Univerza v Ljubljani
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SiN-EV SLOVENIAN NETWORK FOR EXTRACELLULAR VESICLES

EXTRACELLULAR VESICLES: FROM FUNDAMENTAL RESEARCH TO CLINICAL APPLICATION

BOOK OF ABSTRACTS

Ljubljana, 20. 06. 2022

SiN-EV SLOVENIAN NETWORK FOR EXTRACELLULAR VESICLES

Univerza v Ljubljani
Medicinska fakulteta

Workshop

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Ljubljana, 20. 06. 2022

Book of abstracts

Workshop **Extracellular vesicles: From fundamental research to clinical application**

Book of Abstracts
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OPENING ADDRESS

Dear Colleagues,

We are pleased to welcome you to the workshop "Extracellular vesicles: From fundamental research to clinical application" taking place on the 20th of June 2022 at the Faculty of Medicine, University of Ljubljana.

Extracellular vesicles (EVs) are a heterogeneous population of membrane vesicles, which are released from cells *in vitro* and *in vivo*, where they accumulate into various body fluids like blood, cerebrospinal fluid, urine, and others. They consist of a lipid bilayer membrane encapsulating a cargo of various typical proteins, lipids and nucleic acids, which mirror the composition and the physiological state of the cell of origin. Thus EVs have great potential for human diagnostic and therapeutic applications. Importantly, EVs were also identified as mediators of physiological and pathological processes.

At this year's workshop, we will first learn about recent advances in fundamental understanding of EV biology (plenary talks), which are partly driven by advances in EV isolation and characterization techniques (session 1). Next, we will hear about the role of EVs in modulating pathological processes and as biomarkers of human disease (session 2). The stimulating atmosphere will hopefully promote new collaborations and research findings.

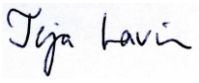
On behalf of the organizing committee, we welcome you to the workshop on the topic of a rapidly expanding field of extracellular vesicles.

Assoc. Prof. Metka Lenassi, PhD

Chair of the organizing committee

Assist. Prof. Katja Goričar, PhD

Assist. Marija Holcar, PhD



Assist. Teja Lavrin

Members of the organizing committee

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Alja Zottel

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PROGRAMME

09.00 – 09.10	**Opening address:** Metka Lenassi, UL MF, SiN-EV president

Plenary talks	**(Chairs: Metka Lenassi, Marija Holcar)**
09.10 – 09.50	Our journey in the field of extracellular vesicles Edit Buzás, Semmelweis University, HU
09.50 – 10.30	ASEV 2.0 - where do Austrian Vesicles go? Wolfgang Holnthoner, Medical University of Vienna, AT
10.30 – 11.10	*Coffee break*

Session 1	**Fundamental discoveries and methodology for extracellular vesicle isolation and characterisation** **(Chairs: Zala Jan, Alja Zottel)**
11.10 – 11.30	Improving extracellular vesicle isolation tools from technical standpoint Matic Resnik, IJS
11.30 – 11.50	Morphology of colloidal vesicles and small cellular particles Veronika Kralj-Iglič, UL ZF
11.50 – 12.15	Quantification of extracellular vesicles after their isolation from body fluids Mario Kurtjak, IJS Investigating the native morphology of extracellular vesicles from human cerebrospinal fluid Mladenka Malenica, Faculty of Medicine in Rijeka
12.15 – 12.35	Extracellular vesicles from the biotechnologically important fungus *Aureobasidium pullulans* Anja Černoša, UL BF
12.35 – 13.35	*Lunch break*

Session 2	**Extracellular vesicles in human disease** **(Chairs: Ula Štok, Saša Koprivec)**
13.35 – 13.55	Calcium ionophore-induced extracellular vesicles mediate cardioprotection against simulated ischemia/reperfusion injury Peter Pečan, KI
13.55 – 14.15	Sonication-packed anti-vimentin nanobody in glioblastoma exosomes shows decreased survival of glioblastoma stem cell line NCH421k Alja Zottel, UL MF
14.15 – 14.35	Extracellular vesicle-bound DNA in urine is indicative of kidney allograft injury Ivana Sedej, UMCL
14.35 – 14.55	Identification of urinary extracellular vesicles derived microRNAs with possible involvement in the development and progression of Fabry nephropathy Tina Levstek, UL MF
14.55 – 15.15	Prediction of feasibility and radicality of pancreatic cancer resection based on plasma EV characteristics David Badovinac, UMCL
15.15 – 15.20	**Closing address:** Metka Lenassi, UL MF

LECTURE ABSTRACTS

Session 1: Fundamental discoveries and methodology for EV isolation and characterisation

Improving extracellular vesicle isolation tools from technical standpoint

Matic Resnik[1], Ita Junkar[1], Veronika-Kralj Iglič[2], Miran Mozetič[1]

[1]Jožef Stefan Institute, Department of Surface Engineering, Ljubljana, Slovenia; [2]University of Ljubljana, Faculty of Health Sciences, Laboratory of Clinical Biophysics, Ljubljana, Slovenia

Introduction: During the isolation procedure, EVs come into contact with numerous surfaces and interact with them. An attempt was made to use gaseous plasma (the fourth state of matter) to treat plastic containers and improve the isolation yields of EVs. The plasma treatment changes surface characteristics drastically but there are considerable differences amongst the containers made by different manufacturers.

Methods: Atmospheric pressure plasma jet was used for the treatment of 1.5 mL polypropylene snap-cap containers, purchased from multiple manufacturers. Surface analyses were performed to assess the treatment effects on surface chemistry (XPS), surface morphology and topography (SEM and AFM), crystallinity, wettability and surface free energy. Subsequently, plasma treated containers were used in the EV isolation process from whole blood and final EV count was performed by flow cytometry.

Results: The surface characteristics of containers are highly dependent on the manufacturer, presumably due to the polymer blend, fillers and production parameters. These parameters change drastically after the plasma treatment process, making the surface nano-rough with improved wettability and increasing the amount of oxygen compared to carbon. The crystallinity also has a direct impact on the modification of the surface. Statistically relevant improvements in EV isolation yields were found. The size distribution of EVs from plasma treated containers also suggests there are less debris and more intact EVs present compared to untreated containers.

Conclusions: The tools used in isolation protocol of EVs are often considered just as standardized interchangeable laboratory accessories. This study resulted in understanding the importance of proper tool surface management and understanding the surface-to-EVs interaction phenomenon. The forces during the centrifugation are high and it can be assumed that contact between EVs and container inner surface can result in EVs decay or they might irreversibly stick onto these surfaces. By an appropriate plasma treatment of inner surfaces of containers, we are able to change surface morphology, surface free energy, surface chemical composition and other characteristics which are believed to help improve in the boundary layer formation, preventing the direct contact between EVs and container surfaces.

Morphology of colloidal vesicles and small cellular particles

Veronika Kralj-Iglič

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Introduction: In order to understand the genesis and destination of small cellular particles (SCPs) as intercellular communication mediators, it is of interest to envisage their morphology and acknowledge evidences of processes in which they may be formed. Furthermore, these evidences are hoped to lead to models which would predict the behaviour of the system and enable its controlled manipulation.

Methods: Electron microscope images of isolates from different samples (obtained by differential ultracentrifugation) and cultured media will be presented. Modeling of shapes is based on the minimization of the membrane free energy at relevant constraints. Rigorous solutions of the variational problem stated by a system of differential equations, approximative solutions by expansion over a set of functions and Monte Carlo simulations have been applied to construct theoretical predictions of the shapes that were compared to the experimentally observed ones.

Results: : It will be shown that membrane-enclosed entities without internal structure tend to attain similar shapes over two orders of dimension magnitudes in isolates from tomato homogenate and blood, erythrocytes and giant phospholipid vesicles. These shapes agree with the ones theoretically predicted on the basis of the minimization of membrane free energy and the corresponding particles are considered as colloidal vesicles. Isolates however contain also considerable amount of particles engineered by cells such as flagellae hairs and scales and viruses found in isolates from flagellate microalgae and plant material infected by viruses.

Conclusions: A part of SCPs found in isolates (colloidal vesicles) is subjected to physical laws as regards their formation, stability and uptake. There is however a large pool of SCPs for which the mechanisms of formation seem more sophisticated and are yet to be explored.

Quantification of extracellular vesicles after their isolation from body fluids

Mario Kurtjak[1], Mladenka Malenica[2], Vedrana Krušić Alić[3], Teja Lavrin[4], Metka Lenassi[4], Karmen Wechtersbach[5], Nika Kojc[5], Marija Vukomanović[1], Siniša Zrna[6], Maša Biberić[6], Kristina Grabušić[3]

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Introduction: Extracellular vesicles (EVs), membranous structures excreted by the cell into the body fluids, carry a potential for disease diagnostics through liquid biopsy [1]. Such application requires their precise and reliable quantification. We compared the concentration and size distribution of EVs obtained by different methods after their isolation from human cerebrospinal fluid (CSF) by size exclusion chromatography (SEC) and evaluated the strengths and weaknesses of each method.

Methods: EVs were isolated from CSF by gravity-driven SEC with Sepharose CL-6B stationary phase and phosphate buffered saline mobile phase [2]. The eluates containing EVs were identified by immunoblot on CD9 and CD81 antibodies and then analysed for size distribution by nanoparticle tracking analysis (NTA), tunable resistive pulse sensing (TRPS), dynamic light scattering (DLS), atomic force microscopy (AFM) in liquid and cryogenic transmission electron microscopy (cryo-TEM). In addition, particle concentration was determined using NTA, TRPS and AFM.

Results and conclusions: Our analysis of the pooled EV-positive eluates generally revealed a wide distribution of particles spanning mainly from 40 to 260 nm. This large polydispersity mainly deteriorated the accuracy of the quantifications, especially in TRPS, where cut distributions and clogging of nanopores occurred. The size distributions could well fit lognormal curves, which yielded the most frequent size of 60–100 nm by methods relying on particle "true" diameters (TRPS, cryo-TEM and liquid AFM) and 150-190 nm by methods measuring particle hydrodynamic diameters (DLS and NTA). AFM determined a smaller mode size (60 nm) than TRPS (100 nm) or cryo-TEM (90 nm), possibly due to 3D-2D transformation, overlapping of particle bases and EV deformations on the positively charged substrate [3]. TRPS detected a significantly higher concentration of particles (around 10^9 particles/mL) than NTA and AFM, which both counted around 10^9 particles/mL. This could be explained by the higher degree of agglomeration in NTA (no surfactant, unlike TRPS) and its lower sensitivity to smaller particles in polydisperse samples, whereas in AFM the lower concentration might be related to a proportion of non-attached EVs, which were not observed and counted. Hence, each method has its pros and cons, so it is best to combine them for a reliable quantification.

[1] S. Rastogi et al. The Evolving Landscape of Exosomes in Neurodegenerative Diseases: Exosomes Characteristics and a Promising Role in Early Diagnosis. *Int. J. Mol. Sci.* **2021**, *22*, 440. https://doi.org/10.3390/ijms22010440.
[2] V. Krušić Alić et al. Extracellular Vesicles from Human Cerebrospinal Fluid Are Effectively Separated by Sepharose CL-6B—Comparison of Four Gravity-Flow Size Exclusion Chromatography Methods. *Biomedicines* **2022**, *10*, 785. https://doi.org/10.3390/biomedicines10040785.
[3] M. Kurtjak et al. Unveiling the Native Morphology of Extracellular Vesicles from Human Cerebrospinal Fluid by Atomic Force and Cryogenic Electron Microscopy. *Biomedicines* **2022**, *10*, 1251. https://doi.org/10.3390/biomedicines10061251.

Investigating the native morphology of extracellular vesicles from human cerebrospinal fluid

Mladenka Malenica[1], Mario Kurtjak[2], Sami Kereïche[3], Simone dal Zilio[4], Marco Lazzarino[4], Hrvoje Križan[1], Marko Perčić[5,6], Damir Klepac[1,6], Kristina Grabušić[7]

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Introduction: To investigate the native morphology of extracellular vesicles (EVs), they should be isolated by a method that preserves their shape and structure. The mildest condition that enables such morphology preservation is gravity-driven size-exclusion chromatography (SEC). After isolation, atomic force microscopy (AFM) and electron microscopy (EM) are mainly used to investigate the presence and purity of EVs, usually by microscopes operating in air or vacuum. However, to reveal the native morphology of EVs, investigations should be performed in liquid.

Methods: To evaluate the native morphology of EVs isolated by SEC from cerebrospinal fluid after traumatic brain injury, we applied AFM in liquid and cryogenic electron microscopy methods (cryo-TEM). The shape, structure, and topography of EVs obtained by AFM were compared to the cryo-TEM detailed morphology and internal structure. In addition, EV images obtained by microscopy in air/vacuum were compared to the ones captured in liquid.

Results: For a detailed insight into the single EV shape and structure, topographic AFM images were investigated and compared to near-native morphology observed by cryo-TEM. The AFM 3D images of individual EV-like particles revealed different shapes of preserved or collapsed lumen (cup-shape). The structures with preserved lumen were mostly round or slightly elongated with distinct features (multilobed, round, elongated bulging, single-lobed flat and flat). The cryo-TEM images of EVs revealed multimembrane structures (onion-like internal structures, one or more vesicles inside one EV and two or more membranes in a single EV) and single-membrane morphology. The images obtained by microscopy methods operating in air/vacuum revealed many forms of artefacts.

Conclusions: By comparing the AFM images of different EV 3D structures to the cryo-TEM images of different EV internal morphologies, we can observe that multimembrane internal structures could support the lumen and prevent its collapse [3]. On the other hand, if only one membrane is present with no internal membranous structures, as seen in cryo-TEM images, it could lead to the lumen collapse. However, cup-shape structures become much more abundant if visualisation in air/vacuum is applied. We demonstrated the usefulness of combining different microscopy methods for characterising the various types of EVs that are present in the CSF.

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[2] M. Kurtjak et al. Unveiling the Native Morphology of Extracellular Vesicles from Human Cerebrospinal Fluid by Atomic Force and Cryogenic Electron Microscopy. *Biomedicines* **2022**, *10*, 1251. https://doi.org/10.3390/biomedicines10061251.
[3] V. Yu. Bairamukov et al. Nanomechanical Characterization of Exosomes and Concomitant Nanoparticles from Blood Plasma by PeakForce AFM in Liquid. *Biochim. Biophys. Acta Gen. Subj.* **2022**, *1866*, 130139. https://doi.org/10.1016/j.bbagen.2022.130139.

Extracellular vesicles from the biotechnologically important fungus *Aureobasidium pullulans*

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Introduction: Fungal extracellular vesicles (EVs) were first isolated 15 years ago. Nevertheless, the field of fungal EVs remained little explored. To date, EVs have been isolated from only 25 different fungal species. Most of them are medically important and only a few are biotechnologically relevant. Here, we present for the first time EVs from the biotechnologically important black yeast-like fungus *Aureobasidium pullulans*, which is used for the biotechnological production of the extracellular polysaccharide pullulan and different industrially relevant extracellular enzymes. In the search for environmentally friendly solutions in various fields, another property of *A. pullulans* comes to the fore – its antagonistic activity against various phytopathogenic fungi, leading to its successful use in biocontrol as a substitute for increasingly problematic chemical fungicides. The biocontrol properties of *A. pullulans* are only partially understood. To fill this knowledge gap, we decided to test the biocontrol potential of EVs isolated from *A. pullulans*.

Methods: To isolate EVs from *A. pullulans* we used and optimized the isolation protocol for fungal EVs from liquid cultures. EVs were pelleted by ultracentrifugation and characterized by transmission electron microscopy (TEM) and nanoparticle-tracking analysis (NTA). Proteomics of EVs was analysed by mass spectrometry. Finally, we tested the biocontrol potential of *A. pullulans* EVs on various phytopathogenic fungi.

Results: TEM showed the typical cup-shaped morphology of EVs in a size range from 100 to 200 nm. These results were also confirmed by NTA. The fungus *A. pullulans* produced 6.1×10^8 EVs per milliliter of culture medium. Proteomic analysis revealed 642 proteins in the EVs of *A. pullulans*. The 30 most abundant proteins found are involved in various biological processes, such as carbohydrate metabolism and stress responses. Enrichment analysis using Gene Ontology (GO) confirmed these results. Testing the biocontrol activity of *A. pullulans* EVs resulted in alterations in the cultures of the selected phytopathogenic fungi.

Conclusions: We report for the first time the successful isolation of EVs from the increasingly biotechnologically important *A. pullulans*. We also show that these EVs can affect phytopathogenic fungi. These results provide the starting point for more specific analyses that will allow further understanding of the biological role of EVs produced by fungi.

Session 2: Extracellular vesicles in human disease

Calcium ionophore-induced extracellular vesicles mediate cardioprotection against simulated ischemia/reperfusion injury

Peter Pečan[1,2], Szabolcs Hambalkó[3], Van Thai Ha[1,2], Csilla T. Nagy[3], Csilla Pelyhe[3], Duško Lainšček[1,4], Bence Kenyeres[3], Gábor B. Brenner[3], Anikó Görbe[3,5], Ágnes Kittel[6], Monika Barteková[7,8], Péter Ferdinandy[3,5], Mateja Manček-Keber[1,4], Zoltán Giricz[3,5]

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Introduction: Cardioprotection against ischemia/reperfusion injury is still an unmet clinical need. The transient activation of Toll-like receptors (TLRs) has been implicated in cardioprotection via the activation of an adaptive response, which may be achieved by treatment with blood-derived extracellular vesicles (EVs). However, since the isolation of EVs from blood takes considerable effort, our aim was to establish a cellular model from which cardioprotective EVs can be isolated in a well-reproducible manner.

Methods: EV release was induced in HEK293 cells with calcium ionophore A23187. EVs were isolated using ultracentrifugation and characterized by DLS, NTA, WB and electron microscopy. Cytoprotection was assessed in H9c2 and AC16 cell lines. Cardioprotection afforded by EVs and its mechanism were investigated after 16 h simulated ischemia and 2 h reperfusion. Changes in gene expression and signaling were determined by dual luciferase assay, qPCR, WB and ELISA. Cytotoxicity after hypoxia/reoxygenation was assessed by LDH activity assay and by calcein staining.

Results and conclusions: The induction of HEK293 cells by calcium ionophore resulted in the release of heterogeneous populations of EVs. In H9c2 and AC16 cells, stressEVs induced the downstream signaling of TLR4 and heme oxygenase 1 (HO-1) expression in H9c2 cells. StressEVs decreased necrosis due to simulated ischemia/reperfusion injury in H9c2 and AC16 cells, which was independent of TLR4 induction, but not that of HO-1. Based on these results, we suggest that calcium ionophore-induced stressEVs may reveal novel avenues for cardioprotective treatments against ischemic cardiac disease such as myocardial infarction.

Sonication-packed anti-vimentin nanobody in glioblastoma exosomes shows decreased survival of glioblastoma stem cell line NCH421k

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Introduction: Glioblastoma (GBM) is extremely aggressive and the most common malignant primary brain tumor in adults. Current standard treatment for GBM includes surgery, radiotherapy and chemotherapy with temozolomide. However, the standard therapy does not prolong survival for more than few months. Low survival can be attributed especially to glioblastoma stem cells, that are resistant to standard therapy and can form a new tumour. One of prospective ways to target glioblastoma cells, including glioblastoma stem cells are nanobodies, the smallest available antigen-binding fragments. In our previous research, we identified anti-vimentin nanobody Nb79. The purpose of this research was to monitor the effect of anti-vimentin nanobody Nb79 packed in glioblastoma exosomes as a potential delivery system for glioblastoma cells.

Methods: Several protocols were tested for loading Nb79 into exosomes, such as incubation of cells with nanobody, passive loading, sonication and incubation in presence of saponin. Exosomes were purified by ultracentrifugation and the presence of Nb79 in exosomes was determined by western blot and/or electron microscopy. The effect of exosomes on selected glioblastoma cell lines was determined by WST-1 reagent.

Results and conclusions: We showed that indirect delivery of Nb79 into exosomes by incubation of cells with Nb79 was not successful or the detection method was below threshold. On the other hand, passive loading, sonication and incubation with saponin were successful and the latter two had comparable success. Due to high cytotoxicity of saponin, we continued with sonication method, where the presence of Nb79 was also confirmed by electron microscopy. At last, the effect of exosomes with Nb79 was determined in U251 differentiated glioblastoma cell line and two glioblastoma stem cell lines, NCH421k and NCH644. The exosomes had cytotoxic effect on NCH421k as they decreased survival for 12%. To conclude, sonication and use of saponin are efficient methods for loading Nb79. The exosomes with Nb79 had cytotoxic effect of one of the glioblastoma stem cells tested and the low effect could also be attributed to the use of exosomes isolated from cancerous cell line. For further experiments, other cell line as a source of exosomes should be validated.

Urinary extracellular vesicle bound DNA for the detection of kidney allograft injury

Ivana Sedej[1,2], Maja Štalekar[3], Magda Tušek Žnidarič[3], Katja Goričar[2], Nika Kojc[4], Polona Kogovšek[3], Vita Dolžan[2], Miha Arnol[1,5], Metka Lenassi[2]

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Introduction: Liquid biopsy has shown great potential to circumvent the limitations of tissue biopsy in the diagnosis of kidney allograft injury (KAI). Several studies support elevated donor-derived cell free DNA (ddcfDNA) in blood and urine as a biomarker of KAI. Here, we aimed to investigate whether DNA is bound to extracellular vesicles in urine (uEVs), and if so, does EV-DNA associate with KAI.

Methods: We obtained second morning spot urine from 41 well-characterized kidney transplant recipients (KTR) who underwent biopsy. As per histopathological findings, the patients were divided into normal histology (NH) and KAI (rejection or non-rejection injury) groups. We isolated EVs (using size exclusion chromatography (SEC)), EV-DNA and cfDNA from urine of KTR. EV concentration and size were determined by nanoparticle tracking analysis, while DNA yield, copy number, and DNA integrity index were characterized by fluorometry, donor-recipient genotyping and digital-droplet PCR. Binding of DNA to EVs was explored by DNase assay and immunogold transmission electron microscopy (TEM). The link between EV-DNA and histological phenotype of KAI was analysed. The study was approved by the National Ethics Committee and all patients signed informed consent.

Results: Using SEC we isolated pure EVs from urine of KTR. The median uEV concentration in the cohort was 8.47 x 1010/mmol U-creatinine, while uEV mode size was 125.8 nm. Regarding mean size, EVs were significantly larger in patients with KAI compared to NH (177.5 nm and 174.1 nm vs. 160.7 nm, P = 0.045, respectively). DNA co-isolated with uEVs and correlated with urine cfDNA in several parameters. EV-DNA and cfDNA yield, DNA copy numbers or ddDNA copy numbers (for cfDNA) levels were significantly increased in patients with KAI compared to NH. Importantly, EV-DNA copy numbers were greater in allograft rejection and differed significantly between the antibody- and cell- mediated rejection. Compared to cfDNA, EV-DNA was less fragmented and was bound to the surface of EVs as shown by TEM after DNA detection. uEV-DNA characteristics correlated with the degree of inflammation in several allograft compartments in KTR.

Conclusions: DNA is bound to the surface of EVs from urine and may be a potential non-invasive biomarker of KAI.

Identification of urinary extracellular vesicles derived microRNAs with possible involvement in the development and progression of Fabry nephropathy

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Introduction: Fabry disease (FD) is a rare X-linked disorder characterised by a deficiency in α-galactosidase A activity. Fabry nephropathy is one of the possible manifestations in FD patients with significant impact on the morbidity and mortality. However, currently available biomarkers lack sensitivity and do not predict disease progression. Urinary extracellular vesicles (uEVs) are secreted from the urinary tract and their cargo may reflect pathophysiological processes. microRNAs (miRNAs) have been shown to play an important role in kidney development, maintenance of kidney function, and progression of renal dysfunction. We aimed to identify candidate uEVs derived-miRNAs associated with the development and progression of Fabry nephropathy.

Methods: Total RNA was extracted from uEVs, which were isolated by a previously optimized method based on size exclusion chromatography. A total of 10 male patients and 10 male age-matched control subjects were included in the discovery cohort and profiled for 87 miRNAs using the miRCURY LNA miRNA Urine Exosomes Focus PCR Panel (Qiagen, Germany). In validation cohort, 33 Fabry patients and their sex- and age-matched control subjects were included. The expression of selected miRNAs was analyzed using quantitative PCR. Patients were divided into two groups according to the progression of nephropathy.

Results: We identified five miRNAs that differed significantly by more than 1.5-fold between patients with stable renal function and control subjects. There were nine such miRNAs between patients with progressive nephropathy and control subjects. Among all differentially expressed miRNAs, we selected seven candidate miRNAs potentially important for the progression and/or development of Fabry nephropathy. miR-21-5p and miR-222-3p were significantly upregulated in patients with stable renal function and in patients with progressive nephropathy compared with control subjects. Additionally, miR-10b-5p, miR-30a-5p, and miR-204-5p were significantly downregulated in patients with progressive nephropathy.

Conclusions: The fold change of miR-21-5p and miR-222-3p in progressive nephropathy was twice that in patients with stable renal function, indicating a possible role in the development of nephropathy. Downregulation of miR-10b-5p, miR-30a-5p, and miR-204-5p in patients with progressive nephropathy compared with control subjects indicates their possible role in the progression of nephropathy. Further studies are needed to confirm our results.

Prediction of feasibility and radicality of pancreatic cancer resection based on plasma EV characteristics

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is among cancers with worst prognosis. In resectable disease, upfront resection is indicated, while in borderline cases neoadjuvant therapy is initiated in order to downstage the tumour and make it resectable. However, even some patients with resectable PDAC could benefit from systemic therapy before surgery due to biology of their disease. Better preoperative characterization of these patients would aid in their treatment optimisation. Liquid biopsy with analysis of extracellular vesicles (EV) is a promising diagnostic modality, largely unexplored in PDAC. Our study aimed to evaluate if plasma EV characteristics are associated with PDAC clinical characteristics and overall survival (OS).

Methods: Our prospective cohort included 83 patients with PDAC who underwent surgery with curative intent. Patient data and plasma samples were collected pre- and intraoperatively, then again one, six and 12 months postoperatively. Small plasma EV concentration and size were determined by nanoparticle-tracking analysis. Mann-Whitney and Fisher's tests were used for comparison between patient groups, Spearman's rho, Kaplan–Meier analysis and Cox regression were used in other statistical analysis.

Results: Preoperatively, patients with resection had significantly higher small plasma EV concentrations compared to patients without PDAC resection. Furthermore, small plasma EV concentration before surgery was significantly higher in patients that underwent radical (R0) resection than in patients with micro- or macroscopic tumour remnant (R1 or R2 resection). Median follow-up was 25.7 months and OS 11.3 months. Association of OS with certain cutoff values of small EV characteristics were determined based on patients' resection status.

Conclusions: Plasma EV concentration before surgery could predict PDAC resectability and it correlated with radicality of tumour resection. Similar studies are needed to further evaluate the clinical value of liquid biopsy EV characteristics, however, such findings could help optimise patient treatment approach and aid in evaluation of the resected specimen.