DANISH SOCIETY FOR EXTRACELLULAR VESICLES

6th ANNUAL SYMPOSIUM 2025

DSEV
The Danish Society for Extracellular Vesicles

Thursday 2nd of October 2025

Aarhus Institute of Advanced Studies (AIAS)
Building 1631, Høegh-Guldbergs Gade 6B, Aarhus C

Program

09.00 – 10.00	Registration and breakfast
10.00 – 10.15	Welcome
10.15 – 11.15	**Session 1: (Chair Per Svenningsen)** **Keynote Speaker: Sophie Rome; INRAE, Dep. Human Nutrition, Lab. of Cardiology, Nutrition and Diabetes, Lyon, France:** "Skeletal Muscle-Derived Extracellular Vesicles: Secrets From Within... and Other Stories "
11.15 – 11.45	Coffee break
11.45 – 12.30	**Commercial sponsor session—Technologies (Chair Ole Østergaard)** BD Biosciences, BioNordika, Oleinitec, Proteintech Group, Repligen, Biotechne, AH Diagnostics and Holtra
12.30 – 13.15	Joint photo and Lunch
13.15 – 14.00	Poster session
14.00 – 15.20	**Session 2: "Clinical biomarkers" (Chair Malene Møller Jørgensen)** **Kirstine Kløve-Mogensen, Aalborg University Hospital:** "Towards a Blood-Based Diagnostic Model for Colorectal Cancer Using Extracellular Vesicle Biomarkers" **Camilla Andersen, Roskilde University:** "Characterization of Extracellular Vesicles Derived from MPN patient Sera and Cell Lines Treated with IFNα" **Katja Lund Cliff, Aalborg University Hospital:** "Extracellular vesicle-based miRNA profiles in obese individuals with Metabolic dysfunction-associated steatotic liver disease" **Hannah Weissinger, Aarhus University:** "Single-particle analysis reveals the presence of RNA on the surface of extracellular vesicles" **Louise Søndergaard Rold, North Denmark Regional Hospital:** "The bacterial profile of gut microbiota-derived extracellular vesicles in third trimester in women with and without gestational diabetes mellitus"
15.20 – 15.50	Coffee break
15.50 – 17.10	**Session 3: "New Developments" (Chair Dominic Guanzon)** **Stefan Vogel, University of Southern Denmark:** "Programmed fusion and cargo loading of Extracellular Vesicles as delivery platform" **Søren Roi Midtgaard, Arla Foods Ingredients Group P/S:** "Industrial production and characterization of a whey fraction enriched in extracellular vesicle material" **Anna Sophia Feix, Aarhus University:** "Immunomodulatory effects of Ascaridia galli EVs on chicken peripheral lymphocytes" **Mariena van der Plas, University of Copenhagen:** "Bacterial Extracellular Vesicles Drive Host Defence Peptide Sequestration: A Mechanism for Antimicrobial Inhibition and Immune Dampening" **Bo Have Brøchner, Aarhus University: "**Single-particle tracking analysis of extracellular vesicles for a deconvoluted understanding of uptake and transfection efficiency"
17.10 – 17.50	General Assembly, poster and oral awards, MOVE award
18.00 –	Social Dinner in Kemisk Kantine, Building 1513, Langelandsgade 138 (10 min walk from the venue)

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ORAL PRESENTATIONS:

Towards a Blood-Based Diagnostic Model for Colorectal Cancer Using Extracellular Vesicle Biomarkers.

Kirstine Kløve-Mogensen(1), Rikke Bæk(1), Charlotte Sten(1), Simon Ladefoged Rasmussen(2,3), Maiken Mellergaard(3,4), Ole Thorlacius-Ussing(2,3), Henrik Krarup(3,4), Aase Handberg(3,4), Malene Møller Jørgensen(1,3)

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BACKGROUND: Screening programs for colorectal cancer (CRC) have proven effective in reducing cancer-related mortality. A variety of molecular biomarkers have been proposed for cancer detection in general, and for CRC specifically. Current screening strategies include fecal blood testing. In this study, we investigated the potential of extracellular vesicles (EVs) as plasma-based biomarkers for detection of CRC.

METHODS: We performed an exploratory study of 84 EV biomarkers in plasma samples from 258 CRC patients and 75 colonoscopy-verified healthy controls. Based on the exploratory study a smaller trainings study was conducted containing 21 EV biomarkers who were re-analyzed in the same cohort. This training dataset was used to develop a prediction model, which was validated with a new cohort containing 90 CRC patients and 25 colonoscopy-verified healthy controls.

RESULTS: The exploratory study of 84 different EV biomarkers in the plasma samples made it possible to generate EV profile signatures for the two cohorts. Based on the training study a six-marker (CD36, CD62P, CD81, CD82, CD151 and c-Met) prediction model giving a 10-fold cross validation gave an AUC of 93% (CI: 90-96%). Validation with a new cohort revealed a prediction rate of 97%.

CONCLUSION: EV Array can serve as a potential diagnostic tool for CRC. The findings will be validated with a new validation cohort with 100 new CRC patients and compared with 100 new healthy controls from the Danish Blood Donor Study.

Characterization of Extracellular Vesicles Derived from MPN patient Sera and Cell Lines Treated with IFNα

Camilla Andersen1)

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This study investigates the pro-inflammatory characteristics of EVs derived from cell lines and serum from patients with myeloproliferative neoplasms (MPNs) and the JAK2v617f mutation, and how EVs respond to IFNα-stimulation. MPNs are classified by uncontrolled proliferation of myeloid cells and chronic inflammation. Protein microarray was performed on serum from two cohorts of MPN patients at baseline, 3, 6 and 12 months. Cohort 1 was treated with IFNα only and cohort 2 received IFNα and Ruxolitinib combination therapy. EVs from three cell lines (UKE1, THP1 and SET2) with and without IFNα were also examined. The analysis revealed distinct clustering patterns in protein expression, with much of the variance apparently treatment dependent and with clear distinctions in protein expression between the cohorts. In serum EVs, proteins including IL27RA, CXCR4,

CXCR7 and TGFβR2 were differentially expressed following treatment. The IFNα monotherapy arm showed upregulation of pro-inflammatory markers in EVs after 12 months, potentially mirroring the IFNα induced stress in the microenvironment. miRNA analysis of cell line EVs showed IFNα-induced changes in miR-155, miR-125b and miR-146a at both the cellular and vesicular level. Expression of these miRNAs was upregulated in UKE1 RNA post-IFNα but downregulated in THP1 cells and in both cell line EVs.

Extracellular vesicle-based miRNA profiles in obese individuals with Metabolic dysfunction-associated steatotic liver disease

Katja Lund Cliff(1), Melissa Razo-Azamar(2), Dominic Guanzon(2), Andrew Lai(2), Carlos Salomon(2), Aase Handberg(1,3), Maiken Mellergaard(1,3).

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2) University of Queensland Center for Clinical Research (UQCCR), Brisbane, Australia.

3) Department of Clinical Medicine, Aalborg University, Denmark.

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most frequent chronic liver disorder with a prevalence of 30% in the population. MASLD associates with obesity and an increased risk of developing type 2 diabetes, cardiovascular disease, and hepatocellular carcinoma. Extracellular vesicles (EVs) and their content such as miRNAs strongly associate with MASLD progression, why miRNA profiles are considered potential novel minimal-invasive biomarkers for MASLD. The aim of this project is to characterize EV-based miRNA profiles in obese individuals with MASLD before and during weight-loss intervention. The study population consist of obese individuals with MASLD (n=36), obese control group (n=25), and a lean control group (n=27). RNA extraction and next generation sequencing was performed on EV-enriched plasma samples. Biostatistical analysis revealed that, the liver-specific and MASLD-related miR-122, was significantly upregulated in the MASLD group compared with the obese control group. Additionally, miR-122 correlated with liver fat and levels of the liver enzyme, ALAT and revealed a solid disease predictive ability at baseline. Although, miR-122 did not change during intervention, we found a significant decrease in four MASLD- or HCC-related miRNAs. These findings underline the potential for EV-based miRNA profiles as future minimal-invasive diagnostic tool to identify obese individuals with MASLD, at risk of developing related complications.

Single-particle analysis reveals the presence of RNA on the surface of extracellular vesicles

Hannah Weissinger (1), Madhusudhan Reddy Bobbili (2, 3), Yan Yan (4), Maria Gockert (1), Johannes Grillari (2, 3), Mette Malle (1), Jørgen Kjems (1, 5)

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Confidential abstract

The bacterial profile of gut microbiota-derived extracellular vesicles in third trimester in women with and without gestational diabetes mellitus

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Introduction: Pregnant women with gestational diabetes mellitus (GDM) have an altered gut microbiota, which may contribute to GDM development, though the mechanisms remain unclear. One proposed mechanism is the release of gut microbe-derived extracellular vesicles (GM-EVs) from the gut bacteria. These GM-EVs can cross the gut barrier and have a systemic effect on the host. The aim of this study was to investigate if women with GDM release a GDM-specific bacterial GM-EV profile that have the potential to contribute to GDM development. Methods: GM-EVs were isolated from faecal samples collected in the third trimester of pregnancy from women in the DANish Maternal and Offspring Microbiome (DANMOM) cohort study. The bacterial composition of the GM-EVs and faecal samples they originated from were analysed with 16S rRNA gene sequencing. Nanoparticle tracking analysis was used to investigate particle number and size in the GM-EV samples. Results: GM-EVs from women with and without GDM were similar in size, number, and bacterial diversity. However, based on 16S rRNA gene sequencing, GM-EVs originating from Tyzzerella, Dialister, and NK4A214 group were lower in relative abundance in women with GDM compared to controls. Conclusion We observed differences in GM-EV profiles between women with and without GDM in third trimester of pregnancy. While GM-EVs may contribute to GDM pathophysiology, more research is needed to determine their potential impact on host metabolism.

Programmed fusion and cargo loading of Extracellular Vesicles as delivery platform

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Membrane anchoring of biomolecules is a natural process and observed for proteins and carbohydrates, and essential to functionalize lipid surfaces of biological membranes such as cells and extracellular vesicles (EVs). To engineer the membrane surface of biological lipid bilayers we present lipidated nucleic acids (LiNAs), introducing

programmable functionality to the surface of lipid membranes. LiNAs can be classified as SNARE mimics, whereas SNARE proteins control biomembrane fusion in eukaryotes, LiNAs provides some of the key functionality of SNAREs in a fully programmable fashion for natural and synthetic lipid bilayer membranes. LiNAs exploit the encoding-potential of hybridization for the fusion of liposomes and biological lipid nanoparticles such as EVs. EVs are of particular interest for delivery applications based on their ability to cross biological barriers. The presented fusion platform is suitable for generation of modified EVs for delivery applications and formulation of therapeutic oligonucleotides prepared by fusion between extracellular vesicles (EVs) with synthetic cargo loaded vesicles (e.g., liposomes loaded with oligonucleotides or proteins) resulting in hybrid-EVs (EVLs).We will present the application of the programmable LiNA fusion platform to a range of different EVs types and exemplify mRNA cargo loading which may enable EVLs as broadly applicable delivery platform for therapeutic oligonucleotides and other biologicals.

Industrial production and characterization of a whey fraction enriched in extracellular vesicle material

Søren Roi Midtgaard (1)*, Maria Stenum Hansen (1), Nikolaj Drachmann (1), Xiaolu Geng (1), Kristine Ingrid Marie Blans (1), Manja Mahmens Fabricius Møbjerg (1), Anny F. Frølund (1), Jan Trige Rasmussen (2), Marie Stampe Ostenfeld (1)

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We report a scalable industrial production protocol for a bovine whey-derived ingredient that is highly enriched in EV material using a large-scale sequential ceramic membrane filtration setup. Furthermore, we demonstrate a robust and generally applicable analytical approach to determine the relative contributions of EVs and Milk Fat Globule Membrane (MFGM) using molar ratios of the membrane bound proteins Butyrophilin (BTN) and CD9 as a surrogate markers for MFGM and EVs respectively. Taken together, our findings provides a basis for comparing bovine milk containing foods and aid in developing specialized ingredients that can minimize the compositional difference between infant formula and human milk. See paper for further details DOI: 10.1002/jex2.70044.

Immunomodulatory effects of Ascaridia galli EVs on chicken peripheral lymphocytes

Anna Sophia Feix(1), Konstantinos Papanikolaou(1), Rikke Brødsgaard Kjærup(1), Tina Sørensen Dalgaard(1)

Ascaridia galli (A. galli) is a parasitic nematode causing ascaridiosis in chickens, leading to reduced growth, egg production, and altered immune function. Rising prevalence and potential drug resistance highlight the need for alternative control strategies. Helminth-derived EVs are thought to modulate host immunity, but their role in poultry remains poorly understood. This study compared EVs from adult and larval A. galli for their immunomodulatory effects on chicken peripheral blood mononuclear cells (PBMCs). Adults (n=20/30 ml) and newly hatched L3 larvae (n=60/30 ml) were cultured in EV-depleted medium, and EVs were enriched by size-exclusion chromatography and analyzed by NTA and electron microscopy. PBMCs (n=6) were mitogen-stimulated in vitro with EV fractions for 48 h, and immune cell activation was assessed by flow cytometry. A. galli EV fractions 5–7, which had the highest EV amount, showed general immunomodulatory potential Larval EVs had stronger effects on B-cells, whereas adult EVs mainly modulated T-cell activation. These findings indicate that A. galli EVs exert life stage-specific immunomodulatory effects, likely reflecting distinct molecular cargo. Future proteomic analyses are needed to identify the components underlying these functions.

Bacterial Extracellular Vesicles Drive Host Defence Peptide Sequestration: A Mechanism for Antimicrobial Inhibition and Immune Dampening

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Host defence peptides (HDPs) are key components of the innate immune system, exhibiting broad-spectrum antimicrobial and immunomodulatory functions. Despite their therapeutic potential as alternatives to conventional antibiotics, HDP efficacy is frequently diminished during infection. In this study, we investigated whether bacterial extracellular vesicles (bEVs) contribute to this attenuation. Using bEVs isolated from Gram-positive Staphylococcus aureus and Gram-negative Pseudomonas aeruginosa, we examined their interactions with three human-derived HDPs. Our results demonstrate that bEVs from both species bind the HDPs, thereby neutralizing their antimicrobial activity. Moreover, HDP-bound bEVs exhibited reduced immunogenicity compared to unbound vesicles, suggesting a dual role in immune evasion. The findings support a conserved mechanism whereby bEVs act as decoys, sequestering HDPs to mitigate their antibacterial effects while simultaneously dampening vesicle-induced immune activation.

Single-particle tracking analysis of extracellular vesicles for a deconvoluted understanding of uptake and transfection efficiency

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Introduction: Synthetic nanoparticles such as liposomes and lipid nanoparticles (LNPs) are widely used for drug delivery but are often associated with toxicity and immune responses. Natural extracellular vesicles (EVs) offer a promising, biocompatible alternative. We explore how the cellular origin of EVs influences their uptake by tracking individual vesicles in live cells. Methods: EVs were isolated from C2C12 cell culture, human serum, mouse plasma, and bovine milk, and characterized according to MISEV2018 guidelines. CY5-labeled siRNA was electroporated into EVs, and uptake was quantified using confocal microscopy. For dynamic analysis, EVs with fluorescently labeled membranes were tracked in live cells using HILO microscopy and analyzed with a custom Python script. Results: Snapshot-based quantification revealed comparable siRNA delivery across EV sources. However, live-cell tracking uncovered changes in spatial and temporal behaviors depending on EV origin and recipient cell type, highlighting differences in intracellular trafficking and interaction dynamics. Conclusion: Single-particle tracking provides direct, high-resolution insight into EV-cell interactions and delivery mechanisms. This approach enables mechanistic comparisons between EV types and represents a valuable tool for advancing targeted, EV-based drug delivery strategies.

POSTER PRESENTATIONS

Inflammatory and Metabolic Modulation by Liver-Derived Extracellular Vesicles in Obesity and Metabolic Dysfunction-Associated Steatotic Liver Disease – MASLD

Javier Donoso-Quezada (1), Maiken Mellergaard (1,2), Melissa Razo-Azamar (3,4), Andrew Lai (3,4), Gunna Christiansen (5), Carlos Salomon (3,4), Aase Handberg (1,2)

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As extracellular vesicle (EV) technologies evolve, the study of tissue-specific EVs has emerged as a powerful tool to explore organ-level communication and regulation. In this study, we analyzed the proteomic profile of circulating liver-derived EVs (L-EVs) in individuals with obesity and MASLD (n=36)—a condition marked by excessive hepatic fat accumulation due to metabolic dysfunction—and compared it with obese (n=25) and lean (n=27) controls without MASLD, to assess their role in metabolic and inflammatory disturbances. Magnetic immunoprecipitation targeting liver-specific proteins ASGR1 and CYP2E1 was developed to enrich L-EVs directly from plasma. Proteomic analysis was performed using LC-MS/MS. Differentially expressed proteins (DEPs) were identified between groups, and enrichment analysis was conducted using Gene Ontology and Reactome databases. L-EVs from individuals with obesity and MASLD were enriched in proteins involved in immune processes, including complement activation and humoral immune response ($p<0.01$). In MASLD, L-EVs also contained DEPs related to metabolic pathways, such as triglyceride catabolism and protein metabolism ($p<0.05$), with apparent improvements after weight loss. Notably, several DEPs significantly correlated with clinical variables, including insulin levels, liver fat content, and fibrosis markers. These findings suggest that L-EVs may contribute to MASLD pathophysiology and hold potential as biomarkers for obesity-related complications.

Lipidomic characterization of extracellular vesicles in plasma from obese individuals with metabolic dysfunction-associated steatotic liver disease

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The prevalence of obesity has doubled from 1975 to 2014 and is still increasing worldwide. Obesity associates with many complications including MASLD (metabolic dysfunction-associated steatotic liver disease) which now affects nearly 33 % of the world's population. The potential of extracellular vesicles (EVs) as biomarkers has already been linked with obesity-complications. Investigating EV content, especially lipid composition, is essential to uncover their function and role in intercellular communication during development of MASLD. The study included three groups: Lean Control, Obese and Obese with MASLD. The MASLD group underwent a 5-

month weight loss intervention. Plasma EVs were enriched using the MagNet method and validated by western blot, nanoparticle tracking analysis, and high-resolution flow cytometry. Moreover, lipid composition of lipids extracted from the EV-preparations was analyzed using LC-MS/MS. Preliminary results showed the presence of EV-markers Flottilin-1, Alix, and HSP70 as well as particles with a mean size of 113 nm and a concentration of 4.9 x 10^9 particles/mL. Finally, the untargeted lipidomic analysis identified lipids commonly present in EVs. These findings will contribute to more knowledge about the lipid composition of plasma EVs in obesity and MASLD, while identifying specific lipid EV-species, which may constitute novel biomarker candidates.

Next generation diagnostic of colorectal cancer using extracellular vesicles from plasma

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BACKGROUND: Colorectal cancer (CRC) is one of the most common cancers in the western world. Screening is an efficient method of reducing cancer-related mortality. Molecular biomarkers for cancer in general and CRC in particular have been proposed, and detection of blood in stool are used for CRC screening. We aimed to test whether extracellular vesicles (EVs) can be used as plasma-based biomarkers for CRC detection. METHODS: We conducted a cross-sectional case-control study of 214 CRC patients and 75 colonoscopy-verified healthy controls. EDTA-plasma samples were used and their content of EVs were extensively phenotyped with the use of the EV Array technology (Joergensen et al., 2013, JEV). The markers were grouped into: EV specific, tissue specific, inflammation, and cancer in general. We used multivariable logistic regression to calculate area under the receiver operating characteristics curve (AUC). RESULTS: Analysis of 84 different EV markers in the plasma samples made it possible to generate EV profile signatures for the two cohorts. Multivariate data analysis was performed and revealed that a four-marker model after a 10-fold cross validation gave an AUC of 0.880 (0.837-0.923) with a sensitivity at 0.827 and specificity at 0.794. The four markers are patent pending and cannot be disclosed. CONCLUSION: Using the EV Array technology, we demonstrate how plasma EVs can serve as a potential diagnostic tool for CRC. The findings will be validated on new samples.

Proteomic profiling of extracellular vesicles from CHMP2B-mediated frontotemporal dementia patient-derived cells

Cecilie Madsen (1), Anders Toft (1), Ole Østergaard (2), Emil. E Henriksen1, Jesper V. Olsen (2), Jørgen Erik Nielsen (1), Patrick Ejlerskov (1)

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Frontotemporal Dementia (FTD) is a progressive neurodegenerative disorder caused by neuropathological changes in the frontal and temporal. Familial forms of FTD cover 30-40% of all cases, where a pathogenic variant in the charged multivesicular body protein 2B-gene (CHMP2B) causes a rare form of familial FTD, FTD-3, affecting behavior and executive functions. CHMP2B is part of the endosomal sorting complex required for transport (ESCRT-III), which is important for the endo-lysosomal system and in formation of multivesicular bodies (MVBs). Knowing that exosomes originate from MVBs, we hypothesized that the CHMP2B mutation may influence the proteome of extracellular vesicles (EVs) in FTD-3, and that the EVs could be involved in propagating the disease between brain cells. We test the hypothesis by proteomic profiling of EVs isolated from FTD-3 induced pluripotent stem cell (iPSC)-derived microglia and neurons obtained from FTD-3 patients. The EV proteomes will be compared to EV proteomes from gene corrected controls and from healthy donors. In addition to EVs, we also look at the secreted non-EV fraction and on the whole cell lysates to obtain a comprehensive picture of the effects of the CHMP2B mutation. Focus is on proteins dysregulated in FTD-3, which could potentially be driving the disease pathology, and comprise potential therapeutic targets. The study will provide new insight into the role of CHMP2B by addressing its impact on the proteome of cells and EVs in FTD-3.

Extracellular Vesicle Biology In Vivo: Shedding Light on Non-coding RNA Regulation of Wound Healing

Danna Vo (1), Rikke Halse Østergaard (1), Morten Trillingsgaard Venø (2), Yan Yan (3), Gilles Vanwalleghem (1,4), Kasper Kjær-Sørensen (1), Claus Oxvig (1), Jan Trige Rasmussen (1), Ken Howard (1,5), Jørgen Kjems (1,5), Guillaume Van Niel (6), Frederik Verweij (7), and Yuya Hayashi (1,5)

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Extracellular vesicles (EVs) are hypothesized to play a critical role in regulating biological processes such as regeneration and inflammation. Using zebrafish as a model organism, this study investigates the physiological role of endothelial cell-derived EVs (EcEVs) in wound healing. We have generated a new transgenic line that allows us to image where EcEVs go in response to injury, and profile their non-coding RNA cargo by immunoprecipitation and subsequent small RNAseq as well as digital PCR. Using bioinformatic approaches, we further predict gene targets based on the enriched miRNA cargo and cell type-specific transcriptomes in the context of EV donor-recipient communication. Our findings suggest a supportive function of EcEVs in acute wound angiogenesis. We are currently working on profiling of endothelial cell-specific translatome to assess whether the predicted gene targets are affected in response to wounding.

Tracing the Fate of Glioblastoma-derived Extracellular Vesicles in a Xenograft Model

Jonas Bolt Hansen (1), Camille Menaceur Vandenbroucke (1), Per Svenningsen (2), and Yuya Hayashi (1,3)

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Extracellular vesicles (EVs) mediate intercellular communication by transferring proteins, RNA, DNA, and lipids, thereby regulating differentiation, proliferation, and metastasis. Exosomes are cen-tral to cancer progression, yet tracking EV dynamics is limited, as conventional fluorescence methods cannot selectively label distinct EV populations over time. To overcome this, we designed a cd63-HaloTag construct containing the pH-sensitive marker pHluorin. HaloTag irreversibly binds a tetra-methylrhodamine-linker (TMR), allowing selective fluorescent labeling. Using Tol2 transgenesis in zebrafish, we applied a HaloTag pulse-labeling strategy with pHluorin, TMR, and the blocking ligand 7-bromoheptane (7BR), enabling time-resolved EV tracking. We observed accumulation patterns in vivo in the caudal vein. Building on this system, we aim to trace tumor-derived EVs, particularly from glioblastoma, which drive tumor spread and pre-metastatic niche formation. Zebrafish embryos are an attractive host for xenografting human cancer cells due to their early lack of adaptive immunity. By transfecting glioblastoma cells with HaloTag constructs prior to xenografting into the zebrafish brain, we will monitor the spatiotemporal dissemination of glioblastoma-derived EVs (GEVs) via circula-tion. This model will provide insight into GEV-mediated tumor propagation and evaluate blood-borne GEVs as potential early biomarkers of glioblastoma.

Is Argonaute Inside or Outside of Extracellular Vesicles?

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Extracellular vesicles (EVs) are central to intercellular RNA transport, yet the role of Argonaute 2 (AGO2), the key protein for miRNA stability and function, in this process remains unclear. We previously observed by transfecting the yolk syncytial later (YSL) of zebrafish embryos that fluorescently tagged AGO2 are released into the bloodstream at a high concentration and is sequestered by macrophages as well as scavenger endothelial cells. Interestingly, this distribution pattern follows what is typically observed for EVs derived from the same donor cell (YSL). The unsolved questions are: is extracellular AGO2 inside or outside of EVs? If outside, is it membrane-bound or present as an independent protein complex? To address these questions, this project uses zebrafish as an in vivo model combined with genetic labeling of AGO2-mCherry for live imaging and biochemical isolation. The purified AGO2, with or without non-RNA binding mutations, will be further characterized by nanoparticle tracking analysis, cryogenic electron microscopy, protein and RNA profiling. Our findings will provide insights into the molecular state of extracellular AGO2 and its potential role in miRNA stability and delivery.

The role of extracellular vesicles in ischemic preconditioning of the heart

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Background: Heart failure (HF) is a clinical syndrome marked by structural and functional myocardial abnormalities, often secondary to myocardial infarction [1]. While early reperfusion remains the most effective strategy to limit myocardial injury, it may paradoxically exacerbate damage via ischemia-reperfusion (I/R) injury [2]. To date, no pharmacological therapies have successfully mitigated I/R injury. Transient, non-lethal ischemia applied remotely via limb occlusion (remote ischemic conditioning, RIC) has shown cardioprotective effects [3,4].

Similar protection may be induced through blood-flow-restricted resistance exercise (BFRRE) [5]. These may act through circulating extracellular vesicles (EVs) enriched with regulatory microRNAs, potentially mediating systemic protection [6]. However, the cardioprotective potential and mechanistic role of EVs in HF remain insufficiently characterized. Objectives: This project aims to:•Determine if plasma EVs from HF patients post-RIC/BFRRE confer protection in in vitro and ex vivo models of HF.•Elucidate whether EV-induced protection involves modulation of mitochondrial function, necrosis, and apoptosis. •Identify EV-associated microRNAs mediating cardioprotection through selective loading of vesicles with differentially expressed microRNAs. Results: Pilot data suggest RIC reprograms HF EVs from harmful to protective, enhancing cell survival post-RIC. Disease severity may modulate this effect.

Extracellular vesicles from morphine-exposed prefrontal cortex carry transcriptomic and proteomic signatures of synaptic dysfunction

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Extracellular vesicles (EVs) released by neurons and glial cells play critical roles in intercellular communication within the brain, influencing synaptic plasticity, neuronal survival, and neuropathological processes. Despite substantial evidence linking chronic opioid exposure to neurobiological adaptations, the specific contributions of brain-derived extracellular vesicles (BDEVs) remain poorly understood. In this study, we characterized alterations in the RNA and protein cargo of BDEVs isolated from the prefrontal cortex of rats chronically exposed to morphine. Using integrative analyses combining total RNA sequencing (with ribosomal RNA depletion) and comprehensive proteomics, we identified significant molecular changes involving pathways related to synaptic plasticity, ER stress, mitochondrial dysfunction, and neurodegeneration. Notably, morphine exposure induced the modulation of the synaptic regulator ARC and the ER stress marker Hspa5 at both the transcript and protein levels in BDEVs. Functional validation in primary neuronal cultures further demonstrated that morphine-derived BDEVs modulate the expression of neuropathology-associated genes, including Ncam1 and Kcnn1, which play established roles in synaptic remodeling and neuronal excitability. Together, these findings suggest that BDEVs may be central players in mediating morphine-induced neuronal adaptation, highlighting their potential as biomarkers or therapeutic targets in opioid-related neuropathologies.

Extracellular vesicles in the aging peripheral nerve

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Biological tissue-derived extracellular vesicles (EVs) represent an interesting tool to investigate the dynamics of complex microenvironments, providing information on the intercellular communication within an organ, and how this changes in pathological processes. Peripheral nerve-derived EVs are a largely unexplored topic. Here, we validated a protocol to isolate EVs from murine sciatic nerves with good purity and yield, and we performed basic characterization of nerve-derived EVs (nEVs), including analyses of sizing and concentration, morphology and markers expression. Aging is a naturally occurring condition characterized by progressive deterioration of the organism and loss of organ functions, including in the peripheral nerves. As senescence affects many aspects of cellular physiology, it might result in a change of nEVs functionalities. We isolated nEVs from young and aged mice, and we compared basic features as well as molecular cargoes of nEVs from the two conditions. We hypothesized possible roles of nEVs, aiming to link changes in nEVs content to changes in peripheral nerve tissue during aging.

Sex- and Genotype-Specific Extracellular Vesicle Signatures in Alzheimer's Disease Risk

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Extracellular vesicles (EVs), including exosomes, are key mediators of intercellular communication in the brain. In Alzheimer’s disease (AD), they facilitate the propagation of amyloid-β and tau pathology while modulating neuroinflammation, synaptic health, and neuronal degeneration. With microglia emerging as central players in AD, recent evidence suggests that the molecular cargo of microglia-derived EVs, including lipids, proteins, small RNAs, and surface molecules, is altered in AD and may vary according to APOE genotype and biological sex.This project aims to characterize EV signatures associated with APOE genotype in high- versus low-risk AD, using isogenic induced pluripotent stem cell (iPSC)-derived microglia carrying APOE2/2, APOE3/3, APOE4/4, and APOE knockout variants. Multi-omic profiling (proteomics, lipidomics, and small RNA sequencing) combined with imaging and biophysical characterization will identify sex- and genotype-dependent EV features. Functional studies will test how microglia-derived EVs influence neuronal survival, synaptic integrity, and the spread of amyloid-β and tau pathology in co-culture and organoid models. Together, this work will provide new insight into the role of microglial EVs in intercellular communication and AD progression, while aiming to identify sex- and genotype-specific biomarkers or therapeutic targets.

Pre-Analytical Optimization for Extracellular Vesicle Analysis: Reducing Platelet-Derived Artifacts.

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Pre-analytical variables influence the reliability of extracellular vesicle (EV) analysis in blood, particularly due to platelet activation and release of EVs during sample handling prior to storage. This study aims to systematically evaluate how different pre-analytical conditions affect EV composition in plasma, with a focus on minimizing platelet-derived EVs. Blood samples will be obtained from six healthy fasting donors to ensure a controlled baseline. EDTA and citrate blood collection tubes will be compared and 3 centrifugation strategies will be assessed. Before centrifugation, samples will be analyzed on a Sysmex routine hematology analyzer to obtain platelet counts as a baseline control. After completion of the centrifugation procedures, platelet counts will be determined using a counting chamber. Additionally, the impact of delayed processing by storing samples at room temperature for 1, and 3 hours prior to centrifugation will be investigated. EVs will be characterized and quantified using high-resolution flow cytometry, with platelet-derived EVs identified by CD41 and Lactadherin, while CD9 and CD81 serve as general EV markers.By correlating EV yield with handling protocols, we aim to establish reproducible workflows that minimize artefacts and enhance the diagnostic utility of EVs. We hypothesize that high-speed or double centrifugation reduces platelet-derived EVs after storage.

Cell Type–Specific Extracellular Vesicles from Human Plasma

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Stroke is a leading cause of death worldwide, with acute ischemic stroke (AIS) accounting for ~85% of cases and intracerebral hemorrhage (ICH) constituting the rest. Approved treatments exist only for AIS and must be initiated within 4.5–6 h, while acute blood pressure lowering benefits ICH. However, therapies for one subtype can be harmful for the other, underscoring the urgent need for reliable diagnostic biomarkers. Extracellular vesicles (EVs) have emerged as promising candidates, as their subpopulations reflect the state and biomolecular signature of their parent cells, offering potential for disease monitoring. Here, we developed and validated a protocol to isolate EV subpopulations from human plasma based on cell origin. Using magnetic bead–based immunocapture targeting cell surface markers, we enriched specific EV subsets and assessed their protein signatures by proteomic analysis. Each EV subpopulation displayed unique cell-specific fingerprints. Enrichment of EV-associated (e.g., CD9) and cell-specific markers (e.g., CD31, CD56) confirmed specificity of the approach, while nanoparticle tracking analysis showed distinct size distributions consistent with different cellular origins. These findings demonstrate selective isolation of cell type–specific EVs from plasma and establish a methodological framework for future studies into stroke-related EV signatures, with potential to support differential stroke diagnosis.

Visualization and Characterization of Neuron-Derived Extracellular Vesicles in Spinal Cord Injury

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Extracellular vesicles (EVs) play diverse roles in cellular communication and have therefore attracted extensive scientific scrutiny. However, isolating tissue-specific EVs remains challenging due to their heterogeneous and fragile nature. To address this, we have developed a method for in vivo tracking and subsequent isolation of tissue/cell-specific EVs in zebrafish embryos. It utilizes a transgene cassette driving expression of truncated tetraspanin cd63 (Δ110-237) fused to mCherry, integrated into the host genome via the Tol2 transposon system. Successful EV labelling was confirmed by tissue-specific transfection, where the yolk syncytial layer (YSL) produced mCherry-positive EVs released into the blood. A TEV-protease recognition site between G4S linkers of the construct enabled cleavable affinity purification using anti-RFP beads, with enrichment and detachment confirmed via nanoparticle tracking analysis. Using this construct coupled to a neuron-specific regulatory element (elavl3), we were able to label neuron-derived EVs (NEVs) in a mosaic pattern through transient transgenesis. We are currently working on imaging the dynamics between NEVs and macrophages during early inflammation in spinal cord injury (SCI), complemented by characterization of affinity-purified NEVs. Our construct is shorter than the Snorkel tag and proves readily applicable to generation of new transgenic zebrafish lines with a fluorescent reporter as a next-generation EV labelling approach in vivo

Development of reporter protein for dynamic labeling of cell type-specific extracellular vesicles.

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Introduction: Emerging evidence suggests that metabolism is modified by organ system crosstalk using extracellular vesicles (EVs). Yet, the mechanisms in living animals are largely unknown. We hypothesized that a reporter protein enabling the tagging of cell type-specific EV could be used to gain insights into the in vivo dynamics of intercellular EV transfer. Methods: We designed two different EV reporter proteins based on CD9, an alfa tag and the peroxidase APEX2. The constructs were investigated by stable transfection of HeLa cells. The labeling of cells and EVs by APEX2 activity was tested by tyramide-biotin. EVs were isolated by polyethylene glycol 6000 (PEG) precipitation and fractionated using Size Exclusion Chromatography (SEC) followed by western blotting. Immunoprecipitation (IP) was performed using ALFA Selector ST magnetic beads or Recombinant anti-ALFA conjugated magnetic dynabeads. Results/Summary: Our result shows the potential of both CD9-APEX2 constructs in vitro as the EVs compose the fusion protein within membrane. Furthermore, we successfully performed IP targeting the ALFA tag, though CD9-APEX2 containing the last domain, showed increased promise as vast greater amount were precipitated upon investigating CD63 expression. Lastly, this indicates the APEX2 resides on the outside of the EV membrane. Our results indicate that CD9-APEX2 allows the labeling and affinity isolation of cell type-specific EVs. Next, we aim to investigate liver derived EVs in vivo

Choosing between Glycolysis and Cholera: new methods for detecting EVs in non-model organisms

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There are currently no universal biomarkers for extracellular vesicles (EVs), delaying progress in the field, especially research using non-model organisms. In an attempt to develop cost-effective options for the detection of small EVs (sEVs) from non-model organisms, we explored both lipid-based and protein-based markers. Hereby, we uncovered that cholera toxin subunit B (CTxb), used to target the glycosphingolipid GM1 in vertebrates, also binds to sEVs from various non-model organisms and can be used to detect these sEVs. However, while promising in detecting EVs from cell culture and parasite media, CTxb is unsuitable for complex biofluids such as serum and plasma due to cross-reactivity with high- and low-density lipoproteins.Additionally, we showed that the universally conserved glycolytic enzyme enolase is found in sEVs from phylogenetically distant species and through its enzymatic activity enables detection of sEVs. Notably, sequence alignment revealed conserved regions across human and several representative non-model organism enolase orthologues, which may permit more general use of antibodies against the human orthologue.Together CTxb and enolase thereby provide a tandem method for detection of sEVs from non-model organisms by targeting different components of the sEV.

DRONE – Delivery of RNA Origami Nanoscaffolds via Exosome

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Extracellular vesicles (EVs) have emerged as promising tools for RNA-based drug delivery. However, loading of RNA into EVs remains a challenge. Using zebrafish as a model system, we genetically engineer EVs to enable endogenous packaging of RNA in vivo. We have designed constructs expressing cd63-mEGFP which anchor the engineered RNA-binding domain PUFe to the inner EV membrane. Furthermore, constructs for transcribing mRNA harbouring specific PUFe binding sites (8-nt motif) have been generated. These components were co-injected into the yolk syncytial layer (YSL) of zebrafish embryos for transfection to test loading of the mRNA into YSL EVs. Preliminary imaging showed partial co-localisation of YSL EVs and the translated product of the loaded mRNA in the caudal tissue, where circulating YSL EVs are sequestered. We are currently working on expression and loading of circularised RNA aptamers, with the ability to bind a specific fluorogen, as a fluorescent tracer and indicator of structural integrity. This is the first step towards a more complex scaffolding of RNA realised by programmable co-transcriptional "origami" folding for highly modular theranostics. The ultimate goal of this project is to harness the natural targeting ability of EVs in combination with RNA origami loading to achieve DRONEs, homing nanomachines inspired by EV biology.

Spatial proteomics of the extracellular vesicle corona

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The concept of protein corona formation around extracellular vesicles (EVs) has given birth to new insights into how cells may recognize EVs by proteins presented at the EV surface. Here we present spatially resolved

proteomics to map proteins interacting at the EV surface, without a need for physical isolation of the EV-corona complexes. Protein-protein interactions can be studied by proximity labelling such that the biotin ligase TurboID promiscuously biotinylates nearby proteins within a few nm distance from the fused "bait" protein. We genetically engineered EVs by modifying CD63 as the bait protein to which TurboID was fused facing outward to map EV corona proteins. Biotinylated proteins were then analyzed by Western blotting and tandem mass spectrometry. Western blots reveal protein patterns that are distinct depending on the localization of the fused TurboID. The preliminary mass spectrometry analysis identified many of the proteins commonly known to form a corona around synthetic nanoparticle, also supporting those previously reported through the physical isolation approaches. We are currently working on EV corona mapping in vivo using zebrafish as a model organism. This approach has the potential to revolutionize our understanding of EV biology by shifting the focus from the EVs themselves to the proteins that make up the corona, or how the cell "sees" them, in analogy with the biomolecular corona extensively characterized for synthetic nanoparticles.

RNA sequencing analysis of human urine extracellular vesicles in response to Na+ diets

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Urine extracellular vesicles (uEVs) originate from the genitourinary system, including the kidney's tubular epithelial cells. These cells control Na+ balance by, e.g., increasing aldosterone-induced Na+ reabsorption in response to a low Na+ intake. We hypothesized that the uEV transcriptome reflects the physiological regulation of the tubular epithelial cells by a Na+ diet. We used previously collected paired samples from young healthy men (median age: 22.8 yr, n = 10) after a 5-day low (70 mmol/day) and high (250 mmol/day) Na+ diet and analyzed the samples by RNA sequencing. Of the 20 samples, 17 produced high-quality data, yielding quantitative data for >10,000 genes. The two Na+ diets only affected the uEV abundance of 9 gene transcripts; 5 were decreased, and 4 were increased, including SLC12A3, encoding the Na+, Cl- transporter NCC, in low-Na+ diet sample uEVs. We used transcriptome deconvolution to estimate the uEVs' tissue and cell type origin. The uEVs were mainly derived from the kidneys and bladder. The low-Na+ diet samples had a ~30% higher kidney-derived uEV abundance, primarily derived from proximal tubular cells. The proximal tubule uEV abundance was negatively correlated with the fractional excretion of Na+, and proximal tubular and principal cell uEVs were positively correlated with urine aldosterone levels. In conclusion, our study demonstrates that the uEV RNA analyses can be used to infer physiological regulation of Na+ reabsorption in the human kidney.

Bead-Based PPLC Gradient Isolation and Characterization of Extracellular Vesicles from Human BA8 Cortex

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Extracellular vesicles (EVs) are key mediators of cell–cell communication and potential biomarkers in neuropsychiatric disorders. Here, we standardized a protocol to isolate EVs from BA8 of the dorsolateral prefrontal cortex in control, substance use disorder (SUD), and SUD+major depressive disorder (MDD) groups

(18–60y, HIV−, without infectious diseases), provided by the University of Texas Health Science Center at Houston Brain Collection. Tissue was digested overnight with Collagenase B and subjected to serial centrifugation. Clarified homogenates were fractionated by PPLC in a 30×1cm Sephadex G10–G100 bead gradient, yielding 192 fractions (200 µL) analyzed by 3D UV-Vis, and pooled in F1–F7, que foram entao characterized by Zetaview. EV-rich fractions (F1–F3) displayed the highest particle concentrations, peaked in F2 (7.17×10^{10}/mL) compared to F1 (1.57×10^{9}), F3 (4.37×10^{9}), F4 (4.50×10^{8}), F5 (2.23×10^{9}), F6 (2.53×10^{8}), and F7 (1.47×10^{8}). Particle sizes ranged 120–210.6 nm, with F2 showing the lowest mean (140 nm) and F7 152.5 nm. Zeta potential was more negative in EV fractions: F1: −32.66, F2: -33.48, F3: -30.82, compared to F4: -21.26, F5: -27.79, F6: -24.85, F7: -23.42. No physicochemical differences were found between control, SUD, and SUD+MDD groups. This bead-based PPLC workflow provides a reproducible and validated approach for isolating EVs from human cortex, enabling downstream molecular studies in psychiatric conditions.

A diagnostic tool to distinguish between acute ischemic stroke and intracerebral hemorrhage in a prehospital setting

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Stroke is a leading cause of death and disability worldwide, requiring rapid and accurate diagnosis to guide treatment. Differentiating between acute ischemic stroke (AIS) and intracerebral hemorrhage (ICH) in the prehospital setting remains a major clinical challenge. This study investigates the potential of extracellular vesicle (EV)-associated biomarkers for early stroke subtype differentiation. Plasma samples from 389 patients with suspected stroke were collected in the ambulance and upon hospital arrival. Using EV Array, a high-throughput protein microarray platform, we analyzed 48 EV-associated biomarkers alongside key patient characteristics. A benchmark model incorporating age, sex, and PreSS scores achieved AUCs above 0.55 for both AIS and ICH. Preliminary results suggest that EV markers may enhance diagnostic accuracy and support prehospital triage. Further validation and refinement of biomarker panels are ongoing. These findings support the development of a rapid diagnostic tool for stroke subtype identification, potentially enabling earlier and more targeted treatment interventions.

Long-Read Profiling of EV and Cell-Free DNA Methylation to Distinguish Normal Glucose Tolerance and Gestational Diabetes Mellitus

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Background and aim: Gestational diabetes mellitus (GDM) develops during pregnancy and is linked to adverse maternal-fetal outcomes, yet current screening typically occurs mid-gestation. Circulating extracellular vesicles (EVs) and cell-free (cf) DNA carry pregnancy and placenta derived epigenetic information. By profiling genome-wide methylation of circulating EVs, we aimed to define methylation signatures in pooled plasma samples that distinguish Normal Glucose Tolerance (NGT) from GDM. Material and methods: EVs were isolated by size-exclusion chromatography and characterised by NTA and BCA. EV-DNA and cell-free DNA (cfDNA) were extracted (Qiagen) from three pooled NGT samples and three pooled GDM samples, with each pool consisting of equal plasma volumes from 10 patients. Oxford Nanopore long-read sequencing quantified 4mC/5mC/5hmC/6mA and mutations (Dorado); differential methylation was analysed in R. Results (if obtained to-date): EV-DNA was comparatively long/intact relative to cfDNA. Distinct methylation profiles separated NGT from GDM in both fractions. cfDNA pools yielded a larger number of differentially methylated sites, consistent with higher input and coverage, whereas EV-DNA showed lower yield/coverage but complementary signals. Conclusion (and perspectives): Long-read methylome profiling of EV-DNA and cfDNA from pooled maternal plasma differentiates NGT and GDM, supporting the development of minimally invasive epigenetic biomarkers for earlier risk stratification.