flow cytometry, nanoparticle tracking analysis (NTA), single-particle interferometric reflectance imaging (SP-IRIS), and transmission electron microscopy (TEM). The optimal LPS dose was first established by Kaplan-Meier survival analysis and subsequently used to compare survival between mice receiving intraperitoneal LPS alone or LPS followed by intraperitoneal ECFC-EV (lEV or sEV) administration. Peritoneal uptake of ECFC-lEVs and -sEVs was assessed in vivo by fluorescence microscopy and flow cytometry. Peripheral blood WBCs, T cells, monocytes, and circulating EVs were quantified by flow cytometry at 6 and 24 hours post-LPS injection. At 24 hours, sEV surface marker profiles from platelet-free plasma (PFP) and cell-free peritoneal fluid were compared by MACSPlex across four groups (control, LPS, LPS+EV, EV only), alongside M1/M2 macrophage polarization by flow cytometry.

Results: lEVs expressed tetraspanins and endothelial surface markers, while sEVs were confirmed to express tetraspanins. ECFC-lEVs and -sEVs were internalized by peritoneal immune cells, as shown by fluorescence microscopy and flow cytometry. Both lEV and sEV were well tolerated by the mice and did not compromise survival over the study period. At 6 and 24 hours, EV treatment was associated with alterations in circulating EV levels and shifts in peripheral immune cell populations. MACSPlex profiling of sEVs from plasma and peritoneal fluid revealed distinct surface marker signatures across groups, together with modulation of macrophage polarization.

Conclusions: ECFC-derived EVs were internalized by peritoneal immune cells, well tolerated in vivo, and associated with alterations in local and systemic immune parameters during LPS-induced systemic inflammation, supporting their potential as a cell-free immunomodulatory strategy in sepsis.

Funding: VEKOP-2.3.2-162016-00002, VEKOP-2.3.3-15-2017-00016, TKP2021-EGA-23, RRF-2.3.121-2022-00003, 2019-2.1.7-ERA-NET-2021-00015, EU Horizon 2020 grant agreement No. 739593, NKFIH 147023, 150767 and 151417.

1st EV4 POSTER ABSTRACTS



CELL BIOLOGY OF EXTRACELLULAR VESICLE RELEASE, UPTAKE AND SIGNAL TRANSDUCTION

1. ENDOTHELIAL-DERIVED EXTRACELLULAR VESICLES LINK CHRONIC ISOLATION STRESS TO CARDIOMYOCYTE GENE EXPRESSION

[1]Kristóf Csont, [1]Luigi Menna, [1]Boglárka Nagy, [1]Hargita Hegyesi
[1]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Hungary

Stress can promote systemic inflammation by altering immune responses and increasing pro-inflammatory cytokine production. Extracellular vesicles (EVs) are important mediators of intercellular communication and may contribute to stress-related cardiovascular alterations. We investigated the role of endothelial-derived EVs in endothelial-cardiomyocyte communication under inflammatory and chronic isolation stress conditions.

Endothelial C166 cells were treated with TNF-α or GDF-15 for 4 or 24 hours. The expression of natriuretic peptide receptors NPR2 and NPR3, p21, IL-1β, IL-6, and TNF-α was assessed by qRT-PCR, while endothelial migration was monitored for 48 hours using a real-time wound-healing assay. EVs were isolated from conditioned media by sequential filtration and ultracentrifugation, characterized by nanoparticle tracking analysis, and their cargo was analyzed by mass spectrometry. HL-1 murine cardiomyocytes were exposed to endothelial-derived EVs for 24 hours, followed by plate-based PCR array analysis of 80 cardiovascular-related genes. In vivo, chronic isolation stress was induced in bitransgenic F1 Cdh5 × mT/mG mice, enabling the flow-cytometric quantification of GFP-positive circulating endothelial-derived EVs. EV surface markers were further assessed using MACSPlex, while plasma TNF-α, GDF-15, and troponin I levels were measured by ELISA.

TNF-α markedly reduced endothelial NPR2 and NPR3 expression, increased the expression of inflammatory cytokines and p21, and significantly impaired endothelial migration, whereas GDF-15 had no clear effect. EVs released by TNF-α-treated endothelial cells altered the expression of several cardiovascular-related genes in HL-1 cardiomyocytes, including genes encoding adrenergic receptors, transcription factors, and troponins. In chronically isolated mice, plasma TNF-α levels and the release of endothelial-derived EVs were significantly increased, while the relative contribution of immune cell-derived EVs was altered.

These findings indicate that chronic isolation stress is associated with elevated TNF-α levels, endothelial activation, and enhanced release of endothelial-derived EVs. By modifying cardiomyocyte gene expression, these EVs may contribute to stress-related cardiac remodeling and provide a mechanistic link between chronic stress, systemic inflammation, and cardiovascular disease.

2. HOW MUCH OF YOUR EV PROTEOME IS ACTUALLY VESICULAR? – DEVELOPING V.I.S.O.R TO RESOLVE PROTEIN ORIGIN

[1,2]Mátyás Bukva
[1]Institute of Biochemistry, HUN-REN Biological Research Centre, Hungary, [2]Department of Immunology, University of Szeged, Hungary

Background: Although extracellular vesicle (EV) preparations from biofluids and conditioned media contain EV-associated proteins, they also contain non-vesicular contaminants. Density-gradient protein correlation profiling has classified thousands of proteins as small EV, co-sedimenting non-vesicular, externally associated, or unclassified; however, converting these protein-level assignments into a quantitative, sample-specific estimate of vesicular composition remains challenging. We are developing VISOR (Vesicular Intensity Separation and Origin Reporting), a browser-based tool for estimating the fraction of measured proteomic signal attributable to vesicles.

Methods: VISOR is being implemented in JavaScript as an HTML application requiring no installation, server or upload; computation will remain local. Classification will follow Morales-Sanfrutos *et al.* (*Nat Cell Biol*, 2026), assigning proteins by iodixanol-gradient elution profiles to sEV, non-vesicular, bimodal externally associated, or unclassified categories in conditioned medium, serum, and urine. Classification will be source-specific: EV preparations will be evaluated using the corresponding reference assignments.

Results: VISOR reports class-specific intensity fractions, protein counts, reference coverage. Counts and intensity-weighted fractions will be shown together because abundant contaminants may cause substantial divergence. Each output will include its underlying arithmetic and protein-level provenance. Optional Gene Ontology cellular-component annotation will enable over-representation analysis with non-redundant leading-term selection. Functions include multisample comparisons, MISEV2023 checks, dose partitioning, and CSV, JSON, and methods-text exports.

Conclusion: By translating source-specific protein classifications into quantitative, intensity-weighted estimates of vesicular composition, VISOR aims to make non-vesicular contributions explicit and enable transparent comparisons across samples and experiments.

3. COMPARATIVE ANALYSES OF PROTEIN PROFILES OF EXTRACELLULAR VESICLES PRODUCED BY MACROPHAGES INFECTED WITH VIRULENT AND AVIRULENT STRAINS OF FRANCISELLA TULARENSIS

[1]Ivona Pávková, [1]Jana Klimentová, [1,2]Radim Čermák, [3]Jaroslav Hanuš, [4]Pavla Molinová
[1]University of Defence, Military Faculty of Medicine, Institute of Molecular Pathology and Biology, Hradec Králové, Czechia, [2]Faculty of Pharmacy, Charles University, Czechia, [3]Department of Chemical Engineering, University of Chemistry and Technology, Prague, Czechia, [4]Institute of Molecular Genetics of the Czech Academy of Sciences, Electron Microscopy Core Facility, Prague, Czechia

Extracellular vesicles (EVs) function as key players in intercellular communication. Mounting evidence indicates that EVs also contribute to the course of infection. EVs produced by cells infected with an intracellular pathogen might function as by either promoting or inhibiting the immune response to pathogens. The knowledge remains still quite limited in this area. *Francisella tularensis* (Ft) is an example of an intracellular pathogenic bacterium with a cytosolic life cycle. This causative agent of severe and even fatal tularaemia disease is one of the most infectious bacteria. Ft primarily invades host phagocytic cells; upon entry, it rapidly escapes the phagosome into the host cell cytosol, where it replicates extensively. The pathogen has evolved diverse so far unrecognized strategies to evade the innate immune mechanisms, with consequence of the delayed onset of the adaptive immunity.

Recently we revealed that primary macrophages infected with full virulent Ft strain produce large quantities of EVs which are loaded with host and bacterial proteins involved in various cellular processes. On the contrary, naïve cells produced only negligible amounts of EVs disabling any comparative analysis. To detect proteins that might potential contribute to the suboptimal host response, we decided to analyse the EVs also from primary macrophages infected with avirulent *F. novicida*. The TEM and NTA characterization revealed that the cells stimulated with *F. novicida* are able to produce EVs of similar size and morphology; however, their yields were significantly lower compared to EVs from cells infected with full virulent Ft strain. The comparative proteome analysis revealed significant differences in protein profiles of EVs produced by macrophages in response to the two bacterial strains with different virulence. Further bioinformatic analyses demonstrated, that protein significantly enriched in EVs induced by *F. novicida* were predominantly involved in terms corresponding to immune processes such as regulation of inflammatory response, immune effector process, leucocyte activation, antigen processing and presentation, whereas EVs induced by the full virulent Ft strain were uniquely enriched in proteins related to ribosomes and mRNA processing. Moreover, several bacterial proteins could be identified in samples from cells treated with both the Ft strains. These preliminary data indicated that macrophage-derived EVs might highly contribute to the course if tularaemia infection and warrants further, more thorough studies.

The work was supported by the Ministry of Defence of the Czech Republic “Long Term Organization Development Plan 1011” – Healthcare Challenges of WMD II of the Military Faculty of Medicine Hradec Kralove, University of Defence, Czech Republic (Project No: DZRO-FVZ22-ZHN II).

4. KNOCKING ON THE SURFACE: TETRASPANIN KNOCKOUTS REVEAL CD81 AS THE STRONGEST DRIVER OF EV CARGO REMODELLING

[1]Anna Juráčková, [1]Martin Smolko, [1]Martina Dhima, [1]Anna Vyhlídalová Kotrbová, [2]Václav Pustka, [2]Zbyněk Zdráhal, [3]Karel Souček, [4]Jiřina Procházková, [1]Vendula Pospíchalová
[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic, [2]Proteomics Core Facility, Central European Institute of Technology, Masaryk University, Czech Republic, [3]Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Czech Republic [4]Preclinical Centre, Department of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic

Tetraspanins are four-pass transmembrane scaffolds that organize tetraspanin-enriched microdomains and have been implicated in extracellular vesicle (EV) biogenesis and cargo sorting, but the individual contribution of each family member to cargo partitioning between cells and EVs remains unresolved.

We performed quantitative proteomic profiling of cells and EVs derived from wild-type (WT) DU145 cells and CRISPR-Cas9-generated CD9, CD63, and CD81 knockout (KO) variants. Since cellular and EV proteomes are too distinct to be normalized jointly, KO effects were estimated separately per fraction and compared at the level of effect sizes: global effect consistency via correlation of KO-vs-WT log2FC between fractions, and per-protein re-wiring via formal interaction tests, complemented by differential expression and surfaceome enrichment analyses.

Surprisingly, the tetraspanin KO with the most altered EV proteome had the fewest protein changes in the cells from which the EVs were released. While CD81 KO cells showed the fewest differentially expressed proteins and did not separate from WT cells in PCA, CD81 KO EVs formed a distinct cluster, separate from other tetraspanin KOs and WT. This separation was accompanied by a pronounced downregulation of proteins, with surfaceome proteins significantly enriched among the downregulated ones (odds ratio = 2.02, Fisher's exact test). Consistently, the cell–EV effect correlation was the lowest for CD81 ($r = 0.32$ vs 0.44 for CD9 KO). Formally, CD81 KO significantly re-wired the cell–EV distribution of 14.6% of the quantified proteome, compared to 12% for CD63 KO and 5.7% for CD9 KO. Cargo re-wiring was not restricted to the surfaceome, indicating a broad effect of CD81 depletion on the total EV proteome.

Our data show that loss of CD81 has the strongest impact on cargo partitioning between cells and EVs, while loss of CD9 leaves cell–EV coupling most intact, revealing protein-specific and fraction-dependent tetraspanin requirements in EV cargo composition.

Acknowledgement: CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042, is gratefully acknowledged for the financial support of the measurements at the CEITEC Proteomics Core Facility. Computational resources were provided by the e-INFRA CZ project (ID:90254), supported by MEYS CR.

5. PHARMACOLOGICAL MODULATION OF AUTOPHAGIC COMPARTMENTS AND EXTRACELLULAR VESICLE-ASSOCIATED PATHWAYS BY NOVEL DRUG CANDIDATES

[1]Lilla Lankovics, [1]Tünde Bárkai, [1]Krisztina V. Vukman, [1]Kelsey Fletcher, [2]Martin Krátky, [1,3]Szilvia Bősze, [1,4]Edit I. Buzás, [1,*]Dorina Lenzinger, [1,*]Tamás Visnovitz
[1]Semmelweis University, Institute of Genetics, Cell- and Immunobiology, Hungary, [2]Department of Organic and Bioorganic Chemistry, Charles University, Czech Republic, [3]HUN-REN-ELTE Research Group of Peptide Chemistry, ELTE Eötvös Loránd University, Hungary, [4]HUN-REN-SU Translational Extracellular Vesicle Research Group, Hungary

Autophagy is a fundamental cellular homeostatic process that regulates the turnover and recycling of intracellular components through highly coordinated membrane trafficking pathways. Beyond canonical autophagosome formation and lysosomal degradation, autophagosomes can fuse with multivesicular bodies and generate amphisomes. These structures provide an important functional interface between autophagy, endosomal trafficking and extracellular vesicle (EV) biology. Our recent findings indicate that amphisomes can undergo ectocytosis and be released as amphiectosomes. Through the “torn bag mechanism”, the amphiectosome membrane ruptures, releasing the intraluminal vesicles into the extracellular space as small EVs. This observation suggests, that autophagy-associated membrane trafficking may contribute to EV release through mechanisms distinct from conventional direct plasma membrane budding or classical exosome secretion.

The aim of the present study was to investigate how pharmacological modulation of autophagy affects the formation and intracellular distribution of autophagic compartments and pathways associated with EV biogenesis. HEK293T-PalmGFP-LC3RFP cells were treated with the established autophagy-modulating compounds (chloroquine and bafilomycin A1) and novel drug candidates (salicylanilide and niclosamide). The size and frequency of LC3-positive intraluminal vesicles were quantified using Leica SP8 confocal microscope. Changes in the expression of selected genes associated with autophagy, endosomal maturation, and vesicular trafficking were evaluated by RT-qPCR.

Both chloroquine and bafilomycin A1 led to the accumulation of autophagosomes and amphisomes. Salicylanilide induced concentration-dependent changes in the number and sizes of these compartments, indicating a dose-dependent effect. In contrast, niclosamid had significantly less impact on these properties. Salicylanilide and niclosamide altered the expression of several genes involved in autophagy and endosomal trafficking. Based on the extracellular fluorescent signal, salicilanilid treatment significantly increased the secretion of membrane derived and autophagy related EVs.

These finding suggest, that the drug candidates affect autophagy through different pathways and modulate extracellular vesicle biogenesis. Further studies integrating high-resolution microscopy, biochemical EV characterization and mechanistic perturbation of candidate trafficking pathways will therefore be necessary to define the cellular mechanisms linking autophagy with EV biogenesis and release.

Funding: NKKP ADVANCED_25 152112, Therapeutic Thematic Programme (TKP2021-EGA-23), RRF-2.3.1-21-2022-00003 and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (T.V.).

6. CAVEOLIN-1 SELECTIVELY DEPLETES EXTRACELLULAR VESICLES OF HEPARIN-BINDING CARGO IN PROSTATE CANCER CELLS: AN INTEGRATED PROTEOMIC AND LIPIDOMIC STUDY OF CAV1 KNOCKOUT

[1]Monika Kratochvílová, [1]Klára Hánělová, [1]Jiří Navrátil, [3]Yoav David Shaul, [2]Ondrej Kováč, [2]Miroslav Machala, [1]Jan Balvan
[1]Michal Masarik´s Cancer Research Lab, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [2]Department of Pharmacology and Toxicology, Veterinary Research Institute, Brno, Czech Republic, [3]Department of Biochemistry and Molecular Biology, Faculty of Medicine, The Institute for Medical Research Israel-Canada, Hebrew University of Jerusalem, Jerusalem, Israel

Caveolin-1 (CAV1) organises caveolae and cholesterol-rich membrane domains, yet its role in selecting the cargo of secreted extracellular vesicles (EVs) remains incompletely defined. We combined data-independent-acquisition proteomics and lipidomics of isogenic PC3 prostate cancer cells - wild-type, CAV1 knockout (KO), and CAV1 re-expression (rescue) - to dissect how CAV1 shapes cellular membranes and EV cargo. Whole cells and ultracentrifugation-isolated EVs were profiled by proteomics, and cells together with phosphatidylserine-fractionated vesicles by lipidomics. Differential testing used limma with batch control. To prevent imputation from driving results, protein testing was restricted a priori to features with genuine peptide-level detection in at least 75% of replicates, and findings were stress-tested by leave-one-out resampling, node-degree-corrected interactome analysis, and compositional controls.

CAV1 loss weakened the canonical EV signature of tetraspanins and ESCRT components ($p = 0.007$) and selectively depleted vesicles of heparin-binding, heparan-sulfate-associated cargo (heparin binding, FDR = 2.5×10^{-4}; including THBS1, VTN, FBN1, SLIT2, PTN and SERPINE2), with cell adhesion, extracellular matrix and proteolytic regulation recurring across compartments. “Caveola assembly” was the single strongest downregulated term, providing internal validation of the analysis. At the cellular level, CAV1 loss reduced total membrane sphingolipid by approximately one third and depleted caveolar structural proteins (CAV1, CAV2, STOM); neither change was explained by biosynthetic-enzyme abundance, indicating a membrane-organisational rather than transcriptional mechanism. Re-expression of CAV1 restored the knockout proteome in proportion to the level of CAV1 recovered (regression slope 0.63, 95% CI 0.56–0.69). Together these data show that CAV1 shapes EV protein cargo - particularly heparin-binding proteins - and supports cellular membrane lipid content, consistent with a caveolin-dependent, heparan-sulfate-associated cargo-sorting route that is dose-dependently reversible. The findings nominate CAV1 as a regulator of EV cargo selection with potential relevance to prostate-cancer vesicular signalling.

7. CHARACTERIZING THE IMPACT OF CPAP THERAPY ON CIRCULATING EVS IN PATIENTS WITH SLEEP-RELATED BREATHING DISORDERS

[1]Nicol Strakova, [1]Martina Hyzdalova, [2]Samuel Genzor, [3]Jirina Prochazkova, [3]Petra Ovesna, [3]Karolina Strakova, [2]Petr Mooz, [2]Radana Brumarova, [2]David Friedecky, [1]Martina Fojtikova, [1]Josef Masek
[1]Veterinary Research Institute, Brno, Czech Republic, [2]University Hospital Olomouc, Olomouc, Czech Republic, [3]Masaryk University, Brno, Czech Republic

Sleep-related breathing disorders comprise a group of conditions characterized by recurrent disturbances in breathing during sleep, with obstructive sleep apnoea (OSA) being the most prevalent form. OSA is characterized by repeated episodes of partial or complete upper airway collapse during sleep, leading to intermittent reductions (hypopnoea) or cessations (apnoea) of airflow. Disease severity is determined by the Apnoea-Hypopnoea Index (AHI), which quantifies the number of breathing interruptions per hour of sleep. OSA is classified as mild (AHI 5-14 events/h), moderate (AHI 15-29 events/h), or severe (AHI ≥30 events/h). OSA is associated with intermittent hypoxia, sleep fragmentation, and systemic inflammation, contributing to an increased risk of cardiovascular, metabolic, and neurocognitive complications. Continuous positive airway pressure (CPAP) remains the gold-standard treatment for OSA.

We hypothesized that intermittent hypoxia promotes systemic inflammation and increases circulating levels of pro-inflammatory cytokines. To test this hypothesis, we assessed a panel of inflammatory markers in patients with sleep-related breathing disorders and subsequently evaluated the effects of CPAP therapy on the inflammatory profile. Patients with OSA underwent CPAP treatment, and follow-up samples were collected after three months of therapy. While circulating pro-inflammatory cytokines did not differ significantly between OSA patients and healthy controls, CPAP treatment was associated with increased levels of anti-inflammatory cytokines, suggesting attenuation of chronic inflammation and gradual restoration of systemic homeostasis.

As the identification of reliable blood-based biomarkers for OSA remains challenging, we further investigated extracellular vesicles (EVs), which mediate intercellular communication and reflect ongoing physiological and pathological processes. EV characterization was performed using multi-angle light scattering and nano-flow cytometry, including analysis of EV surface marker expression. Nano-flow cytometry revealed that a substantial proportion of circulating EVs originated from the monocyte-macrophage lineage, indicating a significant contribution of myeloid cells to the circulating EV pool.

In conclusion, conventional circulating inflammatory markers showed limited utility in distinguishing OSA patients from healthy controls. In contrast, circulating EVs represent promising minimally invasive biomarkers that may better reflect the inflammatory and metabolic alterations associated with OSA and their modulation following CPAP therapy.

Supported by AZV No. NW25J-01-00013 and DKRVO RO 0526.

8. CANINE MESENCHYMAL STEM CELLS-DERIVED EXTRACELLULAR VESICLES: CHALLENGES FROM ISOLATION TO CHARACTERIZATION

[1]Alexandra Kešeľáková, [1]Natália Nosálová, [1]Mykhailo Huniadi, [2]Monika Žilková and [1]Daša Čížková,
[1] Laboratory of Experimental and Clinical Regenerative Medicine, Small Animal Clinic, The University of Veterinary Medicine and Pharmacy in Košice, Slovakia, [2] Institute of Neuroimmunology, SAV, Bratislava, Slovakia

Extracellular vesicles (EVs) have emerged as promising therapeutic tools due to their ability to transport bioactive molecules and promote tissue regeneration. Despite extensive research in human medicine, EV-based approaches remain underexplored in veterinary medicine. MSC-derived EVs represent a promising cell-free alternative to MSC transplantation, potentially overcoming limitations associated with cell survival, engraftment, immune responses, and effective delivery to target tissue. Therefore, this study aimed to optimize the isolation and characterization of EVs from canine MSCs, focusing on methodological challenges associated with their analysis.

Canine adipose tissue-derived mesenchymal stem cells (AT-MSCs) were isolated from adipose tissue obtained from dogs undergoing ovariohysterectomy and cultured in serum-free DMEM-HG after reaching full confluence. The conditioned medium was collected, centrifuged to remove debris and filtered through a 0.22 mm filter. EV-enriched fractions were isolated from at least 25mL of conditioned medium using the exoEasy kit (QIAGEN), according to the manufacture´s instructions and stored at -80 °C until analysis. Particle concentration and size distribution were assessed by nanoparticle tracking analysis (NTA) using NanoSight, while particle-associated surface markers were evaluated by nano-flow cytometry. Total EV protein concentration was determined using the Micro BCA Protein Assay Kit.

NTA revealed a heterogeneous population of extracellular particles with a modal diameter of 92.5 nm and a mean diameter of 304 ± 3 nm. The particle concentration was 7.48×10^{10} particles / mL and the total protein concentration was 153.7 µg / mL, corresponding to a particle-to-protein ratio of $4.87.10^{8}$ particles/ µg protein. Nano-flow cytometry confirmed the presence of the tetraspanin CD9. These findings support the presence of EVs in the isolated fraction, but do not confirm EV purity or exclude other extracellular particles and non-vesicular components. The presence of a prominent population around 100 nm was consistent with small EV-sized particles, whereas, the broad particle size distribution and relatively high mean diameter indicated considerable heterogeneity, potentially reflecting the presence of larger vesicles and/or aggregated particles. Further optimization and complementary methods are needed to improve EV characterization and reproducibility.

Overall, these findings demonstrate the feasibility of recovering an EV-enriched fraction from canine MSC-conditioned medium while highlighting several challenges associated with canine samples, including sample variability and the possible presence of non-EV particles and protein contaminants. The difference between the modal and mean particle sizes highlights the need for additional methods to complement NTA.

Funding: This work was supported by the Slovak Research and Development Agency under Contract No. APVV-25-0491.

EV-BASED DIAGNOSTIC APPLICATIONS

9. MATERNAL SERUM PROGESTERONE-INDUCED BLOCKING FACTOR (PIBF) IN THE PREDICTION OF PREECLAMPSIA

[1*]Andrea Balogh, [2*]Tímea Csabai, [1,3]Emese Farkas, [1,4]Dániel Györffy, [2]Zoltán Bognár, [1,5*]Nándor Gábor Than, [2†*]Júlia Szekeres-Barthó

[1]Systems Biology of Reproduction Research Group, Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary, [2]National Laboratory on Human Reproduction, Universit011y of Pécs, Pécs, Hungary, [3]Doctoral College, Károly Rácz Conservative Medicine Division, Semmelweis University, Budapest, Hungary, [4]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary, [5]Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary

Preeclampsia (PE) is a major obstetrical syndrome associated with impaired maternal-fetal immune tolerance. It affects 3-8% of pregnancies worldwide, with significant maternal and perinatal morbidity. Identifying early pathologic pathways and predictive biomarkers is critical for timely, effective, and personalized interventions. Progesterone-Induced Blocking Factor (PIBF) is a key mediator of maternal-fetal immune tolerance, secreted by progesterone-primed lymphocytes, decidual stromal cells, and trophoblasts. Reduced maternal blood PIBF has been documented in PE. Its association with extracellular vesicles (EVs) offers a promising avenue for non-invasive, first-trimester risk stratification. Here, we aimed to evaluate whether maternal serum PIBF$^+$ EV levels, quantified in the first trimester, could predict the later development of various subtypes of PE.

PIBF$^+$ EV levels were measured by flow cytometry in gestational age-matched first trimester serum samples from women with singleton pregnancies who subsequently developed preterm PE (<37 weeks; n=10), term PE (≥37 weeks; n=10), and healthy controls (≥37 weeks; n=42). Group differences were analyzed using the Mann-Whitney U test or the Kruskal-Wallis test followed by Dunn's post hoc test. Receiver-operating characteristic (ROC) curves were generated to assess diagnostic performance based on the area under the curve (AUC).

Baseline demographic characteristics were comparable among groups. Significant differences were found in gestational age at delivery and mean birth-weight percentile between preterm PE group and the term PE or control groups. PIBF$^+$ EV counts were significantly lower in the PE group compared to controls [median (IQR): 1427 (972.7-2801) vs. 4785 (3305-9175) events/µL, p<0.0001]. Subgroup analysis confirmed significantly lower PIBF$^+$ EV numbers in both the preterm PE [1695 (990.1-3073) events/µL, p=0.0013] and term PE [1323 (923.0-2643) events/µL, p=0.0002] groups compared to controls. ROC analysis revealed high predictive accuracy for all PE cases (AUC=0.883), preterm PE (AUC=0.868), and term PE (AUC=0.898). At a fixed 80% specificity, the sensitivities were 80%, 80%, and 90%, for all PE, preterm PE, and term PE, respectively.

First-trimester maternal serum PIBF$^+$ EV levels are significantly reduced in pregnancies that later develop preterm or term PE, reflecting early immune tolerance disruption. These proof-of-concept findings highlight PIBF$^+$ EVs as promising non-invasive biomarkers for early PE risk assessment, warranting validation in larger prospective cohorts.

10. BIOMOLECULAR CORONA MODULATES THE ACCESSIBLE SURFACE PHENOTYPE AND GLYCAN-MEDIATED RECOGNITION OF EXTRACELLULAR VESICLES

[1]Veronika Solovicova, [1]Tomas Bertok, [1]Eduard Jane, [1]Veronika Pinkova Gajdosova, [1,2]Andrea Furdova, [3]Matej Micusik, [4]Monika Sramkova, [4]Katarina Kozics, [1,2]Jan Tkac
[1]Department of Glycobiotechnology, Institute of Chemistry, Slovak Academy of Sciences, Slovak Republic, [2]Glycanostics Ltd., Slovak Republic, [3] Polymer Institute, Slovak Academy of Sciences, Slovak Republic, [4]Cancer Research Institute, Biomedical Research Centre, Slovak Academy of Sciences, Slovak Republic

Extracellular vesicles (EVs) carry molecular information that reflects their cellular origin and represent promising targets for biomarker discovery and liquid biopsy applications. However, the experimentally accessible EV surface is not determined solely by intrinsic membrane components. Following exposure to complex biological environments, EVs acquire a protein corona that may modify the accessibility of surface molecules, thereby affecting both analytical detection and interactions with biological interfaces.

Here, we investigated how protein-corona reduction affects EV surface recognition and explored the interaction behaviour of EVs at extracellular-matrix-mimicking glycan interfaces. EVs derived from MDA-MB-231 malignant cells and non-malignant RWPE-1 cells were processed using tangential flow filtration and size-exclusion chromatography. The protein corona was substantially reduced by a high-ionic-strength treatment followed by repeated size-exclusion chromatography. Accessibility of EV-associated markers was evaluated by antibody-based detection, while EV interactions with heparan-sulfate-based nanobiointerfaces were investigated using surface plasmon resonance (SPR).

Reduction of the protein corona markedly improved recognition of EV surface markers, with particularly pronounced changes in CD81 accessibility, demonstrating that corona-associated biomolecules can substantially influence analytical detection of membrane-associated epitopes. Glycan-based SPR analysis further revealed distinct interaction phenotypes between malignant-derived and non-malignant-derived EVs. Notably, RWPE-1-derived EVs generated substantially higher SPR responses than MDA-MB-231-derived EVs, indicating that particle concentration alone does not determine interaction behaviour and highlighting the importance of surface composition and ligand accessibility.

Together, these findings demonstrate that the accessible EV surface represents a dynamic interface shaped by intrinsic vesicular properties and acquired biomolecules. Considering protein-corona effects alongside interaction-based surface profiling may therefore improve the interpretation of EV biomarkers and contribute to the development of more robust EV-based liquid biopsy strategies.

Acknowledgements: The authors wish to acknowledge the financial support received from the projects of the Slovak Research and Development Agency (APVV-24-0065, APVV-24-0529, APVV-22-0345, and APVV-22-0496).

11. IMMUNO-qPCR-BASED DETECTION OF EXTRACELLULAR VESICLE BIOMARKERS FOR OVARIAN CANCER DIAGNOSIS

[1,2]Klára Mrázová, [3]Lucia Ciglar, [1]Roman Hrstka
[1]Research Centre for Applied Molecular Oncology (RECAMO), Masaryk Memorial Cancer Institute, Czech Republic, [2]Department of Biochemistry, Faculty of Science, Masaryk University, Czech Republic, [3]AIT Austrian Institute of Technology GmbH, Center for Health & Bioresources, Competence Unit Molecular Diagnostics, Austria

Ovarian cancer (OC) has the highest mortality rate among gynecological malignancies, largely due to delayed diagnosis and complications associated with therapy resistance. This highlights the urgent need to identify novel biomarkers and develop innovative approaches for OC diagnostics. Current diagnostic techniques lack sufficient sensitivity and specificity, underscoring the need for novel minimally invasive biomarkers. In this regard, liquid biopsy represents a viable approach, with extracellular vesicles (EVs) emerging as a promising source of OC biomarkers. EVs are a heterogeneous group of particles delimited by a lipid bilayer abundantly present in all body fluids. Their molecular cargo reflects the pathophysiological state of the cell of origin and contains numerous macromolecules, which may harbor potential biomarkers. Therefore, EVs have shown to be a promising candidate for minimally invasive liquid biopsy applications. Here, we present a comprehensive strategy for identification and validation of novel EV-based biomarkers of OC using a multi-omics approach. EVs obtained from serum samples of OC patients and healthy controls will undergo high-resolution mass spectrometry analysis and RNA sequencing. Analysis of integrated multi-omics data will provide a set of potential diagnostic and prognostic biomarkers. Selected biomarkers will be validated using multiple sensitive targeted methods, including Immuno-qPCR. Immuno-qPCR is an advanced technique that combines the high specificity of antibody detection and the high sensitivity of the PCR method. The low concentration markers can be detected in complex samples by employing antibodies with conjugated DNA probes. DNA probes are then amplified and detected through a classical qPCR system. In this preliminary work, we show the development of the Immuno-qPCR method. We demonstrate its ability to detect canonical EV markers across different biological sources. Furthermore, we present initial results on the detection of selected OC biomarkers. The Immuno-qPCR shows potential for early disease diagnosis and monitoring, surpassing the limit of detection of other traditional methods (e.g., ELISA).

12. IMPROVING THE SELECTIVE ANALYSIS AND MASS SPECTROMETRY-BASED CHARACTZERIZATION OF CIRCULATING TUMOR-DERIVED EXTRACELLULAR VESICLES

[1,2]Júlia Németh, [3,4,5]Agustin Enciso-Martinez, [1,2]Alexandra Molnár, [6]Ali Salanti, [1]Boglárka Cziráki, [3]Emma P E M Boerrigter, [7]Krisztina V Vukman, [1]Márkó Grabarics, [6]Mette Ørskov Agerbæk, [1,2]Mirjam Balbisi, [8]Tamás Langó, [9]Judit Moldvay, [1,2]Lilla Turiák

[1]MTA-HUN-REN TTK Lendület Glycan Biomarker Research Group, HUN-REN Research Centre for Natural Sciences, Hungary, [2]Semmelweis University Doctoral College, Hungary, [3]Oncode Institute, Department of Cell and Chemical Biology, Leiden University Medical Center, The Netherlands, [4]Biomedical Engineering & Physics, University of Amsterdam, the Netherlands, [5]Laboratory of Experimental Clinical Chemistry, Laboratory Specialized Diagnostics & Research, Department of Laboratory Medicine, University of Amsterdam, the Netherlands, [6]Centre for Translational Medicine and Parasitology, Department of Immunology and Microbiology, University of Copenhagen, Denmark, [7]Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Hungary, [8]Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Hungary, [9]National Korányi Institute of Pulmonology, Hungary

Extracellular vesicles (EVs) have emerged as promising biomarkers, as their cargo reflects the molecular composition of their parent cell. Tumor-derived EVs (TEVs) display molecular profiles distinct from those of non-tumor-derived EVs that may contribute to cancer development, metastasis, and treatment response. Selective analysis of TEV subpopulations may therefore provide valuable insights into cancer progression and immunotherapy response.

Despite rapid advances in EV research, their mass spectrometry (MS)-based analysis remains challenging. EVs isolated from complex biological samples originate from diverse cell types, potentially obscuring tumor-specific molecular signatures. In addition, the formation of the protein corona (PC) can hinder the detection of EV surface molecules.

The aim of this study was to characterize plasma-derived TEV subpopulations from lung cancer patients using mass spectrometry-based (glyco)proteomic approaches, thereby establishing their potential as biomarkers. To achieve this, we developed and compared strategies for reducing the PC and enriching TEVs through immunocapture.

Small EVs (30-200 nm) were isolated from platelet-depleted plasma of lung cancer patients with size exclusion chromatography (SEC), using Sepharose CL-2B column as solid phase. For immunocapture, we employed the C9 antibody fragment conjugated to streptavidin magnetic beads targeting oncofetal chondroitin sulfate (ofCS), a recently discovered TEV-marker epitope. To elute TEVs from the magnetic bead solid phase, three methods were compared, 0.1 M glycine, chondroitinase ABC, and on-bead digestion using 0.1% RapiGest.

To investigate strategies for the PC reduction, methods based on partial tryptic digestion and high-salt washing were compared. Following the isolation of small EVs by SEC, a range of trypsinization conditions was evaluated by varying enzyme concentration, incubation time, and temperature. In parallel, a 1.5 M NaCl high-salt wash was assessed. To determine the effectiveness of the PC reduction methods, flow cytometry analysis was performed following fluorescent labelling of ofCS epitopes.

Chondroitinase ABC proved to be the most effective approach for eluting TEVs from C9 antibody-coated magnetic beads. Among the tested conditions, trypsinization at 37 °C for 1 h using an enzyme-to-protein ratio of 1:20 resulted in the highest accessibility of the ofCS epitope.

The integration of PC reduction with C9 antibody-mediated immunocapture of SEC-isolated EVs may facilitate the selective characterization of TEV subpopulations in plasma samples of cancer patients. Comprehensive (glyco)proteomic profiling of TEV surface molecules could further elucidate TEV-specific alterations associated with cancer progression and therapeutic intervention. Accordingly, TEVs may also serve as biomarkers of response to immunotherapy.

13. ENRICHMENT OF BRAIN-DERIVED EXTRACELLULAR VESICLES FROM HUMAN PLASMA BY IMMUNE-CAPTURE METHODS

[1]Stanislava Sladeček, [1]Šárka Hrachovinová, [2]Kateřina Veverová, [2]Martina Laczó, [2]Jan Laczó, [1,#]Vendula Pospíchalová
[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic, [2] Memory Clinic, Department of Neurology, Second Faculty of Medicine, Charles University and Motol and Homolka University Hospital, Czech Republic

Thanks to their relative variety, abundancy and easy isolation from different biofluids, extracellular vesicles (EVs) have been considered a potentially good tool for detecting biomarkers of different diseases. However, their heterogeneity in plasma also has its downsides, as the EV volume emerging from different organs, can mask EVs carrying signals coming from organs of interest. One of the means to overcome this information heterogeneity is to enrich the EV sample for certain EV subpopulation before their downstream analyses. Good example of small EV population present in blood plasma, are EVs originating from different cells of neural system. Enrichment of these populations could therefore increase the detection sensitivity of biomarkers connected to neurodegenerative diseases.

Our study focuses on isolation of EVs carrying neural markers from patient plasma by employing an immune-capture approach targeting three surface markers: CD171 (neurons), GLAST (astrocytes), and TMEM119 (microglia). This strategy enables the enrichment of brain-derived EV subtypes based on cell of origin; however the sample enrichment has its pitfalls, which should be considered. Method of EV isolation, immune-capture approach or used antibody heavily affects the successfulness of sample enrichment. Following the MISEV guidelines we have optimized workflow for brain-derived EV sample enrichment and show the example of their potential as biomarkers in neurodegenerative diseases based on samples from patients diagnosed with limbic-predominant age-related TDP-43 encephalopathy (LATE).

Clinically, LATE often mimics Alzheimer's disease (AD) due to its progressive impairment of episodic and semantic memory. Unlike AD, however, LATE currently lacks established biomarkers for reliable diagnosis. One of LATE hallmarks is pathological TDP-43, particularly found in neuronal and glial cells in the brain, which can be detected in brain-derived EVs.

By improving the specificity of EV enrichment, this methodology can be used as a piece of puzzle, leading to more accurate differential diagnosis of LATE and neurodegenerative diseases associated with other pathologies.

Funding: This work was supported by Ministry of Health of the Czech Republic in cooperation with the Czech Health Research Council under project no. NW25-04-00337.

EVS AS THERAPEUTICS, INCLUDING EFFICACY, SAFETY AND EVS AS DELIVERY SYSTEMS

14. ENHANCING EXTRACELLULAR VESICLE-MEDIATED GENE DELIVERY USING CELL-PENETRATING PEPTIDES

Mohamed A. Fattah[1], Bernadett R. Bodnár[1], Sayam Ghosal[1], Brachyahu Meir Kestecher[1], Edit I. Buzás[1], Xabier Osteikoetxea[1]
[1]Institute of Genetics, Cell and Immunobiology, Semmelweis University, Hungary

Extracellular vesicles (EVs) are lipid-bilayer-delimited particles released by all cell types. Among many roles, they mediate intercellular communication through the transfer of proteins, lipids and nucleic acids. Their biocompatibility and capacity to carry diverse biomolecular cargo make engineered EVs attractive candidates for non-viral gene delivery. However, inefficient cellular entry and intracellular trafficking, including sequestration within endolysosomal compartments, remain major barriers to functional cargo delivery. This study aims to address this problem directly by utilising cell-penetrating peptides (CPPs). CPPs are short peptides that promote membrane translocation or endocytic uptake and may therefore improve EV delivery. Here, we engineer EVs to display CPPs on their surface and investigate whether this improves their cellular uptake and functional delivery of gene-editing cargo.

We selected 11 CPP candidates from the literature to create expression cosntructs with molecular cloning techniques. Subsequently, we generated a panel of CPP-modified EV constructs in HEK293T producer cells and isolated them by ultracentrifugation and size-exclusion chromatography. EV preparations were characterized by nanoparticle tracking analysis and evaluated in HEK293T recipient cells. CPP modified EV were loaded with Sleeping Beauty (SB) transposase and an eGFP-Puromycin transposon construct as a functional cargo-delivery model. Following EV treatment, flow cytometry was used to assess eGFP cargo transfer to recipient cells followed by puromycin selection to assess transposase mediated integration of the transposon construct. Preliminary screening at a standardised EV dose revealed construct-dependent differences in GFP-associated fluorescence and survival under puromycin selection, identifying candidate CPP-EV designs for further validation. These findings are being followed by dose-response experiments to distinguish CPP conditions that improve EV uptake and productive cargo delivery.

Our group previously demonstrated functional EV-mediated delivery of CRISPR/Cas9 and editing of the therapeutically relevant PCSK9 locus. Building on this platform, the present study aims to identify CPPs and lead CPP-EV candidates to be subsequently evaluated for PCSK9-targeted genome editing as a potential strategy for reducing atherosclerotic cardiovascular risk. Together, this work seeks to establish surface-engineered EVs as more efficient vehicles for functional gene delivery.

15. GENE EXPRESSION DYNAMICS OF CHONDROCYTES CULTIVATED IN 2D AND 3D TREATED WITH PLASMA EXTRACELLULAR VESICLES

[1]Lívia K. Fecskeová, [1]Jana Bzdiľová, [1]Hedviga Košuthová, [1]Jana Matejová, [1]Lucia Slovinská, [1]Tímea Špaková, [1]Denisa Harvanová
[1]Associated Tissue Bank, Faculty of Medicine, P. J. Šafárik University and L. Pasteur University Hospital in Kosice, Slovakia

Osteoarthritis (OA) is characterized by progressive cartilage degradation and impaired function of chondrocytes, the cells responsible for maintaining extracellular matrix homeostasis in articular cartilage. Due to the limited regenerative capacity of cartilage, extracellular vesicles (EVs) have attracted considerable interest as potential cell-free therapeutic agents capable of modulating tissue repair. EVs mediate intercellular communication by transferring biologically active molecules to recipient cells and can influence processes relevant to OA, including inflammation, extracellular matrix metabolism, cell survival, and regenerative signalling. Growing evidence indicates that EVs from different cellular and biological sources can promote chondroprotective and anabolic responses in chondrocytes, supporting their potential application in cartilage regeneration and OA therapy.

This study evaluated the regenerative effects of EVs isolated from platelet-poor plasma of healthy donors on human OA chondrocytes, with particular focus on culture dimensionality, temporal response, and treatment regimen.

Chondrocytes were cultivated in conventional 2D monolayer and 3D culture systems and treated with PPP-EVs. Early transcriptional responses were assessed at 6, 12, and 24 h after EVs administration by analysing the expression of chondrogenic marker genes *COL2A1*, *ACAN*, *SOX-9* and *COMP* relative to untreated controls. In addition, the effect of repeated EV exposure was evaluated by comparing a single dose of EVs with five repeated doses over a 2.5-week cultivation period. Functional matrix production was assessed by measuring sulphated glycosaminoglycan (sGAG) accumulation in 2D and 3D cultures after repeated EV treatment.

PPP-EVs treatment induced culture-dependent and time-dependent changes in chondrogenic gene expression. In 2D culture, the response was generally strongest at early time points and declined over time, whereas 3D-cultivated chondrocytes showed a more delayed and sustained increase of chondrogenic markers. Repeated EVs administration induced an even stronger chondrogenic transcriptional response than a single EV treatment, with significant increases in *SOX-9* and *COMP* and positive trends for *COL2A1* and *ACAN*. Repeated EV treatment also increased sGAG production in both culture systems.

Together, these findings indicate that PPP-EVs-mediated effects on chondrocytes are not fixed but depend on culture architecture, exposure time, and dosing regimen. The results suggest that 3D culture provides a more favourable environment for sustained EV-induced chondrogenic activation, while repeated EV exposure may be required to achieve stronger transcriptional and functional matrix-related responses.

This work was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic (VEGA), project number 1/0146/24.

16. FROM DESIGN TO FUNCTION: A DUAL-STUDY ON ENGINEERED EXTRACELLULAR VESICLES FOR TARGETED ALZHEIMER'S DISEASE THERAPY

[1]Raja Natesan Sella
[1]Membrane-Protein Interaction Laboratory, Department of Genetic Engineering, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu-603203, India

Alzheimer's disease (AD) remains a major therapeutic challenge owing to amyloid-β (Aβ) aggregation, progressive neuronal dysfunction, and the limited ability of therapeutic molecules to efficiently reach neuronal targets. Here, we developed and functionally validated a surface-engineered extracellular vesicle (EV) platform for targeted delivery of the anti-amyloid peptide H102. The study was designed as an integrated computational-to-functional approach comprising rational peptide engineering, in silico validation, and evaluation of the resulting EV-based therapeutic system. A 22-residue chimeric peptide, H102-CP05, was designed by conjugating the β-sheet breaker peptide H102 with the CD63-targeting anchor CP05 to facilitate its incorporation onto the EV surface. In silico analyses demonstrated favourable physicochemical characteristics, non-allergenic potential, and low predicted immunogenicity under chronic exposure. HADDOCK docking identified stable CD63-H102-CP05 interactions involving salt bridges, hydrogen bonds, and non-bonded contacts, while 100 ns molecular dynamics simulations in aqueous and EV-mimetic lipid bilayer environments further confirmed the stability of the complex. Experimental evaluation demonstrated the safety of H102-CP05 in HEK-293 cells and zebrafish, while Thioflavin-T assays revealed a dose-dependent inhibition of Aβ fibrillation, with near-complete suppression at 100 µM. To translate this peptide design into a targeted delivery system, EVs derived from NIH/3T3 cells stably expressing Lamp2b-RVG were surface-modified with H102-CP05 through CP05-CD63 affinity binding. Successful peptide incorporation and preservation of EV integrity were confirmed by ATR-FTIR, SERS Raman spectroscopy, HR-TEM, nanoparticle tracking analysis, and Western blotting. In Aβ25-35-induced neuronal toxicity in NGF-differentiated PC-12 cells, surface-modified EVs (SME) demonstrated time-dependent cellular uptake and markedly improved neuronal outcomes, restoring cell viability to 80-90% of control levels while reducing LDH release and intracellular ROS by more than 50%. Furthermore, SME treatment modulated key AD-associated molecular markers by reducing APP, Bax, and Stat1 expression and increasing the neuroprotective deacetylase Sirt1. Collectively, these findings establish a rationally designed, computationally validated, and experimentally characterized EV platform that combines neuronal targeting with anti-amyloid and neuroprotective functions, highlighting H102-CP05-decorated EVs as a promising multifunctional nanocarrier for targeted AD therapy.

Keywords: Extracellular vesicles, Alzheimer's disease, H102-CP05, surface engineering, molecular dynamics, neuroprotection, targeted delivery

17. CHLORELLA SOROKINIANA-DERIVED EXTRACELLULAR VESICLES EXERT ANTIFIBROTIC EFFECTS IN PULMONARY FIBROSIS

[1,2]Domonkos Pap, [1,2]Beáta Szebeni, [1]Mária Bernáth, [1]Péter Bokrossy, [3,4]Csaba András Schwarcz, [5]Dorina Lenzinger, [5,6]Tamás Visnovitz, [7]Marcell Pálmai, [7]Judith Mihály, [7,8,9]Zoltán Varga, [10]Gergely Maróti, [1,2]Apor Veres-Székely, [1]Csenge Szász, [1]Jamila Raufi, [5,11]Edit Buzás, [1,2]Attila J. Szabó and [1,2]Ádám Vannay
[1]Pediatric Center, MTA Center of Excellence, Semmelweis University, Budapest, Hungary, [2]HUN-REN-SU Pediatrics and Nephrology Research Group, Budapest, Hungary, [3]Institute of Clinical Pathophysiology, Semmelweis University, 1094 Budapest, Hungary, [4]HUN-REN-SU Cerebrovascular and Neurocognitive Diseases Research Group, Budapest, Hungary, [5]Institute of Genetics, Cell - and Immunobiology, Semmelweis University, Budapest, Hungary, [6]Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Budapest, Hungary, [7]Biological Nanochemistry Research Group, Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary, [8]Department of Physical Chemistry and Materials Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Budapest, Hungary, [9]Department of Biophysics and Radiation Biology - HUN-REN Office for Supported Research Groups, Faculty of Medicine, Semmelweis University, Budapest, Hungary, [10]Institute of Plant Biology, HUN-REN Biological Research Centre, Szeged 6726, Hungary, [11]HUN- REN-SU Translational Extracellular Vesicle Research Group, Budapest, Hungary

Idiopathic pulmonary fibrosis (IPF) is a severe, progressive lung disease that leads to irreversible scarring of the lung tissue with a median survival of approximately 3 years after diagnosis. Chronic inflammation plays a key role in the pathomechanisms underlying IPF, contributing to excessive proliferation and extracellular matrix production by lung fibroblasts. Chlorella sorokiniana (Cs) is an edible microalgal species that has been reported to exert anti-inflammatory, antioxidant, and antiproliferative properties. Our aim was to investigate the therapeutic potential of Cs-derived extracellular vesicles (EV) in IPF.

Cs-derived EVs (Cs_EV) were purified from the Cs culture medium using tangential flow filtration (TFF), followed by size-exclusion chromatography (SEC) and characterized using complementary biophysical and molecular approaches. The effect of Cs_EV on activation of primary lung fibroblasts (P-FB) was investigated by MTT cell-proliferation, Sirius Red collagen accumulation and migration assays, respectively. The antifibrotic relevance of Cs_EV was further investigated in a bleomycin-induced mouse model of lung fibrosis.

The isolated Cs_EV exhibited a spherical morphology, with mean diameter approximately 140 nm. In vitro Cs_EV inhibited PDGF-B induced proliferation, TGF-*ß* induced collagen production, and EGF induced migration of P-FB. In vivo intratracheal and intravenosus Cs_EV treatment reduced the mRNA expression of bleomycin induced Col1a1 and Fibronectin.

Our findings demonstrate the antifibrotic effects of Cs_EV and provide insights into their potential as a therapeutic agent for the treatment of IPF.

Grants: K-142728, K-131594, K-145998, ADVANCED 150767, ADVANCED-150077, ADVANCED-152698, ADVANCED- 152112, TKP2021-EGA-24, TKP2021-EGA-31, STARTING 150629, 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, Hungarian Academy of Sciences, János Bolyai Research Scholarship.

18. SPIRULINA MAXIMA-DERIVED EXTRACELLULAR VESICLES ATTENUATE PERITONEAL FIBROSIS: POTENTIAL INVOLVEMENT OF PARK7 AND HSP70

[1,2]Beáta Szebeni, [1]Mária Bernáth, [1]Péter Bokrossy, [1,2]Domonkos Pap, [3]Zoltán Molnár, [4,5,6]Tamás Mészáros, [7]Dorina Lenzinger, [7,8]Tamás Visnovitz, [9]Marcell Pálmai, [9]Judith Mihály, [9,10,11]Zoltán Varga, [7]Nóra Fekete, [7]Éva Pállinger, [1,2]Apor Veres-Székely, [1]Csenge Szász, [7,12]Edit Buzás, [1,2]Attila J. Szabó and [1,2]Ádám Vannay
[1]Pediatric Center, MTA Center of Excellence, Semmelweis University, Budapest, Hungary, [2]HUN-REN-SU Pediatrics and Nephrology Research Group, Hungary, [3]Department of Plant Sciences, Albert Kázmér Faculty of Agricultural and Food Sciences in Mosonmagyaróvár, Széchenyi István University, Hungary, [4]Department of Translational Medicine, Nanomedicine Research and Education Center, Semmelweis University, Hungary, [5]SeroScience Ltd., Hungary, [6]Heart and Vascular Center, Faculty of Medicine, Semmelweis University, Hungary, [7]Institute of Genetics, Cell - and Immunobiology, Semmelweis University, Hungary, [8]Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Budapest, Hungary, [9]Biological Nanochemistry Research Group, Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Hungary, [10]Department of Physical Chemistry and Materials Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Hungary, [11]Department of Biophysics and Radiation Biology - HUN-REN Office for Supported Research Groups, Faculty of Medicine, Semmelweis University, Hungary, [12]HUN- REN-SU Translational Extracellular Vesicle Research Group, Hungary

Peritoneal fibrosis is a major pathological complication of long-term peritoneal dialysis and is characterized by mesothelial injury, fibroblast activation, excessive extracellular matrix deposition, and progressive loss of peritoneal membrane function. Extracellular vesicles derived from biologically active organisms have emerged as potential mediators of intercellular communication and may represent a novel therapeutic approach to fibrotic disease. Here, we investigated the antifibrotic properties of small extracellular vesicles derived from a blue-green microalga, *Spirulina maxima* (*Sm*-sEVs).

Sm-sEVs were isolated from conditioned culture medium by tangential flow filtration followed by size-exclusion chromatography and characterized using complementary biophysical and molecular approaches. Their uptake by primary human peritoneal mesothelial cells and peritoneal fibroblasts was assessed using fluorescent labeling. The effects of *Sm*-sEVs on TGF-β-induced mesothelial-to-mesenchymal transition and PDGF-B and TGF-β -driven fibroblast activation were evaluated in vitro by cellular and extracellular matrix-related assays. The antifibrotic activity of *Sm*-sEVs was subsequently examined in a chlorhexidine digluconate-induced mouse model of peritoneal fibrosis. Candidate bioactive proteins associated with the vesicles were also investigated.

Sm-sEVs displayed a vesicular morphology and a mean size of approximately 150 nm and were efficiently internalized by both mesothelial cells and fibroblasts. Treatment with *Sm*-sEVs attenuated TGF-β-induced mesenchymal transformation of mesothelial cells and suppressed fibroblast proliferation and collagen production stimulated by PDGF-B and TGF-β respectively. In mice with experimental peritoneal fibrosis, *Sm*-sEV administration reduced submesothelial thickening and diminished the accumulation of fibronectin and collagen type I, while preserving the mesothelial phenotype. Molecular characterization further identified PARK7 and HSP70 within *Sm*-sEVs, suggesting that these stress-response and cytoprotective proteins may contribute to the observed antifibrotic activity.

Our findings demonstrate that *Sm*-sEVs exert multifaceted antifibrotic effects by targeting key cellular processes involved in mesothelial dysfunction, fibroblast activation, and extracellular matrix deposition. The identification of PARK7 and HSP70 within *Sm*-sEV cargo provides a potential mechanistic

link between vesicle-mediated signaling and suppression of fibrotic responses. These findings support further investigation of *Sm*-sEVs as a naturally derived EV-based strategy for the prevention or treatment of peritoneal fibrosis.

This project was supported by K-142728, K-131594, ADVANCED 150767, ADVANCED-150077, ADVANCED-152698, ADVANCED-152112, TKP2021-EGA-24, TKP2021-EGA-23, TKP2021-EGA-31, STARTING 150629, 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, HAS János Bolyai Research Scholarship

19. EFFECTS OF DENTAL PULP MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES ON BONE CELLS

[1]Radmila Žižková, [1]Barbora Voltrová, [2]Kateřina Budková, [2]Olga Šebestová Janoušková, [1]Eva Filová
[1]Institute of Experimental Medicine of the CAS, Czech Republic, [2]Faculty of Science, Centre of Nanomaterials and Biotechnology, Jan Evangelista Purkyně University in Ústí nad Labem, Czech Republic

Extracellular vesicles (EVs) released by mesenchymal stem cells have emerged as key mediators of intercellular communication and represent a promising cell-free approach for bone regeneration. Dental pulp mesenchymal stem cells (dpMSCs) are a particularly attractive source of EVs due to their accessibility, proliferative capacity, and osteoregulatory potential. Previous studies suggest that dpMSCs-derived EVs can modulate osteogenesis, angiogenesis, and inflammation; however, the biological response may depend strongly on the applied EVs dose. The concentration-dependent effects of dpMSCs-derived EVs on bone and immune cells are an essential prerequisite for the development of EVs-functionalized biomaterials intended for bone tissue engineering.

In this study, dpMSCs from adult patients were isolated, and EVs produced by dpMSCs were collected using the tangential flow filtration method. Concentration and size of the yielded EVs was analyzed by nanoparticle tracking analysis. Subsequently, bone cells were cultured in the presence of different concentrations of dpMSCs-derived EVs. Cell behavior in response to added dpMSCs-derived EVs was analyzed by measuring metabolic activity, proliferation, and osteogenic markers. While metabolic activity increased with increasing EVs concentrations, all other markers remained comparable, regardless of the concentration of the added EVs.

The effect of the added dpMSCs-derived EVs on cells may have been masked by the fetal bovine serum added to the medium, which is known to contain EVs. Despite the serum being added at a lower concentration than is typically used. However, long-term serum-free cell culture was not possible; therefore, another way must be found to study the effects of dpMSCs-derived EVs on bone cells.

The project was supported by the Ministry of Health of the Czech Republic, project OPJAK CZ.02.01.01/00/22_008/0004562.
This work/participation was supported by the Czech Society for Extracellular Vesicles (CzeSEV) through a CzeSEV Travel Grant.

20. ENGINEERING ANTIGEN PRESENTING EXTRACELLULAR VESICLES FROM THP-1 CELLS FOR CELL FREE NANO-IMMUNOTHERAPY

[1]Shafaq Gulfam,_[1]Majeda Muluhie, [1]Loris Rizzello
[1]Department of Pharmaceutical Sciences, University of Milan, Italy

Dendritic cell (DCS) based immunotherapies elicit strong antigen-specific immune responses, but their clinical application is constrained by their complex production and unstable nature. Extracellular vesicles (EVs) offer a viable cell-free alternative that is more stable and simpler to store while retaining key immunological features of their parent cells. Using lentiviral transduction, we generated a THP-1 monocytic cell line stably expressing a single chain construct in which the immunodominant human cytomegalovirus (CMV) pp65 peptide is covalently linked to HLA class I and β2-microglobulin, together with the co-stimulatory molecule CD80 and CD86, to antigen-presenting EVs driving antigen specific T-cell activation.

Flow cytometry confirmed effective engineering: HLA-ABC expression was consistently high in both lines, and transduced THP-1 cells expressed CD80 and CD86 in over 99.9% of cells (MFI approximately 31,000 and 60,000, respectively), compared to 3.3% and 1.7% in wild-type (WT) cells ($p < 0.0001$). GM-CSF, IL-4, TNF-α, and ionomycin were used to differentiate transduced and WT THP-1 cells into immature and mature dendritic cells (iDCs, mDCs), and PMA was used to differentiate them into M0 macrophages. In both lines, maturation markers CD40, CD83, CCR7, and HLA-DR gradually increased throughout differentiation, indicating the acquisition of a mature antigen-presenting phenotype that was supported by morphological changes that were visible under a light microscope.

EVs were isolated by differential ultracentrifugation from WT and transduced THP-1 cells, their macrophage/DC progeny, and M0 polarization states were characterized by nanoparticle tracking analysis. In all cell stages, particles exhibited mean diameters of 110-195 nm, mode diameters of 76-159 nm, and concentrations ranging from 1.14×108 to 1.24×1011 particles/mL, which are consistent with small-EV populations.

These findings establish a standardized engineered THP-1 platform for the generation of antigen-presenting EVs. To determine the therapeutic potential of this cell-free immunotherapy platform, ongoing research will describe the EV protein corona and evaluate antigen-specific CD8+ T-cell activation.

21. CANCER-TARGETED EXTRACELLULAR VESICLES FUNCTIONALIZED WITH UNIVERSAL NANOBODY ADAPTERS

[1]Aida de la Cruz-Gambra, [1]Jiri Zahradnik, [2]Dominik Havlicek, [3]Ziv Porat, [2]Daniel Jirak, [1,2]Andrea Galisova
[1]BIOCEV, First Faculty of Medicine, Charles University, Czech Republic, [2]Department of Imaging Methods, Institute for Clinical and Experimental Medicine, Czech Republic, [3]Biotech a.s., Czech Republic, [4]Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel

Extracellular vesicles (EVs) are natural carriers of biomolecules and have emerged as promising therapeutic delivery systems. However, their limited tissue specificity can lead to off-target effects, reducing their therapeutic efficacy. To overcome this limitation, EVs can be genetically engineered to display specific targeting proteins on their surface, enabling more selective delivery to target tissues. However, conventional EV surface engineering strategies require generating a dedicated producer cell line for each targeting ligand, from which the engineered EVs are subsequently isolated, limiting scalability and multiplexed design. To address this limitation, we developed the Nanobody-Tag-Ligand (NaTaLi) platform, which exploits high-affinity engineered eALFA nanobodies displayed on the EV surface to facilitate the rapid and modular loading of ALFA-tagged functional proteins.

NaTaLi enables stable and high-affinity attachment of targeting proteins to the EV surface. To demonstrate its translational potential, NaTaLi EVs were functionalized with cancer-targeting proteins (RGD targeting integrins overexpressed on cnacer cells and LinTT1 targeting p32 protein overexpressed in tumor cells and vessels), which enhanced their uptake by breast cancer cell lines (4T1) both *in vitro* and *in vivo* in mice. Notably, targeting functionalization selectively enhanced EV accumulation in the tumor while maintaining comparable distribution across healthy organs.

The therapeutic potential of NaTaLi EVs was evaluated by loading them with the chemotherapeutic agent doxorubicin (DOX) using microporation combined with incubation. 4T1 cells treated with DOX-loaded NaTaLi EVs showed significantly reduced cell viability compared with cells treated with DOX-loaded control EVs. Indeed, DOX-loaded NaTaLi EVs functionalized LinTT1 peptide showed the highest cytotoxicity toward 4T1 cancer cells compared with control EVs, demonstrating that targeted EV functionalization can enhance the therapeutic efficacy of encapsulated cargo.

Overall, NaTaLi enables efficient and adaptable engineering of EVs for targeted cargo delivery, highlighting its potential as a versatile platform for the development of advanced EV-based therapeutics.

22. EFFECTS OF CULTURING ON CARDIOPROTECTIVE PROPERTIES OF EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STEM CELLS

[1]Rácz Richárd, [1]Hambalkó Szabolcs, [1]Wagner Nóra, [2]Jessica Whelan, [2]Junpeng Luo, [1]Dr. Giricz Zoltán
[1]Semmelweis University, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, Budapest, 1089, Nagyvárad square 4
[2]University College Dublin, Biomanufacturing Research Group, Belfield, Dublin 4, Ireland

Chemotherapeutic agents 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.

Mesenchymal stem cells are exclusively adherent; they can proliferate only once attached to a surface. For this reason, they can grow in 2D flask-based environments, 3D microcarrier-based spinner flasks, or bioreactors. 3D microcarrier systems allow for increased cell proliferation and improved EV release while still potentially maintaining the quality and functionality of the 2D systems.

We aim to validate and understand the therapeutic potential and differences between 2D and 3D 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 while also measuring and comparing the health of the MSCs, their proliferation, EV production and the quality of their EVs (TP, TL, NTA, WB).

In conclusion, MSC-derived EVs represent a promising therapeutic strategy for reducing the cardiotoxic side effects of chemotherapeutics. Utilising the advantages of the 3D microcarrier-based systems, we can improve the proliferation of our MSC cells, increase their EV production, while the EVs still maintain their quality and functionality. 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.

23. INTER-DONOR VARIABILITY IN MSC-DERIVED EVs: IMPLICATIONS FOR PERSONALIZED CELL-FREE THERAPIES

[1]Monika Rytel, [2]Sylwia Katarzyna Król, [1]Anna Sarnowska
[1]Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, PAS, Poland,
[2]Department of Neurooncology, Mossakowski Medical Research Institute, PAS, Poland

Mesenchymal stem/stromal cells (MSCs) possess immunomodulatory, regenerative, and neuroprotective properties, making them promising candidates for the treatment of various disorders. These unique properties of MSCs appear to be closely linked to the release of extracellular vesicles (EVs) — membrane-bound nanoparticles that serve as key mediators of intercellular communication by transferring bioactive cargo to recipient cells, thereby modulating downstream signalling pathways and contributing to many of the paracrine effects attributed to MSCs.

We hypothesized that MSCs derived from different sources secrete varying numbers of EVs and may therefore differ in their therapeutic potential. Thus, we compared two types of MSCs - WJ-MSCs and ASCs. Human umbilical cords (n=5) were obtained from full-term deliveries by caesarean section and MSCs were then mechanically isolated from Wharton's jelly (WJ-MSCs). Adipose tissue-derived MSCs (ASCs) were isolated enzymatically with collagenase from human adipose tissue (n=5). Next, both WJ-MSCs and ASCs were cultured until 3rd passage, at 5% O2, 5% CO2, 37°C, mimicking physiological normoxia. Extracellular vesicles were then isolated from cell culture medium by ultracentrifugation (300xg for 10 min. 4°C, 2 000xg for 30 min. 4°C, and 100 000xg for 1h 30 min. 4°C). The obtained EVs were suspended in PBS and their number and size was determined by NTA (Nanoparticle Tracking Analysis) on Nanosight NS300. EV morphology was analyzed by TEM (Transmission Electron Microscopy) and flow cytometric analysis was performed to evaluate the distribution of the specific EV markers - CD9, CD81, and CD63.

EVs isolated from both cell types represented a typical extracellular vesicle-like morphology according to TEM analyses. EV phenotyping demonstrated that CD81 was the predominant single-positive marker across EVs derived from both WJ-MSCs and ASCs, reaching approximately 21–35% depending on the cell source and marker combination. CD9-only EVs were less abundant, whereas CD63-only EVs generally represented a smaller fraction of the EV population. The proportion of double-positive EVs was consistently lower than that of the corresponding single-positive populations. Quantitative analyses revealed substantial donor-dependent variability in EV secretion by both WJ-MSCs and ASCs, which may influence the consistency and therapeutic efficacy of cell-free therapies and should therefore be considered in the development of personalized therapeutic strategies.

This research was funded by National Science Centre grant number 2022/47/O/NZ3/01739.

24. POTENTIAL OF EXTRACELLULAR VESICLES AS A SYSTEM FOR DELIVERING ANTI-CANCER DRUGS

[1]Mateusz Bąk, [1]Jakub Gliński, [1]Patrycja Wińska
[1]Chair of Drug and Cosmetics Biotechnology, Warsaw University of Technology, Poland

The rising incidence and mortality of cancer worldwide necessitate the development of novel and more effective therapeutic strategies. One innovative approach is to use extracellular vesicles (EVs) as drug delivery vehicles. EVs are lipid bilayer nanostructures characterized by low immunogenicity, high biocompatibility, the ability to accumulate in target tissues, and a natural capacity to transport bioactive cargo. These properties make them promising candidates for the targeted delivery of chemotherapeutic agents and functional RNA molecules. Depending on the cargo type, various EVs loading methods can be employed, including electroporation, sonication, and incubation.

In this study, we investigated the potential of EVs derived from PC3 (androgen-independent prostate cancer) and MCF-7 (hormone-dependent breast cancer) cell lines as carriers of chemotherapeutic agents for delivery to homologous recipient cells. A major challenge in EVs-based research is the lack of a standardized isolation protocol. A better understanding of differences arising from various isolation techniques is essential for interpreting EVs function, particularly in vivo. Moreover, optimizing EVs production and isolation is crucial for their large-scale therapeutic application. We evaluated the ability of MCF-7 and PC3 cells to produce EVs under culture conditions without EVs-depleted fetal bovine serum. To assess the impact of isolation methods on EVs function, we compared samples obtained by ultrafiltration, ultracentrifugation, precipitation, and lyophilization. EVs size distribution and particle concentration were analyzed using nanoparticle tracking analysis (NTA), while sample purity and protein contamination were evaluated by electrophoretic analysis. Subsequently, we investigated the effects of EVs isolated using different methods on homologous recipient cells and compared their uptake efficiency. EVs internalization was assessed using fluorescence microscopy and flow cytometry after labeling with the fluorescent dye VesiDye.

In addition, EVs derived from MCF-7 cells isolated by ultrafiltration and precipitation were subjected to proteomic characterization by liquid chromatography-mass spectrometry (LC-MS). EV-associated proteins, including tetraspanins such as CD9, CD63, and CD81, were identified, and their distribution across samples obtained by different isolation methods was compared.

Our findings confirm the potential of MCF-7- and PC3-derived extracellular vesicles as carriers of therapeutic agents. Furthermore, the isolation method was shown to significantly influence EVs purity, protein composition, and uptake efficiency by target cells. These results contribute to ongoing efforts to standardize EVs isolation procedures and support the future application of extracellular vesicles as targeted drug delivery systems in cancer therapy.

This work was funded by the National Science Centre, Poland under the OPUS call in the Weave programme, 2024/55/I/NZ5/00401.

EVS IN SPECIFIC DISEASE CONDITIONS, INCLUDING CANCER, IMMUNE- AND CARDIOVASCULAR DISEASES

25. DISEASE-SPECIFIC LIPID CARGO AND CD1 EXPRESSION PATTERN IN THE LUNGS IMPLY INVOLVEMENT OF BALF-DERIVED SMALL EXTRACELLULAR VESICLE-MEDIATED LIPID PRESENTATION IN LUNG PATHOLOGY

1Reza Abouali, [2]Michal Mlynarczyk, [1]Aleksandra Malgorzata Siedlar, [3]Alicja Janowicz, [4]Karolina Kita, [5]Graham Ogg, [2]Weronika Hewelt-Belka, [1]Danuta Gutowska-Owsiak
[1]Laboratory of Experimental and Translational Immunology, University of Gdansk and Medical University of Gdansk, Intercollegiate Faculty of Biotechnology, Gdansk, Poland, [2]Department of Analytical Chemistry, Faculty of Chemistry, Gdansk University of Technology, Gdansk, Poland, [3]Department of Pulmonology and Allergology, Medical University of Gdansk, Gdansk, Poland, [4]Department of Allergology, Medical University of Gdansk, Gdansk, Poland, [5]MRC Human Immunology Unit, Weatherall Institute for Molecular Medicine, University of Oxford, Oxford, UK

Background: Small extracellular vesicles (sEVs) mediate immune regulation in the lungs, also, by facilitating antigen presentation. While immune responses mediated by proteins isolated from bronchoalveolar lavage fluid (BALF) have been extensively studied, the lipid cargo of BALF-derived sEVs and their functional role in CD1-restricted T cell responses remain largely unexplored. This study aims to determine lipid composition of the BALF and lung sEVs and investigate their involvement in lipid presentation pathways.

Methods: Publicly available lung single-cell RNA-sequencing data were analyzed for CD1A–E expression across lung cell populations. Differences between allergic asthma and allergic controls were assessed using targeted donor-level pseudobulk analysis. For the experimental analysis, BALF samples were collected from patients with inflammatory lung diseases, including interstitial lung disease and asthma. sEVs were isolated by serial ultracentrifugation (30 min at 10,000 × g, followed by two 2h at 100,000 × g) and characterized using nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and Western blot. Lipidomic analysis was performed on eight samples using LC-QTOF-MS. Lipid abundance was further compared with a published CD1 capture dataset to assess predicted CD1-binding potential.

Results: Bioinformatic analysis of publicly available lung single-cell RNA-sequencing data confirmed the expression of CD1A, CD1B, CD1C, CD1D and CD1E transcripts in lung dendritic cells. Among these genes, CD1B, CD1D and CD1E showed significantly higher expression in allergic asthma than in allergic controls. Experimentally, we confirmed the presence of sEVs of the expected size, expression of CD9, CD63 and Flotillin-1, and absence of ApoA1 and Calnexin, confirming their purity in fractions obtained from BALF. Lipidomics confirmed enrichment of relevant lipid classes in BALF-derived sEVs. High levels of lysophosphatidylcholine (LPC 16:0 and LPC 18:0) and elevated sphingomyelin (SM) 42:2 were observed across all samples, while SM 40:1 was higher in sarcoidosis compared with the remaining conditions. Combined analysis of our BALF-sEV lipidomic results, CD1 capture profiles and lung transcriptomic data showed preferential capture of SM 42:2 by CD1d, together with an increased population of CD1d-expressing lung dendritic cells in allergic asthma compared with allergic controls.

Conclusions: Our findings demonstrate the presence of CD1A–E transcripts in lung dendritic cells. Lipidomic profiling characterized the lipid composition of BALF-derived sEVs across different inflammatory lung diseases. Integrating lipid abundance with published CD1 capture profiles revealed BALF-sEV lipids with CD1-binding potential. Their disease-specific profiles suggest differential activation of CD1 isoform-specific T-cell responses in the lung.

26. EXTRACELLULAR VESICLE HETEROGENEITY REFLECTS HISTOLOGICAL AND BIOLOGICAL DIVERSITY IN PLEURAL MESOTHELIOMA

[1]Nikolett Kitti Dobos, [1]Vanda Dévényi, [2]Krisztina Vukmann, [2]Tamás Visnovitz, [2]Dorina Lenzinger, [3]Natalie Baldes, [3]Servet Bölükbas, [3]Luca Hegedűs, [3]Balázs Hegedűs, [1,4]Tamás Garay
[1]Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary, [2]Semmelweis University, Faculty of Medicine, Institute of Genetics, Cell- and Immunobiology, Budapest, Hungary, [3]University Medicinea Essen - Ruhrlandklinik, University Duisburg-Essen, Department of Thoracic Surgery, Essen, Germany, [4]Semmelweis University, Division of Oncology, Department of Internal Medicine and Oncology, Budapest, Hungary

Pleural mesothelioma (PM) is an aggressive malignancy characterized by marked histological and molecular heterogeneity. Extracellular vesicles (EVs) are important mediators of intercellular communication and represent promising candidates for liquid biopsy-based biomarkers. We investigated whether EV characteristics reflect biological differences among PM cell lines and whether EV phenotypes are associated with underlying transcriptomic and migratory features.

Eight PM cell lines were characterized according to histological subtype, biomarker expression, migratory phenotype, and EV production. EVs isolated from conditioned cell culture media were characterized by particle concentration, protein content, CD63 expression, and Annexin V (AV) positivity. The CD63/AV ratio was used as a relative measure of the abundance of CD63-positive versus AV-positive vesicles. Associations between EV characteristics and cellular phenotypes were assessed using Wilcoxon rank-sum tests, while transcriptomic data were integrated using Spearman correlation analysis.

EV characteristics varied substantially across PM cell lines. The CD63/AV ratio differed significantly between epithelioid (n=5) and biphasic (n=3) cell lines ($p=0.0357$), indicating histotype-associated differences in EV surface marker profiles. EV particle output also differed between PD-L1-positive (n=2) and PD-L1-negative (n=6) cell lines, with a trend toward higher vesicle shedding in PD-L1-positive lines ($p=0.0714$). Importantly, donor-cell migratory phenotype was associated with EV production: cell displacement correlated positively with particle output per cell ($\rho=0.86$, $p=0.011$) and negatively with protein content per particle ($\rho=-0.81$, $p=0.022$). Thus, highly migratory PM cell lines tended to release more particles per cell, while individual particles contained less protein. Transcriptomic analysis further identified associations between the CD63/AV ratio and genes involved in cell adhesion, glycosylation, cytoskeletal organization, and tumor cell plasticity, including *FSCN1*, *ST3GAL1*, and *NCAM1*.

PM cell lines display distinct EV phenotypes associated with histological subtype and donor-cell migratory properties. The observed relationships between EV marker profiles, vesicle production, cellular migration, and transcriptional programs suggest that EV heterogeneity may reflect intrinsic biological differences among PM subtypes. The CD63/AV ratio and associated adhesion- and cytoskeleton-related transcriptional signatures warrant further investigation as potential biomarkers of PM tumor biology and aggressiveness.

27. FUNCTIONAL AND PROTEOMIC PROFILES OF CD3(+) PLASMA-DERIVED SMALL EXTRACELLULAR VESICLES DIFFERENTIATE CANCER PATIENTS FROM HEALTHY DONORS

[1]Piotr Widlak, [2]Jie Han, [2,3]Alicja Gluszko, [4]Justyna Mika, [5]Aneta Zebrowska, [2]Sujan Kumar Mondal, [5,6]Marta Gawin, [7]Yana G. Najjar, [4]Joanna Polańska, [5,6]Monika Pietrowska, [2,7]Theresa L. Whiteside
[1]2nd Department of Radiology, Medical University of Gdańsk, Gdańsk, Poland, [2]Department of Pathology, University of Pittsburgh School of Medicine,Pittsburgh, Pennsylvania, USA, [3]Department of Biochemistry, Medical University of Warsaw, Warsaw, Poland, [4]Department of Data Science and Engineering, Silesian University of Technology, Gliwice, Poland, [5]Center For Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice, Poland, [6]Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Magdalenka, Poland, [7]UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Plasma of cancer patients contains a heterogeneous mix of small extracellular vesicles (sEV) derived from tumor cells (tumor-derived extracellular vesicles, TEX) and various tissue-resident and circulating nonmalignant cells, including immune cells. Separation from plasma of CD3(+)sEV that are products of T lymphocytes allows for assessments of their molecular and functional characteristics in addition to enabling studies of the TEX-enriched CD3(−)sEV fraction. Emerging data suggest that T lymphocytes in melanoma (and other cancers) are reprogrammed by the tumor and acquire functional characteristics that differentiate them from T cells in healthy individuals. As T cells in the tumor-bearing hosts frequently have compromised immune competence, the examination of T cell-derived CD3(+)sEV, which mimic parent T cells, could inform about the phenotypic and functional status of patients' T cells.

In the presented study, we applied the immune capture strategy to separate CD3(+)sEV from the plasma of melanoma patients (MPs). Blood samples were obtained from 10 consenting healthy donors (HDs) and 10 melanoma patients treated at the UPMC Hillman Cancer Center. Vesicles were isolated from the precleared plasma by ultrafiltration followed by size exclusion chromatography.

The functional studies performed suggest that CD3(+)sEV obtained from MPs have acquired some functional attributes that are usually associated with MTEX, including pro-tumor effects such as promotion of tumor growth and suppression of T cells or fibroblast migration. The obtained results support our hypothesis that in MPs, T cells are reprogrammed by MTEX produced by the tumor and acquire the ability to produce and release CD3(+)sEV that differ functionally from CD3(+)sEV of HD's and recapitulate certain functional features of MTEX. Comparison of mass spectrometry-based proteomic profiles of CD3(+)sEV isolated from the plasma of MPs and HDs revealed differences, especially in the abundance of species involved in the function of the immune system, including increased levels of immunoregulatory proteins in CD3(+)sEV in MPs (e.g. CD36, CD47, and CD59). On the other hand, among proteins downregulated in MPs' CD3(+)sEV were heat shock proteins, which are general stimulators of immune response. Moreover, among downregulated processes associated with proteins discriminating MPs from HDs was the response to pathogens. Overall, the obtained data suggest that CD3(+)sEV may be potentially valuable for molecular, functional, and metabolic monitoring of T cells in patients with melanoma, and likely in patients with other cancers.

The study was supported in part by grants # R01-CA256068 from the National Institutes of Health, USA, and # ME210061 from the USA DOD to T.L.W. Support to M.P. was provided by the National Science Centre (Poland), OPUS 23 Grant Number: 2022/45/B/NZ5/03510. J.P. and J.M. received support from the Silesian University of Technology Grant No: 02/070/BK_25/0068.

28. TUMOR CELL DERIVED SIGNALS SUPPRESS STROMAL ENDOPLASMIC RETICULUM STRESS RESPONSES

[1]Ákos Kovács, [1]Benedek Papp, [1]Ferenc István Nagy, [1]Mohana Krishna Gopisetty, [1]Lili Marczinka, [1]Mónika Kiricsi
[1]Department of Biochemistry and Molecular Biology, University of Szeged, Hungary

Introduction: The tumor microenvironment (TME) poses significant challenges for the survival of the resident cells, endoplasmic reticulum stress (ERS) is unquestionably part of these difficulties. Tumor cells are genetically plastic but stromal cells are only able to adapt epigenetically, which according to our hypothesis is at least partially due to reprogramming by tumor cells and extracellular vesicles (EVs) may also play a role in this process.

Methods: In our study breast adenocarcinoma cell lines (MCF-7; MDA-MB231) and stromal cell lines (THP-1 differentiated into macrophages, MRC-5 fibroblast cells, MCF-10A2 immortalized breast epithelial cells) are utilized. For ER stress induction tunicamycin is used in different concentrations and treatment schedules (1-500ng/ml, 1-3 days of treatment). Epigenetic reprogramming is investigated using co-culture of tumor and stromal cells using separable membrane inserts or tumor cell derived EV treatment for which size exclusion chromatography isolated EVs are used. EV. ERS response of the cells is analyzed by qPCR or western blot targeting ERS marker RNAs or proteins.

Results: Tunicamycin induces ERS in stromal cells, in a concentration and time dependent manner. The ERS marker levels of tunicamycin treated stromal cells are decreased by tumor cell co-culturing and tumor cell derived EV treatment. This effect is most prominent in the macrophages and in tumor-stromal cell co-cultured setups.

Conclusions: Tunicamycin induced ERS of stromal cells is mitigated by cancer cells. This effect is observed both in tumor-stromal cell co-cultures and tumor cell derived EV treated stromal cells although being more prominent in co-cultures and macrophage cells. The results imply that tumor cells may contribute to the survival of stromal cells by attenuating their ERS response through paracrine signaling and EVs play a role in this process.

29. SORLA DYSFUNCTION LINKS ENDOSOMAL TRAFFICKING DEFECTS TO ALTERED EXTRACELLULAR VESICLE HOMEOSTASIS IN ALZHEIMER'S DISEASE

[1]Petra Riegerová, [2]Petr Fojtík, [1]Šárka Pokorná, [2]Dáša Bohačiaková, [1]Martin Hof
[1]Heyrovský Institute, Prague, Czech Republic, [2]Faculty of Medicine, Masaryk University, Brno, Czech Republic

Alzheimer's disease (AD) is characterized by progressive neurodegeneration associated with impaired protein sorting and clearance. SORLA, encoded by *SORL1*, is an endosomal sorting receptor that plays an important role in AD by regulating amyloid precursor protein (APP) trafficking and processing, as well as Aβ clearance. Moreover, loss of *SORL1* function disrupts neuronal endosomal trafficking and causes enlargement of early endosomes, one of the earliest phenotype observed in AD.

To investigate how SORLA dysfunction contributes to AD pathology, we analyzed human iPSC-derived neurons carrying the AD-associated *SORL1* p.D1105H variant, located in the first complement-type repeat (CR1) domain, and *SORL1*-knockout cells. Super-resolution fluorescence microscopy was used to assess the subcellular distribution of key components of the intracellular trafficking machinery, with particular focus on early endosomes and multivesicular bodies. Quantitative analysis revealed that SORLA dysfunction significantly altered the abundance and cargo loading of vesicular compartments involved in extracellular vesicle (EV) biogenesis and was associated with enhanced release of CD63-positive EVs. In addition, dysfunctional SORLA displayed altered subcellular localization, accompanied by impaired retrograde trafficking of APP. These changes were associated with altered size and biophysical properties of isolated EVs, as well as increased levels of Aβ and phosphorylated tau (pTau), both known to accumulate in EVs during AD progression.

In summary, our findings demonstrate that SORLA dysfunction disrupts early stages of the endolysosomal pathway, promotes abnormal EV biogenesis, and enhances amyloidogenic APP processing, ultimately contributing to the accumulation of pathogenic species and impaired proteostasis in neurons.

30. PROTEOMIC AND GLYCOMIC SIGNATURES OF TUMORIGENIC AND NON-TUMORIGENIC CELL LINE-DERIVED SMALL EXTRACELLULAR VESICLES

[1,2]Alexandra Molnár, [1]Mirjam Balbisi, [3]Tamás Langó, [1,2]Júlia Németh, [3]Gábor Tusnády, [4]Gitta Schlosser, [3,5] Beáta G. Vértessy, [1]Lilla Turiák
[1]HUN-REN Research Centre for Natural Sciences, MTA-HUN-REN TTK Lendület (Momentum) Glycan Biomarker Research Group, Budapest, Hungary, [2]Semmelweis University Doctoral College, Budapest, Hungary, [3]HUN-REN Research Centre for Natural Sciences, Institute of Molecular Life Sciences, Budapest, Hungary, [4]MTA-ELTE Lendület (Momentum) Ion Mobility Mass Spectrometry Research Group, Department of Analytical Chemistry, Institute of Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary, [5]Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, Faculty of Chemical Technology and Biotechnology, Budapest, Hungary

Lung cancer remains a major global health burden, highlighting the need for sensitive and minimally invasive biomarkers. Extracellular vesicles (EVs) represent promising biomarker candidates; however, their glycosylation and glycosaminoglycan (GAG) profiles remain poorly characterized. Terefore, our aim was to develop a methodology examining the proteomic, *N*-glycopeptide, and chondroitin sulfate (CS) glycosaminoglycan profiles of small EVs isolated from A549 lung adenocarcinoma and BEAS-2B non-tumorigenic cell lines using nanoflow ultrahighperformance liquid chromatography tandem mass spectrometry (nanoUPLCMS/MS). A549 cells were cultured in non-completed F12 medium, while BEAS-2B cells were cultured in BEGM medium for 72 hours. The supernatant was then harvested and centrifugation and filtration steps were performed followed by isolation of small EVs using mini-size exclusion chromatography on in-house prepared columns. EV size distribution was characterized by microfluidic resistive pulse sensing and shapes by transmission electron microscopy. Small EVs were lysed and digested with trypsin and chondroitinase ABC enzymes to produce peptides and chondroitin sulfate disaccharides, respectively. The peptides obtained were enriched for *N*-linked glycopeptides, 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).

Altogether 408 proteins were found to be differentially expressed between small EVs of the cell lines investigated, among them 5 CS proteoglycan core proteins. Versican and testican-1 were upregulated in A549, while aggrecan, syndecan-4 and CSPG4 were downregulated. In glycoproteomics, a total of 302 N-glycoforms were tested for statistical differences, and 176 were dysregulated in cancer, including *N*-glycoforms of EV marker proteins (e.g., galectin-3-binding protein and thrombospondin-1) and proteoglycan core proteins (e.g., versican). Chondroitin sulfate analysis successfully identified the most abundant disaccharides and significant changes were observed between 4S and 6S sulfation in case of EVs isolated from the tumorigenic and non-tumorigenic cell lines. Our results highlight the importance of exploring the glycosylation of EV proteins from cancerous and non-cancerous cell lines in understanding the mechanisms behind disease progression.

31. SURFACE PROTEOME PROFILING OF PANCREATIC CANCER CELL LINE-DERIVED EXTRACELLULAR VESICLES TO PRIORITIZE PLASMA BIOMARKER CANDIDATES FOR EARLY DETECTION

[1]George Mickhael Harinck, [1,2]Masood Kamali-Moghaddam
[1]Department of Immunology, Genetics and Pathology, Uppsala University, Sweden, [2]Science for Life Laboratory (SciLifeLab), Uppsala University, Sweden

Pancreatic ductal adenocarcinoma (PDAC) is usually diagnosed at an advanced stage, and no blood-based biomarker is currently validated for early detection or for distinguishing PDAC from other pancreatic conditions such as intraductal papillary mucinous neoplasm (IPMN). Extracellular vesicles (EVs) carry tumor-derived surface proteins that may serve as a source for such biomarkers. Within EU PANCAID consortium, we combined an EV surface proteome discovery step in pancreatic cancer cell lines with a plasma-based proximity barcoding assay (PBA) planned across clinical spectrum of pancreatic disease, aiming to build a prioritized list of candidate surface markers for early detection and differential diagnosis.

Intact EVs were isolated by differential ultracentrifugation from serum-free conditioned media of three PDAC cell lines (Capan1, Capan2, Panc1) and one normal pancreatic ductal epithelial line (HPDE), surface-labeled with sulfo-NHS-SS-biotin, captured on neutravidin resin, and the eluted proteins were analyzed by LC-MS/MS (two replicates per line). After cross-referencing with Cell Surface Protein Atlas with high-confidence annotations, we found 81 surface proteins common to all four lines and 95 candidate cancer-associated surface proteins, comprising 77 proteins detected in at least one PDAC line but absent from HDPE, and 18 significantly enriched in PDAC lines relative to HPDE (Welch's t-test or Wilcoxon rank-sum as appropriate, BH adjusted $p<0.05$). The candidates included established tumor-associated antigens such as mesothelin, CD133 and CEACAM1.

In parallel, 346 plasma samples collected through PANCAID consortium, spanning healthy controls, individuals at risk, IPMN (branch duct, benign, high-grade dysplasia, invasive carcinoma) and PDAC (stage T1 to T4), have been received for EV profiling by PBA on a 550-protein panel. Cross-referencing the 95 candidate surface proteins against this panel, we identified 34 shared targets, which would be of interest to see whether a similar trend could be observed or not.

This two-step strategy moves from cell line-derived EV surfaceome discovery to targeted single EV-profiling in plasma. It is intended to yield a prioritized candidate marker set for early PDAC detection and for distinguishing PDAC from IPMN and at-risk patients, to be taken forward for validation in this consortium cohort.

32. IMMUNOMODULATORY ACTIVITY OF STAPHYLOCOCCUS AUREUS-DERIVED EXTRACELLULAR VESICLES IN MACROPHAGE MODELS RELEVANT TO INFECTIVE ENDOCARDITIS

[1,2]Veronika Mlcekova, [2]Miriam Dutkova, [3,4]Hana Obrucova, [3,4]Tomas Freiberger , [1,2]Julia Orlovska, [1,2,5]Lukas Kubala, [1,2]Gabriela Ambrozova , [2,5]Kristyna Turkova
[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic, [2]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic, [3]Centre of Cardiovascular and Transplantation Surgery, Brno, Czech Republic, [4]Department of Clinical Immunology and Allergology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [5]International Clinical Research Center, St. Anne´s University Hospital, Brno, Czech Republic

Infective endocarditis (IE) is a life-threatening infection of the heart's inner lining. The infection is predominantly caused by bacteria from the genera *Streptococcus* and *Staphylococcus*, including *Staphylococcus aureus*, as one of the most common causative agents. Among other mechanisms involved in bacterial virulence and IE pathogenesis, extracellular vesicles produced by bacteria are emerging as important mediators of host-pathogen crosstalk, yet their contribution to inflammatory responses during IE remains incompletely understood. Therefore, we aimed to isolate and study the effects of bacterial extracellular vesicles from *S. aureus* (SaEVs) on various macrophage models.

SaEVs were isolated from a clinical *S. aureus* strain 3.496, previously characterized by whole-genome sequencing using Oxford Nanopore technology. Vesicle purification was achieved through sequential centrifugation, membrane filtration, ultracentrifugation, and size exclusion chromatography. Structural and biochemical characterization was performed by nanoparticle tracking analysis, cryo-electron microscopy, FTIR spectroscopy, Western blotting, and total protein quantification. The biological activity of purified SaEVs was evaluated in multiple macrophage models, including RAW264.7 cells, primary murine bone marrow-derived macrophages (BMDMs), and murine BMDM-derived cell line cultures. The characterization confirmed a successful isolation of SaEVs with a characteristic lipid bilayer structure and an average diameter of approximately 80 nanometers. Functional experiments showed immunomodulatory properties of the isolated vesicles. Specifically, the stimulation of RAW 246.7 macrophages and BMDMs significantly increased the production of proinflammatory mediators, namely TNF-α, IL-6, nitric oxide, and inducible nitric oxide synthase. Additionally, SaEVs may play a role in inflammasome activation in BMDMs, as evidenced by enhanced secretion of IL-1β and IL-18.

To conclude, the findings support a role of SaEVs in modulating macrophage-driven inflammatory signalling, highlighting their potential contribution to the virulence of the strain and immunopathology of IE. However, additional experiments are required to shed light on the precise mechanisms, including confirming inflammasome activation and identification of the specific inflammasome pathway involved.

33. MALIGNANT ASCITES-DERIVED EXTRACELLULAR VESICLES AS LIQUID BIOPSY BIOMARKERS IN OVARIAN CANCER

[1]Šárka Hrachovinová, [1]Stanislava Sladeček, [2]John Atanga, [3]Michal Felsinger, [4]Markéta Bednaříková, [5]Jitka Hausnerová, [6]Václav Pustka, [6]Zbyněk Zdráhal, [2]Irina Nazarenko, [3]Vít Weinberger, [1]Vendula Pospíchalová
[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic, [2] Institute for Infection Prevention and Hospital Epidemiology, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany, [3] Department of Obstetrics and Gynecology, University Hospital Brno and Medical Faculty, Masaryk University, Brno, Czech Republic, [4] Department of Internal Medicine - Hematology and Oncology, University Hospital Brno and Medical Faculty, Masaryk University, Brno, Czech Republic, [5]Department of Pathology, University Hospital Brno and Medical Faculty, Masaryk University, Brno, Czech Republic, [6]Proteomics Core Facility, Central European Institute of Technology, Masaryk University, Brno, Czech Republic

Background: High-grade serous carcinoma (HGSC) represents the deadliest gynecological malignancy worldwide. Standard first-line therapy relies on platinum-based chemotherapy; however, it is currently administered as a standard protocol to all patients regardless of their individual chemosensitivity. Malignant ascites, routinely drained during cytoreductive surgery, represents an underestimated and clinically accessible source of the tumor microenvironment, specifically extracellular vesicles (EVs). This study aims to identify ascites-derived EV biomarkers to stratify patients prior to the initiation of chemotherapy into three clinical categories based on time to disease recurrence: platinum-sensitive (SE), partially resistant (RP), and totally resistant (RT), thereby shifting clinical decision-making from retrospective assessment of treatment failure to upfront predictive stratification and personalized therapeutic intervention.

Methods: We established a clinical cohort of 56 ascites samples from HGSC patients. EVs were isolated by size-exclusion chromatography using qEV1 70 nm columns (Izon Science). Phenotypic characterization of EV surface markers (specifically EpCAM) at single-particle resolution is performed using nano-flow cytometry (NanoFCM), complemented by comprehensive global profiling via tandem mass spectrometry (MS/MS)-based proteomics.

Results: High-resolution NanoFCM analysis enabled single-particle quantification and surface marker profiling of ascites-derived EVs across patient groups. Combining this single-EV phenotyping with downstream proteomic datasets allows for the characterization of molecular signatures associated with varying degrees of platinum resistance, identifying candidate biomarker panels across the SE, RP, and RT cohorts.

Conclusion: Ascites-derived EVs represent a promising liquid biopsy tool for capturing tumor heterogeneity and predicting treatment response. Integrating single-particle NanoFCM analysis with proteomic profiling provides a robust framework for pre-treatment patient stratification, with the potential to guide personalized therapeutic strategies in HGSC.

Funding: This work was supported by Ministry of Health of the Czech Republic, grant nr. NW26-03-00442.

34. LIPOTOXIC STRESS INDUCES AN IMMUNOPROTEASOME-ASSOCIATED EXTRACELLULAR VESICLE SIGNATURE ACCOMPANIED BY CD8⁺ T-CELL AND MONOCYTE ALTERATIONS IN A MULTICELLULAR MASLD MODEL

[1]Zuzana Vince Kážmérová, [2]Jakub Janko, [3]Peter Baráth, [3,4]Veronika Lukáčová, [5]Radka Obořilová, [5]Jan Přibyl, [2]Roman Gardlík
[1]Biomedical Research Center, Slovak Academy of Sciences, Slovakia, [2]Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Slovakia, [3]Institute of Chemistry, Slovak Academy of Sciences, Slovakia, [4]MediXbank, n.o., Slovakia, [5]CEITEC Masaryk University, Czech Republic

Keywords: MASLD; extracellular vesicles; immunoproteasome; CD8⁺ T cells; monocytes

Metabolic dysfunction-associated steatotic liver disease (MASLD) involves hepatocyte steatosis, fibrosis, and chronic inflammation driven by liver–immune interactions. Extracellular vesicles (EVs) may contribute to this intercellular communication and reflect disease-associated molecular changes. We established a multicellular liver–immune model to investigate EV-associated protein signatures and immune-cell alterations induced by lipotoxic stress.

HepG2 hepatocytes, human hepatic stellate cells (HSCs), and THP-1-derived macrophage-like cells were co-cultured, with peripheral blood mononuclear cells (PBMCs) placed in transwell inserts to enable paracrine communication without direct cell contact. Lipotoxic stress (LTS) was induced using palmitic acid, high glucose, and insulin, and fibrogenic activation by TGF-β. Lipid accumulation and HSC activation were assessed by staining, immunofluorescence, and Western blotting. PBMC and THP-1 phenotypes were analyzed by flow cytometry. EVs were isolated from conditioned medium by size-exclusion chromatography (SEC) and analyzed by LC–MS/MS proteomics and gene ontology (GO) enrichment analysis.

LTS induced hepatocellular lipid accumulation, while TGF-β promoted HSC activation. PBMC phenotyping revealed LTS-associated changes, including an increased CD8⁺ T-cell fraction and a shift within the CD14/CD16 monocytic compartment toward a CD16-high/CD14-low-to-intermediate phenotype, whereas the CD4⁺ T-cell fraction remained largely unchanged. LC–MS/MS revealed significant LTS-associated changes in SEC-isolated EV fractions with a prominent proteasomal/immunoproteasomal signature. Identified proteins included all seven 20S proteasome α-subunits, multiple β-subunits, all three inducible catalytic immunoproteasome subunits PSMB8/LMP7, PSMB9/LMP2, and PSMB10/MECL-1, and the PA28αβ activator subunits PSME1 and PSME2. GO analysis revealed enrichment of proteasome core/accessory complexes, proteasome activation and protein catabolism, together with type I/II interferon responses. Immune-related processes included CD8⁺ T-cell homeostasis, Th1/Th17 differentiation, Treg differentiation regulation, and IL-2 production.

LTS induces a coordinated immunoproteasome–PA28 signature in the EV-associated proteome of a multicellular MASLD model, accompanied by alterations in CD8⁺ T-cell and CD14/CD16 monocytic populations. The associated enrichment of interferon and adaptive immune pathways suggests a potential link between lipotoxic hepatic stress, EV-associated proteostasis, and immune dysregulation in MASLD.

Funding/Acknowledgments:
This work was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project No. 09I03-03-V04-00463, A4L_Bridge grant agreement No. 101136453, and Support of the projects Seal of Excellence No. 101278822.

35. APOPTOTIC NEUTROPHIL EVS ATTENUATE EXPERIMENTAL ARTHRITIS

[1]Mátka Nagy, [1]Éva Kovács-Kemecsei, [2]Dorina Lenzinger, [1]Zoltán Jakus, [1]Erzsébet Ligeti, [1,3]Ákos M. Lőrincz
[1]Department of Physiology, Semmelweis University, Budapest, Hungary, [2]Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, [3]Second Department of Internal Medicine, Szent György Hospital, Székesfehérvár, Hungary

Neutrophilic granulocytes (PMNs) release distinct populations of extracellular vesicles (EVs) according to their functional state and microenvironment. In our *in vitro* studies, EVs released spontaneously (spEVs) or during apoptosis (apoEVs) exhibited anti-inflammatory properties, whereas EVs generated upon activation with opsonized zymosan (ozEVs) exerted pro-inflammatory effects on neutrophils and monocytes. Rheumatoid arthritis is a chronic autoimmune disease characterized by persistent joint inflammation, in which myeloid cells contribute to its initiation, amplification and progression.

We investigated whether functionally distinct PMN-EV populations retain their differential immunomodulatory effects *in vivo* in a serum-transfer arthritis model.

Wild-type mice received either arthritogenic K/BxN serum or non-arthritogenic control BxN serum on day 0. Three well-characterized EV populations were generated from bone marrow-derived neutrophils. EVs were isolated by two-step centrifugation and gravity filtration and injected intra-articularly into the right ankle on day 1. Arthritis severity was monitored for eight days by clinical scoring, caliper-based measurement of ankle thickness and plethysmometric assessment of ankle swelling. Hind limbs were subsequently processed for paraffin-based histology analysis. Ongoing analyses reveal local immune cell accumulation by flow cytometry and inflammatory cytokine profiles by ELISA.

All three PMN-EV populations showed comparable size distributions by NTA, with particle diameters predominantly ranging from 100 to 400 nm. However, apoEVs exhibited approximately sixfold higher particle counts by flow cytometry and markedly higher particle concentrations by NTA than spEVs and ozEVs. ApoEV preparations also contained higher total protein and lipid levels. Following intra-articular administration, EVs caused no overt signs of inflammation in BxN serum-treated control mice, although ozEV treatment resulted in a minor increase in ankle thickness. K/BxN serum induced pronounced arthritis, which was not further exacerbated by pro-inflammatory ozEVs. In contrast, spEVs and apoEVs significantly reduced ankle thickening and swelling by approximately 50%. The anti-inflammatory effect was already detectable one day after EV administration, with apoEVs markedly reducing ankle thickening compared with PBS-treated arthritic mice. ApoEVs produced a stronger early response than spEVs and remained the most effective EV population throughout the observation period.

The functional differences previously observed among PMN-EV populations *in vitro* were demonstrated *in vivo*, with apoEVs displaying the most prominent anti-inflammatory activity. These findings demonstrate a rapid and sustained modulation of joint inflammation by neutrophil-derived apoEVs, supporting their further investigation in inflammatory arthritis.

FUNDING: EFOP-3.6.3-VEKOP-16-2017-00009, NKFIH FK 137770, TKP2021-EGA-24

36. DECODING TUMOR MICROENVIRONMENT CROSSTALK VIA PHOSPHATIDYLSERINE-POSITIVE EXTRACELLULAR VESICLES

[1]Klára Hánělová, [1]Jan Balvan, [1,2]Jiří Navrátil, [2]Martina Raudenská, [2]Monika Kratochvílová, [2,3]Tomáš Vičar, [1,4,5]Michal Masařík
[1]Department of Pathophysiology, Faculty of Medicine, Masaryk University Brno, Czech Republic, [2]Department of Physiology, Faculty of Medicine, Masaryk University Brno, Czech Republic, [3]Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, VUT Brno, Czech Republic, [4]International Clinical Research Centre (ICRC), St. Anne's University Hospital Brno, Czech Republic, [5]BIOCEV, First Faculty of Medicine, Charles University Vestec, Czech Republic

Cancer-associated fibroblasts (CAFs) have emerged as important regulators of cancer progression and the tumor microenvironment. Beyond their ability to persist and expand within tumors, CAFs often exhibit remarkable resistance to conventional therapies, highlighting their potential as therapeutic targets. One key mechanism through which CAFs influence tumor behavior is the release of extracellular vesicles (EVs). By transferring diverse molecular cargo between cells, EVs can shape the tumor microenvironment and influence cancer progression. Therefore, their molecular content holds considerable promise as a source of diagnostic and prognostic biomarkers. Phosphatidylserine (PS), traditionally regarded as a hallmark of apoptotic cells, can also be exposed on the surface of EVs, where it may influence their interactions with recipient cells and shape their biological effects. Despite this distinctive surface feature, the molecular characteristics and functional relevance of phosphatidylserine-positive EVs (PS-EVs) remain largely unexplored.

In our work, we focused on the potential role of PS-EVs released by CAFs in modulating cancer cell behaviour in head and neck squamous cell carcinoma (HNSCC). Proteomic analysis revealed several components associated with the YAP signalling pathway within these EVs, suggesting a potential link between CAF-derived PS-EVs and YAP-mediated signalling in HNSCC cells. YAP acts as a central regulator of cell fate and controls diverse cellular processes, including proliferation, survival, migration, stemness, and epithelial-mesenchymal plasticity. The presence of YAP-associated proteins within CAF-derived PS-EVs suggests that PS-EVs may contribute to the modulation of YAP-related signalling in recipient tumor cells, as indicated by elevated nuclear localisation of YAP in such cancer cells after PS-EV treatment. These changes in YAP signalling may, in turn, contribute to alterations in cancer cell behaviour. Together, this work aims to provide insight into the potential role of PS-EVs as mediators of CAF-cancer cell communication and highlights YAP-associated signalling as a potential mechanism underlying their effects.

37. Targeted proteome analysis of extracellular vesicles from gastric cancer cell lines

[1]Tanay Kumar Sinha, [1]George Mickhael Harinck, [1]Ehsan Manouchehri Doulabi, [1]Radiosa Gallini, [1]Liza Löf, [3]Sara K. Lindén, [2]Anders Larsson, [1]Qiujin Shen, [1]Masood Kamali-Moghaddam
[1]Department of Immunology, Genetics & Pathology, Science for Life Laboratory, Uppsala University, Sweden, [2]Department of Medical Sciences, Clinical Chemistry, Uppsala University, Sweden, [3]Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Sweden

Small extracellular vesicles (sEVs) carry proteins that may inform gastric cancer (GC) research, but profiling limited vesicle material remains challenging. We developed a proximity extension assay (PEA) workflow to characterise GC sEV protein profiles and prioritise candidates for further investigation.

sEVs from Kato III, AGS, MKN45 and MKN7 cells were isolated by differential centrifugation, filtration and ultracentrifugation. Transmission electron microscopy, nanoparticle tracking analysis and western blotting characterised the preparations. Five Olink PEA panels covered 427 distinct proteins across 460 assays, using 1 µL per sample per panel, with one replicate per condition. Profiles were compared with parental cell lysates and seminal-fluid EVs (prostasomes) as an EV-rich reference. Background-corrected data were analysed by principal component analysis and STRING pathway enrichment. EphA2 and cMet were evaluated using solid-phase proximity ligation assay (SP-PLA), extracellular vesicle proximity ligation assay (EV-PLA) and western blotting.

EV preparations contained CD9, CD63 and CD81, with no detectable calnexin. Principal component analysis separated sEVs from parental cells and distinguished Kato III sEVs from the other GC sEV profiles. After removing duplicate targets, 71 proteins showed medium or high relative abundance in at least one GC sEV sample but low or undetectable levels in prostasomes. These descriptive candidates included EphA2, cMet, IL-6, SCF, AXIN1, NOTCH1 and CAV1. STRING analysis identified enrichment of cancer-related, cytokine–cytokine receptor and PI3K–Akt pathways. SP-PLA and EV-PLA detected EphA2 and cMet on AGS and MKN45 sEVs. Western blotting supported EphA2 detection in both preparations and cMet in MKN45 sEVs; cMet was not detected by western blotting in AGS sEVs.

Combining multiplex PEA with orthogonal proximity assays enables targeted profiling and follow-up detection of GC sEV-associated proteins. The 71 candidates provide a basis for further EV characterisation and biomarker investigation. The cell-line design, single-replicate profiling, preselected panels and non-gastric EV reference limit biological and clinical inference. Validation in patient biofluids with appropriate healthy and benign-disease controls is required to establish diagnostic relevance.

EXTRACELLULAR VESICLES ORIGINATING FROM NON-MAMMALIAN AND NON-ANIMAL SOURCES

38. STAPHYLOCOCCUS AUREUS EXTRACELLULAR VESICLES INFLUENCE GROWTH DYNAMICS OF INFECTIVE ENDOCARDITIS-ASSOCIATED PATHOGENS

[1,2]Magdaléna Bočánová, [1,2]Veronika Mlčeková, [1,2]Julia Orlovská, [3,4]Hana Obručová, [3,4]Tomáš Freiberger, [1,2,3]Lukáš Kubala, [1,2]Gabriela Ambrožová, [1,5]Kristýna Turková
[1]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czechia, [2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czechia, [3]Department of Clinical Immunology and Allergology, Faculty of Medicine, Masaryk University Brno, Czechia, [4]Centre of Cardiovascular and Transplantation Surgery, Brno, Czechia, [5]International Clinical Research Center, St. Anne´s University Hospital, Brno, Czechia

Infective endocarditis (IE) is a life-threatening infection affecting the endocardial surface characterized by the formation of bacterial biofilms within platelet–fibrin vegetations. Roughly 80–90% of IE cases are caused by Gram-positive bacteria, predominantly *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, and streptococci. Bacterial extracellular vesicles (bEVs) have emerged as important mediators of bacterial virulence and host–pathogen interactions; however, their role in interbacterial interactions during polymicrobial IE remains largely unexplored. This study aims to characterize bEVs released by *S. aureus* (SaEVs) isolated from a clinical IE strain and to investigate their potential role in interactions with other clinically relevant IE-associated bacteria.

SaEVs were isolated from the clinical *Staphylococcus aureus* strain 3.496, characterized by whole-genome sequencing using Oxford Nanopore Technologies. SaEVs were purified by sequential centrifugation, filtration, ultracentrifugation, and size-exclusion chromatography, and comprehensively characterized using nanoparticle tracking analysis, cryo-electron microscopy, Raman spectroscopy, Western blotting, and protein quantification. Purified SaEVs exhibited a characteristic lipid bilayer morphology with a mean diameter of approximately 80 nm, confirming the successful isolation of intact vesicles. As an initial step towards understanding their biological role, the effects of SaEVs on the growth of clinically relevant IE-associated bacteria were evaluated, while analyses of biofilm formation and virulence-associated gene expression are currently ongoing. Preliminary results demonstrate that exposure to purified SaEVs alters the growth dynamics of all tested indicator strains, including *S. aureus* 3.496, *E. faecalis* CCM7000, and *E. faecium* CCM7167, compared with untreated controls. These findings suggest that SaEVs influence bacterial physiology and may contribute to intra- and interspecies interactions among Gram-positive bacteria. Ongoing proteomic and functional analyses aim to identify vesicular cargo components involved in bacterial cross-talk and to further elucidate the contribution of SaEVs to polymicrobial interactions during infective endocarditis.

39. ZEBRAFISH-DERIVED EXTRACELLULAR VESICLES: ESTABLISHING A NOVEL IN VIVO MODEL FOR EXPOSURE-RESPONSIVE EVS

[1]Csilla Pelyhe, [1]Nina Mokrackova, [1]Ondrej Adamovsky
[1]Mechanistic Environmental Toxicology, RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic

Zebrafish (Danio rerio) is widely used as an in vivo vertebrate model in developmental biology, toxicology and environmental toxicology, yet extracellular vesicles (EVs) remain poorly characterized in this organism. Establishing zebrafish as a source of EVs could provide a valuable whole-organism model for investigating EV-mediated responses during development and environmental-born chemical exposure.

To establish a robust isolation workflow, zebrafish at 5 days post fertilization (5 dpf) were enzymatically digested, followed by low-speed centrifugation and filtration. EV-enriched preparations were obtained either as a 10,000 × g centrifugation or by size-exclusion chromatography (SEC) using qEVoriginal Gen2 35 nm and 70 nm columns. The EV-enriched preparations were characterized by nanoparticle tracking analysis (NTA), protein quantification and cryo-electron microscopy to assess particle abundance, protein content, size distribution and morphology. SEC fractionation further enables identification of particle-rich fractions separated from the major protein-containing fractions, while the 10,000 × g preparation yields a complementary EV-enriched population with comparable particle numbers but substantially lower purity.

The comparison of isolation approaches identified qEVoriginal Gen2 70 nm columns as the most suitable method for downstream zebrafish EV studies, providing a favorable balance between particle recovery and sample purity. This optimized workflow will support subsequent EV cargo molecular characterization, including small-RNA profiling, and its application to exposure studies.

This study was funded by the European Union under the Marie Skłodowska-Curie Actions (Project No. 101153260). This work was also supported by the EU Horizon 2020 programme, grant No. 857560 (CETOCOEN Excellence). This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014. RECETOX RI (No. LM2023069).

40. LRV2-ASSOCIATED REMODELING OF EXTRACELLULAR VESICLE CARGO IN LEISHMANIA MAJOR

[1,2]Liudmyla Hrebenyk, [1]Marta Férová, [1]Vyacheslav Yurchenko
[1]Laboratory of trypanosomatid biology, Life Science Research Centre, University of Ostrava Czech Republic, [2] Academic and Research Medical Institute, Sumy State University, Ukraine

Leishmania RNA virus 2 (LRV2), an endosymbiotic RNA virus found in some Leishmania major strains, is associated with altered parasite virulence and host inflammatory responses. Leishmania-derived extracellular vesicles (EVs) carry virulence-associated molecules involved in host-parasite communication, but the association of LRV2 with EV production and cargo composition remains insufficiently understood. We investigated the temporal dynamics of EV-associated lipophosphoglycan (LPG), an important Leishmania surface glycoconjugate involved in parasite-host interactions, in LRV2-positive and LRV2-negative L. major strains.

LRV2-negative Th24 and LRV2-positive Tg44 strains were analysed on days 4 and 7 of cultivation. EVs were obtained from fixed numbers of parasites by polyethylene glycol precipitation. EV concentration was assessed by nanoparticle tracking analysis (NTA), while size distribution and surface charge were characterized by dynamic light scattering (DLS) and zeta-potential measurements. LPG in EV preparations and corresponding cellular lysates was quantified by semi-quantitative Dot Blot analysis. Digital densitometry was performed using Image Lab software (Bio-Rad). EV-associated LPG was evaluated using two complementary normalization ratios: EV-LPG/lysate-LPG (EV/LL) and EV-LPG/lysate-α-tubulin (EV/LT). EV/LL reflects the relative proportion of cellular LPG associated with EVs, whereas EV/LT relates EV-associated LPG to cellular material.

EVs from both strains showed a relatively stable size range of 150–170 nm, while zeta potential remained within a comparable range between strains and time points. In LRV2-positive Tg44, the EV/LL ratio increased markedly on day 7, accompanied by a more moderate increase in EV/LT. NTA independently confirmed increased EV concentration during stationary-phase growth. The greater change in EV/LL than in EV/LT suggests that the increase in EV-associated LPG was not attributable solely to increased EV abundance and is consistent with enhanced LPG enrichment in EVs. In contrast, both normalized LPG ratios decreased in LRV2-negative Th24 on day 7, accompanied by reduced EV-associated output.

These results reveal strain- and growth-phase-dependent changes in EV-associated LPG in L. major. The combined NTA and dual-ratio analysis indicates that LRV2-positive Tg44 undergoes both increased EV output and altered relative incorporation of LPG into EVs during stationary-phase growth, whereas LRV2-negative Th24 shows the opposite temporal pattern. Together with previous evidence linking LRV2 with virulence-associated phenotypes, these findings support the hypothesis that LRV2-associated remodeling of EV cargo may contribute to parasite adaptation and pathogenicity.

This work was co-funded by the European Union under the LERCO project (No. CZ.10.03.01/00/22_003/0000003) and the MSCA4Ukraine scheme.

41. PREVENTION AND INNOVATIVE THERAPEUTIC TREATMENT OF INTESTINAL BOWEL DISEASES: POTENTIAL USE OF BACTERIAL EXTRACELLULAR VESICLES.

[1]Monika Kvaková, [1]Jana Štofilová, [1,2]Natália Kertésová, [1]Veronika Benetinová, [1]Anna Kamlárová
[1]Center of Clinical and Preclinical Research MEDIPARK, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia
[2]Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University, Košice, Slovakia

In this study we optimized the isolation and characterization of bacterial extracellular vesicles (BEVs) from novel probiotic strains of Limosilactobacillus fermentum (LF-AK1, LF-AQ3), as well as evaluated the potential use of BEVs compared to their parental cells in the prevention and treatment of inflammatory bowel diseases (IBD) by comparing their protective effect against inflammation-induced damage to the complex intestinal barrier in vitro.

BEVs are non-living and non-replicating biological entities that can be purified, quantified, and administered at defined particle concentrations, potentially facilitating product standardization and quality control based on vesicle size, cargo composition, and biological activity. BEVs selectively encapsulate a wide range of bioactive molecules, including proteins, metabolites, DNA, and regulatory RNAs. The composition of this cargo depends on the bacterial strain and its physiological state, thereby determining the specific biological effects exerted on the host. Because BEVs do not contain live microorganisms, they do not pose a risk of microbial infection and may therefore represent a safer therapeutic strategy, particularly for immunocompromised patients. More broadly, postbiotics, including BEVs, contribute to the maintenance of intestinal homeostasis, preservation of intestinal barrier integrity, and establishment of stable microbial communities within the gut microbiota.

LF-AK1- and LF-AQ3-derived BEVs exhibited concentration-dependent inhibitory activity against the common human pathogen Escherichia coli. Preliminary results indicate that increasing BEVs concentrations progressively reduced the viability of E. coli. Monitoring the cytotoxic effects of the tested BEVs on colon cancer cell lines using the xCELLigence system demonstrated that BEVs exerted a more pronounced anti-proliferative effect compared with their parental bacteria. In addition to the concentration-dependent effect, a strain-dependent response was also observed. The protective effect of the isolated BEVs on intestinal barrier integrity was further demonstrated in vitro, using a complex three-layer intestinal barrier model. A significant increase in transepithelial electrical resistance (TEER) following BEVs treatment suggests that, at the tested concentrations, BEVs contribute to the maintenance of homeostasis between epithelial and immune cells without inducing pro-inflammatory activation of macrophages.

In conclusion these bioactive compounds have emerged as promising therapeutic candidates for the prevention and management of IBD.

This work was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic and Academy of Sciences VEGA no. 1/0253/25 and no. 1/0232/25.

42. EXTRACELLULAR VESICLES IN CANCER THERAPY: ANTITUMOR POTENTIAL OF CYANOBACTERIA-DERIVED VESICLES

[1]Boglárka Schilling-Tóth, [2]Gábor Andócs, [1]András Újvári, [1]Dávid Sándor Kiss, [1]István Tóth, [1]Krisztián Németh[1]Gergely Jócsák
[1]University of Veterinary Medicine Budapest, Department of Physiology and Biochemistry, H-1078 Budapest, István u. 2, Hungary, [2]University of Veterinary Medicine Budapest, Department of Microbiology and Infectious Diseases, H-1143 Budapest, Hungária krt. 23-25. Hungary

Background: Extracellular vesicles (EVs) serve as crucial mediators of intercellular communication and play a significant role in the regulation of tumor–immune interactions. Bacteria-derived EVs contain biologically active components that can stimulate innate immune responses, whereas EVs from healthy mammalian tissues primarily facilitate cellular communication and contribute to tissue homeostasis. This investigation aimed to compare the effects of cyanobacteria-derived extracellular vesicles (BEVs), used as an adjuvant in cancer therapy, with EVs isolated from healthy mouse tissues (MEVs) on various tumor cell lines, as well as to assess the cytotoxicity and biological effects of BEVs.

Materials and methods: BEVs were isolated through size exclusion chromatography utilizing conditioned media from Spirulina Sp., while MEVs were derived from primary mouse fibroblasts using the same chromatography method. Cell viability was evaluated in vitro using a Resazurin-based assay with syngeneic tumorous and normal fibroblast cell lines. The in vivo efficacy of these treatments was examined in adult BalB/c mice as an additional adjuvant treatment. After 2 weeks, the mice were executed, the tumors isolated, and the blood collected. From the blood PBMC-s were isolated for flow cytometric analysis.

Results: In vitro analyses assessed the effects of BEVs and MEVs on C26, 4T07, 4T1, and Renca tumor cell lines using a resazurin-based viability assay. BEV treatment resulted in approximately 20% reduction in cell viability within C26 and non-metastatic 4T07 cells, whereas no significant effects were observed in 4T1 or Renca cell populations. MEVs did not exhibit cytotoxic effects across any of the tumor cell lines examined. The initial phase of in vivo experiments employed a syngeneic tumor model established with 4T1 cells. Although BEV treatment did not significantly reduce tumor size, it induced structural modifications in the tumor tissue architecture. Flow cytometry analysis revealed no substantial differences in the distribution of immune cell populations among the experimental groups.

Conclusions: The findings demonstrate that responses to BEV treatment depend on the specific tumor cell line. The absence of notable suppression of tumor growth in the 4T1 model aligns with the low in vitro sensitivity observed in this cell line. These results underscore the importance of careful tumor model selection and provide a foundation for future investigations with alternative tumor models that exhibit greater responsiveness to BEV-based therapies in vitro.

Acknowledgment: The project is supported by the University of Veterinary Medicine Budapest Strategic Fund, grant RRF-003.

NEW TECHNOLOGIES RELATED TO EXTRACELLULAR VESICLES, INCLUDING ISOLATION, CHARACTERISATION, ENGINEERING, AND SYNTHETIC EV-MIMETICS

43. COMPARISON OF CITRATE- AND EDTA-ANTICOAGULATED PLASMA FOR EXTRACELLULAR VESICLE ISOLATION USING DENSITY GRADIENT ULTRACENTRIFUGATION FOLLOWED BY SIZE EXCLUSION CROMATOGRAPHY

[1,2]Yavuz Artuner, [1,2]Dóra Kapui, [1,2]Orsolya Szalai, [1,2]Eszter Cserni, [1,2,3]Péter Ferdinandy, [1,2,3]Zoltán Giricz, [1,2]Csenger Kovácsházi
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Budapest, Hungary, [3]Pharmahungary Group, Szeged, Hungary

Pre-analytical factors, including the anticoagulant used for blood collection, may influence extracellular vesicle (EV) measurements and complicate comparisons between clinical cohorts. To investigate the effect of anticoagulant used on general EV sample characteristics, we performed a preliminary comparison of citrate- and ethylenediaminetetraacetic acid (EDTA)-anticoagulated plasma samples processed with the same EV enrichment workflow in patients with cardiovascular diseases.

Citrated plasma samples were obtained from patients with acute myocardial infarction, whereas EDTA plasma samples were obtained from patients with advanced heart failure. EVs were enriched from both sample types using iodixanol density-gradient ultracentrifugation (DGUC) followed by size-exclusion chromatography (SEC) with Sepharose 4 Fast Flow columns. Samples were characterized by nanoparticle tracking analysis (NTA), total protein measurement, and Western blotting.

Median particle size for Citrated samples was 156.6 ± 22.0 nm (mean ± SD), whereas it was 144.0 ± 20.2 nm for EDTA samples. Particle concentrations were $1.80 \pm 1.90 \times 10^{10}$ particles/mL in the citrate (AMI) group (n=17) and $3.43 \pm 2.15 \times 10^{10}$ particles/mL in the EDTA group (n=3), with substantial inter-individual variability. Western blot profiles were similar between citrated and EDTA samples with the presence of EV-associated proteins in both sample types, while lipoprotein-associated proteins, primarily Apolipoprotein A, were also detectable, indicating potential residual co-isolation. Total protein measurements did not identify differences between the sample types.

Overall, the results indicate that citrate- and EDTA-anticoagulated plasma can yield EV-enriched preparations with similar concentration and purity characteristics when processed using the same DGUC-SEC workflow.

44. DIRECT COMPARISON OF DENSITY GRADIENT ULTRACENTRIFUGATION-BASED EXTRACELLULAR VESICLE ENRICHMENT FROM RAT, MOUSE AND HUMAN PLASMA

[1,2]Eszter Cserni, [1,2]Dóra Kapui, [1,2]Yavuz Artuner, [1,2]Orsolya Szalai, [1,2]Sophie Lebedovszkij, [1,2,3]Péter Ferdinandy, [1,2,3]Zoltán Giricz, [1,2]Csenger Kovácsházi
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary; [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Budapest, Hungary; [3]Pharmahungary Group, Szeged, Hungary

Extracellular vesicle (EV) enrichment from blood is essential to reduce contamination from plasma proteins, lipoproteins and other non-EV components. The combination of density gradient ultracentrifugation (DGUC) and size-exclusion chromatography (SEC) is an efficient approach for obtaining relatively pure EV isolates. However, the efficiency and comparability of EV isolation methods may differ between species, which is particularly important for the translational application of EV research. Therefore, the aim of this study was to compare different DGUC protocols for mouse, rat and human plasma samples and to evaluate the comparability of EV and lipoprotein distribution across the species.

Blood samples from mice, rats and humans were processed to obtain platelet-free plasma by sequential centrifugation. The plasma samples were then subjected to 20 hours 10-50% iodixanol DGUC using 10 mL or 5 mL columns, termed as high- and low-volume protocols respectively. High-volume protocol was performed with 1 mL plasma sample, while how-volume protocol was performed with 0.5 mL plasma sample. DGUC columns were separated to 11 (high-volume) or 10 (low-volume) equal-volume fractions and were analyzed by Western blotting using EV and lipoprotein markers.

In the high-volume protocol, EV markers were detected mainly in fractions 1–8 in mouse samples and fractions 1–5 in rat samples, while lipoprotein contaminants were predominantly detected in fractions 1–4 in both species. In the low-volume protocol, EV enrichment was observed mainly in fractions 3–7 in mouse samples and fractions 2–7 in rat samples, whereas contaminants remained mainly in fractions 1–6. In human high-volume protocol, EVs were predominantly detected in fractions 2–6 and 8 and lipoprotein markers were detected mainly in fractions 1–8.

Overall, the results suggest that EV markers show comparable distribution patterns across mouse, rat, and human DGUC fractions, however, species-specific differences in the distribution of lipoprotein contaminants, were observed. Therefore, optimization of DGUC protocol is needed for all species, that may affect direct translatability of the results.

45. EXTRACELLULAR VESICLE – GOLD NANOPARTICLE INTERACTIONS: IMPLICATIONS FOR INFRARED SPECTROSCOPIC ANALYSIS

[1]Tímea Farkasné Bebesi, [1]Marcell Pálmai, [1,2]Ádám Takács, [1]Attila Bóta, [1]Zoltán Varga, [1]Judith Mihály
[1]Biological Nanochemistry Research Group, Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Hungary, [2]Hevesy György PhD School of Chemistry, Eötvös Lóránd University, Hungary

Extracellular vesicles (EVs) are increasingly investigated as biomarkers and therapeutic carriers because their molecular composition reflects key aspects of cellular physiology. Their membranes, composed primarily of phospholipids, cholesterol, sphingolipids, and membrane proteins, can interact with gold nanoparticles (AuNPs) through electrostatic, hydrophobic, and ligand-mediated interactions. These interactions may influence membrane organization and the molecular environment at the EV–AuNP interface. Infrared (IR) spectroscopy provides a label-free approach for characterizing EVs by probing their lipid, protein, and nucleic-acid signatures. AuNPs can enhance the sensitivity of IR spectroscopy through plasmonic enhancement of molecular vibrational signals, resulting in surface-enhanced infrared spectroscopy (SEIRS). The proximity and orientation of EV membrane components within the localized plasmonic field can determine the magnitude and selectivity of spectral enhancement. Consequently, SEIRS spectra may reflect not only the intrinsic molecular composition of EVs but also changes arising from their interaction with AuNPs.

Negatively charged citrate-capped and positively charged cysteamine-capped gold nanoparticles were synthesized and tested with blood-derived EVs. Because EV–AuNP interactions are critical for achieving effective SEIRS enhancement, changes in the plasmonic properties of the nanoparticles and their potential aggregation were investigated using UV–Vis spectroscopy, dynamic light scattering (DLS), and freeze-fracture transmission electron microscopy (FF-TEM). Understanding the relationship between EV membrane chemistry, AuNP surface properties, nanoparticle aggregation and plasmonic enhancement is therefore essential for the reliable interpretation of SEIRS spectra and the development of sensitive, label-free approaches for EV characterization.

The research was funded by the National Research, Development and Innovation Office (NKFIH) under grants ADVANCED-152698, ADVANCED-150077 and STARTING-150629.

46. SYSTEMATIC CATEGORIZATION OF ANTIMICROBIAL PEPTIDE-EXTRACELLULAR VESICLE INTERACTIONS: PROTEIN CORONA REMOVAL AND MEMBRANE ACTIVITY IN RED BLOOD CELL-DERIVED VESICLES

[1,2]Tasvilla Sonallya, [1]Tünde Juhász, [1]Imola Cs. Szigyártó, [2,3]Kinga Ilyés, [1]Priyanka Singh, [4]Delaram Khamari, [4,5,6]Edit I. Buzás, [3,7]Zoltán Varga, [1]Tamás Beke-Somfai*

[1]Biomolecular Self-assembly Research Group, Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary, [2]Hevesy György PhD School of Chemistry, Eötvös Loránd University (ELTE), Budapest, Hungary, [3]Biological Nanochemistry Research Group, Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary, [4]Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, Hungary, [5]HCEMM Extracellular Vesicle Research Group, Semmelweis University, Budapest, Hungary, [6]HUN-REN-SU Translational Extracellular Vesicle Research Group, Budapest, Hungary

Host antimicrobial peptides (AMPs) and extracellular vesicles (EVs) are important components of innate immunity. Recent evidence indicates that EVs can serve as natural carriers for AMPs, while certain AMPs can remove the protein corona (PC), the externally adsorbed protein layer on EV surfaces, enabling subtractive proteomics. However, AMP-EV interactions remain poorly characterized. Here, we investigated 26 AMPs, including several cell-penetrating peptides (CPPs), and their interactions with red blood cell-derived vesicles (REVs) using flow-linear dichroism spectroscopy, flow cytometry, nanoparticle tracking analysis, freeze-fracture electron microscopy, and zeta-potential measurements.

Based on lytic activity, surface protein clearance, and effective concentration range, the peptides were classified into eight mechanistic categories. LL-37 and lasioglossin III emerged as the most efficient corona-removing peptides, clearing the EV surface protein layer at low concentrations while causing minimal membrane disruption, making them attractive tools for EV surface engineering. In contrast, octa-arginine, penetratin, buforin II, KLA, bradykinin, and histatin-5 tolerated high peptide loads with minimal lytic damage, consistent with membrane-translocating or cargo-transporting behavior. A third group, including melittin and buforin I, exhibited pronounced lytic activity at low concentrations, identifying them as candidates for vesicle-disrupting cargo-release applications.

Zeta-potential analysis indicated that both corona removal and cationic peptide adsorption contribute to loss of the native negative surface charge, with peptide charge, length, and membrane engagement mode governing the extent of this shift. This categorization framework provides a practical guide for selecting AMPs for EV-engineering applications, from minimally invasive corona clearance to controlled cargo loading or vesicle disruption. Building on these findings, lasioglossin III has since been applied to additional EV types as a corona-stripping reagent for subtractive proteomic characterization, demonstrating broad utility for EV surface protein removal.

47. LABELLING OF EXTRACELLULAR VESICLES USING FLUORESCENT ANTIBODIES: EFFECT OF PROTEIN CORONA

[1,2]Kinga Ilyés,[1,2] Tasvilla Sonallya,[1]Tünde Juhász,[1] Zoltán Varga,[1] Tamás Beke-Somfai
[1]HUN-REN Research Centre for Natural Sciences, Institute of Materials and Environmental Chemistry, Hungary,[2] Hevesy György PhD School of Chemistry, ELTE Eötvös Loránd University, Hungary

A key challenge in extracellular vesicle (EV) research is developing a specific, sensitive labelling strategy. Red blood cell-derived EVs (REVs) are ideal models as they are homogenous in size and contain the erythrocyte-specific surface marker protein CD235a. Fluorescent antibodies are widely used for EV labelling, however, the biological environment such as protein corona, may influence binding efficiency.

We have systematically investigated labelling of REVs with CD235a antibodies (Ab) conjugated to three fluorophores of various chemical nature and thereby physicochemical properties. Antibody binding was quantified using various methods such as the widely used flow cytometry (FCM), the less frequently used size exclusion chromatography (SEC-HPLC), and microscale thermophoresis (MST), not yet used to this goal.

Despite basic differences between the methods, FCM and SEC-HPLC derived data showed good correlation providing comparable apparent binding efficacies. In complementary MST assays, Ab concentration-dependent fluorescence and MST response changes were obtained. Moreover, Ab-dependent differences were revealed with all three techniques yielding variable apparent affinities.

Partial protein corona removal upon high-salt treatment affected labelling efficacy at various extent and a method-dependent way.
Our results contribute to optimizing EV staining strategies.

Acknowledgements: This work was funded by the Ministry of Innovation and Technology of Hungary from the National Research, Development, and Innovation Fund, grant no. TKP2021-EGA-31, KKP22 144180, and 2020-1-1-2-PIACI-KFI 2020-00021.

48. TOWARD CLINICAL STANDARDIZATION OF EXTRACELLULAR VESICLE WORKFLOWS: BENCHMARKING AUTOMATED SEPARATION PLATFORMS AGAINST MANUAL SEC

[1]Anna Maria Zimbo, [1]Valentina Crapella, [1]Selena Mimmi, [2]Elisabetta Pingitore, [1]Annarita Scardamaglia, [1,3]Andrea Quattrone, [2]Enrico Iaccino, [1,3]Aldo Quattrone
[1]Department of Medical and Surgical Sciences, University Magna Graecia of Catanzaro, Italy, [2]Department of Experimental and Clinical Medicine, University Magna Graecia of Catanzaro, Italy, [3]Neuroscience Research Center, University Magna Graecia of Catanzaro, Italy

Clinical translation of extracellular vesicle (EV)-based diagnostics is constrained by preanalytical variability in separation workflows. We benchmarked two automated EV separation platforms against manual size-exclusion chromatography (SEC), utilizing matched membrane cutoffs (30–35 nm) and quality indicators aligned with International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and Minimal Information for Studies of Extracellular Vesicles 2023 (MISEV2023) standards. This study provides an evidence-based framework for standardizing EV separation methods in clinical laboratory practice.

Paired serum and plasma from 10 healthy donors were processed by ultrafiltration-oscillation (EXODUS), automated SEC (Summit), and manual gravity-flow SEC (qEV). Endpoints included nanoparticle tracking analysis (NTA) yield and size distribution, particle-to-protein ratio (PPR), intra-matrix reproducibility (CV%), and tetraspanin preservation (CD9, CD63, CD81) by single-particle interferometric reflectance imaging sensing (SP-IRIS).

EXODUS resulted in significantly higher particle concentrations and PPR compared with SEC-based methods in both matrices, while no significant difference was observed between Summit and qEV. Summit was the only platform achieving CV% values below <20% in both serum and plasma. Particle size distributions were similar across all platforms. Transmission electron microscopy confirmed the presence of typical round-shaped, membrane-delimited particles (~100 nm) across all methods. SP-IRIS showed comparable tetraspanin detection across EXODUS and Summit, whereas qEV exhibited lower signal intensity. Workflow analysis showed Summit's superior scalability, allowing the processing of 4–48 samples per run with only 10–15 min of hands-on time.

No single platform optimizes all parameters. EXODUS maximizes yield for sensitivity-limited analyses; Summit satisfies preanalytical robustness for analytical standardization; manual SEC remains a reference method for morphological assessment. Therefore, method selection should be based on the intended analytical and clinical application rather than solely on particle yield.

49. OPTIMIZATION OF FLOW CYTOMETRIC METHODS FOR EXTRACELLULAR VESICLE ANALYSIS

[1]Zsófia Fodor, [1]Kelsey Fletcher, [1,2]Tamás Visnovitz, [1]Tünde Bárkai, [1]Dorina Lenzinger, [13]Edit I. Buzás, [1]Krisztina V. Vukman
[1]Semmelweis University, Department of Genetics, Cell- and Immunobiology, Nagyvárad tér 4, 1089 Budapest, Hungary, [2]ELTE Eötvös Loránd University, Department of Plant Physiology and Molecular Plant Biology, Pázmány Péter sétány 1/c, 1117 Budapest, Hungary, [3] HUN-REN-SU Translational Extracellular Vesicle Research Group, Nagyvárad tér 4, 1089 Budapest, Hungary

Extracellular vesicles (EVs) have gained increasing attention in biomedical research because of their essential functions in cellular homeostasis and intercellular communication. However, their small size, heterogeneity, and variable concentration make reliable detection and characterization challenging. To address these challenges, researchers are actively developing protocols for EV measurement and analysis. 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 contribute to the development of a standardized flow cytometry protocol for EV analysis.

EVs derived from HEK293 and GFP-expressing HEK293 cell lines were used in this study. EVs were isolated from conditioned media using differential centrifugation and filtration. The samples were stained with the novel fluorescent dye BioxML Red. The effects of dye and sample concentrations, buffer composition, washing procedures, and instrument settings on EV detection were investigated.

The sensitivity of EVs to different detergents was investigated using Triton X-100, saponin, Tergitol and SDS. Our results demonstrate that both instrument settings and EV concentration are critical factors for obtaining reproducible measurements. Among the detergents tested, Triton X-100 and Tergitol effectively disrupted lipid membranes and showed good compatibility with lipid dye-based EV detection. In contrast, saponin showed lower membrane-lysis efficiency and resulted in less consistent measurements.

Overall, this study contributes to the development of standardized protocols for EV characterization by flow cytometry. These findings enhance methodological rigor and support a deeper understanding of EV structure-function relationships.

Funding: STARTING_25_No.152115, ADVANCED_No.152112, ADVANCED_No.150767, VEKOP-2.3.2-16-2016-00002, VEKOP-2.3.3-15-2017-00016, TKP2021-EGA-23, RRF-2.3.1-21-2022-00003, János Bolyai Research Scholarship

50. STANDARDIZATION OF INSTRUMENT PREPARATION AND SAMPLE ACQUISITION FOR THE ANALYSIS OF EXTRACELLULAR VESICLES USING IMAGING FLOW CYTOMETRY

[1,2]Irma Hostnik, [1]Ana Kolenc
[1]Slovenian Institute for Transfusion Medicine, [2]Biotehnical Faculty, University of Ljubljana

Extracellular vesicles (EVs) are small membrane-enclosed vesicles released by various cell types and are increasingly studied as potential indicators of cellular processes and changes in biological samples. Due to their small size and low fluorescence intensity, standardized pre-analytical and analytical procedures are essential to ensure reproducible and comparable measurements. Imaging flow cytometry combines quantitative fluorescence analysis with image acquisition, enabling additional morphological characterization of detected events. The aim of our work was to establish a standardized and reproducible procedure for instrument preparation, sample acquisition, quality control, and data analysis of EVs in blood and blood components using the Cytek Amnis ImageStream Mk II. Prior to sample acquisition, instrument calibration, fluidics, laser operation, and overall instrument performance were checked. Synthetic/plastic beads were used as controls to assess sample flow and instrument performance, and biological samples were measured only after satisfactory control results had been obtained. An acquisition protocol was prepared using Amnis INSPIRE software and included laser and fluorescence channel selection, SSC settings, sample flow rate, image acquisition parameters, 60× magnification, and High Gain amplification. Appropriate signal amplification is particularly important for EV analysis due to their low fluorescence intensity. Fluorescent markers such as FITC, PE, and APC can be used according to the experimental design; CD9-FITC was used as an example for the identification of EVs expressing CD9. During acquisition, sample flow, signal stability, and image quality were monitored. The acquired data were subsequently analyzed using Amnis IDEAS software. EV populations were identified using a predefined and standardized gating strategy, allowing assessment of EV number, size, fluorescence intensity, morphology, and potential cellular origin based on fluorescent markers. Events originating from synthetic/plastic beads were excluded from subsequent biological analysis. The established protocol provides a standardized framework using imaging flow cytometry. Consistent measurement and analysis conditions are particularly important for EV characterization because of their small size and weak fluorescence signals. The protocol provides a methodological basis for reproducible characterization of EVs in blood and blood components and supports further studies of their number, size, fluorescence, morphology, and potential cellular origin.

51. ENGINEERING EXOSOMES AS A DECOY PLATFORM THROUGH SYNTENIN-MEDIATED RECEPTOR SORTING

[1,2]Kateřina Budková, [2]Olga Šebestová Janoušková, [2]Stanislav Vinopal, and [2]Jan Malý
[1]Faculty of Science, Department of Cell Biology, Charles University in Prague, Czech Republic, [2]Faculty of Science, Centre of Nanomaterials and Biotechnology, Jan Evangelista Purkyne University in Usti nad Labem, Czech Republic

Extracellular vesicles (EVs), particurarly exosomes, are widely investigated as natural carriers for therapeutic cargo; however, their membrane itself may provide a functional therapeutic interface. In this study, we genetically engineered donor cells to produce exosomes displaying non-signalling transmembrane receptor modules that can function as molecular decoys by capturing extracellular ligands.

Our engineering strategy exploits the syntenin–ALIX pathway involved in exosome biogenesis and membrane cargo sorting. Physiologically, syntenin acts as an adaptor within the syndecan–syntenin–ALIX axis. Its interaction with ALIX provides a functional link to the ESCRT machinery involved in inward budding of the endosomal membrane and formation of intraluminal vesicles within multivesicular bodies. Based on this mechanism, we incorporated an N-terminal syntenin-derived sorting module into a transmembrane receptor construct to enhance its incorporation into exosomal membranes.

We selected a key receptor from a signalling axis implicated in chronic inflammatory disorders as the core of our fusion proteins. The engineered constructs retain the extracellular receptor region required for molecular recognition and the transmembrane region, while intracellular signalling regions are replaced by mEGFP to generate a minimal, non-signalling easily detectable receptor module. Constructs containing or lacking the syntenin-derived sorting module were expressed in donor cells, and the resulting EV populations were isolated and characterized. Receptor display at the single-vesicle level was assessed by nano-flow cytometry, enabling comparison of the proportion and fluorescence characteristics of receptor-positive particles between individual fusions. Functional accessibility of the displayed receptor is further evaluated using receptor-specific binding proteins.

Our ongoing study establishes an experimental framework for evaluating syntenin-assisted generation of receptor-decorated exosomes and their potential use as extracellular decoy systems. Importantly, the strategy is designed as a modular platform rather than a specific system. Adaptation to other transmembrane receptors could enable the generation of engineered EVs displaying defined ligand-binding domains. Morever, modifications of the extravesicular part of the fusion protein could allow for alternative purification methods. Together, this platform provides a versatile approach for sequestration soluble mediators and modulating pathological intercellular signalling.

Acknowledgement:
This work was supported by the Ministry of Education, and by the project MATBIOMED—Materials and Technologies for Bioapplications and Medicine, reg. no.: CZ.02.01.01/00/23_021/0008398, funded by OP JAK

52. 10 YEARS LATER: WHAT'S NEW IN EXTRACELLULAR VESICLE STORAGE?

[1]Mátka Nagy, [2]Balázs Kondoros, [2]Tamás Kiss, [3]Dorina Lenzinger, [3]Tamás Visnovitz, [1]Erzsébet Ligeti, [2]Tamás Sohajda, [1,4]Ákos M. Lőrincz
[1]Department of Physiology, Semmelweis University, Budapest, Hungary, [2]CarboHyde Zrt, Budapest, Hungary, [3]Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, [4]Second Department of Internal Medicine, Szent György Hospital, Székesfehérvár, Hungary

The long-term storage of extracellular vesicles (EVs) remains challenging, as environmental conditions may compromise their integrity, cargo and biological function. Optimized preservation protocols are therefore required to ensure reproducible research outcomes and support future clinical applications. Based on our previous findings, storage at −80 °C provides better EV preservation than storage at room temperature, 4 °C or −20 °C. However, substantial alterations may still occur at −80 °C, highlighting the need for improved preservation strategies.

We aimed to investigate the long-term preservation of EVs by comparing storage at −80 °C with lyophilization and evaluating the protective effects of selected carbohydrate-based cryoprotectants.

Neutrophil-derived EVs were generated and isolated by differential centrifugation and filtration. EVs were stored either at −80 °C or lyophilized and kept for one month with or without carbohydrate-based cryoprotectants. Particle counts were determined by flow cytometry, while size distribution was assessed by nanoparticle tracking analysis. EV membrane integrity was examined using a lactate dehydrogenase (LDH) release assay and protein content with BCA measurements, while morphology was evaluated by transmission electron microscopy (TEM). Biological activity was tested by measuring the viability of EV-treated leukocytes using flow cytometric Annexin V/propidium iodide staining and cellular LDH release assay.

Compared with fresh controls, particle counts were reduced under all storage conditions, including storage at −80 °C and lyophilization. Cryoprotectants significantly mitigated this loss in lyophilized samples, resulting in higher detectable EV counts and improved membrane integrity, as indicated by reduced LDH release from the vesicles. TEM imaging showed that the characteristic EV morphology was retained under all storage conditions, however, increased aggregation and a reduced abundance of larger vesicles were observed after storage. Storage also decreased protein content, this reduction was consistently detected in samples stored at −80 °C, whereas lyophilization in the presence of cryoprotectants improved protein preservation. Preliminary functional studies demonstrated reduced LDH release from leukocytes treated with cryoprotectant-preserved EVs, suggesting that the cryoprotectants contributed to the preservation of EV biological activity during storage.

Our comparative findings indicate that carbohydrate-based cryoprotectants mitigate storage-induced alterations in neutrophil-derived EVs, particularly during lyophilization. Their application improved EV recovery, membrane integrity, protein preservation and biological activity of stored EVs. These results support lyophilization with carbohydrate-based cryoprotectants as a promising approach for the long-term preservation of EVs.

FUNDING: EFOP-3.6.3-VEKOP-16-2017-00009, NKFIH FK 137770, TKP2021-EGA-24, MSCA SE: Bicyclos, 2024-2.1.2-EKÖP-KDP-2024-00002

53. ONE-STEP SEPARATION OF EXTRACELLULAR VESICLES FROM BLOOD PLASMA BY IODIXANOL DENSITY-GRADIENT ULTRACENTRIFUGATION: COMPARING HUMAN AND EQUINE SPECIMENS

[1,2]Vadym Pelypenko, [1]Aiden Blumer, [1,3]Julian Krause, [2]Jessika-Maximiliane Cavalleri, [1]Silvio Kau-Strebinger
[1]Department of Biological Sciences and Pathobiology, Unit of Morphology, University of Veterinary Medicine Vienna, Austria, [2]Department for Companion Animals and Horses, Clinical Unit of Equine Internal Medicine, University of Veterinary Medicine Vienna, Austria, [3]Department of Biological Sciences and Pathobiology, Unit of Medical Physics and Biophysics, University of Veterinary Medicine Vienna, Austria

Current EV enrichment techniques are distinguished by imbalanced trade-off between yield, purity and structural integrity, implying the need to compromise for each individual set of further experiments desired. Such compromise limits the fraction of the total EV population to be enriched, narrowing the range of downstream applications. The described study aims to develop a MISEV-compliant workflow of improved reproducibility and particle recovery, universally suitable for a variety of downstream processing, analysis, and cross-species comparisons.

Samples of human and equine blood from healthy donors were depleted of cellular components within one hour of venipuncture. Pre-cleared plasma was processed by 24-hour top-bottom iodixanol density ultracentrifugation (pre- and post-centrifugation SEC and/or UC steps were skipped, in contrast to currently reported studies). Fractions of a self-formed continuous density gradient (controlled by refractometry measurements) were characterized using NTA, TEM, immunoblotting, total protein measurements, and quantitative fluorescence microscopy.

Post-centrifugation comparison of density distributions between plasma-exposed and sample-free trials showed formation of a spatial density gradient in previously reported ranges for EVs and lipoproteins without being disturbed by sample application. NTA, TEM and Western Blot data confirmed size-density re-distribution, without pre-processing steps, of mentioned particle populations and dynamic changes depending on iodixanol pre-layering configurations. Differential staining (Nile Red and Bodipy493/503 + CMDR) of density fractions for confocal microscopy confirmed these outcomes and the possibility of exploiting broader structural features for discriminating between EVs and co-enriched components, avoiding human-specific immunogenicity-based methods.

Obtained results set the bases for improvements in surfaceome profiling (including attached but not integrated cargo) of extracellular vesicles and interaction studies of EVs with components of media. Such EV fractions enriched with low-to-no loss in a minimum number of steps would be suitable for a wide range of possible modifications and cross-species comparisons for human and veterinary medicine.

The research was funded by Sandgrueb foundation. The authors thank Curtis Goding and Dr. Peter Dungel (Ludwig Bolzmann Institute for Traumatology, Vienna, Austria) for providing the samples of equine and human blood respectively in accordance with a local ethics agreement, Olga Olszanska, Waltraud Tschulenk, Sarah Schlosser and Elisabeth Clifford for their technical assistance in laboratory analysis. Julian Krause is funded by the One Health PhD Programme of the University of Veterinary Medicine Vienna, Austria.

54. FROM SAMPLE TO INSIGHT: A ONE-STOP MULTIDISCIPLINARY PLATFORM FOR EXTRACELLULAR VESICLE RESEARCH AND TRANSLATION

Avraham Dayan
Multidisciplinary Vesicle Program, Life Sciences Core Facilities, Weizmann Institute of Science, Israel

Extracellular vesicle (EV) research increasingly requires the integration of complementary expertise in vesicle production, isolation, characterization, imaging, functional analysis and omics. However, these capabilities are often distributed across independent laboratories as well as technological platforms, creating challenges in experimental continuity, standardization and data integration. The Multidisciplinary Vesicle Program (MVP) at the Weizmann Institute of Science was established as a one-stop scientific platform connecting researchers with multidisciplinary technologies and expertise across the entire EV and lipid nanoparticle (LNP) workflow.

The platform integrates EV production and separation approaches with nanoparticle tracking analysis, zeta potential measurements, atomic force microscopy, cryo-electron microscopy, super-resolution imaging, high-sensitivity flow cytometry, functional assays, proteomics, metabolomics and next-generation sequencing. Rather than operating as an isolated facility, the MVP coordinates collaborative projects across multiple Core Facilities and research groups, enabling complementary technologies to be combined within a single framework.

Several ongoing collaborative studies illustrate this model. A comparative EV isolation project integrating TFF, ultracentrifugation, nanoparticle analysis, AFM, cryo-EM and flow cytometry demonstrated method-dependent differences in particle recovery, colloidal properties as well as vesicle morphology, with TFF producing higher particle recovery and cleaner preparations in the tested system. A second multi-unit project addresses EV cryostorage and standardization through longitudinal analysis using ZetaView, NanoSight, AFM and cryo-TEM. In this workflow, 10% sucrose preserved 72–88% of baseline EV concentration after 14 days, compared with 21–34% in PBS, highlighting the importance of integrated analytical validation for EV biobanking.

In parallel, collaboration with the Flow Cytometry Unit is establishing standardized EV labeling and high-sensitivity detection workflows. Optimization of Acoerela dyes identified 2.5 µM as a practical staining condition for routine analysis, with ongoing extension toward fluorescent EV populations, imaging flow cytometry, cargo-sensitive labeling and cellular uptake studies.

Together, these examples demonstrate how a coordinated one-stop EV research model can transform distributed technological expertise into reproducible multidisciplinary workflows, facilitate cross-unit and international collaborations as well ad accelerate EV research from methodological development to translational applications in diagnostics, drug delivery and precision medicine.

55. PROBING EXTRACELLULAR VESICLE MEMBRANE ORGANIZATION WITH A NOVEL TRES-PHASOR APPROACH

Šárka Pokorná[1], Petra Riegerová[1], Vendula Hlaváčková Pospichalová[2], Martin Hof[1]
[1]Department of Biophysical Chemistry, J. Heyrovský Institute of Physical Chemistry, Prague, Czech Republic,
[2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

The lipid organization of extracellular vesicle (EV) membranes influences their physical properties and potentially their biological functions. The environment-sensitive fluorescent probe Laurdan provides a convenient tool for probing membrane lipid organization.[1] Here, we compared commonly used steady-state Laurdan readouts, including generalized polarization and spectral phasor analysis, and developed a novel approach based on model-free analysis of the time-resolved emission spectra (TRES) – TRES-phasor.[2]

We applied these approaches to EVs isolated from wild-type cells and three single-tetraspanin knockout cell lines lacking CD9, CD63, or CD81. Direct comparison showed that TRES-phasor analysis provides higher sensitivity to subtle differences in EV membrane properties than steady-state approaches. EVs also displayed pronounced heterogeneity and behavior distinct from model lipid membranes. Finally, fluorescence-based readouts were correlated with lipidomic data to relate EV membrane biophysics to lipid composition. TRES-phasor therefore provides a sensitive, model-free approach for probing EV membrane organization and heterogeneity.

56. CORRELATION BETWEEN SPECTROSCOPIC AND STOICHIOMETRIC PROTEIN TO LIPID RATIOS IN ERYTHROCYTE-DERIVED EXTRACELLULAR VESICLES AND NANOERYTHROSOMES

A. Bóta[1], K. Ilyés[1], A. Wacha[1], Z. Varga[1,2], J. Mihály [1,3] and, B. Petricevic Ristic[4], I. Drvenica[4] Rados Stefanovič[4]
[1]Research Centre for Natural Sciences, Institute of Materials and Environmental Chemistry, Biological Nanochemistry Research Group, Magyar tudósok körútja 2, H-1117 Budapest, Hungary, [2]Department of Physical Chemistry and Materials Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Műegyetem rkp. 3., H-1111 Budapest, Hungary, [3]Department of Chemistry, Eszterházy Károly Catholic University, , Leányka utca 12, H-3300 Eger, Hungary, [4]Institute for Medical Research, University of Belgrade, Dr Subotića 4, SRB-11029 Belgrade, Republic of Serbia

The erythrocyte-derived extracellular vesicles and nanoerythrosomes show high similarities due to their same origins supporting their prosperous applicability for new generations of nanocarrier systems. The addition of a representant erythrocyte-membrane lipid (dipalmitoyl-phosphocholine, DPPC), different morphological and structural changes occurred in function of the increasing DPPC ratio. The integration of guest DPPC molecules, expressed in weight ratio extending approximately to an order of magnitude, yields the formation of standalone particles in nanoerythrosomes (NERYs), while the integration of DPPC molecules is significantly reduced with red blood cell derived extracellular vesicles (RBCEVs) resulting aggregations of these EVs. Despite the induced severe and different changes in morphological and structural characteristics, both systems show systematic changes in spectroscopical signals displaying quasi proportional alterations in their spectroscopical protein to lipid ratios. The study of series with controlled DPPC amount results in the observance of the correlation between spectroscopic and stoichiometric protein to lipid ratios. Independently from the selected spectroscopic lipid-determination (regarding to carbon-hydrogen and carbonyl vibrations), a relatively simple approximation for the stoichiometric protein to lipid ratio can be achieved in the knowledge of protein content or in case of its estimation. Based on the observed linear correlation the determination of an approximative lipid concentrations of natural NERYs and EVs comes to be possible without any lipid analytic.

57. ADVANCES AND CHALLENGES IN VESICULAR MARKER ANALYSIS USING NANO-FLOW CYTOMETRY

Olga Šebestová Janoušková[1], Simona Grabovská[1], Oksana Batkivska[1], Jan Malý[1]
[1]Centre for Nanomaterials and Biotechnology, Faculty of Science, Jan Evangelista Purkyně University in Ústí nad Labem, Czech Republic

Extracellular vesicles (EVs) are now widely recognized as important mediators of intercellular communication. Their potential as biomarkers reflecting pathological conditions, including cancer and chronic inflammatory diseases, is receiving increasing attention. In plants, EVs may likewise reflect responses to environmental stress. At the same time, EVs continue to be investigated as promising drug-delivery vehicles.

For EV characterization and their subsequent use in research and clinical applications, it is important to detect both canonical EV-associated markers and, depending on the vesicle population under investigation, specific protein or other markers that may reflect changes in tissue or organismal physiology. Due to the nanoscale size of EVs and the need for appropriate particle isolation, conventional methods such as light microscopy and standard flow cytometry have limited applicability. Western blotting and enzyme-linked immunosorbent assays (ELISAs) are commonly used; however, they do not provide information at the single-vesicle level, and the required sample preparation may affect both marker detectability and signal intensity. Nano-flow cytometry is emerging as a powerful approach that substantially accelerates the detection of protein and other molecular markers while also enabling the determination of particle concentration in EV samples.

In our studies, we used nano-flow cytometry with the Flow NanoAnalyzer (NanoFCM) to characterize the protein-marker profile of EVs, monitor EV–liposome fusion, and follow the removal of the protein corona from plasma-derived EVs. We also applied nano-flow cytometry to optimize the detection of canonical EV-associated markers, including CD63 and CD9, together with tumor-associated protein markers.

Nano-flow cytometry requires the careful development of an analytical protocol and meticulous sample preparation. Nevertheless, it represents a major advance in the detection of protein structures on individual EVs and enables concurrent monitoring of vesicle aggregation and degradation.

Acknowledgemet:
This work was supported by the Ministry of Education, and by the project MATBIOMED—Materials and Technologies for Bioapplications and Medicine, reg. no.: CZ.02.01.01/00/23_021/0008398, funded by OP JAK

OTHER TOPICS RELATED TO EXTRACELLULAR VESICLES

58. CONNECTING EARLY-CAREER RESEARCHERS IN THE EXTRACELLULAR VESICLE FIELD THROUGH SNEV

Reza Abouali
Student Network on Extracellular Vesicles (SNEV) Executive Committee

Findings on extracellular vesicles (EV) are made by a global community of researchers, including students and postdoctoral researchers working across different disciplines and environments. Despite the significant efforts from the field to connect ECRs outside individual laboratories and established scientific conferences, a centralized approach was desperately needed. The Student Network on Extracellular Vesicles (SNEV) was established in 2020 and entered into a formal partnership with the International Society for Extracellular Vesicles (ISEV) in 2021. In 2024, SNEV became ISEV's official ECR arm. SNEV is coordinated by an international team of volunteers working across events, media, design, newsletters and podcasts. Since its establishment, SNEV has organised over 110 online events and two international virtual conferences, while its newsletter has reached 61 issues. Participation in the ISEV annual meetings has expanded from representation within organising committees to dedicated Meet the Experts sessions, satellite events and informal networking activities. Importantly, the ISEV–SNEV Mentorship Program has completed two editions, connecting ECRs with researchers from different career stages.

The SNEV Interconnect Program extended this network to national and regional EV societies. Through 29 SNEV ambassadors representing societies worldwide, the Program connects ECR communities across countries and supports international activities such as SNEV Ambassadors' Day. SNEV has also been introduced at scientific meetings across Europe, Asia and America, while its social media platforms currently reach a combined audience of more than 8,000 followers. Additional initiatives, including the EV Graphical Abstract Competition, provide opportunities for ECRs to present their research, develop important communication skills and participate in major EV meetings.

Together, these activities show how a virtual, volunteer-led network can complement established scientific societies by maintaining continuous support for ECRs between major meetings. SNEV provides an accessible route for young researchers to share their work, access career information, develop professional connections, and participate in the wider EV community. Its integration with ISEV and national and regional EV societies supports the continued expansion of ECR involvement and cross-border collaboration within the EV field.

59. ISOLATION AND COMPREHENSIVE CHARACTERIZATION OF OUTER MEMBRANE VESICLES FROM ESCHERICHIA COLI

[1,2]Omaima Barkaoui, [1,3]Sonallya Tasvilla, [1,4,5]Zoltán Varga, [6,7]Judit Tóth, [1]Tamás Beke-Somfai
[1]Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Hungary, [2]Doctoral School of Biology, ELTE Eötvös Loránd University, Hungary, [3]Doctoral School of Chemistry, ELTE Eötvös Loránd University, Hungary, [4]Department of Physical Chemistry and Materials Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Hungary, [5]Faculty of Medicine, Department of Biophysics and Radiation Biology - HUN-REN Office for Supported Research Groups, Semmelweis University, Hungary, [6]Department of Applied Biotechnology and Food Science, BME Budapest University of Technology and Economics, Hungary, [7]Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Hungary

Outer membrane vesicles (OMVs) released by Gram-negative bacteria play roles in host–pathogen interactions and microbial communication, contribute to antimicrobial resistance, and are being explored as vaccine and drug delivery platforms. Yet a recent literature survey found that only about 25% of OMV studies combine biochemical and biophysical characterization. We set out to build a platform for isolating and thoroughly characterizing OMVs from a hypervesiculating ΔnlpI Escherichia coli strain.

Starting from a 500 mL bacterial culture, we removed cells and debris by low-speed centrifugation (2,500 × g, twice), concentrated the supernatant by 100 kDa ultrafiltration, pelleted vesicles by ultracentrifugation (150,000 × g), and separated them by size-exclusion chromatography on Sepharose CL-4B. The two main SEC fractions were then examined by transmission electron microscopy (TEM), dynamic light scattering (DLS), Bradford protein assay, and Fourier-transform infrared spectroscopy (FTIR).

TEM showed spherical, membrane-bound vesicles matching OMV morphology. DLS gave mean hydrodynamic diameters of 39.9 nm and 211.6 nm for Fraction 1 and 29.4 nm for Fraction 2. Bradford assay measured protein concentrations of 0.4 mg/mL in Fraction 1 and 1.05 mg/mL in Fraction 2, enough for downstream work, and FTIR spectra showed the lipid, protein, and lipopolysaccharide signatures expected for outer-membrane material.

This establishes a scalable, reproducible pipeline for isolating and characterizing bacterial OMVs. Building on this platform, we next plan to add nanoparticle tracking analysis, multi-parameter resistive pulse sensing, and flow cytometry with vesicle-specific antibodies, moving toward functional assays on the role of OMVs in quorum sensing and their interactions with antimicrobial peptides.

60. INVESTIGATION OF EXTRACELLULAR VESSICLE CORONA FORMATION WITH LABEL-FREE OPTICAL WAVEGUIDE LIGHTMODE SPECTROSCOPY METHOD

[1]Péter Bokrossy, [1,2]Beáta Szebeni, [3,4]Tamás Visnovitz, [3]Dorina Lenzinger, [3]Éva Pálinger, [3]Nóra Fekete, [3]András I. Försönits, [5,6,7]Zoltán Varga, [5]Judith Mihály, [8]Balázs Fórizs, [1,2]Domonkos Pap, [1,2]Apor Veres-Székely, [1]Csenge Szász, [1]Jamila Raufi, [3,9]Edit I. Búzás, [1,2]Attila J. Szabó, [1,2]Ádám VannAY, and [10]Nóra Adányi
[1]Pediatric Center, MTA Center of Excellence, Semmelweis University; Budapest, Hungary;
[2]HUN-REN–SU Pediatrics and Nephrology Research Group, Budapest, Hungary; [3]Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary; [4]Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Budapest, Hungary; [5]Biological Nanochemistry Research Group, Institute of Materials and Environmental Chemistry, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary; [6]Department of Physical Chemistry and Materials Science, Budapest University of Technology and Economics, Budapest, Hungary; [7]Semmelweis University, Faculty of Medicine, Department of Biophysics and Radiation Biology - HUN-REN Office for Supported Research Groups, Budapest, Hungary; [8]Cantabio Pharmaceuticals Kft. Budapest, Hungary; [9]HUN-REN SU, Translational Extracellular Vesicles Research Group, Budapest, Hungary; [10]Insulinde BT. Budapest, Hungary

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.

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.

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 4.5×10^{10} 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.

Because the EV corona also includes non-protein components — such as nucleic acids like double-stranded DNA and microRNA — we further investigated their attachment to the EV surface. For this purpose, we used a purified PCR product of the PARK7 gene, along with miR-494, a microRNA known to downregulate DJ-1 expression.

The newly developed OWLS-based method for the non-specific immobilization of EVs enables detailed investigation of EV interactions with proteins and other biomole-cules, providing a robust platform for studying molecular behaviour through real-time, label-free monitoring of corona formation.

The presented study was supported by: 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.

61. LIPID-BASED FLUORESCENT PROBES AS A TOOL FOR LABELING AND TRACKING OF EXTRACELLULAR VESICLES

[1]Martina Hyzdalova, [1]Nicol Strakova, [2]Michal Jurasek, [2]Roman Effenberg, [1]Josef Masek
[1]Veterinary Research Institute, Brno, Czech Republic, [2]University of Chemistry and Technology, Prague, Czech Republic

Extracellular vesicles (EVs) are membrane-bound nanostructures (30–150 nm) secreted by most cell types, contain biologically active substances, and are found in body fluids. They may therefore serve as key mediators of intercellular communication. EVs are believed to play a crucial role in physiological processes as well as in pathological conditions, including cancer, neurodegenerative diseases, immune responses, and inflammation in diseases such as rheumatoid arthritis. Consequently, their potential as diagnostic biomarkers and drug carriers have sparked considerable interest in their molecular characterization and functional studies.

The labeling of EVs is essential for their reliable detection and subsequent characterization. Improving labeling methods and implementing rigorous quality control measures are essential for generating reproducible and biologically relevant data, and for translating EV research findings into clinical diagnostics and therapeutic applications. Given their nanoscale dimensions, direct observation of EVs is extremely challenging; as a result, fluorescent labeling and other chemical labeling methods have become indispensable in EV research. Labeling strategies enable not only the detection of EVs but also provide insights into their membrane composition, uptake mechanisms, and biodistribution in both *in vitro* and *in vivo* contexts. Among the most commonly used methods are lipophilic fluorescent dyes, which incorporate into the phospholipid bilayer of the vesicles; a second method involves the use of nucleic acid-specific dyes, which enable the detection of RNA content within EVs; another possible approach is immunological labeling using antibodies directed against typical proteins associated with EVs, such as the tetraspanins CD9, CD63, and CD81.

In this report, we describe the use of new lipid-based fluorescent (Cy-7) probes for labeling of EVs, emphasizing the efficiency of the labeling and the stability of the labeled EVs for further applications. Using flow cytometry and confocal microscopy with accessories for live-cell imaging, we demonstrate the utility of these probes in intracellular tracking of EV dynamics and their homing in real time. This work represents a significant step toward expanding the tools for extracellular vesicle research and paves the way for their use in clinical diagnostics and drug development.

This work was supported by the Ministry of Agriculture of the Czech Republic (project No. RO0523), Ministry of the Ministry of Health of the Czech Republic (project No. NU22-03-00290) and the Ministry of Education, Youth and Sports OPVVV CEREBIT CZ.02.1.01/0.0/0.0/16_025/0007397.

62. PLACENTA-DERIVED EXTRACELLULAR VESICLE-ASSOCIATED GALECTIN-14 AS LIQUID-BIOPSY BIOMARKER FOR TROPHOBLAST DYSFUNCTION AND MISCARRIAGE

[1,2]Emese Farkas, [1,3]Orsolya Oravecz, [1,2]Máté Posta, [4]Éva Pállinger, [4]Gábor Seregélyes, [5]János Matkó, [6]Petronella Hupuczi, [6]Zoltan Papp, [1,7,8]Nándor Gábor Than, [1]Andrea Balogh
[1]Systems Biology of Reproduction Research Group, Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary; [2]Doctoral College, Károly Rácz Conservative Medicine Division, Semmelweis University, Budapest, Hungary; [3]Doctoral School of Biology, Institute of Biology, ELTE Eötvös Loránd University, Budapest, Hungary; [4]Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary; [5]Department of Immunology, Eotvos Lorand University, Budapest, Hungary; [6]Maternity Private Department, Kutvolgyi Clinical Block, Semmelweis University, Budapest, Hungary; [7]Maternity Private Clinic of Obstetrics and Gynecology, Budapest, Hungary; [8]Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary

Placental galectins (e.g., Gal-13, Gal-14) play key immunoregulatory roles at the fetomaternal interface and are primate-specific proteins predominantly expressed by the syncytiotrophoblast (STB) and released into maternal circulation via STB-derived extracellular vesicles (STB-EVs). However, their biomarker utility is hindered by high sequence homology within the chromosome 19-galectin cluster. Here, we aimed to validate high-affinity recombinant monoclonal antibodies against Gal-14, establish a flow cytometric platform to quantify intravesicular Gal-14 across human gestation, and explore EV-associated Gal-14 changes in spontaneous miscarriages.

Lacking commercial, well-validated antibodies distinguishing Gal-14 from homologous family members, candidates were screened from Human Combinatorial Antibody Libraries (HuCAL). Clone 32 was selected, fluorescently conjugated, verified by Western blot, and validated for flow cytometry using STB-differentiated (5-azacitidine- and forskolin-treated) BeWo cells. Intravesicular Gal-14 in plasma EVs was quantified by flow cytometry across healthy pregnancies (all trimesters; n=10 each), spontaneous miscarriages (n=24), and elective terminations (n=25).

Clone 32 demonstrated strict Gal-14 specificity with negligible cross-reactivity. Simultaneous 5-azacitidine and forskolin treatment increased intracellular Gal-14 in BeWo cells, as detected using the Clone 32. In clinical samples, we characterized maternal blood EV-associated Gal-14 dynamics across normal gestation. Furthermore, the proportion of circulating Gal-14-positive EVs was significantly elevated in spontaneous miscarriages compared to gestational age-matched controls.

The apparent paradox of previously reported lower placental tissue expression alongside the elevated peripheral EV-associated Gal-14 levels observed here suggests elevated vesicle release during trophoblast distress, mirroring Gal-13 dynamics in preeclampsia. Establishing flow cytometric liquid-biopsy thresholds for STB-EV-associated Gal-14 provides a promising, minimally invasive approach for the early detection and monitoring of trophoblast dysfunction and pregnancy loss.

63. CHOLESTERYL ESTERS AND HIGH PROTEIN-TO-LIPID RATIOS DISTINGUISH NON-VESICULAR EXTRACELLULAR PARTICLES FROM EXTRACELLULAR VESICLES

Sayam Ghosal [1 2], Rita Leporati [3], Árpád Varga [4], Petra Susanszki [4], Nóra Fekete [2], Tamás László [5], Tünde Bárkai [2], Fülöp Károly Grébecz [2], Delaram Khamari [2], Tárek Zoltán Magyar [6], Marcus Hoering [7], Krisztina V Vukman [2], Bernadett R Bodnár [1 2 8], Brachyahu M Kestecher [1 2 8], Mohamed A Fattah [2], Csaba Bödör [5], József Maléth [4], Gerhard Liebisch [7], Evelyn Orsó [2], Edit I Buzas [1 2 8], Xabier Osteikoetxea [1 2]
[1]HCEMM-SU Extracellular Vesicle Research Group, Budapest, 1089, Hungary, [2]Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, 1089, Hungary, [3]Division of Medical Oncology, Modena University Hospital, Modena, 41124, Italy, [4]First Department of Internal Medicine, University of Szeged, Szeged, 6725, Hungary, [5]Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, 1085, Hungary, [6]Institute of Behavioural Sciences, Semmelweis University, Budapest, 1089, Hungary, [7]Institute of Clinical Chemistry and Laboratory Medicine, Regensburg University Hospital, Regensburg, 93053, Germany, [8]HUN-REN-SU Translational Extracellular Vesicle Research Group, Budapest, 1089, Hungary.

Non-vesicular extracellular particles (NVEPs), including exomeres and supermeres, represent a distinct component of the extracellular secretome that can carry disease-associated cargo previously attributed solely to extracellular vesicles (EVs). Their biogenesis and compositional relationship to canonical EV subtypes remain poorly defined, owing to a lack of systematic multiparametric comparisons. To address this, large and small EVs (14k-lEV, 100k-sEV), as well as NVEPs (167k-NVEP) were isolated from HEK293 cells by differential ultracentrifugation with filtration and characterized using transmission electron microscopy (TEM), nanoparticle tracking analysis, multiplexed flow cytometry, SP-IRIS, protein/lipid assays, Raman spectroscopy, quantitative lipidomics, and super-resolution microscopy. Particle biogenesis was probed via EGFP-tagged trafficking markers (Arf6, CD63, LC3b, MysP, Rab7a) using a custom confocal membrane-association assay.

TEM confirmed a lipid bilayer in 14k-lEV (190.8±26.9 nm) and 100k-sEV (125.2±3.2 nm), whereas NVEPs showed non-membranous, electron-dense morphology. NVEPs displayed a markedly elevated protein-to-lipid ratio (0.72±0.09) versus 100k-sEV (0.103±0.033) and 14k-lEV (0.0094±0.0047), corroborated by Raman spectra enriched in phenylalanine and Amide I signatures, with near-complete PCA separation of populations. Lipidomics revealed NVEP enrichment in short-chain cholesteryl esters and triglycerides, contrasting with sphingomyelin- and plasmalogen-enriched, longer-chain lipids in sEVs, consistent with an endosome-lysosome/lipid-droplet origin rather than the Golgi-associated exosomal route. Super-resolution microscopy showed NVEPs were predominantly single-tetraspanin-positive (67.1%), with fewer triple-positive particles (23.5%) than sEVs (43.2% single-, 39.4% triple-positive), alongside Arf6/CD63 enrichment and negligible Annexin V binding, indicating a non-bilayer, protein-dense surface. NVEPs further showed dose-dependent cargo transfer, with CD63-EGFP-enriched NVEPs achieving 54.6% EGFP-positive recipient cells at the highest dose tested (500 µg protein).

These findings establish a framework distinguishing NVEPs from canonical EVs by their non-membranous, protein-dense composition and non-canonical biogenesis, supporting their role as mediators of intercellular communication and candidate vehicles for targeted delivery and liquid biopsy biomarkers of metabolic disease.

64. COMPARATIVE EVALUATION OF EXTRACELLULAR VESICLE ISOLATION METHODS FROM EV-DEPLETED FBS-SUPPLEMENTED CELL CULTURE MEDIUM

[1]Valeriia Husieva, [1]Helena Kavcova, [2]Zuzana Kozovska, [3,4]Andreas Nicodemou, [1]Zdena Sulova, [1,5]Albert Breier, [1]Kristina Simonicova, and [1,5]Lucia Messingerova
[1]Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Slovakia, [2]Cancer Research Institute, Biomedical Research Center of Slovak Academy of Sciences, Slovakia, [3]Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University, Slovakia, [4]GAMMA-ZA s.r.o., Slovakia, [5]Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Slovakia

Isolation of extracellular vesicles (EVs) from cell culture medium supplemented with EV-depleted fetal bovine serum (FBS) remains challenging due to residual serum-derived components that may affect the purity and composition of EV preparations. We compared four commonly used EV isolation methods—Amicon ultrafiltration (AM), polyethylene glycol (PEG) precipitation, the Total Exosome Isolation Kit (TE), and the ExoEasy membrane affinity system (EE)—using conditioned medium from SKM-1 cells cultured in RPMI supplemented with commercially available EV-depleted FBS. All methods recovered particles detectable by nanoparticle tracking analysis, but the degree of cell-derived EV enrichment varied substantially. EE yielded similar particle concentrations in conditioned and cell-free control media and showed limited enrichment of EV-associated proteins, suggesting a considerable contribution of medium-derived particles. AM co-isolated abundant medium-associated proteins. In contrast, PEG and TE showed the most consistent evidence of cell-derived EV enrichment and demonstrated comparable performance. These findings indicate that particle concentration alone is insufficient to reliably assess EV enrichment. Seven protein extraction methods yielded comparable total protein measurements in PEG-enriched preparations, whereas RNA extraction workflows produced marked differences in small RNA-associated signals. Overall, our results emphasize that EV isolation and downstream extraction methods should be selected according to the starting material and intended application. The use of matched cell-free controls, together with complementary particle, protein, and RNA analyses, is essential for a reliable evaluation of EV preparation.

This research was funded by VEGA 2/0063/26, EU NextGeneration EU through the Recovery and Resilience Plan for Slovakia under project No. 09I03-03-V02-00020.

65. GLYCOPROFILING OF EXTRACELLULAR VESICLES (EVs) BY USING SURFACE PLASMON RESONANCE

[1]Muhammad Umair Khan, [1]Lucia Paulovičová, [1]Jaroslav Katrlík
[1]Institute of Chemistry, Slovak Academy of Sciences, Dúbravska cesta 5807/9, 845 38 Bratislava, Slovakia

The extracellular space contains a heterogeneous population of lipid bilayer-enclosed particles, collectively termed extracellular vesicles (EVs), released by virtually all cell types. EVs mediate intercellular communication in physiological and pathological processes [1]. EV surface glycans critically regulate vesicle biodistribution, cellular recognition, and uptake [2]. Alterations in cellular glycosylation are mirrored in EV glycan signatures, making EV glycome profiling a promising source of diagnostic biomarkers [3]. EVs can be isolated from multiple biofluids obtained through non-invasive or minimally invasive procedures [4].

In this study, the interaction of mannose-binding glycans on the surface of EVs from *Candida albicans* strain CCY 29-3-100 with mannose-binding lectin was investigated using label-free surface plasmon resonance (SPR). In addition to providing a well-defined experimental model, fungal EVs are biologically relevant carriers of surface glycoconjugates involved in interactions with the host and may contribute to fungal recognition and pathogenicity. Their glycoprofiling may therefore provide insight into biologically relevant features of fungal EV surfaces and their interactions with glycan-binding proteins.

Yeast-derived EVs were directly immobilized on the polycarboxylated SPR sensor chip and their interaction with lectin concanavalin A (ConA) was monitored in real time. Efficient EV immobilization enabled reproducible lectin binding measurements and determination of the apparent equilibrium dissociation constant (K_D) for the ConA-EV interaction. The stability of the immobilized EV layer was also evaluated and was found to be limited to the order of few days, identifying an important practical constraint of the direct EV immobilization format.

Complementary SPR configurations involving covalently immobilized lectins with EVs flowed over the sensor surface, as well as a sandwich format based on antibodies to capture EVs followed by lectin probing are being developed to expand the possibilities for EV glycoprofiling.

The developed SPR strategy provides a label-free approach for probing glycan-dependent interactions at the surface of intact EVs and may contribute to the characterization of EV glycosylation in both microbial and biomedical applications.

Acknowledgement: Funded by the project VEGA 2/0141/26.

References:
[1] Berumen Sánchez et al. Cell Commun. Signal. 2021;19:1–18. doi:10.1186/s12964-021-00787-y.
[2] van Dongen et al. Microbiol. Mol. Biol. Rev. 2016;80:369–386. doi:10.1128/mmbr.00063-15.
[3] Williams et al. Sci. Rep. 2019;9:1–14. doi:10.1038/s41598-019-48499-1.
[4] Khan et al. IJMS 2026;27:1–38. doi:10.3390/ijms27104298.

66. PROFILLING OF VOLATILE ORGANIC COMPOUNDS FROM EXTRACELLULAR VESICLES USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY, A METHODOLOGICAL DEVELOPMENT

Quynh-Mai Nguyen Le[1], Anrui Ge[1], Nayab Nadeem Abdul Kader[1], Sanooj Soni[1] and Ilaria Belluomo[1]
[1]Department of Surgery and Cancer Imperial College London, London, UK

Volatile organic compounds (VOCs) are carbon-based chemicals generated as metabolic by- or end-products of cellular and microbial processes. VOCs can enter the circulation and be excreted via the lungs, making breath VOC profiles potentially informative of endogenous processes and diseases. However, the biological mechanisms by which VOCs are transported throughout the body remain poorly understood. Extracellular vesicles (EVs), which mediate intercellular communication and transport diverse biomolecules, may provide a mechanism for VOC transport and represent a potential source of biomarkers. Determining whether VOCs are associated with EVs could therefore clarify how cellularly and microbially derived VOCs reach the circulation and breath while establishing EV-associated VOCs as a potential diagnostic target.

This study investigated whether VOCs can be detected in association with EVs using in vitro and clinically relevant models. Mechanical, chemical, and osmotic EV disruption strategies were evaluated. Disruption efficiency was assessed using flow cytometry, while released VOCs were analysed using headspace thermal desorption gas chromatography–mass spectrometry (HS-TD-GC-MS) and selected ion flow tube mass spectrometry (SIFT-MS). Water-mediated hypotonic disruption combined with sonication emerged as the most promising approach, providing a favourable balance between EV disruption and minimal background VOC contamination. Targeted analysis detected VOCs following EV membrane disruption, indicating that measurement of EV-associated VOCs is feasible. However, concentrations varied across chemical groups, limiting quantitative interpretation.

These findings provide preliminary evidence that EVs carry VOCs and establish foundational methods for their analysis, while highlighting challenges in reproducible quantification. Further methodological development is required to establish detection thresholds. Reliable profiling of VOCs in patient-derived EVs could ultimately enable volatile signatures to be linked to the cellular or microbial origins of EVs, providing new insight into the biological sources, transport pathways, and mechanisms underlying VOC production. Such knowledge could improve the biological interpretation of volatile biomarkers and enhance their diagnostic specificity.

67. EXOSOME BIOGENESIS: META-ANALYSIS OF INTRALUMINAL VESICLE SIZE ACROSS SPECIES

Sayam Ghosal[1,2], Rita Leporati[3], Bora Yilmaz[1,2], Brachyahu M. Kestecher[1,2,4], Bernadett R. Bodnár[1,2,4], Mohamed A. Fattah[2], Luigi Menna[2], Angéla Takács[2], Hargita Hegyesi[2], László Kőhidai[2], Edit I. Buzás[1,2,4], Xabier Osteikoetxea[1,2]
[1]HCEMM-SU Extracellular Vesicle Research Group, Budapest, Hungary; [2]Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary; [3]Division of Medical Oncology, Modena University Hospital, Modena, Italy; [4]HUN-REN-SU Translational Extracellular Vesicle Research Group, Budapest, Hungary

Exosomes, a major subpopulation of small extracellular vesicles (sEVs), arise as intraluminal vesicles (ILVs) within multivesicular bodies, yet the size of these endosomal precursors has never been systematically quantified across species or imaging modalities, and current guidelines assign sEVs a sub-200 nm range without citing primary supporting data. To address this gap, two independent PRISMA-based meta-analyses of published ILV measurements were performed, one using transmission electron microscopy (TEM) data and one using cryogenic electron microscopy (cryo-EM) data, spanning diverse eukaryotic taxa. ILV diameters were extracted from micrographs using scale bar calibration and Feret diameter quantification in ImageJ. sEVs secreted by HEK293T cells were then isolated by differential ultracentrifugation and characterized in parallel by TEM, nanoparticle tracking analysis (NTA), and direct stochastic optical reconstruction microscopy (dSTORM) to test whether ILV size conservation is reflected in secreted vesicles.

The TEM meta-analysis included 40 studies across 13 species (human, goat, rat, mouse, turtle, fish, C. elegans, insects, isopods, crustaceans, fungi, plants, and green algae), yielding a mean ILV diameter of 112.05±26.79 nm, with the smallest ILVs in C. elegans (approximately 25 nm) and the largest in plants (up to 197 nm); no ILV exceeded 200 nm. The cryo-EM meta-analysis, comprising 8 studies across 5 species, gave a lower mean diameter of 70.4±14.4 nm, with only one particle (of 86 measured, in monkey tissue) marginally exceeding this threshold at 205.5 nm. Combined across 48 studies, the overall mean ILV diameter was 100.5±39.12 nm. TEM-derived ILVs were significantly larger and more variable than cryo-EM-derived ILVs, consistent with known fixation-related artifacts of conventional EM. HEK293T-derived sEVs, characterized orthogonally, showed mean diameters of 96.00±14.47 nm by TEM and 123.7±9.507 nm by NTA, with no significant difference between TEM-derived sEV and TEM-derived ILV diameters, while dSTORM confirmed co-localization of the tetraspanins CD9, CD63, and CD81 with phosphatidylserine-based membrane labeling on individual particles. Together, these findings establish, for the first time, a quantitative cross-species and cross-modality framework confirming an ILV size ceiling of approximately 200 nm, supporting the notion that EVs larger than this threshold arise via ectosomal shedding rather than the endosomal pathway, with direct relevance for standardizing MISEV size criteria and refining EV biogenesis models.

STRATEGIC SPONSORS

ANAMET
ANALYTICAL & MEASURING & TESTING

PARTICLE METRIX

ACCELA

IZON

EXODUS

Secretech Biosciences

PREMIUM SPONSORS

BIO-SCIENCE
www.bio-science.hu

BECKMAN COULTER

AURO-SCIENCE CONSULTING

KROMAT

ZEISS

BIO-RAD

RWD

greiner BIO-ONE

ANIMALAB DISCOVERY PARTNER®

everest biolabs

SPONSORS

HSEV Hungarian Section for Extracellular Vesicles

WACŁAW FELCZAK ALAPÍTVÁNY

CzeSEV

SEMMELWEIS UNIVERSITY 1769

SlovSEV SLOVAK SOCIETY FOR EXTRACELLULAR VESICLES