DANISH SOCIETY FOR EXTRACELLULAR VESICLES

5th ANNUAL SYMPOSIUM 2024

DSEV
The Danish Society for Extracellular Vesicles

Thursday 3rd of October 2024

Aalborg University (AAU INNOVATE)
Thomas Manns vej 25, 9220 Aalborg Ø

Program

09.00 – 10.00	Registration and breakfast
10.00 – 10.15	Welcome
10.15 – 11.15	**Session 1: (Chair Malene Møller Jørgensen)** **Keynote Speaker: Cecilia Lässer; Gothenburg University, Sweden:** "Detangling the heterogeneity of extracellular vesicles"
11.15 – 11.45	Coffee break
11.45 – 12.30	**Commercial sponsor session—Technologies** Acousort, Izon, BioXpedia, Omiics, International Flavors & Fragrances
12.30 – 13.15	Joint photo and Lunch
13.15 – 14.00	Poster session
14.00 – 15.20	**Session 2: "Clinical biomarkers" (Chair Suzette Sørensen)** **Christian Mirian, Copenhagen University: "**PECAM1/CD31 in cerebrospinal fluid-derived extracellular vesicles is associated with childhood central nervous system lymphoblastic leukemia" **Johann Mar Gudbergsson, Aarhus University: "**Malignant ascites-derived extracellular vesicles present myeloid cell signatures in metastatic ovarian cancer" **Malene M. Jørgensen, Aalborg University Hospital:** "A Promising Novel Microarray Analyzer for Prediction of Obstetric Syndromes using Multi Surface Biomarkers on Extracellular Vesicle from Maternal Plasma" **Lee-Ann Clegg, Aalborg University Hospital:** "EV biomarker discovery for ultra-early differential diagnosis of stroke"
15.20 – 15.50	Coffee break
15.50 – 17.10	**Session 3: "Technical advancement" (Chair Jaco Botha)** **Jørgen Kjems, Aarhus University:** "Programmable RNA loading of extracellular vesicles" **Javier Donoso-Quezada, Aalborg University Hospital:** "High-resolution flow cytometry approach to evaluate the recovery yield of extracellular vesicles during isolation" **Mads Zippor, Aarhus University:** "One-step precipitation for removal of low-density lipoproteins from extracellular vesicle preparations" **Yuya Hayashi, Aarhus University:** "Decrypting the "Blood-Streamed" RNA Communication in Tissue Regeneration"
17.10 – 17.50	General Assembly, poster and oral awards, MOVE award
18.00 –	Social Dinner in Faculty Club

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ORAL PRESENTATIONS:

PECAM1/CD31 in cerebrospinal fluid-derived extracellular vesicles is associated with childhood central nervous system lymphoblastic leukemia.

Christian Mirian[1,2], Ole Østergaard,[2] Maria Thastrup[1], Signe Modvig[3,4], Hanne Vibeke Marquart[3,4], Bodil Als-Nielsen[1], Birthe Lykke Thomsen[1,5], René Mathiasen[1,4], Jesper Velgaard Olsen[2*] and Kjeld Schmiegelow[1,4*]

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Introduction: The mechanisms that enable migration and subsequent tropism of acute lymphoblastic leukemia cells (ALL) in the central nervous system (CNS) are not well understood. We aimed to investigate the cerebrospinal fluid (CSF) extracellular vesicle (EV) proteome in pediatric patients with vs without CNS leukemia.

Malignant ascites-derived extracellular vesicles present myeloid cell signatures in metastatic ovarian cancer

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Malignant ascites occurs in most patients with high grade serous ovarian cancer (HGSOC) and correlates with poor disease prognosis. The primary tumor located in the ovaries starts spreading to the peritoneal cavity and omentum, ultimately resulting in late-stage dissemination to other organs via transcoelomic routes. The peritoneal cavity is a fluid-filled lymph-immune environment thought to participate in HGSOC progression, including resistance to treatment and metastasis. In this study, we interrogate the small extracellular vesicles (sEVs) present in peritoneal fluid and malignant ascites from mice with advanced ovarian cancer and human HGSOC patients using mass spectrometry. We find that some of the predicted functions and the origin is associated to the innate immune system with several macrophage-specific markers present.

A Promising Novel Microarray Analyzer for Prediction of Obstetric Syndromes using Multi Surface Biomarkers on Extracellular Vesicle from Maternal Plasma

Marei Sammar[1], **Malene Møller Jørgensen[2,3]**, Rikke Bæk[2], Jenni K. Sloth[2], Rami Sammour[4], Manu Vatish[5], Hamutal Meiri[6]

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6) TeleMarpe Ltd, Tel Aviv, Israel

Background: Placental-derived EVs are facilitate intercellular communication between the feto-placental unit and the mother. The main objective was to evaluate a novel microarray analyzer for profiling the differential expression of surface biomarkers on circulating plasma EVs with a power to predict preterm delivery (PTD) and preeclampsia (PE) compared to term delivery (TD) controls.

Methods: Maternal blood was collected from pregnant women with PTD, PE and matched TD controls. Plasma samples were overlayed on the microarray glass slides previously printed with a wide panel of antibodies to EV surface receptors. For detection, captured EVs were marked with antibodies specific to EVs (CD9, CD63, CD81) or to placental EVs (PLAP and PP13). The fluorescent signal was measured and analyzed for sensitivity and specificity using area under the receiver operating characteristic curves (AUROCs). Results were validated by comparison with EVs purified using standard procedures.

Results: Distinct profile of surface receptors expressed on total EVs and PEVs of PE, PTD, and TD were identified. The multiple marker analysis generated high prediction accuracy with 90% sensitivity and specificity according to surface receptor relevant to inflammation (TNF RII), relaxation (PP13), and immune-modulation (LFA1), cell adhesion (ICAM), immune suppression and general EV markers (CD81, CD82, and Alix), the complement activation cascade (C1q) and autoimmunity.

EV biomarker discovery for ultra-early differential diagnosis of stroke

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Today, stroke cannot be diagnosed based on a physical examination alone but requires a brain scan at the hospital. This delay in treatment initiation may lead to progression of brain injury and long-term disability. Therefore, time between stroke onset and treatment should be minimized to improve the treatment outcome. Several EV-based biomarkers for stroke detection have been investigated, however, none are applied in clinical practice. In this study we investigated specific EV biomarkers potential use as an ultra-early stroke diagnostic with the goal to speed up stroke treatment initiation. These biomarkers were identified on EVs derived from 1200 plasma samples from stroke patients with EV Array. Three samples from each patient were also used for investigation of biomarker distribution over time. The samples were drawn at <4 hours from stroke onset in the ambulance, repeated at the hospital and again 24 hours after stroke. The biomarker abundance was used to create a stroke prediction model that may distinguish between intracerebral hemorrhage from acute ischemic strokes and non-vascular diseases.

Programmable RNA loading of extracellular vesicles

Mette G. Malle[1], Ping Song[1], Philipp M. G. Löffler[2], Yan Yana, Nazmie Kallisi[2], Julián Vakero [1], Stefan Vogel[2] and **Jørgen Kjems [1]**

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Liposomes and lipid nanoparticles (LNPs) are extensively utilized for drug delivery, yet they suffer from drawbacks such as toxicity and adverse immune reactions. Natural extracellular vesicles (EVs) offer promise as native, safe, and versatile carriers for drugs. However, incorporating large molecules into EVs remains challenging. In this study, we introduce a method for loading EVs by utilizing DNA-mediated fusion between EVs and liposomes loaded with mRNA. We assess loading and fusion efficiency at the individual particle level using real-time TIRF microscopy.

Liposomes containing mCherry mRNA are fused with EVs extracted from C2C12 cell culture medium. The EVs are anchored to a surface by lipidated DNA strand complementary to a biotin-DNA handle on the surface. This enables liposome fusion with surface-tethered EVs, followed by simple removal of non-fused liposomes by thoroughly washing. Fused EVs can then be released and collected using a DNA sequence for toehold release, providing an inherent fusion and purification strategy.

To evaluate the transfection efficiency of the hybrid EV-liposome particles, we delivered the fused hybrid EV-liposome encapsulated mRNA encoding mCherry to HEK cells. Significant mCherry expression was observed in the hybrid particles, whereas control groups with pure liposomes containing mCherry showed no expression.

We anticipate that this method represents a significant advancement for targeted EV-mediated therapy and natural drug delivery.

High-resolution flow cytometry approach to evaluate the recovery yield of extracellular vesicles during isolation

Javier Donoso-Quezada[1], Rikke Bülow Eschen$_1$, Ayad Babaee[1], Malwina Ulanowska[1], Claus Vinter Bødker Hviid[1,2], Maiken Mellergaard[1,2], Aase Handberg[1,2].

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As applications of extracellular vesicles (EVs) advance in biomedical research, standardizing high-throughput methods for obtaining high-purity EV preparations from biological fluids remains challenging, particularly for multi-omics studies. Various isolation methods have been developed, including differential centrifugation, ultrafiltration, and commercially available options like polymer precipitation, size exclusion chromatography (SEC), and immunoprecipitation (IP). However, choosing the ideal method for a particular application is complicated by limited available data on EVs recovery yields.

In this study, we evaluated the recovery yield of EVs from human plasma using high-resolution flow cytometry (hFCM). EVs were labeled with fluorescent-conjugated CD9 and CD81 antibodies directly in plasma and after SEC and magnetic IP isolation. Stained samples were analyzed by hFCM, estimating EV concentrations in the initial plasma, different SEC fractions, and post-IP isolation. The total number of CD9+/CD81+ EVs was calculated to determine the isolation yield.

Our results demonstrate that hFCM is a reliable and robust method for assessing EV concentration in both plasma and isolated EVs preparations. Moreover, SEC and IP methods result in significant EVs losses, highlighting the importance of carefully selecting the isolation method to achieve the necessary EV concentration and purity for downstream analyses.

One-step precipitation for removal of low-density lipoproteins from extracellular vesicle preparations

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When studying extracellular vesicles (EVs), the co-purification of low-density lipoproteins (LDL) represents a challenge to determining EV biology. LDL is present in plasma at concentrations several orders of magnitude greater than EVs and shares similarities with EVs regarding size, density, and lipid composition. Size exclusion chromatography (SEC), one of the most widely used isolation techniques for EVs, is effective at removing HDL, but not LDL. Problematically, contamination with LDL interferes with downstream applications such as proteomics and functional assays. Therefore, we aimed to develop a protocol to selectively remove LDL from plasma samples without the loss of EVs.

Our initial studies aimed to deplete LDL via selectively targeting EV enriched sphingolipids for EV capture. Capture and detection assays using a combination of immunoblotting and Leprechaun SP-IRIS EV analysis revealed significant cross reactivity between EVs, LDL and HDL when using ligands specific for GM1 gangliosides, which are enriched in lipid rafts in EVs. Therefore, a second approach was developed that enabled the precipitation of LDL directly from plasma using a simple one-step procedure prior to SEC.

Using our precipitation buffer, we were able to eliminate LDL to undetectable levels in the supernatant, as assessed by Western blot. Importantly, CD63 positive EVs were retained in the supernatant, although some were lost to the pellet.

Decrypting the "Blood-Streamed" RNA Communication in Tissue Regeneration

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We have previously presented in vivo imaging of endothelial cell-derived extracellular vesicles (EcEVs) using a zebrafish model. EcEVs, visualized by a fluorescent reporter, immediately accumulate at a wound created by a needle-stick injury of blood vessels. Striking is the rapidity of this process observed already at 2 min post-

wounding, leading us to formulate a hypothesis where physiological EcEVs provide first aid to cells of the same kind without tuning of the cargo.

Here we have developed methods to isolate EVs from zebrafish embryos, with or without immunoprecipitation to enrich EcEVs, for miRNA cargo profiling and mRNA target prediction. We first show selective enrichment of 81 miRNAs in crude EVs that in general have more, and often overlapping, potential mRNA targets than those enriched in the cellular pool. If a certain set of miRNAs are selectively packaged to have shared targets, it may provide a predefined problem-specific support beyond basal gene regulation. To explore this possibility on the basis of wound-induced EcEV sequestration, we affinity-purified EcEVs, in which 21 miRNAs were enriched versus the flow-through. The context was also given to mRNA target prediction by filtering the output with the endothelial cell transcriptome extracted from Zebrahub (a single-cell RNAseq database). We have thus identified candidates of several miRNAs and their shared targets that may play a role in acute wound angiogenesis.

POSTER PRESENTATIONS

Extracellular vesicles to predict outcome in traumatic brain injury

Ayad Babaeea[a,b], Thea O Wichmann[b], Mikkel M Rasmussen[a,c], Ole Brink[d], Dorte Aalund Olsen[e], Lars Borris[d], Maj Lesbo[f], Rikke Wehner Rasmussen[g], Maiken Mellergaard[g,h], Aase Handberg[g,h], Claus VB Hviid[a,b,g,h]

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Traumatic brain injury (TBI) is a leading cause of mortality and morbidity among young adults. The potential for survival and recovery is notoriously difficult to predict, which has fueled search for biochemical biomarkers. Extracellular vesicles (EVs) are shed from the injured brain tissue and could have biomarker potential in TBI. However, large-scale longitudinal studies that explore this are not yet available.

In this study, trauma patients with TBI (n=79) and trauma patients without TBI (n=21) were included and blood samples collected 0h, 15±2, and 72±4 hours after hospital admission. EVs were harvested from citrated plasma, using 70 nm qEVoriginal size exclusion columns. The EV cargo and plasma levels of the nerve-derived biomarkers Tau, Neurofilament Light Chain, Glial Fibrillary Acidic Protein, and Ubiquitin C-Terminal Hydrolase L1 was analyzed using the Neurology 4-plex assay on the ultra-sensitive Single Molecule Array (Simoa)™ platform.

Levels of nerve-derived biomarkers in EVs and plasma will be compared between the groups. Moreover, we will assess the association between biomarker levels in EVs and plasma with clinical TBI severity, neuroradiological findings, medical and surgical interventions will be analyzed. Finally, the prognostic ability of EVs and plasma biomarkers for 1-year mortality and 6–12 months functional outcomes will be analyzed. This study explores the potential of EVs as biomarkers in TBI to better understand their role in prognosis and outcome.

What the Cell "Sees" in Extracellular Vesicle Biology

Camille MENACEUR-VANDENBROUCKE [(1)], Yuya HAYASHI [(1)].

1) Molecular biology and Genetics department

Protein-protein interaction occurs at the surface of extracellular vesicles (EVs) forming a dynamic "corona" that is known for ages in the field of synthetic nanoparticles. The concept of the EV-protein corona has emerged as a very important topic because the proteins that make it up, not the EVs themselves, may be at the origin of cellular processes and induce responses in the cell targeted by these EVs. The question is: how can we study the EV-protein corona? This project proposes a combination of emerging technologies: 1) enzyme-catalysed proximity labelling for protein-protein interactions, 2) protein labelling on the extracellular loop of a transmembrane protein on EVs, and 3) zebrafish embryos as a model for studying tissue- or cell-type-specific EVs in vivo. Initial results show the effectiveness of TurboID technology expression on EVs, making it possible to analyze the protein-protein interaction. The technology behind this project is a unique idea that seeks to redefine the biology of EVs with a new dimension, fundamentally from the point of view of the cell.

Cytoprotective effect of conditioned extracellular vesicles in stroke

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Stroke is the second leading cause of death globally, with 85 % of cases being ischemic. Ischemic stroke leads to oxygen and glucose deprivation in the brain, causing rapid cell death and tissue injury. Human brain microvascular endothelial cells, which line the blood-brain barrier, are first affected, compromising the barrier's integrity and function.

Remote ischemic conditioning (RIC) has emerged as a non-invasive stroke treatment promoting neuroprotection. RIC induces the release of extracellular vesicles (EVs). EVs are crucial for intercellular communication and hold therapeutic potential due to their ability to cross the blood-brain barrier. Preliminary findings show the effect of RIC-EVs in vitro and in vivo with higher concentrations of EVs from endothelial and red blood cells post-RIC. However, the precise protective mechanism of these EV subpopulations remains unclear.

This research aims to elucidate the cytoprotective mechanisms of RIC-induced EVs in stroke by isolating and characterizing specific RIC-EV subpopulations. EVs from endothelial and red blood cells are isolated from RIC plasma using cell type-specific antibodies with MACS nanobeads. Their biological effect is evaluated in an oxygen-glucose deprivation assay, mimicking stroke conditions, using immortalized human brain microvascular endothelial cells. Understanding these mechanisms may lead to novel therapeutic strategies to attenuate stroke-related damage and improve patient outcomes.

Extracellular vesicles in milk as biological modulators of gut development and function during early life

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In milk, the load of miRNAs, enclosed in extracellular vesicles (EVs), is reported to be involved in maturation of the gut and immune system in newborns. Research shows that, compared to human milk, nourishment with infant formula increases the risk of intestinal pathologies – particularly in preterm infants. Given the large resemblance between the miRNA profiles in human and bovine milk, we hypothesize that bovine milk EVs have a gut protective role across species. Today, industrial processes used in infant formula production diminish the bovine EV and miRNA content. Within the GutBioMod project, we hypothesize that supplementing infant formulas with a bovine milk EV and miRNA-containing ingredient will improve infant gut development and reduce the potential negative effects of formula feeding.

To validate cellular uptake of EVs and examine the effects of miRNA in the developing gut, feeding and gut loop studies are performed in preterm and newborn piglets. Collected gut tissue samples will be used for a transcriptomics-based analysis, to provide insights into the mechanism of action of milk EV-derived miRNAs in the gut. The aim is to use the acquired knowledge, to develop an ingredient for infant formulas, with an EV-enclosed miRNA content closely mimicking that of human milk.

A Reporter System to Study Adipocyte-Derived Extracellular Vesicles in vivo

Didde Riisager Hansen[(1)], Mikkel Ørnfeldt Nørgård[(1)], Jannik Hjortshøj Larsen[(1)], Lasse Bach Steffensen[(1)], Jan-Wilhelm Kornfeld[(2)], Per Svenningsen[(1)]

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Adipocytes secrete extracellular vesicles (EVs), but their effect on whole-body metabolism is unclear due to tracking and isolation limitations1. We have developed a Cre-dependent EV reporter model with CD63 fused to Nanoluciferase (NanoLuc) and hemagglutinin (HA). We hypothesized that viral delivery of CD63-NanoLuc-HA to adiponectin-Cre mice induces adipocyte-specific NanoLuc expression, creating a sensitive reporter system. We designed an AAV vector with a CD63-NanoLuc-HA construct, and a control with an IgK-NanoLuc-HA construct, reflecting normal cellular release. Both vectors were packed in AAV9 capsid and given to adiponectin-Cre mice via intraperitoneal injection (5*1011 vg). After two weeks, organs (e.g., adipose tissues, liver), and plasma and urine were harvested for evaluation of adipocyte-specific NanoLuc and HA expression by western blot, luciferase assay and qPCR. The HA-Tag was not detected in tissues by western blot (n=1). NanoLuc activity was found in plasma (n=3), urine (n=1) and tissues (n=3) with highest levels in eWAT, BAT and iWAT. The EV reporter showed higher tissue levels than the control, which had the highest levels in plasma and urine. qPCR confirmed Cre-activation in adipose tissues with highest levels in eWAT, iWAT and iBAT (n=3). In conclusion, our model enables adipocyte-specific Cre-dependent EV labeling, providing a tool to study adipocyte-derived EVs in vivo to unveil their role in normal physiology and during metabolic disturbances.

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

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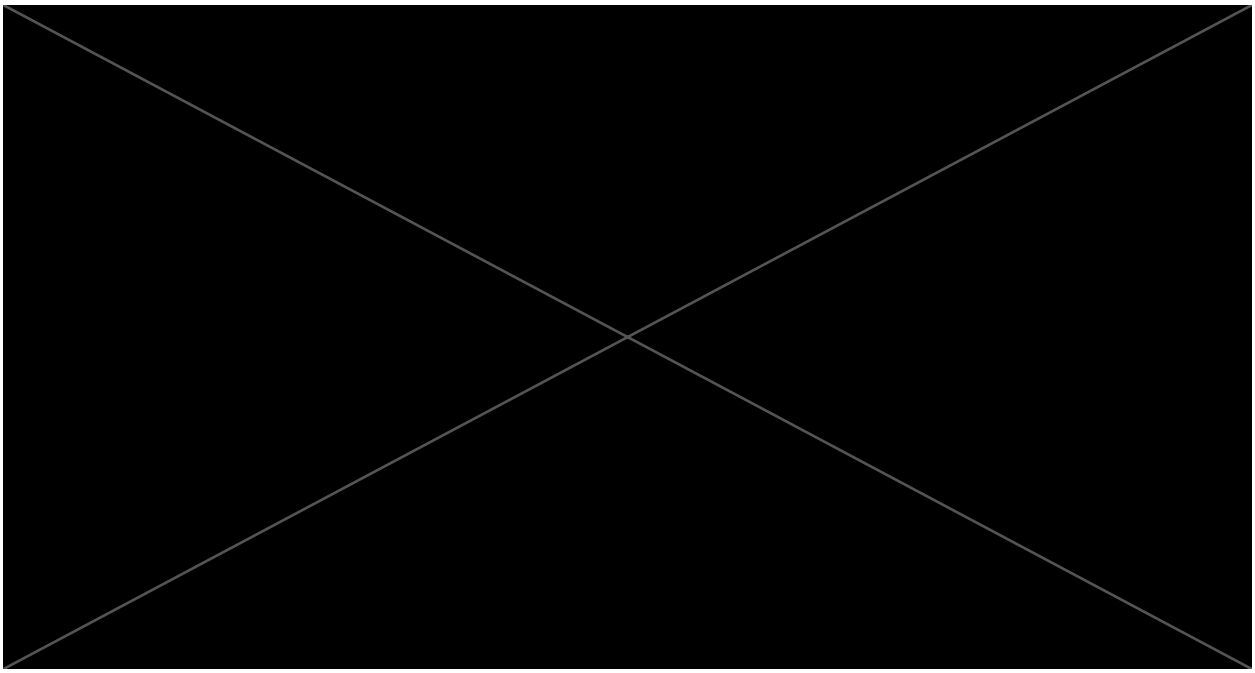

Storage, uptake and bioactivity of milk derived extracellular vesicles

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Milk extracellular vesicles (MEVs) are suggested to be relatively stable and capable of surviving the harsh conditions of the gastrointestinal tract. The action of MEVs may function across species, as indicated by the high similarity between the major miRNA expressed in human, bovine, and caprine milk. For MEVs to exert their suggested bioactivity, they must be taken up by the recipient, not in any way stressful to the exposed tissue/organism and stored optimally for study.

The results presented show that the cellular uptake of MEVs is only mildly affected by storage at -70°C after quick freezing in liquid nitrogen, whereas storage at -20°C should be avoided. We aim at studying the gastro-intestinal MEV uptake and derived effects using zebrafish embryos as a model organism. So far, it has not been possible to document the in vivo uptake of fluorescently labeled MEVs in zebrafish embryos.

This might be due to technical issues, but a new study is planned to investigate MEV uptake orally via microgavage. Lastly, uptake and migration assays with the Ca9-22 cell line (of oral origin) have been conducted with MEVs, using milk fat globule membrane material/proteins and proteoliposomes as control samples.

Extracellular vesicles as biomarkers for non-alcoholic fatty liver disease (NAFLD)

Katja Lund Cliff [(1)], Maiken Mellergaard [(1)(2)], Javier Donoso-Quezada [(1)], Nahuel Aquiles Garcia [(3)], Rikke Bæk [(4)], Rikke Wehner Rasmussen [(1)], Malene Møller Jørgensen [(2)(4)], Aase Handberg [(1)(2)]

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Non-alcoholic fatty liver disease (NAFLD) is defined by hepatic steatosis (>5% ectopic liver fat). NAFLD is present in up to 50% of obese individuals and is associated with the metabolic syndrome (MetS) and related complications. Current diagnostic tools are invasive and expensive, highlighting the need for new minimal-invasive methods. The imminent potential of extracellular vesicles (EVs) as circulating biomarkers along with their reported key roles in NAFLD development makes them promising candidates.

The aim of this project is to develop liver-specific EV biomarker panels for early detection of NAFLD in obese individuals.

Our MULTISITE study contains three groups: Lean, Obese, and Obese with NAFLD+MetS. Plasma samples were analysed by EV-Array testing the presence of 90 selected NAFLD-related markers on EVs, identified by CD9, CD81, CD63. Preliminary analysis has revealed promising candidate markers, that are significantly different between Obese and Obese with NAFLD+MetS. These are related to lipid accumulation, inflammation, and NAFLD progression. High-resolution flow cytometry (hFCM) will be used to validate the top candidates by investigating source of origin, with liver-specific marker Asialoglycoprotein receptor 1 (ASGRP1).

These findings have potential as a future minimal-invasive diagnostic tool to identify obese individuals at risk of developing NAFLD and related complications.

Towards proteome analysis of single EVs

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Introduction: Single-cell proteomics (SCP) is revolutionizing the study of cellular heterogeneity by enabling the analysis of individual cells. Our lab developed the OneTip protocol, combining efficient sample preparation with data-independent acquisition to quantify over 5,000 proteins from a Hela cell. We used this protocol to see how few extracellular vesicles (EVs) can be analyzed by mass spectrometry and still give good results.

Methods: EVs were isolated from human body fluids or cancer cell lines using ultracentrifugation and EV concentrations were then estimated using nanoparticle tracking analysis. Dilution series of EVs were processed with the OneTip protocol, involving lysis, digestion, and peptide concentration on C18 resin. The samples were analyzed using an EvoSep One LC with Orbitrap Astral mass spectrometer, and data processed with Spectronaut v18.6.

Preliminary Data: EVs were isolated from human body fluids and cell lines. From 16 million EVs, over 5,000 proteins were quantified, and even with just 63,000 EVs (similar to one HeLa cell's protein content), over 1,000 proteins were detected. Below 12,000 EVs, detection of EV-specific proteins was limited. Analysis of EVs from cancer cell lines confirmed the retention of cell-type characteristics, with the Her2/ErBB2 protein detected in as few as 250,000 EVs. The OneTip-based method shows promise for characterizing low quantities of EVs, enabling deeper insights into their heterogeneity after fractionation.

Peripheral nerve-derived extracellular vesicles: an identikit

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Isolating extracellular vesicles (EVs) from solid tissues entails several challenges, such as the choice of the most proper tissue dissociation and EV enrichment technique as well as the evaluation of the sample purity and yield. However, tissue-EVs are representative of all the intercommunicating cell types, providing a snapshot of the complex biological dynamics in a tissue. We apply an ultracentrifugation-based approach to extract EVs from murine peripheral nerves and we adopt several techniques – Western blotting, mass spectrometry, transmission electron microscopy and nanoparticle tracking analysis – to thoroughly characterize EVs from this tissue type, searching for both common EV features and signature properties that will help the investigations and possible medical applications of nerve-EVs. We combine the isolation approach with an ex vivo study of the biodistribution of genetically tagged Schwann cell-derived EVs in peripheral nerves, based on tissue clarification and immunostaining. We critically assess the limitations of the used EV isolation protocol and we describe the perspectives for future research.

Elucidating the molecular mechanisms of remote ischemic conditioning in acute ischemic stroke: insights from extracellular vesicle surface markers and microRNA regulation in a randomized-controlled-trial

Rebecca Best Jensen [(1)], Maria Kjølhede [(2,3)], Jesper Just [(1,4)], Lee-Ann Clegg [(5)], Rikke Bæk[(5)], Erik Kaadt[(6)], Betina Elfving[(6)], David C Hess[(7)], Grethe Andersen[(3)], Malene Møller Jørgensen[(5)], Rolf Ankerlund Blauenfeldt[(2,3)], Kim Ryun Drasbek[(1)]

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Remote ischemic conditioning (RIC) has demonstrated neuroprotective effects, yet translating this into clinical application has proven challenging. This study aims to uncover the molecular mechanisms underlying RIC's protective effects, which could potentially identify new therapeutic targets.

We conducted the ENOS study, a patient-assessor blinded, sham-controlled clinical trial, to investigate the molecular changes in patients diagnosed with acute ischemic stroke (AIS). Patients were assigned to undergo either RIC, involving transient ischemia and reperfusion of the arm, or a sham-placebo treatment. We collected plasma samples at three time points: within 24 hours of hospital admission, two hours post-initial RIC, and after seven days, with RIC administered twice daily. Analysis focused on stroke and brain biomarkers; extracellular vesicle (EV) surface markers and circulating microRNAs (miRNAs).

We identified significant differences in the regulation of CD62 on EVs and five specific miRNAs between the RIC and sham groups. Several of these miRNAs were correlated to improvements in red blood cell (RBC) deformability and aggregation during shear stress. We observed significant increases in CD62 on EVs at both the two-hour and seven-day follow-ups in RIC group. This suggests that RIC induces specific changes in EV surface markers, circulating miRNAs that potentially could affect RBC aggregation, which could serve as biomarkers for the protective effects in future studies.

The phenotypical changes of plasma EVs over time in healthy donors

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Introduction: To use EVs in clinical settings, it is of great importance to establish whether EV phenotypes and numbers change over time in a healthy cohort. The aim of this study was to determine both the long-term (week-to-week) as well as the short-term (day-to-day) variation of EV concentration and composition in plasma from healthy donors and to compare the findings with blood cell counts.

Methods: Venous peripheral blood (citrate and EDTA) was obtained from 4 fasting healthy donors once a week over a period of 6 weeks. Furthermore, blood samples were drawn daily for one week. Blood cell counts were measured using a Sysmex XN-1000. EVs were analyzed by the EV Array (Jørgensen et al., 2013, JEV) using antibodies against 23 selected surface-markers for capture in combination with detection by antibodies against CD9, CD63, CD81 or a cocktail of these. Capture antibodies included antibodies against EVs in general and immunological and inflammatory markers. In comparison, hFCM (Sanden et al., 2018) was used for analyzing EV populations based on the expression of CD9, CD63, and CD81 as well as colocalization of these markers. NTA was performed to determine size and numbers of EVs.

Results: Large inter-individual variation was found in EVs analyzed by EV Array and hFCM. For certain blood cell types, the long-term intra-individual variation varied significantly over time, which was not seen in the context of small EVs.

Impact of increased glucose concentrations on extracellular vesicles (EVs) derived from INS-1E clonal beta cells

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It is currently unknown how glucose concentrations of culture media affect EVs from pancreatic beta cells. In this study, we examined the impact of different levels of glucose on INS-1E cells and their derived EVs.

INS-1E cells were cultured for 48hrs with 1% EV-depleted serum in either 5 or 20mM glucose RPMI1640 medium, or with 5mM glucose/15mM mannitol. EVs were isolated from conditioned medium by ultracentrifugation and characterized for protein content, dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). To confirm appropriate cellular response to glucose, MTS assays were conducted to assess cell viability and proliferation. RT-qPCR was used to measure gene expression of the glucose-responsive TXNIP mRNA relative to TFIIB. Data were analyzed using ANOVA followed by Tukey's post-hoc test.

Our findings confirmed that a higher concentration of glucose (20mM) significantly increase cell viability and TXNIP gene expression in beta cells. EV protein yield was significantly higher at elevated glucose level. While DLS showed no differences in size or polydispersity between conditions, NTA showed EV preparations to contain a significantly greater number of particles and larger particle sizes at high glucose levels, unrelated to the medium osmolality.

The study indicates that high glucose concentrations (20mM) impact EV characteristics and suggests a glucose-dependent change in EV release kinetics. Further studies are ongoing to determine composition of EVs in relation to media glucose concentration.