HSEV Magyar EV kutatók szimpóziuma

HSEV symposium of Hungarian EV researchers

24th June 2022, Friday

Semmelweis University, Nagyvárad Téri Elméleti Tömb (NET), Tanácsterem

1089 Budapest, Nagyvárad tér 4.

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Magyar Kísérletes és Klinikai Farmakológiai Társaság

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Introduction

Kedves Résztvevők!

A szervezők nevében üdvözöljük Önöket a HSEV első szimpóziumán!

A szimpózium célja a magyar EV kutatásban tevékeny műhelyek egymással való megismerkedése, a szinergiák feltárása, a jelenleg kutatott témák egymásnak való felvázolása, valamint a közösségépítés. Meggyőződésünk, hogy a nagyszámú rövid előadás és a jelentős poszterszekció segítségével a hazai EV kutatás legtöbb szegmensét sikerült megszólaltatnunk, amivel reményeink szerint hozzájárulunk a közösségünk fejlődéséhez, láthatóságának növeléséhez.

Reméljük, hogy az összejövetel elnyeri tetszésüket, és hogy megtisztelő részvételük által a szimpózium a magyar EV kutatást előre mozdításának hatásos eszköze lesz.

Dear Participants,

On behalf of the Organizers, we welcome you to the first symposium of the HSEV!

The aim of the symposium is to get to know each other's Hungarian EV research workshops, to explore synergies, to outline the topics currently being researched, and to build community. We believe that the high number of lectures and the significant poster section covers most avenues of the Hungarian EV research, which will contribute to the development and to the increased visibility of our Community.

We hope that you enjoy the symposium and that with the help of your esteemed contribution the meeting will be an efficient tool to accelerate the Hungarian EV research field.

Organizers:

Dr. Zoltán Giricz

Prof. Dr. Edit Buzás

Krisztina Kecskés

Márta Szabó

Nóra Tatár

Judit Táncsics

Schedule

11:00 - 11:20 Welcome speech, introduction of the Hungarian Extracellular Vesicle Section (HSEV)

Dr. Zoltán Giricz, Prof. Dr. Edit Buzás

11:20 – 12:20 – Oral Session 1

12:20 – 13:20 – Lunch break, poster viewing

13:20 – 14:20 – Oral session 2

14:20 – 15:00 – Coffee break, poster viewing

15:00 – 16:30 – Oral session 3

16:30 – 16:45 – Closing remarks, group photo

17:00 – Meeting of senior scientists

18:00 – Social program. Venue: Buttler Terasz, Orczy kert, paid by the participant, separately

Scientific program

11:00 - 11:20 Welcome speech, introduction of the Hungarian Extracellular Vesicle Section (HSEV)

Dr. Zoltán Giricz

Section Secretary for the Extracellular Vesicle Section, Hungarian Society for Experimental and Clinical Pharmacology

Department of Pharmacology and Pharmacotherapy, Semmelweis University

Prof. Dr. Edit Buzás

President of ISEV, Section Secretary for the Extracellular Vesicle Section, Hungarian Society for Experimental and Clinical Pharmacology

Department of Genetics, Cell- and Immunobiology, Semmelweis University

11:20 - 12:20 – Oral Session 1

11:20: Tímea Bebesi

Infra red spectroscopy of storage-induced red blood cell derived extracellular vesicles

Research Centre for Natural Sciences, Biological Nanochemistry Group

11:35: Gabriella Dobra

Small extracellular vesicles, the promising serum biomarkers for central nervous system tumors

Institute of Biochemistry, Biological Research Center, Doctoral School of Interdisciplinary Medicine, University of Szeged

11:50: Tasvilla Sonallya

Categorization of antimicrobial and cell penetrating peptides based on affinity to interact with vesicles

Research Centre for Natural Sciences, Institute of Materials and Environmental Chemistry

12:05: Árpád Sebestyén

Introduce the microhub era: the Leica MICA system

Biomarker Kft.

12:20 – 13:20 – Lunch break, poster viewing

Poster 1: József Arany

Experimental dermatology – A molecular and cellular physiology approach

Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen

Poster 2: Tímea Böröczky

Donor cell-specific aspects of small extracellular vesicle production

Institute of Biochemistry, Biological Research Centre- Eötvös Loránd Research Network, Szeged, Hungary

Poster 3: Mátyás Bukva

Raman spectral signatures of plasma-derived extracellular vesicle-enriched isolates may support the diagnosis of different cancerous diseases

Laboratory of Microscopic Image Analysis and Machine Learning, Institute of Biochemistry, Biological Research Centre, Szeged, Hungary

Poster 4: Kitti Garai

Serum isolated exosomal miRNAs as predictive markers for Avastin® induced haemorrhage in non-small cell lung cancer

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pecs, 2 Rokus Str, Pecs, H-7624, Hungary

Poster 5: Anna Koncz

Investigation of extracellular vesicles derived from cardiac cell lines under normoxia and hypoxia

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

Poster 6: Csenger Kovácsházi

Helium conditioning increases cardiac fibroblast migration which effect is not propagated via soluble factors or extracellular vesicles

Semmelweis University, Department of Pharmacology and Pharmacotherapy, Budapest, Hungary

Poster 7: Krisztina Pálóczi

Circulating small EV-related alterations in early stage of atherosclerosis

Department of Genetics, Cell- and Immunobiology, Semmelweis University

Poster 8: Zóra Szilovics

Examining the interaction between oral pathogenic bacteria and Candida species at the level of extracellular vesicles

Department of Microbiology, University of Szeged, Szeged, Hungary

Poster 9: Éva Veres

Effect of Candida-derived extracellular vesicular treatment on oral squamous cell carcinoma cells

Department of Microbiology, University of Szeged, Szeged, Hungary

Poster 10: Hegyesi Hargita

Identification of cardiomyocyte-derived circulating extracellular vesicles in the bloodstream

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

13:20 – 14:20 – Oral session 2

13:20: Tamás Visnovitz

Development of lipid-based assays for standardization of EV preparations

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

13:35: Xabier Osteikoetxea

Engineered Cas9 extracellular vesicles as a novel gene editing tool

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

13:50: Krisztina Németh

Dynamics of hepatic extracellular vesicle release and uptake under normolipemia and hyperlipidemia

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

14:05: Zoltán Wiener

Intra-tumoral cellular heterogeneity for EV uptake in colorectal cancer

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

14:20 – 15:00 – Coffee break, poster viewing

15:00 – 16:30 – Oral session 3

15:00: Zoltán Ádám

Acute exercise induces alteration in the microRNA content and abundance of plasma derived microvesicles and exosomes- A pilot study

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pécs

15:15: Attila Oláh

Cannabinoids and extracellular vesicles (EVs) in the skin: (r)EVolution of experimental dermatology?

Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

15:30: Beáta Szebeni

Extracellular vesicles from peritoneal dialysate in the regulation of peritoneal fibrosis

ELKH-SE, Pediatrics and Nephrology Research Group, Budapest, Hungary

15:45: Viktória Szeifert

Role of Mac-1 receptor clustering in the formation of neutrophil granulocytes derived extracellular vesicles

Department of Physiology, Semmelweis University, Budapest, Hungary

16:00: Ferenc Kolonics

Neutrophilic granulocytes release custom-made extracellular vesicles

Department of Physiology, Semmelweis University, Budapest, Hungary

16:15: Zoltán Giricz

Extracellular vesicles as therapeutic tools in cardiovascular diseases

Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

16:30 – 16:45 – Closing remarks
17:00 – Meeting of senior scientists

Acute exercise induces alteration in the microRNA content and abundance of plasma derived microvesicles and exosomes- A pilot study

Zoltán Ádám [1,2], *Kitti Garai* [1,2], *Judit E. Pongrácz* [1,2], *Márta Wilhelm* [3], *Krisztián Kvell* [11,2]

1Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pécs, Pécs, Hungary;
2Wnt Signaling Research Group, Szentagothai Research Center, University of Pécs, Pécs, Hungary;
3Institute of Sport Sciences and Physical Education, Faculty of Science, University of Pécs, Pécs, Hungary

Background: Regular physical activity (PA) has multi-systemic benefits and attenuates the physiological impairments associated with aging. Emerging evidence suggests that circulating extracellular vesicles (EVs) contribute to those benefits via the transfer of microRNAs (miRNA) between tissues. However, the long-term adaptation to physical exercise and its impact on the circulating EV subtypes and their miRNA content (EVmiR) in older populations remains unknown.

Methods: In our study, we examined plasma-derived EV distribution and miRNA content collected from healthy, the past 25 years physically active male participants (n = 9; age: 62 ±6 years) before and after performing an incremental exercise protocol until volitional exhaustion. Following the isolation and enrichment of microvesicles (MV) and exosomes (Exo) from platelet-free plasma, the isolates were characterized and EVmiR levels were determined by qRT-PCR. The effect of acute exercise on the release of EV subpopulations and their miRNA cargo was assessed by comparing the baseline and post-exercise states.

Results: Acute exercise altered the concentration of different circulating EV subgroups. We observed increased size and concentration in the MV population and a slight decrease in the Exo population after acute exercise. The relative quantitation of miRNAs revealed, that of the expressed miRNAs 52% was shared between the two EV types, while 34% was unique to MVs and 14% was Exo specific.

Conclusion: Taken together, our study revealed that acute physical activity potentially increases the release of EVs in the MV size range in physically active senior individuals. The differences found in the miRNA content between MVs and Exos highlight the importance of dealing with these subtypes separately. Further analysis of the targeted molecular pathways will get us closer to enhancing our understanding of how PA contributes to overall health maintenance.

Acknowledgement: The work was supported by the UNKP-21-3 New National Excellence Program of the Ministry for Innovation and Technology.

Experimental dermatology – A molecular and cellular physiology approach

József Arany [1,2], *Dorottya Ádám* [1,2], *Attila Oláh*[1]

1Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary;
2Doctoral School of Molecular Medicine, University of Debrecen, Debrecen, Hungary

Skin is a vital organ, and its diseases are among the most frequent human pathological conditions. By using molecular and cellular physiology approaches, our group mainly focuses on the investigation of the pathophysiology of the pilosebaceous unit (acne, skin dryness, hair growth disorders, etc.) as well as certain inflammatory skin conditions (e.g., atopic dermatitis). Our key goal is to unveil so-far hidden aspects of the physiological regulation of cutaneous homeostasis, with a special emphasis on the role of the complex cannabinoid signaling (i.e., the "c[ut]annabinoid" system), mitochondrial biology, as well as extracellular vesicle (EV)-mediated intercellular communication.

In our experiments, we usually follow a stepwise approach, namely, we investigate basic phenomena first in cell lines, and then in more complex model systems, including primary human cells (derived from healthy individuals as well as from patients), reconstructed 3D human skin equivalents, as well as different organ cultures (i.e., hair follicle and full-thickness human skin organ cultures). Among others, changes in viability, proliferation, lipid production, intracellular ion homeostasis, ROS generation, gene expression (Q-PCR, western blot, immunolabeling; fluorescent, confocal and superresolution microscopy followed by quantitative immunohistomorphometry), and mediator release (ELISA) are monitored. We routinely influence gene expression by siRNA-mediated selective gene silencing, while in case of genomic and lipidomic studies, we rely on the expertise of our collaborators.

Although we have several well-established model systems to study key cellular aspects of several skin diseases (e.g., acne), and we are capable of isolating EVs, we currently lack the tools for appropriate characterization of the vesicles, and our EV-yield is rather low. Thus, we hope to build up mutually beneficial collaborations with the members of the HSEV community by investigating the putative cutaneous effects of EVs isolated and characterized by others as well as by providing EVs isolated in our skin-relevant model systems.

Infra red spectroscopy of storage-induced red blood cell derived extracellular vesicles

Tímea Bebesi

Természettudományi Kutatóközpont Biológiai Nanokémia Kutatócsoport

Extracellular vesicles (EVs) are nano- and micro-sized structures of cellular origin located in the extracellular space and bounded by a double phospholipid membrane. Because EVs also play a significant role in intercellular communication, they may also be potential biomarkers or drug carriers.
Red blood cells emit EVs both in vivo and in vitro; the latter are also formed in blood products during storage and may have inflammatory-inducing adverse effects, for example during transfusion. My experiments were aimed at investigating the effect of storage medium and storage time on the size and composition of EVs under dynamic light scattering (DLS), freeze-fracture transmission electron microscopy (FF-TEM), microfluidic resistive pulse sensing (MRPS), and attenuated total reflection infrared spectroscopy (ATR-IR) techniques. During short storage times, in addition to EVs, I also proved the formation of protein-rich particles (<100 nm). In isotonic buffer solution (PBS), the amount of vesicles formed and the hemoglobin content are orders of magnitude higher than in the medium used in blood preparations (SAGM). By detailed evaluation of the second derivative spectra, I pointed out that the IR spectra is also suitable for the identification of metabolites in EV: the presence and the change in concentration over time was detected of ATP, lactose, glucose, and oxidized hemoglobin.
IR spectroscopy, using ATR-IR technique, is a fast, non-destructive technique that provides valuable novel biochemical insight by using only small sample volumes (3uL) and minimal sample preparation. Combined with other methods, dedicated to determine the size and morphology, a complex characterization of EVs might be possible.

Donor cell-specific aspects of small extracellular vesicle production

Tímea Böröczky[1,2,3,*], *Mária Harmati*[1,*], *Mátyás Bukva*[1,2,3], *Gabriella Dobra*[1,3], *Edina Gyukity-Sebestyén*[1], *Krisztina Buzás*[1,2,3]

[1]Institute of Biochemistry, Biological Research Centre- Eötvös Loránd Research Network, Szeged, Hungary; [2]Department of Immunology, University of Szeged, Szeged, Hungary; [3]Doctoral School of Interdisciplinary Medicine, University of Szeged, Szeged, Hungary; [*]equal contributions

Introduction

It is widely demonstrated that extracellular vesicle (EV) release depends on the donor cells and the microenvironmental stimuli. Today, there are several quantitative methods to measure EV production, but comparative studies are still limited. Here, we investigated different mammalian cell cultures – as in vitro models of various healthy cells and tumors – to compare their capacity for EV release and vesicular protein production.

Methods

We set up a heterogeneous cell culture panel (n=17) paying attention to the diverse origin, morphology and disease state (normal-tumor cells). Small EVs (sEVs) were isolated by differential filtration and ultracentrifugation, then subjected to nanoparticle tracking analysis and BCA protein assay. Primary mouse mesenchymal stem cell (MSC) isolation was performed in accordance with the national and European animal ethics guidelines (clearance no.: XVI./78/2018).

Results

The average number of produced sEVs was higher and more variable in the group of tumor cells compared to the normal cells. In contrast, the protein content of the tumor cell-derived sEVs was lower. Therefore, the protein content of the sEVs (fg/sEV) negatively correlated (r_S=-0.515, $p<0.034$) with the sEV production rate. For instance, THP-1 human monocytic leukemia cells produced the most sEVs and the primary MSCs released the highest amount of vesicular protein. In general, the vesicular protein production correlated with the population doubling time (r_S=0.729, $p<0.001$) and the cell size (r_S=0.578, 0.015).

Conclusion

These experiments further evidence that the ability for EV production depends on several internal and external parameters providing a unique and continuously adapting communication route for all cells. This quantitative data may provide valuable information for future studies, e.g. high production favors the sEV downstream analyses. At the same time, exploring the quantitative differences in sEV production between cells may provide valuable information for describing the pathophysiology of different tumors or assess the efficacy of sEV-based therapies

Raman spectral signatures of plasma-derived extracellular vesicle-enriched isolates may support the diagnosis of different cancerous diseases

Matyas Bukva[1,2], *Edina Sebestyen-Gyukity*[1], *Timea Boroczky*[1,2,12], *Yasmin Ranjous*[3], *Maria Harmati*[1], *Gabriella Dobra*[1,2], *Adrienn Jenei*[4], *Laszlo Szivos*[5], *Katalin Hideghety*[6,7], *Krisztina Budai*[8], *Judit Olah*[7], *Peter Horvath*[1], *Gyorgy Lazar*[9], *Tamas Biro*[10,11], *Zoltan Konya*[3], *Pal Barzo*[5], *Almos Klekner*[4], *Krisztina Buzas*[1,12,13]

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Introduction

Thorough examination of the molecular composition of small extracellular vesicles (sEVs) by spectroscopic methods is a promising but hitherto barely explored approach for diagnosing cancerous diseases, especially central nervous system tumors. We attempt to reveal the potential role of plasma-derived sEVs in diagnosing seven different patient group through Raman spectroscopic analyses using a relevant number of clinical samples.

Methods

The study is conducted in accordance with the Declaration of Helsinki, informed consent forms are collected and the study was approved by national ethics committee. Up to 490 plasma samples will be obtained from seven patient groups (glioblastoma multiforme, meningioma, melanoma and non-melanoma brain metastasis, colorectal tumors, melanoma and a control group). SEV isolation is performed through differential centrifugation. The isolates are characterized by Western Blot, transmission electron microscopy and nanoparticle tracking analysis. Principal Component Analysis–Support Vector Machine algorithm is performed on the Raman spectra for classifications. Classification accuracy, sensitivity, specificity and the Area Under the Curve (AUC) value are used to evaluate the performance of classification.

Results

According to preliminary results, the patient groups are distinguishable with 80–95% sensitivity and 80–90% specificity. AUC scores of 0.82–0.9 suggest excellent classification performance.

Conclusion

Our results support that Raman spectroscopic analysis of sEV-enriched isolates from plasma is a promising approach for developing non-invasive, cost-effective methods for clinical diagnosis of different cancerous diseases.

Funding

Supported by GINOP-2.2.1–15-2017–00052, ÚNKP-20–3-New National Excellence Program of the Ministry for Innovation and Technology from the National Research, Development and Innovation Fund; University of Szeged, Faculty of Medicine, Szent-Györgyi Albert Research Fund (2021); MEC-R 141410, MEC-R 141412.

Small extracellular vesicles, the promising serum biomarkers for central nervous system tumors

Gabriella Dobra*[1, 2]*, Matyas Bukva*[1,2]*, Maria Harmati*[1]*, Edina Gyukity-Sebestyen*[1]*, Zoltan Szabo*[3]*, Zoltan Konya*[4]*, Peter Horvath*[1]*, Almos Klekner*[5]*, Krisztina Buzas*[1,6]**

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Investigating the molecular composition of small extracellular vesicles (sEVs) for tumor diagnostic purposes is becoming increasingly popular, especially for diseases where diagnosis is challenging, such as central nervous system (CNS) malignancies. We attempt to reveal the potential role of serum-derived sEVs in the monitoring of CNS tumors using a relevant number of clinical samples.

A total of 230 serum samples were obtained from four patient groups (glioblastoma, non-small-cell lung cancer brain metastasis, meningioma and lumbar disc herniation as control). After the isolation and characterization of sEVs, LC-MS was performed on two different sample types (whole serum and serum sEVs). Moreover, ELISA was carried out on the MMP9 level of serum sEVs, and the whole molecular content was also examined by Raman spectroscopy. Statistical analyses were completed to compare the proteome of the two different sample types, the MMP9 levels and the Raman spectral signature of serum sEVs derived from the four patient groups.

From the 311 identified proteins, 10 whole serum proteins and 17 sEV proteins showed the highest intergroup differences, and sEVs were better at distinguishing between the patient groups as compared to the whole sera. Significant differences were observed in the MMP9 levels between the patient groups with a correlation with tumor aggressiveness. Under determining the effect of MMP9 levels on survival, patients with low MMP9 level (<30 ppm) presented with a significant OS benefit, which translated into a median increase in OS of 8 months. Using Raman spectra, the compared groups were distinguishable with AUC scores in the range of 0.82-0.9 suggest excellent and outstanding classification performance.

Our results support that sEVs have greater potential to distinguish CNS tumors, than whole sera, their MMP9 levels correlate with tumor aggressiveness and patient survival, and Raman spectroscopic analysis of serum sEVs is a promising method for diagnosing CNS tumors.

Serum isolated exosomal miRNAs as predictive markers for Avastin® induced haemorrhage in non-small cell lung cancer

Garai, Kitti [1,2]*; Herczeg, Robert; Torok* [1,3]*, Zsofia; Adam* [1,2]*, Zoltan; Kvell* [1,2]*, Krisztian; Kajtar* [4]*, Bela; Sarosi* [3]*, Veronika;Pongracz* [1,2]*, JuditE*

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The first approved angiogenesis inhibitor, Avastin® (bevacizumab) is used in combination with cancer chemo- and immunotherapy. Despite all the precautions, in some patients Avastin® can cause serious adverse reactions that have no reliable predictive biomarkers. Avastin®, in combination with carboplatin and pemetrexed or paclitaxel, is indicated for first-line treatment of patients with unresectable, locally advanced, recurrent, or metastatic non–squamous non–small cell lung cancer (NSCLC). Non-squamous is an important distinction, as haemorrhaging is about 30% more likely in squamous cell non-small cell lung cancer (LUSC). In the present study we used serum derived exosomal miRNA profiles of lung adenocarcinomas (LUAD), LUSC and age matched healthy controls to identify cancer subtype specific miRNA profiles as well as clinical treatment response to recognise potential biomarkers for adverse effects triggered by Avastin®.

Extracellular vesicles as therapeutic tools in ischemic heart diseases

Zoltán Giricz

Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, Pharmahungary Ltd, Szeged, Hungary

The development of interventions and pharmaceutical tools to limit detrimental effects of ischemic heart diseases, such as acute myocardial infarction, has not yet resulted in a solution that can be translated into clinical practice successfully, thus novel approaches and tools are continuously sought after. Extracellular vesicles (EVs) have been recognized as potential therapeutic tools in different pathologies very early in their research. We have evidenced that EVs may mediate infarct size-limiting effect of a robust cardioprotective intervention, the remote ischemic conditioning in an ex-vivo setting. To enable our examinations in in-vivo systems and ultimately in human subjects, we developed and characterized different methods to isolate EVs from blood plasma, and launched the VEZICS program to develop equipment providing means to automated, efficient and reproducible EV isolation. For the development of therapeutic tools robust and reproducible manufacturing methods are quintessential. To this end, we investigated several cellular systems with chemical or genetic induction of EV release and assessed therapeutic potential of the purified EVs in cellular models relevant to ischemic heart diseases. We evidenced that Ca-ionophore-induced EVs released from HEK293 cells may limit hypoxic damage to cardiomyocytes, that EVs released from adipose derived mesenchymal stromal cells expressing TERT and myocardin proteins may transfer pro-angiogenic miRNAs and that helium conditioning of cardiac fibroblasts does not induce the release of EVs with cardioprotective potential. These findings evidence that EVs might bear therapeutic potential in ischemic heart diseases, however, systems to produce, purify and characterize efficacious EVs need further investigation.

Identification of cardiomyocyte-derived circulating extracellular vesicle in the bloodstream

***Hargita Hegyesi**, Annamária Huszár, Éva Pallinger, Balázs Hornyák, Csenger Kovácsházi, Gábor B Brenner, Zoltán Giricz, Krisztina Pálóczi, Delaram Khamari, Péter Ferdinandy, Edit I Buzás*

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Extracellular vesicles (EVs) play a role in regulating the physiological and pathological functions of cardiomyocytes and it gives the potential to monitor cardiovascular diseases. The release of EVs is increased under cellular stress and cardiomyocyte damaging conditions. However, whether cardiomyocyte-derived EVs eventually reach the systemic circulation and whether their number in the bloodstream reflects cardiac injury, remains unknown. Conditional transgenic mice (MerCreMer/mTmG) expressing green fluorescent protein (GFP) by cardiomyocytes were studied in lipopolysaccharide (LPS)-induced systemic inflammatory response syndrome (SIRS). We aimed to detect cardiomyocyte-derived EVs in the blood. EVs were separated both from platelet-free plasma and from the conditioned medium of isolated cardiomyocytes of the left ventricular wall. Size distribution and concentration of the released particles were determined by Nanoparticle Tracking Analysis. The presence of GFP + cardiomyocyte-derived circulating EVs was monitored by flow cytometry and cardiac function was assessed by echocardiography. In LPS-treated mice, systemic inflammation and the consequent cardiomyopathy were verified by elevated plasma levels of TNFα, GDF-15, and cardiac troponin I, and by a decrease in the ejection fraction. Furthermore, we demonstrated elevated levels of circulating small- and medium-sized EVs in the LPS-injected mice. Importantly, we detected GFP+ cardiomyocyte-derived EVs in the circulation of control mice, and the number of these circulating GFP+ vesicles increased significantly upon intraperitoneal LPS administration. The cardiomyocyte-derived GFP+ EVs were also positive for intravesicular troponin I (cTnI) and muscle-associated glycogen phosphorylase (PYGM). These data provide direct evidence that cardiomyocyte-derived EVs are present in the circulation and that the increased number of cardiac-derived EVs in the blood reflects cardiac injury in LPS-induced systemic inflammation. Grant: NVKP_16-1-2016-0017

Neutrophilic granulocytes release custom-made extracellular vesicles

Ferenc Kolonics[1], *Erika Kajdácsi*[2], *Sebastian Mrosik*[1], *Veronika J. Farkas*[3], *Delaram Khamari*[4], *Ákos M. Lőrincz*[1,5], *Erzsébet Ligeti*[1]

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5. Second Department of Internal Medicine, Szent György Hospital, Székesfehérvár, Hungary

Introduction: Extracellular vesicles (EVs) are released by every known cell type and represent a novel way of intercellular communication. Neutrophilic granulocytes (PMN) are the principal phagocytes of the innate immune system and play a crucial role in immunity. A plethora of different effects of PMN EVs have been described in the last 20 years. In this study, we investigated under comparative conditions the thrombo- and immunomodulatory effects of three different well-characterized PMN-derived EV populations.

Methods: Human PMN were stimulated with opsonized zymosan or left unstimulated for 20 min. Other PMN were incubated in unstimulated conditions for 24 h. Cells were eliminated and the medium-sized EV fraction was pelleted via differential centrifugation and filtration. EVs derived from these three different conditions were co-incubated with leukocytes, endothelial cells or pooled human plasma. We evaluated the uptake of the vesicles and their acute and long-term effects on phagocytosis, cell migration, endothelial activation markers, reactive oxygen species (ROS) production, coagulation and cytokine production.

Results: We show that EVs released from resting PMN exert anti-inflammatory action by reducing production of ROS and proinflammatory cytokine release from PMN while enhancing their TGF-β production. In contrast, pretreatment with EVs generated upon encounter of PMN with opsonized particles promotes proinflammatory processes as they increase production of ROS and cytokine secretion from PMN and activate endothelial cells. EVs released from apoptosing cells were mainly active in promoting coagulation and the TGF-β production of PMN. These specific effects of EVs disappear and emerge to be a general, rather anti-inflammatory effect in the acute setting.

Conclusions: We propose that PMN-derived EVs are custom-made and can have divergent, selective, and sometimes even antagonistic effects depending on the environmental conditions prevailing at the time of the EV production.

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Investigation of extracellular vesicles derived from cardiac cell lines under normoxia and hypoxia

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Introduction: Myocardial dysfunctions are one of the leading cause of death globally. Extracellular vesicles (EVs), released by these cells may help in early diagnostics and therapy. Cardiac cell lines (such as H9c2, AC16 and HL1) are widely used in cardiovascular EV research as *in vitro* models. Increasing number of studies are using these cell lines, although they have significant limitations. We have decided to perform comparative characterization of EVs released by differentiated H9c2, AC16 and HL1 cells. The aim of the recent project was to compare cardiac cells derived EVs under normal and hypoxic conditions.

Methods: Cell lines, AC16 (human), HL1 (mouse) and H9c2 (rat) cardiomyoblast cells were used in this study. Following differentiation protocols, small- medium- and large-sized EVs (sEV, mEV, lEVs) were collected from conditioned medium of normal and hypoxia treated cells. EVs separated by a combination of gravity filtration, differential centrifugation and tangential flow filtration (TFF). EVs were characterized according to MISEV 2018 by MicroBCA, SPV-Lipid assay, Nanoparticle Tracking Analysis and transmission electron microscopy (TEM). The protein profile of sEV and mEV were determined by mass spectrometry. Some of the proteomic hits were validated by Western blotting, flow cytometry and immunogold TEM. EV release was examined by fluorescent live-cell imaging using GFP-fusion protein transfected cells.

Results: One of our most relevant proteomic hits was endoplasmin (ENPL, grp94, gp96). It was specifically present in EVs released by hypoxia-treated cells. Even though ENPL is an endoplasmic reticulum resident chaperon, its presence in EVs were confirmed by Western blotting, flow cytometry and Immunogold TEM. Secretion of ENPL containing EVs was analyzed by confocal microscopy using fixed and living HL1 cells.

Conclusions: We provide evidence that ENPL-containing EVs are released by cardiac cell lines. We suggest that ENPL may take part in cardio-protection during hypoxia through reducing ER stress.

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Helium conditioning increases cardiac fibroblast migration which effect is not propagated via soluble factors or extracellular vesicles

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Background: Helium inhalation induces cardioprotection against ischemia/reperfusion injury, of which cellular mechanism is not fully elucidated. Extracellular vesicles (EVs) are cell-derived, nano-sized membrane vesicles which play role in cardioprotective mechanisms, but their function in helium-conditioning (HeC) is not elucidated.
Purpose: To investigate, how HeC affects cardiac fibroblasts and if their HeC-induced EVs or other secreted factors mediates remodeling of the cardiac tissue.
Methods: Neonatal rat cardiac fibroblasts (NRCF) were exposed to glucose deprivation and HeC rendered by four cycles of 95% helium + 5% CO2 for one hour, followed by one hour of normal culturing conditions. 40 hours later, NRCF migration was analyzed and Western Blot and quantitative PCR were used to analyze the expression of fibroblast to myofibroblast transformation markers. From the cell supernatant, medium-sized extracellular vesicles (mEVs) were isolated with differential centrifugation and analyzed with WB, transmission electron microscopy and nanoparticle tracking analysis. Supernatant of HeC-treated NRCF was transferred to naïve NRCF or immortalized human umbilical vein endothelia cells (HUVEC/TERT2) and migration and in vitro angiogenesis assay was performed.
Results: HeC accelerated the migration of NRCF. Meanwhile, HeC did not increase the expression of myofibroblast markers. HeC tended to decrease mEV secretion of NRCFs, but supernatant of HeC-NRCF neither accelerate the migration of naïve NRCF, nor affect the angiogenic potential of HUVEC/TERT2.
Conclusion: Since HeC increased the migration of NRCF but HeC-NRCF mEVs did not affect the function of remote cells, HeC may exert its cardioprotective effect via NRCFs, but not affect cardiac remodeling remotely, via NRCF mEVs.
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The functional heterogeneity of neutrophil-derived extracellular vesicles reflects the status of the parent cell

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Introduction: Similar to other cell types, neutrophilic granulocytes (PMN) release extracellular vesicles (EVs), mainly medium-sized EVs. Laboratories working on PMN-derived EVs have reached a consensus on the physical parameters (size, density) and chemical composition (surface proteins, proteomics) of neutrophil-derived EVs. In contrast, there is large diversity and even controversy in the reported functional properties. We believe that these discrepancies lie in the different activation status and viability of examined cells. Our work group investigates the functional properties of EVs generated by differently activated neutrophils under comparable conditions. Earlier we presented, that opsonized pathogens elicit antibacterial EV production from activated cells, however resting neutrophils produce non-antibacterial EVs spontaneously. The aim of our later experiments was to reveal the background of this significant switch of functional properties of EVs.

Methods: We isolated PMN EVs from peripheral human blood neutrophils and from mice bone marrow PMNs by two-step centrifugation and gravitational filtration validated by size exclusion chromatography. We characterized the EVs by flow cytometry, Bradford protein assay, proteomics, NTA, and TEM. We tested their functional properties in bacterial survival assays, endothelial activation assay, coagulation assay, reactive oxygen species (ROS) production, and cytokine production of resting neutrophils.

Results: Our results prove that Mac-1 integrin signaling is a key factor that switches non-antibacterial, anti-inflammatory spontaneous EV generation into pro-inflammatory and antibacterial EV production. On the other hand, our data reveal that intact Ca^{2+} signaling is also crucial for pro-inflammatory EV production but not sufficient alone to initiate pro-inflammatory EV biogenesis.

Conclusions: The EV production of neutrophils is not a ‘yes or no’ function but a constitutive process of the cells through that neutrophils are able to communicate with their partner cells. The biological effect of the produced EV reflects the status of the parent cell and the environmental conditions prevailing at the time of EV genesis.

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Dynamics of hepatic extracellular vesicle release and uptake under normolipemia and hyperlipidemia

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Extracellular Vesicle Research Group, Budapest, Hungary 7ELKH-SE Immune-Proteogenomics Research Group Introduction Liver plays a central role in elimination of circulating extracellular vesicles (EVs), and it also significantly contributes to EV release. However, the involvement of the different liver cell populations remains unknown. Methods Here, we investigated EV uptake and release both in normolipemia and hyperlipidemia. C57BL/6 mice were kept on high fat diet for 20-30 weeks before circulating EV profiles were determined. In addition, mice were injected intravenously with fluorescent EVs, and an hour later, liver cell types were isolated and analysed. In vitro, liver cell types were tested for EV release and uptake with/without prior fatty acid treatment. Results We detected an elevated circulating EV number after the high fat diet. To clarify the differential liver cell involvement, we carried out in vitro experiments. We found an increased release of EVs by primary hepatocytes at concentrations of fatty acids comparable to what is characteristic for hyperlipidemia. When investigating EV uptake, upon injection of medium EVs (326.3±19.8 nm) intravenously to mice, we detected their presence primarily in isolated Kupffer cells. In vitro, we found that medium sized and small sized (130.5±5.8 nm) EVs were preferentially taken up by Kupffer cells, and liver sinusoidal endothelial cells, respectively. Finally, we demonstrated that in hyperlipidemia, there was a decreased EV uptake both by Kupffer cells and liver sinusoidal endothelial cells. Conclusions Our data suggest that hyperlipidema increases the release and reduces the uptake of EVs by liver cells. We also provide evidence for size-dependent differential EV uptake by the different cell types of the liver. Funding NVKP16-1-2016-0017, ÚNKP19-4-SE-09, János Bolyai Research Scholarship, Hungarian Scientific Research Fund (K120237), VEKOP2.3.2-16-2017-000002, VEKOP2.3.3-15-2017-00016, H2020- MSCA-ITN-2017-722148 TRAIN EV, FIKP-Therapeutic Thematic Programme, Horizon 2020 Research and Innovation Programme (739593)

Cannabinoids and extracellular vesicles (EVs) in the skin: (r)EVolution of experimental dermatology?

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Hair growth disorders, skin dryness, acne, as well as other inflammatory skin conditions are among the most prevalent diseases worldwide. While most of the skin diseases are not directly life-threatening conditions, they can severely impair quality of life of patients. Thus, there is an unmet demand from the medical community, pharmaceutical industry, and society to find new, efficient, yet safe solutions to treat them.
Pharmacological exploitation of the complex cannabinoid signaling appears to be a valuable asset in this field, since it contributes to the regulation of a number of (patho)physiological processes in human skin. These include, but are not limited to, local inflammatory processes, sebum production, pigmentation, hair growth, or differentiation of epidermal keratinocytes. Notably, cannabinoid signaling was shown to contribute to the regulation of the release of extracellular vesicles (EVs), and EVs were demonstrated to carry functional endocannabinoids. Thus, there are obvious (yet mostly unexplored) interactions between the cannabinoid signaling and EV-mediated intercellular communication. Importantly, although skin is relatively less studied from this perspective, the available evidence strongly supports the concept of the existence of an EV-based cutaneous communication network. Intriguingly, although EV profiles of epidermal keratinocytes and sweat gland cells have already been characterized in certain conditions, only scant evidence is available about the EVs produced by the major members of the pilosebaceous unit (i.e., hair follicles and sebaceous glands), or about the effects of EVs (produced by other, extracutaneous cells) on the human pilosebaceous unit.
Thus, our team aims to study the role of EVs in the skin, with a special emphasis on their interactions with the cannabinoid signaling. Moreover, we also intend to investigate the effects of modified EVs loaded by naturally occurring endo- and phytocannabinoids, as well as by semi-synthetic phytocannabinoid-derivatives in hair growth disorders, acne, and other inflammatory skin diseases.

Circulating small EV-related alterations in early stage of atherosclerosis

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Introduction - Atherosclerosis is a disease with complex genetic background. Multiple environmental and genetic factors are responsible for its development. Here we focused on circulating extracellular vesicles (EVs) and studied their changes in atherosclerosis.

Methods - Atherosclerosis was assessed by carotid and femoral artery ultrasonography. Blood samples were collected from healthy controls (n=21), from patients with early-stage atherosclerosis (=15) and from individuals with more advanced atherosclerosis (n=18). ACD anticoagulated blood samples were used to prepare platelet free plasma. The PFP samples were subjected to 18,000g centrifugation to remove the medium sized EVs. Then the supernatant was filtered by gravity through a 0.22 micron filter, and was concentrated by ultrafiltration (100kDa cut-off). The filtrate was further purified by SEC (qEV-70), and the EVs were analyzed by Western blotting, NTA, ELISA and by flow cytometry (MACSPlex Exosome Kit). We also measured the blood plasma Lp(a), MMP12 and MCP1 levels, and analyzed the ApoE polymorphism.

Results - We found that MMP12 concentrations were significantly elevated both in the PFP samples and in the small EV preparations in the advanced atherosclerosis group as compared to healthy subjects. Importantly, we also found a significant elevation of MMP12 in small EVs of early-stage atherosclerotic patients, while there was no elevation in the PFP samples of the same patients at this early stage of the disease. We also detected early-stage atherosclerosis-related increase in the number of CD14+ and CD142+ sEVs, while the CD146+ sEVs were significantly elevated only in the advanced atherosclerotic group.

Summary/Conclusion - Our data suggest that by analyzing circulating small EVs, certain atherosclerosis-related alterations can be detected at an earlier stage of the disease than by the analysis of blood plasma samples.

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Engineered Cas9 extracellular vesicles as a novel gene editing tool

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Extracellular vesicles (EVs) have shown promise as biological delivery vehicles, but therapeutic applications require efficient cargo loading. Here, we developed new methods for CRISPR/Cas9 loading into EVs through reversible heterodimerization of Cas9-fusions with EV sorting partners. Cas9-loaded EVs were collected from engineered Expi293F cells using standard methodology, characterized using nanoparticle tracking analysis, western blotting, and transmission electron microscopy and analyzed for CRISPR/Cas9-mediated functional gene editing in a Cre-reporter cellular assay. As we have shown earlier, light-induced dimerization using Cryptochrome 2 combined with CD9 or a Myristoylation-Palmitoylation-Palmitoylation lipid modification resulted in efficient loading with approximately 25 Cas9 molecules per EV and high functional delivery with 51% gene editing of the Cre reporter cassette in HEK293 cells. New data shows that this approach could also reach 25% editing if the Cre reporter cassette in HepG2 cells as well as targeting knock-down of the therapeutically relevant PCSK9 gene with 6% indel efficiency. Importantly, Cas9 transfer was detergent-sensitive and associated with the EV fractions after size exclusion chromatography, indicative of EV-mediated transfer. Considering the advantages of EVs over other delivery vectors we envision that this study will prove useful for a range of therapeutic applications, including CRISPR/Cas9 mediated genome editing.

Keywords: extracellular vesicles, exosomes, CRISPR/Cas9 delivery, gene editing, optogenetics.

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Categorization of antimicrobial and cell penetrating peptides based on affinity to interact with vesicles

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Host defense peptides (HDP) are promising biomaterials with antimicrobial and anticancer applications. They exert their biological function via perturbing or lysing the cell membrane. These peptides shows numerous types of membrane interaction mechanisms i.e. carpet, toroidal pore, and barrel stave. The interactive mechanism of these peptides has been studied widely with model membranes however our knowledge with extracellular vesicles (EV) is scarce. There are several aspects where EV – HDP interactions could be relevant, ranging from cooperative presence on infection sites functions to EV cargo loading.

Based on initial research, we selected 24 antimicrobial peptides (such as Penetratin, Transportan, LL-37, Polybia MPI, Histatin 5, CM15, Buforin II, Bactenecin, Macropin I, Lasioglossin LL-III, Melittin, Tempo-La etc.) and investigated their interactions with red blood cell-derived EVs (REVs) to acquire a better understanding of their interaction mechanisms. Biophysical techniques such as Linear Dichroism spectrocopy, Transmission electron microscopy, Infrared spectroscopy and Dynamic light scattering were used to investigate these interactions.

The results reveal that some HDP mechanisms were vesicle penetrating, whereas others were lytic or resulted in protein corona removal. More specifically, CM15 effectively removes surface proteins; Melittin has a high membrane disrupting action, whereas Tempo-La, Polybia MPI, and Bactenecin show a different mechanism that has a lower disruptive affinity on the REV membrane.

These results provide an overview of the surface interactions of peptides with REVs, allowing us to gain a wide perspective on the molecular level interactions, which may be useful in tailoring the surface and interior of EVs with short HDPs.

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Extracellular vesicles from peritoneal dialysate in the regulation of peritoneal fibrosis

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Introduction, aim: There is a growing interest regarding the isolation and characterization of the extracellular vesicles (EVs). However, much less is known about their biological functions. Therefore in the present study we investigated the role of peritoneal dialysis effluent (PDE) derived EVs (PDE-EVs) on the pathomechanism of peritoneal fibrosis.

Methods: PDEs were collected from children receiving continuous ambulatory peritoneal dialysis treatment in the Ist Department of Pediatrics, Semmelweis University, Hungary. PDE-EVs were isolated by size exclusion chromatography and were characterized according the recommendations of the International Society for Extracellular Vesicles. Their significance on the viability and LDH release of human primary peritoneal mesothelial cells (HPMC) and human umbilical vein endothelial cells (HUVEC) treated with methylglyoxal (MGO) or H2O2 was tested by MTT or LDH assay, respectively. Expression of antioxidant genes was measured by real-time RT-PCR. Effect of EVs was also studied in a MGO induced mice model of peritoneal fibrosis in vivo. Submesothelial thickness was analysed after Masson's Trichrome staining and peritoneal transport was monitored using tetramethylrhodamine isothiocyanate-dextran.

Results: PDE-EVs showed typical EV features and penetrated into the cytoplasm of HPMC and HUVEC cells, as well. According to the MTT and LDH assays PDE-EVs prevented H2O2- or MGO-induced cellular damage. Furthermore, PDE-EVs reduced the expression of antioxidant genes, including hHO1 and hGCLC of MGO treated cells. In vivo intraperitoneally administrated PDE-EVs entered into the peritoneal cells, reduced the MGO treatment induced peritoneal thickness, and improved ultrafiltration capacity of the peritoneal membrane of C57BL/6J mice.

Conclusions: In summary, PDE-EVs reduce the harmful effect of H2O2 or MGO treatment in vitro, moderate submesothelial thickening and ameliorate the ultrafiltration capacity of the peritoneal membrane in vivo.

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Role of Mac-1 receptor clustering in the formation of neutrophil granulocytes derived extracellular vesicles

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Introduction: Previously, our group characterized different types of extracellular vesicles (EV) released from neutrophilic granulocytes (PMN). The three characterized populations are the EVs formed spontaneously (spEV), during apoptosis (apoEV), and upon activation with opsonized Zymosan (oZEV). The oZEVs show a rather pro-inflammatory effect on resting neutrophils and an antibacterial effect on bacteria and fungi, while the spEVs tend to mediate anti-infammatory effects. We also found that the Mac-1 integrin receptor is crucial for the oZEV formation. We aimed to examine how the selective activation of the Mac-1 and the calcium signal affects the production of neutrophils EV.

Methods: We isolated medium-sized PMN EVs from peripheral human blood neutrophils by two-step centrifugation and gravitational filtration validated by size exclusion chromatography. We characterized the EVs by flow cytometry, Bradford protein assay, NTA, and TEM. We tested the effect of Ca^{2+} ionophore and examined the EV production on C3bi and fibrinogen surface and in soluble form. We also followed the cluster formation of Mac-1 by TIRF microscopy.

Results: On C3bi-coated surface, we observed an increased EV production, these EVs possessed antibacterial capacity. However, in soluble condition, C3bi did not induce EV production. We found that ionophore initiated EV formation, but these EVs were ineffective in functional tests. We observed EV production increase after Ca^{2+} ionophore treatment both in the presence and in the absence of extracellular Ca^{2+}.

Conclusions: The calcium signal is crucial, but not sufficient alone in the generation of oZEVs. The selective Mac-1 activation and receptor clustering is not just crucial, but sufficient in initiation of the oZEV biogenesis that shows a completely different biological activity on other cells than spEV or apoEV. This observation suggests that neutrophils are able to change their EV production according to the environmental conditions detected by their receptors.

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Examining the interaction between oral pathogenic bacteria and Candida species at the level of extracellular vesicles

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The human oral cavity is colonized by more than 700 microbes, such as bacteria, viruses, fungi, known as the oral microbiota. As a result of environmental effects, such as smoking or infections, the microbial composition may change, which can result in dysbiosis that may lead to diseases, such as oral candidiasis. Oral candidiasis is most commonly caused by Candida albicans, which can alter the bacterial diversity.

To examine the nature of such fungal-bacterial interactions, we aim to investigate the interaction between Candida species and oral pathogenic bacteria at the level of extracellular vesicles (EV).

For our experiments we used the C. albicans SC5314 and C. parapsilosis CLIB214 strains, along with Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aeruginosa as pathogenic bacterial counterparts. We optimized the fungal and bacterial EV isolation protocol from liquid media. The characterisation of the EVs by transmission electron microscopy and NanoSight showed round shaped particles with diameters between 50 and 250 nm.

We examined the effects of EVs released by C. parapsilosis and the yeast and hyphae form of C. albicans on the growth and biofilm formation efficiency of S. aureus, P. aeruginosa and E. faecalis and vica versa. As a results, we found that EVs from C. albicans and C. parapsilosis had different effects on the growth of S. aureus. Fungal EVs also affected the biofilm formation efficiency of bacterial species.

Regarding the effect of bacteria, the bacterial EV treatment altered the biofilm formation efficiency of C. albicans and C. parapsilosis in a species dependent manner.

Using fluorescence microscopy we found that fungal cells and bacterial EVs colocalize after 4 hours of incubation, and fungal cells form hyphae with various efficiency, while bacterial cells and fungal EVs do not colocalize.

Altogether these results suggest the presence of an active interaction between fungal and bacterial cells at the level of EVs.

Effect of Candida-derived extracellular vesicular treatment on oral squamous cell carcinoma cells

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Globally, 90% of oral cancer cases are oral squamous cell carcinoma (OSCC). In relation to OSCC, the normal oral microbiota is often altered that might set the ground for developing local infections or conditions such as oral candidiasis. A previous study of our laboratory showed that the presence of *Candida albicans* increases the progression of OSCC *in vitro* and *in vivo* by increasing the activity of genes and signaling pathways involved in tumor progression, the production of oncometabolites and matrix metalloproteinases activity (MMP). Interaction between *Candida* and tumor cells not only occur directly, but also indirectly at the level of extracellular vesicles (EV).

To investigate the interaction between oral squamous cell carcinoma (OSCC) and *Candida* cells we used HSC-2 human OSCC cell line and EVs derived from *C. parapsilosis,* and the yeast and hyphae forms of *C. albicans*. We examined the uptake of the fungal EVs by the OSCC cells with flow cytometry and confocal microscopy. Our results showed that both tumor cell lines can take up the fungal EVs with high efficiency. We examined the effect of the fungal EV treatment on the viability and proliferation of tumor cells. As a result, we found that EV treatment did not cause any major changes in the mentioned cell functions. Live cell imaging system was used for the examination of the effects of fungal EVs on the migration of OSCC cells. During the experiments we observed that *Candida* EV treatment affected the migration of the OSCC cells, because we detected single migrating elongated tumor cells. We also found that the total secreted MMP activity of OSCC cells significantly increased after EV treatment.

These results suggest the presence of an active interaction between OSCC cells and *Candida albicans* also at the level of extracellular vesicles.

Development of lipid-based assays for standardization of EV preparations

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Despite the recent technological progress, quantification of extracellular vesicles (EV) remains challenging. EVs are typically standardized on the basis of their protein content sometimes also combined with the determination of the particle number. Even with this approach, errors in the determination of EV concentration should be expected. Lipid bilayers are defining components of EVs. A lipid-based quantification, in combination with the determination of protein content and/or particle count, appears to be a straightforward approach for quantification of EVs. We developed, optimized and patented sulfo-phospho-vanillin based lipid assay with increased sensitivity for EV detection.

An aqueous phase liposome standard (DOPC) was introduced to replace the previously used purified lipid standards dissolved in organic solvents. The results were compared with those obtained by the previously described ATR-FTIR spectroscopy-based lipid quantification. The SPV lipid assay was validated with an EPIC biosensor system, tunable resistive pulse sensing (qNano), commercially available lipid standards and LDL.

Elimination of organic solvents from the reaction mixture abolished the background color that interfered with the assay. Comparison with a commercial lipid determination kit (based on the original SPV lipid assay) showed an approximately one order of magnitude increase in the sensitivity of our new assay. The novel SPV assay provides a quick, reliable and sensitive test that may fill an existing niche in EV standardization. When using our optimized total lipid assay, EV lipid measurements can be as easy as measuring proteins with a simple BCA test.

Intra-tumoral cellular heterogeneity for EV uptake in colorectal cancer

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The majority of colorectal cancer (CRC) patients carry mutation in the *APC* gene, which leads to the unregulated activation of the Wnt pathway. Extracellular vesicles (EV) are considered as potential therapeutic tools. Although CRC is a genetically heterogeneous disease, the significance of the intra-tumor heterogeneity in EV uptake of CRC cells is not yet known. By using mouse and patient-derived organoids, the currently available best model of capturing cellular heterogeneity, we found that *Apc* mutation induced the expression of interferon-induced transmembrane protein 1 (Ifitm1), a plasma membrane protein that is critical in inhibiting virus uptake. Importantly, organoids derived from IFITM1^{high} CRC cells contained more proliferating cells and they had a markedly reduced uptake of fibroblast EVs as compared to IFITM1$^{low/-}$ cells. In contrast, there was no difference in the intensity of EV release between CRC subpopulations with high and low IFITM1 levels. Importantly, the difference in cell proliferation between these two subpopulations disappeared in the presence of fibroblast-derived EVs, proving the functional relevance of the enhanced EV uptake by IFITM1^{low} CRC cells. Furthermore, inactivating IFITM1 by CRISPR-Cas9 resulted in an enhanced EV uptake, highlighting the importance of this molecule in establishing the cellular difference for EV effects. Collectively, we identified CRC cells with functional difference in their EV uptake ability that must be taken into consideration when using EVs as therapeutic tools for targeting cancer cells.