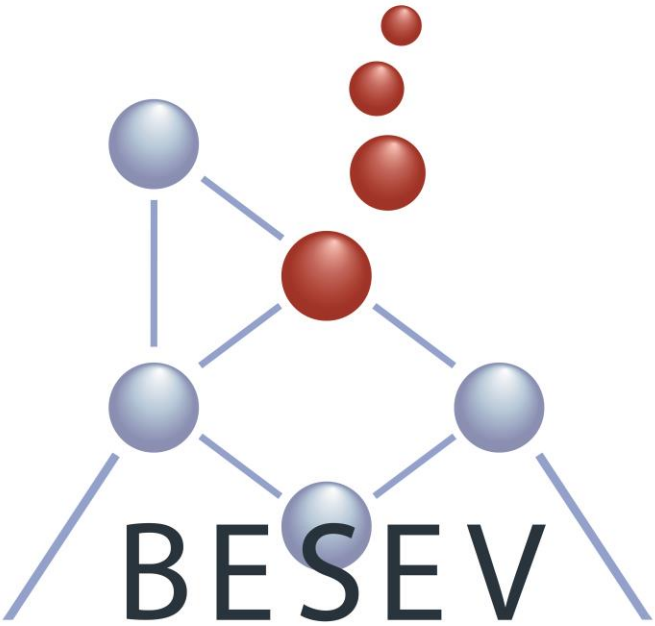
First Annual meeting

September 14th 2018



Belgian Society for Extracellular Vesicles

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Program

8h30 – 9h00:	Registration
9h00 – 9h15:	Opening and welcome
9h15 – 10h10:	Prof. Haiying Zhang (Meyer Cancer Center, Weill Cornell Medicine, New York, NY, USA) Identification of exomeres, a novel nanoparticle, and distinct exosome subsets using AF4
10h10 – 10h25:	Edward Geeurickx Recombinant extracellular vesicles: biological reference material to standardize extracellular vesicle research
10h25 – 10h45	PI session: Annelies Bronckaers - Morphology Research Group, Biomedical Research Institute (BIOMED), Hasselt University, Hasselt, Belgium Nick Geukens - PharmAbs, the KU Leuven Antibody Center, KU Leuven, Leuven, Belgium An Hendrix - Laboratory of Experimental Cancer Research, Ghent University, Ghent, Belgium Joy Irobi - Neurofunctional genomics Group, Biomedical Research Institute (BIOMED), Hasselt University, Hasselt, Belgium
10h45 – 11h15:	**Coffee break**
11h15 – 11h30:	Eline Oeyen Determination of biological and technical variability at protein and lipid level in isolated urinary extracellular vesicles: a crucial step in biomarker discovery studies
11h30 – 11h55:	Brief oral session: Adam Ceroi Extracellular vesicles, engulfment, and airway inflammation Sarah Deville High-resolution flow cytometry of extracellular vesicles released upon nanoparticle exposure of alveolar macrophages Joeri Tulkens Identification of the Content, Corona, Contaminants and Clinical potential of circulating extracellular vesicles by reproducible size- and density-based plasma fractionation Question & Answer session
11h55 – 12h15:	PI session:

Inge Mertens - Sustainable Health, VITO nv (Flemish Institute for Technological Research), Mol, Belgium & Centre for Proteomics, University of Antwerp, Antwerp, Belgium.

Luc Michiels - BioNanoTechnology (BNT) research group, Biomedical Research Institute (BIOMED), Faculty of Medicine and Life Sciences, Hasselt.

Inge Nelissen - Sustainable Health, VITO nv (Flemish Institute for Technological Research), Mol, Belgium

Christophe Pierreux - CELL Unit, de Duve Institute, Université Catholique de Louvain, Brussels, Belgium

12h15 – 13h30: **Lunch break & poster session**

13h30 – 14h25: Dr. Jacky Goetz (The Goetz Lab for Tumor Biomechanics, INSERM U1109, Institut d'Hématologie et d'Immunologie, Strassbourg, France)
In vivo tracking of tumor extracellular vesicles at high spatio-temporal resolution

14h25 – 14h40: Makon-Sébastien Njock
Sputum exosomal miR-142-3p suppresses fibrogenesis by targeting TGF-β pathway in Idiopathic Pulmonary Fibrosis

14h40 – 15h05: PI session:

Dragana Spasic - MeBioS-Biosensors group, KU Leuven, Leuven, Belgium

Basile Stamatopoulos - Laboratory of Clinical Cell Therapy, Université Libre de Bruxelles (ULB), Jules Bordet Institute, Belgium

Ingrid Struman - Molecular Angiogenesis Laboratory, GIGA research center, ULiège, Liège, Belgium

Roosmarijn Vandenbroucke - Barriers in Inflammation lab, VIB Center for Inflammation Research, Ghent, Belgium & Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

Pascale Zimmermann - University of Leuven, Leuven, Belgium & Cancer Research Center, Marseille, France

15h05 – 15h35: **Coffee break**

15h35 – 15h50: Baharak Hosseinkhani
Extracellular vesicles work as a functional inflammatory mediator between vascular endothelial cells and immune cells

15h50 – 16h05: Charysse Vandendriessche
Protective effects of caloric restriction on Alzheimer's disease progression: role for choroid plexus derived extracellular vesicles?

16h05 – 16h30: Closing remarks + discussion

16h30 – 18h30: **Reception**

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Prof. Haiying Zhang (Meyer Cancer Center, Weill Cornell Medicine, New York, NY, USA)

Identification of exomeres, a novel nanoparticle, and distinct exosome subsets using AF4

Abstract 2

Dr. Jacky Goetz (The Goetz Lab for Tumor Biomechanics, INSERM U1109, Institut d'Hématologie et d'Immunologie, Strassbourg, France)

In vivo tracking of tumor extracellular vesicles at high spatio-temporal resolution

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Makon-Sébastien Njock

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Sarah Deville

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Joeri Tulkens

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Pascale Zimmermann - University of Leuven, Leuven, Belgium & Cancer Research Center, Marseille, France

Abstract 1

Identification of exomeres, a novel nanoparticle, and distinct exosome subsets using AF4

Prof. Haiying Zhang (Meyer Cancer Center, Weill Cornell Medicine, New York, NY, USA)

Abstract

Exosome research has grown exponentially due to the recognition of the potential roles of exosomes in pathophysiological processes, including cancer. However, due to technical challenges, isolated nanovesicles constitute a heterogeneous population and this has hindered our understanding of their biogenesis, molecular composition, biodistribution, and functions in vivo. By employing asymmetric-flow field-flow fractionation (AF4) and examining the nanovesicles derived from various cell lines and human specimens, we have identified two distinct exosome subpopulations (large exosome vesicles, Exo-L, 90-120 nm in diameter; small exosome vesicles, Exo-S, 60-80 nm in diameter) and have discovered a novel, non-membranous nanoparticle population termed “exomeres” (<50 nm with an average of 35 nm in diameter). Biophysical characterization of these nanoparticles demonstrated they are remarkably distinct in terms of morphology, size, and biophysical properties such as zeta potential and stiffness. Proteomic profiling revealed an enrichment of proteins related to specific pathways, such as glycolysis and mTOR signaling for exomeres, endosomal function and secretion pathways for Exo-S, mitotic spindle and IL-2/STAT5 signaling for Exo-L, and multiple additional signaling transduction pathways for both exosome subpopulations. Each particle subset contained a distinct N-glycosylation profile and showed genetic diversity in DNA and RNA prevalence. Furthermore, these nanoparticle subsets demonstrated distinct organ biodistribution patterns in animal models, suggesting their functional differences in vivo. This study suggests that AF4 in combination with sensitive molecular assays can serve as an improved analytic tool for isolation of specific nanoparticle subpopulations, thereby addressing the complexities of vesicle heterogeneity.

URL:

vivo.med.cornell.edu/display/cwid-haz2005

Abstract 2

In vivo tracking of tumor extracellular vesicles at high spatio-temporal resolution

Dr. Jacky Goetz (The Goetz Lab for Tumor Biomechanics, INSERM U1109, Institut d'Hématologie et d'Immunologie, Strassbourg, France)

Abstract

Tumor extracellular vesicles (tumor EVs) mediate the communication between tumor and stromal cells mostly to the benefit of tumor progression. Notably, tumor EVs have been reported to travel in the blood circulation, reach specific distant organs and locally modify the microenvironment. However, visualizing these events in vivo still faces major hurdles. Here, we show a new method for tracking individual circulating tumor EVs in a living organism: we combine novel, bright and specific fluorescent membrane probes, MemBright, with the transparent zebrafish embryo as an animal model. We provide the first description of tumor EVs' hemodynamic behavior and document their arrest before internalization. Using transgenic lines, we show that circulating tumor EVs are uptaken by endothelial cells and blood patrolling macrophages, but not by leukocytes, and subsequently stored in acidic degradative compartments. Finally, we prove that the MemBright can be used to follow naturally released tumor EVs in vivo. Overall, our study demonstrates the usefulness and prospects of zebrafish embryo to track tumor EVs in vivo.

URL:

www.goetzlab.com

Abstract 3

Recombinant extracellular vesicles: biological reference material to standardize extracellular vesicle research

Authors:
Edward Geeurickx (1,12), Joeri Tulkens (1,12), Bert Dhondt (1,12,17), Jan Van Deun (1,12), Lien Lippens (1,11,12), Glenn Vergauwen (1,12), Elisa Heyrman (1), Delphine De Sutter (2), Kris Gevaert (2,12,16), Francis Impens (2,12,15,16), Ilkka Miinalainen (3), Pieter-Jan Van Bockstal (4), Thomas De Beer (4), Marca Wauben (5), Esther Nolte-'t-Hoen (5), Katarzyna Bloch (6), Johannes V. Swinnen (6), Edwin van der Pol (7,13,14), Rienk Nieuwland (7,14), Geert Braems (9), Nico Callewaert (8,12,16), Pieter Mestdagh (10,12), Jo Vandesompele (10,12), Hannelore Denys (11,12), Sven Eyckerman (2,12,16), Olivier De Wever (1,12) and An Hendrix (1,12*).

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Abstract

Introduction

Extracellular vesicles (EV) derived from liquid biopsies are emerging as potent biomarkers in health and disease. However, the complexity of liquid biopsies and the plethora of isolation and detection methods introduce variability that impedes interlaboratory concordance and clinical application. To evaluate and mitigate this variability, we developed recombinant EV (rEV) as biological reference material with unique trackability, and physical and biochemical similarity to EV.

Methods

We studied the similarity of rEV and EV using electron microscopy, zeta potential analysis, nanoparticle tracking analysis (NTA), lipidomics and proteomics. We assessed the trackability, stability and commutability of rEV using fluorescent NTA (fNTA), flow cytometry (FC), quantitative

real time PCR (RT-qPCR) and ELISA. rEV was spiked in plasma to calculate the recovery efficiency of EV isolation methods and to normalize EV numbers in plasma using fNTA and ELISA.

Results

rEV shows biophysical and biochemical similarity to EV such as morphology, zeta potential, size distribution, density, and protein/lipid content. rEV can be accurately quantified by fNTA and FC in EV-comprising samples. In addition, rEV behaves linearly with ELISA concentrations, and semi-logarithmic with RT-qPCR for eGFP mRNA. rEV is stable during multiple freeze-thaw cycles at -80°C and can be lyophilized without changes in morphology and concentration. EV recoveries from plasma for size-exclusion chromatography, differential ultracentrifugation, and DG were respectively 100%, 10% and 30%. For the first time, we could calculate the normalized EV concentration for breast cancer patients, which was significantly higher than healthy individuals (1.77E11 vs 6.51E10 particles/mL plasma).

Conclusion

We developed rEV, a biological reference material for EV research which can be used as positive control, spike-in material or calibrator to ensure standardized EV measurements in various applications

Abstract 4

Determination of biological and technical variability at protein and lipid level in isolated urinary extracellular vesicles: a crucial step in biomarker discovery studies

Authors:
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Abstract

The origin and function of extracellular vesicles (EVs) and their presence in easily accessible body fluids make EVs a promising source of biomarkers. The cargo of urinary EVs provide a targeted view into the urogenital tract to enhance the ability to detect urological diseases or tumors as they are released by the epithelia of the complete urogenital tract. In biomarker discovery studies, determining the variability is necessary for a correct experimental design with sufficient statistical power. We determined the variability at protein and lipid level in urinary EVs of healthy individuals.

Urine samples of healthy individuals (+50 years) were collected in compliance with the Declaration of Helsinki. Informed consents were obtained and the study was approved by the medical ethics committee of the University Hospital of Antwerp. EVs were isolated from urine using ultrafiltration in combination with size exclusion chromatography. Different experimental set-ups of variation were used to determine the total variation, including interbiological, intrabiological, technical and instrumental variation.

Proteins were identified using shotgun LC-MS/MS and lipids were identified using targeted lipidomics. Log normalization was applied on all data. 90% of the peptides and lipids do not have a standard deviation that exceeds 2 and 1.2, respectively. This information can be used in a sample size calculation in order to define a valid and powerful study design which results in a biologically significant biomarker identification.

Abstract 6

Sputum exosomal miR-142-3p suppresses fibrogenesis by targeting TGF-β pathway in Idiopathic Pulmonary Fibrosis

Authors:
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Abstract

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrosing interstitial lung disease of unknown etiology which leads rapidly to death. Although dysregulation of some exosomal microRNA (miR) from plasma has been shown to contribute to the pathophysiological processes of IPF, the role of altered exosomal miRs from sputum of IPF patients has never been studied. Consequently, we sought to characterize miR content of exosomes from sputum of IPF patients compared to healthy donors in order to identify novel biomarkers of the disease, and to study the roles of altered miRs in the pathogenesis of IPF. Exosomes were isolated from induced sputum samples of 16 IPF patients diagnosed following ATS/ERS recommendations and 14 healthy donors with standard ultracentrifugation protocol. Using miRNA qPCR array, we found a substantial dysregulation of sputum exosomal miR levels between IPF patients and healthy subjects, and identified for the first time an IPF-specific miR signature from sputum exosomes, among which miR-142-3p and miR-33a-5p present an upregulation (FC>3, $p<0.01$), whereas let-7d-5p a downregulation (FC<0.5, $p<0.01$). Interestingly, miR-142-3p is dysregulated similarly in exosomes from sputum and plasma of IPF patients, suggesting a major role in the pathophysiological processes of IPF. Furthermore, we found a positive correlation between miR-142-3p and monocytes/macrophages from sputum of IPF patients, and an enrichment of the miR in these immune cells. The overexpression of miR-142-3p was able to suppress transforming growth factor (TGF)-β-induced fibrogenesis in lung fibroblasts and epithelial cells via targeting TGFβ-R1. Taken together, our experiments demonstrated the antifibrotic effects of miR-142-3p and suggested that macrophage-derived exosomes from IPF sputum may slow pulmonary fibrosis progression via the delivery of miR-142-3p in lung fibroblasts and epithelial cells.

Abstract 7

Extracellular vesicles work as a functional inflammatory mediator between vascular endothelial cells and immune cells

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Abstract

Introduction: EV-mediated intercellular networking between monocytes (MC) and endothelial cells (EC) plays an active role at the crossing of inflammation and development of vascular disease. This study aims to unravel the immunomodulatory content of EV bulk-derived from control (uEV) and TNF-α induced inflamed endothelial cells (tEV) and to define their capacity to affect the inflammatory status of recipients monocytes (THP-1) and endothelial cells (HUVEC) .

Methods: TEM, NTA and western blot were used to confirm the presence of EV in the culture supernatant of a HUVEC, either untreated (uEV) or treated with TNF-α (tEV). In addition, protein arrays were used to discover the immunomodulatory content of both uEV and tEV. Relevant pro/anti-inflammatory markers were evaluated on protein level (ELISA, protein arrays) in both cell types exposed to the uEV and tEV. The functionality of uEV and tEV were assessed using migration and adhesion cell -based assays.

Results: EV derived from inflamed vascular EC (tEV) was readily taken up by HUVEC and THP-1 in culture. tEV were verified as packages of pro-inflammatory markers. Accordingly, the expression of pro-inflammatory markers (IL-6, IL-8 and ICAM-1) in tEV treated- HUVEC was increased. In the case of THP-1, EC-EV do induce a mixed of pro and anti-inflammatory response as indicated by the elevated expression of ICAM-1, CCL-4, CCL-5 and CXCL-10 proteins. At the functional level, EC-EV mediated inflammation and promoted the adhesion and migration of THP-1 [1].

Conclusion: Taken together, our current findings proved that an inflammatory cross-talk between inflamed EC and their neighboring EC and circulating MC is established via EV-carrying corresponding mediators.

This work was co-financed by the EU through the Interreg IV Flanders-the Netherlands project Interreg V Flanders-the Netherlands project Trans Tech Diagnostics (TTD).

[1] B. Hosseinkhani, et al. (2018), Frontiers in Immunology (9) 1789.

Abstract 9

Extracellular vesicles, engulfment, and airway inflammation

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Abstract

Extracellular vesicles (EV) represent a heterogeneous collection of membrane-enclosed structures secreted by cells. Broadly, EVs can comprise the larger apoptotic bodies (>1µm) and microvesicles (>100µm), as well as smaller exosomes (<100µm). Recent studies implicate EVs as mediators of intercellular communication, in part mediated via receptor/ligand interactions, and via transfer of contents from EVs into target cells. EVs are implied in intracellular communication during homeostasis as well as during inflammatory processes and thus have been considered as potential therapeutics for transfer of bioactive compounds into target cells. However, a key gap in our understanding is how the EVs interact with inflammatory components and influence responses in the context of ongoing inflammation, and hence disease outcomes. Previously, we have shown that microvesicles can be anti-inflammatory. In our current work we find that while the total bronchoalveolar lavage fluid from asthmatic patients or HDM-treated mice is pro-inflammatory, purified microvesicles from the BALF of patients and HDM-treated mice still possess anti-inflammatory properties. This suggests that the normally 'protective' effects imparted by microvesicles is modified by their interaction with other components in BALF. Mechanistically, this dampening of the anti-inflammatory effect of microvesicles is in part due to decreased uptake of these microvesicles by the bronchial epithelial cells. While intracellular content release is a hallmark of several inflammatory diseases, our work highlights a potential contribution of factors present in the inflammatory environment in regulating the balance between the anti-inflammatory/pro-inflammatory effects of EVs. This study thus sheds light on the importance of considering the inflammatory environment during the use of EVs as potential therapeutics.

Abstract 12

Underlying the exosomal export of the microRNA, miR-503, and its implication in the resistance to chemotherapy

Authors:
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Abstract

The communication between cancer cells and their environment is an essential aspect of cancerology. In this study, we focus on the communication between the endothelium and breast cancer cells during neoadjuvant chemotherapy by extracellular vesicles (EVs). EVs are small vesicles released by cells that mediate cell-cell communication. In the context of cancer, EVs are potentially secreted by all cell types that compose the tumor environment. Previously, we identified miR-503 which exhibited upregulated levels in EVs released from endothelial cells under neoadjuvant chemotherapy. In this study, we highlighted an exosome-dependent transfer of microRNAs from endothelial to tumor cells. This suggests that there might be a specific mechanism that sorts miR-503 into EVs. The aim of this study is to identify the proteins involved in the export of miR-503, the impact on tumor cells and how epirubicin, a chemotherapeutic agent, could affect it. In order to identify miR-503 partners, we pulled-down a biotinylated form of this microRNA and we determined the proteins attached to it using mass spectrometry. Five proteins, named miR-EXO proteins, were identified: ANXA2, hnRNPA2B1, VIM, TSP-1 and FN1.Then, we investigated which proteins were involved in the export of miR-503. We showed that the treatment with epirubicin induces the release of hnRNPA2B1 from the complex and, thus, allow the sorting of the microRNA. We also showed that the chemotherapy affects the localization of hnRNPA2B1. Furthermore, the proteins were silenced in endothelial cells and we determined the impact on breast cancer cells. The hnRNPA2B1 silencing decreased the proliferation of cancer cells. According to these results, we showed that hnRNPA2B1 inhibits the export of miR-503 into EVs. Future work will attempt to evaluate the impact of this export during tumor growth and the clinical relevance for breast cancer patients.

This work is supported by the FNRS, the Télévie, the fonds Léon Frédéricq and ULiège.

Abstract 13

Extracellular vesicles from human dental pulp stem cells as proangiogenic strategy in tooth regeneration

Authors:
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Abstract

Tooth loss remains a major health issue since current therapies cannot regenerate damaged dental tissues such as pulp and enamel. Successful pulp regeneration depends on angiogenesis, which is key for oxygen and nutrient supply. Proangiogenic features have already been assigned to mesenchymal stem cells (MSC) of the dental pulp. So far, paracrine factors, including VEGF, have been identified as responsible angiogenic mediators. However, more recent studies indicate that extracellular vesicles (EVs) produced by bone marrow MSC (BMMSC) also have the potential to induce neovascularisation. Therefore, we compared the angiogenic properties of EVs from dental pulp stem cells (DPSC) with those of BMMSC.

EVs were isolated from serum-free conditioned medium (CM) of DPSC and BMMSC after 48h by differential ultracentrifugation. EV size and concentration were measured by Nanoparticle Tracking Analysis (NTA) and purity was confirmed by Western blot with enrichment of classical EV markers CD9, CD63, CD81 and TSG101 and absence of non-EV marker mitochondrial complex V.

The functional effect of EVs on the migration of endothelial cells (HUVEC), as a key step in angiogenesis, was studied in a transwell system. Preliminary data show that EVs from DPSC induce HUVEC migration (n=8). However, this effect was less compared to BMMSC EVs (n=6), which might be caused by the lower EV yield from DPSC as measured by NTA. An antibody array revealed lower expression of proangiogenic factors (e.g. VEGF, MCP1 and Ang-1) and enrichment of antiangiogenic factors (e.g. TIMP1) in EVs compared to EV-depleted CM.

Our preliminary data show promising in vitro proangiogenic effects of DPSC EVs. In the future, we will analyse the potential of DPSC and BMMSC EVs to induce blood vessel growth in ovo and ultrastructural analysis of both EV types willl be performed.Acquired insights have positive implications for pulp regeneration and diseases associated with insufficient angiogenesis, such as stroke.

Abstract 14

Necroptosis, MLKL and the role of Extra-cellular Vesicles

Authors:
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Abstract:

For a long time, apoptosis was considered as the only form of regulated cell death, and necrosis was considered as accidental. Over the 2 past decades, necrosis was shown to be driven by different pathways. Nowadays, necroptosis, a necrotic regulated and caspase-independent cell death is intensively studied. Moreover, an increasing number of studies underline an important role of regulated necrosis in diseases (e.g. Cröhn disease, ischemia-reperfusion injuries…). TNF-induced necroptosis is relying on two kinases – RIPK1 and RIPK3 – and a pseudo kinase, MLKL. Since its discovery in 2012, the implication of MLKL in necroptosis accomplishment was constantly reinforced. However, even if most studies agreed on its crucial role at the plasma membrane, confusion remains and a new MLKL function in EV production was recently unveil (Gong et al., 2017; Yoon et al., 2017). This cell death modality was recently described as a process where EV production is increased. We are focusing on analysis of EVs produced during TNF-induced necroptosis and are determining the MLKL implication in this process. Our results indicate an increase in EVs production in necroptotic and apoptotic conditions when compare to survival condition. The EVs production can be blocked completely by GW4869 in apoptosis but only partially in necroptosis. Concerning MLKL dynamics during necroptosis, we developed and validate a cell line that enables us to monitor MLKL behavior upon TNF-induced necroptosis and visualized MLKL in real-time at the single living cells level. Using combination of light microscopy and electron microscopy, we witness for the very first time in TNF-induced necroptosis, MLKL punctuate structure formation few minutes before plasma membrane rupture. According to our preliminary results, some of these punctuates co-localize with exosomal markers, enhancing the MLKL function in the EV field.

Abstract 15

Cell communication via microRNA exchange between endothelial and tumour cells during anti-cancer neoadjuvant therapy

Authors:
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Affiliations:
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Abstract

The interaction between tumor cells and their microenvironment is an essential aspect of tumor development. Understanding how this tumor microenvironment communicates with tumor cells is crucial for the development of new anti-cancer therapies. Extracellular vesicles (EV) are small vesicles released by cells in the extracellular environment that mediate cell-cell communication. In the context of cancer, EVs are potentially secreted by all cell types in the tumor microenvironment. The aim of this study is to identify microRNAs (miRNA) mediating tumor-endothelial cell (HUVEC) communication and involved in tumor response to neoadjuvant chemotherapy. In particular, we focus on the transfer of miRNAs in endothelial EVs. We first determined that when the HUVECs were treated for 2h with paclitaxel 20 ng/ml or epirubicin 1 µg/ml, half of the cells survive after 72h. Similar treatment does not lead to endothelial cell apoptosis. We then purified EVs by differential centrifugation. We found that the treatments didn't modify the size of the vesicles using dynamic light scattering analysis. By Western Blotting, analyses did not reveal any modification on exosomal marker TGS101, CD63, CD81 and CD9, nor the endothelial cell specific marker CD31. We isolated RNA from EVs and from producing cells to make a profiling of their miRNA content. Four miRNAs (miR-373-3p, miR-887-3p, miR-122-5p and miR-129-5p) have been selected for further studies, based on their increased level in EVs from chemotherapy-treated HUVECs. We performed functional assays with MDA-MB-231 cells and epirubicin- or paclitaxel-resistant MDA transfected with pre-miRNAs. Interestingly we found that the effects on normal MDAs were lost in resistant cells. In parallel, we also found that EVs from HUVECs treated with epirubicin or paclitaxel affected the expression of genes known to participate in drug resistance. Further work will focus on evaluating the miRNA targets in MDAs and their modifications in resistant cells.

Abstract 16

A path to the Standardization of exosome isolation & recent solutions for the characterization of EVs

Authors:
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(4): Beckman Coulter GmbH Germany

Abstract

Exosome characterization and analysis comprise a fast, evolving research area even though their biological function has yet to be completely elucidated. Exosomes contain proteins, lipids, and microRNA capable of regulating an assortment of target genes. Recent studies have suggested that exosomes can serve as biomarkers for future clinical and diagnostic use in not only cancer but many other human diseases4. Exciting new findings have implicated exosomes in cardiovascular diseases, autoimmune syndromes, and neurodegenerative disorders such as Alzheimer's and Parkinson's disease, in addition to infectious diseases such as tuberculosis, diphtheria, and even HIV.

An improved and more efficient isolation and characterization protocol for exosomes and other EVs is critical to advancing this exciting field and experts have recently called for the establishment of standardized methods. EV isolation is particularly tedious, requiring several rounds of differential centrifugation and a density gradient centrifugation step to obtain highly pure vesicles. Downstream challenges involve a standardized method for genetic profiling of encapsulated miRNA. Here, we discuss the theory behind the proper techniques to isolate EVs and their contents and describe a workflow using automated Biomek methods for centrifugation layering and fractionation, total RNA extraction, and cDNA amplification and clean-up for next generation sequencing. Characterization of exosome size and morphology will also be presented.

The presentation also includes an update on recent development of characterization techniques for exosomes and Exosomes & EV subpopulations.

Abstract 17

Au@Ag Nanoparticles as a Colloidal SERS Substrate for Identification of Exosome-Like Vesicles

Authors:
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Abstract

Exosome-like vesicles (ELVs) are small vesicles actively released by cells carrying various biomolecules such as proteins, lipids and nucleic acid. As their content reflects the composition of the parent cell and because they are easy accessible, ELVs are envisioned as non-invasive biomarkers for cancer detection and follow-up. Several methods have been developed in an attempt to characterize and discriminate healthy from cancer cell-derived ELVs. In particular, one method that stands out is surface enhanced Raman spectroscopy (SERS), which is a plasmonic phenomenon based on the optical properties of noble metal nanoparticles (NPs), associated with the localized surface plasmon resonance.

In a previous work, we demonstrated that the identification of individual ELVs derived from different cell types is feasible by SERS after complete functionalization with 10 nm Au NPs by electrostatic interactions. For this end, Au NPs were previously functionalized with 4-dimethylaminopyridine (DMAP), being able to interact with the ELV membrane due to positive zeta potential. Although this method allowed to functionalize and obtain information from individual vesicles, the analysis of the SERS spectra had to take into account the strong SERS signals from the coating molecule. In the here presented work, we introduce a step forward on this colloidal strategy for identification of ELVs by performing a chemical modification to the NPs, generating a Au@Ag core@shell system, which entails two main advantages: 1)The Ag coating demonstrated to get rid of the background signals of the surface ligands of the Au NPs allowing to have a naked fingerprint of the vesicular composition improving the identification of ELVs. This was corroborated by using partial least squares discriminant analysis on the obtained SERS spectra. 2)The modification allows the biomolecules to be in direct contact with the metal surface being able to experience higher near field enhancements (higher SERS signals).

Abstract 19

Extracellular Vesicle-associated miRNAs indicate the presence of activated HSCs in the fibrotic liver

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(2) Department of Gastroenterology and Hepatology, Algemeen Stedelijk Ziekenhuis, Aalst, Belgium

Abstract

Chronic liver damage, due to various damage-causing agents such as alcohol abuse, viral infection, and obesity, may lead to the development of liver fibrosis, cirrhosis and even hepatocellular carcinoma. A key event in such fibrosis initiation and progression is the transdifferentiation of quiescent hepatic stellate cells (HSCs) towards an activated myofibroblastic state, characterized by an excessive production and deposition of extracellular matrix by these cells. Till date, the gold standard for diagnosis of liver fibrosis remains the invasive liver biopsy, which is however associated with multiple drawbacks. Non-invasive diagnostic tools such as serum markers and imaging modalities, have been proposed, but lack sensitivity and specificity. Our aim was to analyze the possibility to link circulating extracellular vesicle (EV)-associated miRNAs to the presence of activated HSCs in the affected liver. To this end, we induced the activation of primary murine HSCs in vitro by plating them on a hard substrate, and isolated their EVs by ultracentrifugation on a quiescent (day 0-2) and activated (day 8-10) culture time point. The miRNA content of these EVs was analyzed by Nanostring analysis, and the differently regulated miRNAs were identified. To analyze the potential use of these EV-associated miRNAs as biomarker for the presence of activated HSCs, and thus progression of liver fibrosis, circulating EVs were extracted from the plasma of healthy, and CCl4-treated mice, a widely used mouse model of liver fibrosis, and expression levels of HSC-associated miRNAs were analyzed and compared to their expression in total plasma. HSC-status specific miRNAs were further validated in plasma samples from patients with fibrosis (HBV, HCV, NAFLD, alcoholic), and their diagnostic value was compared to widely used clinical scoring systems of liver damage and fibrosis.

Abstract 21

Isolation and characterization of extracellular vesicles from culture media individually conditioned by bovine blastocysts and by bovine or human degenerate embryos

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(3) Department for Reproductive Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium.

Abstract

Efficient communication between cells and tissues is paramount in many physiological processes. For a successful pregnancy, an appropriate communication is required between the mother and the embryo. Recently, we have identified extracellular vesicles (EVs) are one of the crucial communication mechanisms among bovine embryos when they are cultured in a group. Extracellular vesicles released by group cultured bovine embryos are nano-sized (25-250nm), with a lipid bilayer, and are functionally active, since they carry and transfer regulatory molecules, such as microRNAs, mRNAs, lipids, and proteins. . Isolating EVs from culture medium conditioned by individual embryos can be useful in order to differentiate between good and bad embryos. Hence, we mainly focused on isolating EVs from bovine embryos cultured individually, which may be more challenging due to the small volume involved (25 µL). We used routine in vitro maturation and fertilization methods. In vitro bovine embryo culture took place in individual droplets of 25-µL, covered with mineral oil and incubated at 38°C in 5% CO2, 5% O2 and 90% N2. On 8 days post insemination (dpi), medium conditioned by bovine embryos was collected individually, while discriminating between blastocysts and degenerated embryos. Similarly, on 5 dpi we also collected culture medium conditioned by degenerated human embryos (10 embryos) from BioBank (Department for Reproductive Medicine, Ghent University Hospital). We used the Izon qEV size exclusion column (Izon qEV, iZON science, Cambridge MA) for isolating EVs from the medium (200 uL) conditioned by 10 degenerated bovine/ human embryos or by bovine blastocysts. The isolated EVs were analyzed with nanoparticle tracking, electron microscopy and western blot (CD9, CD63).We have confirmed that human embryos do release EVs into the culture media, which shows EVs can be a potential source of communication during embryo-maternal interactions.

Abstract 23

Exploring the neuroprotective function of extracellular vesicles-rich small heat shock proteins upon neuroinflammation.

Authors:
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Abstract

Currently, the repair mechanisms of Multiple Sclerosis (MS) is still unknown. However, it is known that small heat-shock proteins (HSPBs), which have protective functions are upregulated in MS lesions. Furthermore, it is shown that mutations in HSPB1 and 8 causes peripheral neurodegeneration. Although the protective intracellular functions are known, the extracellular functions remains unclear. One way cells secrete HSPBs is by releasing extracellular vesicles (EV). In MS, HSPB1/8 is upregulated in astrocytes but downregulated in oligodendrocytes and microglia cells during MS lesion development. We hypothesize that extracellular HSPBs exhibit neuroprotective roles which are altered upon inflammation in oligodendrocytes.

To determine the protective activity of intracellular and extracellular HSPBs in oligodendrocytes, we establish HSPB overexpressing cell-lines for the production and characterization of HSPB positive EV under normal and inflamed conditions. These purified HSPB-EV are further applied to measure their role in cell survival.

Pilot data shows that in oligodendrocyte, upon heat shock, there is a slight increase in early apoptosis. Striking, when oligodendrocytes were stimulated with inflamed EV, they exhibited a similar level of apoptosis comparable to known inflammatory mediators. In addition, immunoblot analysis of oligodendrocyte showed low expression of monomeric HSPB1/8 in non-stressed cells.

Summary: we observed low monomeric HSPB1/8 protein expression in non-stressed oligodendrocytes. Interestingly, inflamed-EV isolated from TNFa stimulated oligodendrocytes, exhibited a similar rate of apoptosis as compared to proinflammatory mediators. Reduction in endogenous expression of HSPB1/8 in oligodendrocytes might impair their cytoprotective activity during early inflammation.

This work was financed by Hasselt University and by EFRO through the Interreg V Grensregio Vlaanderen Nederland project Trans Tech Diagnostics

Abstract 25

EV-TRACK knowledgebase: evaluation, updates and future plans

Authors:
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Affiliations:
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Abstract

Background

Transparent reporting is vital to facilitate interpretation and replication of extracellular vesicle (EV) experiments. In March 2017, the EV-TRACK consortium launched a resource to improve the rigor and interpretation of experiments, record the evolution of EV research and create a dialogue with researchers about experimental parameters.

Methods

The EV-TRACK database is accessible at http://evtrack.org, allowing online deposition of EV experiments by authors pre- or post-publication of their manuscripts. Submitted data are checked and an EV-METRIC is calculated, which is a measure for the completeness of reporting of information necessary to interpret and repeat an EV experiment. When the EV-METRIC is obtained at the preprint stage, it can be implemented by authors, reviewers and editors to help evaluate scientific rigor of the manuscript.

Results

Between March 2017 and January 2018, data on 150 experiments (unpublished: 49%; published: 51%) were submitted by 74 unique users. The average EV-METRIC for all experiments was 43% (unpublished: 57%; published: 31%).

For 7 experiments, authors added additional data that were not included in the original article to the corresponding EV-TRACK entry, improving transparency of their experiments. These data included isolation protocol details such as ultracentrifugation rotor type, and antibody clone and dilution as used in immunoassays.

Based on user comments, a new submission system was developed. The new system has been modified to make it more complete, intuitive and user-friendly.

The usability of the EV-TRACK platform as a database is being improved, allowing more versatile search options and easier data download.

Conclusion

The EV-TRACK knowledgebase is a unique resource devoted to EV research, with a focus on transparent reporting of experimental data. Through constant evaluation and interaction with its users, new developments will ensure that the platform remains up-to-date, user-friendly and relevant.

Abstract 26

Isolation and proteomic analysis of high-purity urinary extracellular vesicles from prostate cancer patients: potential for biomarker discovery

Authors:
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(3) Cancer Research Institute Ghent (CRIG), Ghent, Belgium
(4) University of Oulu, Dept. of Pathology, Oulu, Finland
(5) University of Turku, Dept. of Biochemistry, Turku, Finland

Abstract

Introduction & Objectives: Urinary extracellular vesicles (uEV) have raised interest as a potential source of biomarker discovery. Contaminants such as Tamm-Horsfall protein (THP) polymers hinder accurate downstream analysis of uEV by masking low abundance proteins or by entrapping non-EV associated extracellular RNA molecules. The isolation of highly purified uEV using a standardized, reproducible technique, is a prerequisite to obtain reliable omics data for biomarker studies. An isolation technique meeting these requirements was applied on urine samples from prostate cancer patients and followed by LC-MS/MS proteomic analysis.

Materials & Methods: Cell-free urine samples from prostate cancer patients were concentrated by ultrafiltration and uEV were isolated using a bottom-up discontinuous Optiprep™ density gradient (ODG) in 6 technical replicates and characterized by nanoparticle tracking analysis (NTA), Coomassie Blue (CB) staining, Western Blot (WB) analysis, Transmission Electron Microscopy (TEM) and unbiased proteomic analysis (LC-MS/MS).

Results: NTA, CB, WB and TEM confirmed the enrichment of uEV in density fractions of 1.1 g/ml (EV-rich fractions) and contaminants in the high-density fractions (SP-rich fractions, containing urinary soluble protein). Unbiased mass spectrometry-based proteomics identified consistent and biologically relevant EV-associated proteins with high repeatability as analyzed by principle component analysis and hierarchical clustering. Volcano plot analysis showed a clear differential protein enrichment between EV- and SP-rich density fractions and gene set enrichment analysis of these proteins demonstrated differential biological functions and cellular origin. Of interest, known prostate cancer markers PSA and PAP were enriched in urine as SP, while PSCA and PSMA were especially EV-derived. Of the 2232 proteins that were identified across all examined ODG fractions,

623 (27.9%) were uniquely detected in the EV-rich fraction, highlighting the potential of highly purified uEV for biomarker discovery.

Conclusion: Bottom-up ODG results in an efficient and reproducible separation of uEV from contaminants, an important step towards EV-based biomarker discovery in prostate cancer patients.

Abstract 27

Annelies Bronckaers

Morphology Research Group, Biomedical Research Institute (BIOMED), Hasselt University, Hasselt, Belgium

Abstract:

Professor Annelies Bronckaers (°1982) is since October 2016 Assistant Professor at the Morphology Group of the UHasselt. This laboratory, started by Prof. Ivo Lambrichts, has a long-lasting experience in histology and electron microscopy and the main research topic is the use of dental pulp stem cells (DPSCs) for (dental) tissue regeneration . The new research group of A. Bronckaers focuses on new strategies to enhance angiogenesis as a therapy for diseases where angiogenesis is insufficient such as ischemic stroke. Her laboratory routinely uses following techniques: stem cell culture and differentiation, western blot, in vitro angiogenesis models, in ovo models of blood vessel formation and mouse model of stroke (dMCAO). In collaboration with the research groups of L. Michiels and J. Irobi, she is comparing the angiogenic activities of EVs of DPSCs and EVs of bone marrow-derived mesenchymal stem cells.

Abstract 28

Nick Geukens

PharmAbs, the KU Leuven Antibody Center, KU Leuven, Leuven, Belgium

Abstract:

PharmAbs (www.pharmabs.org) is an innovation, incubation and valorization platform at KU Leuven, clustering the antibody expertise of five different KU Leuven groups. It is the mission of PharmAbs to deliver innovative research tools and translate fundamental biomedical research carried out by universities into lead products for new medicines or into novel diagnostic solutions.

PharmAbs has state-of-the-art facilities for antibody selection and characterization and medium-scale production and has deep knowledge in customized antibody engineering and immunoassay development. Therapeutic activities are mainly focused on pre-clinical development (discovery, in vitro & in vivo proof-of-concept in relevant animal models), whereas for diagnostic development our activities can extent to retrospective and prospective clinical studies.

PharmAbs has the expertise to design and develop customized tools for detection and profiling of extracellular vesicles and a wide industry network for advancing this research to the market.

Keywords:

Monoclonal antibody, Immunoassay, Antibody therapeutics

URL:

www.pharmabs.org

PHARMABS SLAAT BRUG TUSSEN WETENSCHAPPELIJK ONDERZOEK EN BEDRIJFSWERELD

Antilichamen? Bij de meeste mensen gaat er wellicht geen belletje rinkelen. Antilichamen zijn eiwitten die je lichaam op een natuurlijke manier aanmaakt als afweersysteem tegen mogelijke indringers, zoals bacteriën of virussen. De KU Leuven doet baanbrekend onderzoek rond zulke antilichamen. En PharmAbs, een platform binnen de universiteit, zet de wetenschappelijke resultaten rond antilichamen om naar de praktijk. Toen ze daar tien jaar geleden mee begonnen, kregen ze 118.500 euro innovatiesteun van de provincie. Vandaag heeft PharmAbs al 15 miljoen euro geïnvesteerd in translationeel onderzoek.

PharmAbs ondersteunt academische groepen en biotechbedrijven bij de valorisatie van projecten rond antilichamen op maat. Het stemt onderzoeksresultaten rond antilichamen af op de markt en ontwikkelt ze economisch.

Professoren Paul Declerck en Hans Deckmyn lagen aan de basis van PharmAbs. In samenwerking met LRD – Leuven Research and Development - dienden ze in 2007 bij de provincie een aanvraag in voor een innovatiesubsidie. Die werd vanaf 2008 onder andere gebruikt om dr. Nick Geukens aan te werven. Zijn taak bestond er in de eerste plaats in om nieuwe fondsen te verzamelen voor PharmAbs.

Hoe belangrijk was de Innovatiesteun van de provincie Vlaams-Brabant voor PharmAbs?

Paul Declerck: "Puur financieel gezien was die bijdrage misschien eerder beperkt, maar ik durf gerust stellen dat PharmAbs er zonder de startsubsidie van de provincie niet zou gekomen zijn. Het was absoluut de helboom voor ons, want daarmee hebben we Nick Geukens als projectcoördinator aangetrokken. Binnen de KU Leuven is zowel bij collega Deckmyn in Kortrijk als hier op de Leuvense campus enorm veel expertise beschikbaar rond antilichamen. Die kennis wilden we zichtbaarder maken en bundelen. Alleen zo zouden we onze knowhow bij externe gebruikers en bedrijven kunnen krijgen. Ook inzake onderzoeksniveau zouden we zo de lat hoger kunnen leggen en ons onderzoek kunnen valoriseren. We wilden een synergie krijgen tussen verschillende laboratoria om zo het succesgehalte voor projecten en onderzoeksresultaten te vergroten."

* Provinciale innovatiesubsidie zorgde voor sneeuwbaleffect

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Abstract 29

An Hendrix

Laboratory of Experimental Cancer Research, Ghent University, Ghent, Belgium

Abstract:

The laboratory of Experimental Cancer Research (LECR, PI De Wever and Hendrix) is an ecosystem to study tumor-environment interactions during metastasis. One important research line is the focus on extracellular vesicles (EV), lipid packaged molecular information, as important mediator of cancer progression.

EV are present in biofluids and their molecular information may guide cancer diagnosis and therapy monitoring. To allow and boost the clinical implementation of EV in daily clinical practice LECR invests in standardization and reproducibility of EV research since this is the foremost important step to initiate biomarker discovery in any type of biofluid for any type of disease. EV-TRACK, an expandable open-source knowledgebase to map methodological specifications of EV-related publications, raises the awareness about the importance of transparency and reproducibility (PMID: 28245209; abstract 25). Comparative evaluation of EV isolation protocols demonstrates that high purity is a prerequisite to obtain reliable omics data and identify EV-associated biomarkers (PMID: 25317274). Isolation of EV from blood and urine is challenging due to abundant proteins (aggregates), free nucleic acids and lipoproteins. Orthogonal biochemical approaches are required to enrich EV from biofluids with minimal remnants of these contaminants (Tulkens et al. and Dhondt et al., unpublished data; abstract 11 and 26). In addition, biofluid complexity and the technical errors that are introduced during sample preparation, isolation and analysis hamper data analysis. To understand and mitigate these errors we design recombinant EV (rEV) that harbor physical and biochemical traits of EV (Geeurickx et al., unpublished data; abstract 3).

Keywords:

cancer, liquid biopsies, EV biogenesis, EV analysis, transparency

URL:

www.ugent.be/ge/oncol/en/research/lecr

Abstract 31

Inge Mertens

Sustainable Health, VITO nv (Flemish Institute for Technological Research), Mol, Belgium & Centre for Proteomics, University of Antwerp, Antwerp, Belgium

Abstract:

It is known for several years now that extracellular vesicles (EVs) hold great potential for improved diagnostics, therapy monitoring as well as drug target identification in several disease areas. The first technical hurdle that needs to be overcome is obtaining pure and well characterized EV from the primary clinical sample.

Together with the VITO technology development team and commercial/external partners, we focus on the isolation and characterization of EVs from various clinical samples, like urine, plasma and cerebrospinal fluid. However, the final goal of our research is to identify and validate EV associated biomarkers for diagnostic and therapeutic applications in the field of oncology and neurodegenerative diseases. we have optimized a workflow to isolate and characterize EVs from urine samples, using ultrafiltration and size exclusion chromatography. In collaboration with commercial parties we also optimized the AF4 technology to characterize the urinary EVs based on size, composition and concentration (Oeyen et al., JEV, published online). After a variation study in a healthy control population, we calculated the sample size for a case-control experiment between bladder cancer patients and a matched healthy control population using mass spectrometry based proteomics and lipidomics. A prospective sample collection is currently performed.

An in-depth knowledge on sample preparation of protein and lipid samples, expertise in LC-MS/MS analytics and a strong knowledge in data science, statistics and MS data quality control are of equal importance to achieve relevant, reproducible and high quality results from EV samples that can serve as target for a novel diagnostic test or therapeutic compound development.

Keywords:

Extracellular vesicles, Liquid biopsies, Clinical proteomics, Mass spectrometry, Biomarkers

URL:

www.uantwerpen.be/centre-for-proteomics

Abstract 32

Luc Michiels

BioNanoTechnology (BNT) research group, Biomedical Research Institute (BIOMED), Faculty of Medicine and Life Sciences, Hasselt.

Abstract:

Within the BioNanoTechnology (BNT) research group at Hasselt University (UH), we apply nanotechnology approaches in molecular biological research, more specifically to study the role of extracellular vesicles (EV) in intercellular communication, in particular in inflammation associated diseases. Our major goal is to isolate intact and functional disease related EV in order to study their functional behavior and intercellular communication using cell based assays. In parallel the specific cargo of these EV subsets are analyzed to explore the mechanistic pathways involved in these processes.

The BNT lab is equipped with basic EV research tools for EV isolation and characterization such as UC, SEC, NTA, immunogold-TEM and WB. In addition we use advanced tools including HR imaging, SPR based biosensing, QPCR, droplet digital PCR and NGS nucleic acid content measurements. Next to the physical and molecular analysis techniques we established functional cell based assays to study the effects of EV on inflammation related processes. Finally, we have access to the UBILIM biobank (Jessa hospital/UH) containing large collections of inflammation related patient samples.

Ongoing projects in the BNT research group focus on the role of EV in cardiovascular disease, in collaboration with CARIM at Maastricht University and VITO (Interrreg IV and V projects VARiA and TTD). The use of EV as therapeutic vesicles is explored in collaboration with the material research IMO institute at UH (FWO PhD project).

Recent EV related publications:

Hosseinkhani B. et al. Direct detection of nano-scale extracellular vesicles derived from inflammation-triggered endothelial cells using surface plasmon resonance, Nanomedicine: Nanotechnology, Biology, and Medicine, 2017, 13, pp 1663-1671.

Hosseinkhani B., et al. Extracellular vesicles work as a functional inflammatory mediator between vascular endothelial cells and immune cells. Frontiers in immunology (2018), https://doi.org/10.3389/fimmu.2018.01789

Abstract 33

Inge Nelissen

Sustainable Health, VITO nv (Flemish Institute for Technological Research), Mol, Belgium

Abstract:

Extracellular vesicles (EVs) derived from various cells and shed into blood, urine and cerebrospinal fluid offer unique potential for providing minimally invasive diagnostics. While being highly attractive for clinical diagnostics, their purification and detection is challenging given the heterogeneity and low abundance of EV populations in primary clinical liquid biopsies, as well as the presence of various other biomolecules with similar characteristics (e.g. size, density, protein markers, etc).

Based on long-term multi-disciplinary expertise in the field of nanomaterials characterization, molecular and cell biology, biochemistry, data integration of various 'omics' technologies, and in vitro assay validation, at VITO we have established a unique translational research platform for development of innovative methods and technologies for EV isolation and detection. Samples of increasing complexity are used, starting with conditioned media of relevant in vitro cell types and ending with liquid biopsies, for which we develop standard collection, processing and storage protocols. To obtain pure subpopulations of EVs we combine different fractionation techniques, including fluorescence-activated EV sorting. Finally, we exploit targeted detection principles based on affinity for specific biomarker profiles and nanotechnology to create novel, easy-to-use devices. These are benchmarked against state-of-the-art technologies for EV-based biomarker detection, such as high-resolution flow cytometry, LC-MS-based proteomics or qPCR.

In this research area we collaborate with various Flemish and European partners, while the market readiness of the technologies and applications developed at VITO are guided through close interactions with end-users (clinics) and industrial stakeholders (technology developers).

Keywords:

Health diagnostics, Nano-biotechnology, Point-of-care devices, High-resolution flow cytometry, Fluorescence-activated EV sorting

URL:

www.vito.be/en/application-area/health-diagnostics

Abstract 34

Christophe Pierreux

CELL Unit, de Duve Institute, Université Catholique de Louvain, Brussels, Belgium

Abstract

The group of C. Pierreux is interested in the paracrine communications between epithelial cells and blood vessels endothelial cells during development and diseases of two endoderm-derived organs, the pancreas and the thyroid. Using ex vivo organ culture, we demonstrated instructive role of endothelial cells on the development and differentiation of the pancreas and thyroid. In the thyroid, we recently demonstrated that endothelial cells instruct thyrocyte progenitors by producing extracellular vesicles (EVs) with exosomal characteristics. Addition of endothelial-derived EVs to thyroid organ stimulated thyrocyte progenitor organization into follicles and lumen expansion. More recently, we have been using a genetically-engineered mouse model, in which the oncogene BRAFV600E can be switched on and trigger papillary thyroid carcinoma (PTC), to identify the miRNAs contained in EVs released during PTC progression and correlate their presence with histological changes and thyroid dedifferentiation. The clinical significances of the identified EV-miRNAs will be evaluated on plasma from patients with thyroid cancer. Similar projects on pancreatic cancer are just starting.

Keywords:

thyroid, pancreas, development, cancer, endothelium

URL:

www.deduveinstitute.be/epithelial-differentiation

Abstract 35

Dragana Spasic

MeBioS-Biosensors group, KU Leuven, Leuven, Belgium

Abstract:

Since its foundation in 2005 (by Prof. Jeroen Lammertyn), the MeBioS-Biosensors group has been focusing on developing novel bio-molecular detection concepts and miniaturized analysis systems to be applied in a broad range of sectors including food and medical diagnostics (e.g. cancer, diabetes, infectious diseases and neurodegenerative diseases). Although relatively divers, all the research activities within the group belong to at least one of the 3 research lines: bioassay development, optical sensors and microfluidics. The last two research lines are related to several technology platforms established in the group over the years, including fiber optic surface plasmon resonance (FO-SPR) biosensors, digital microfluidics with microwell array technology for implementation of digital bioassays, optical tweezers, as well as iSIMPLE lab-on-a-chip technology for point-of-care (POC) applications.

Over the past years, the Biosensors group has been working on the development of (1) technology platforms (e.g. microfluidic chips and FO-SPR biosensor) to selectively capture (cancerous) extracellular vesicles (EVs) and (2) bioassays for ultrasensitive detection and molecular profiling of (cancerous) EVs (e.g. in combination with another in-house developed optical tweezers technology). This work is being performed in the framework of different (inter)national projects, such as Vlaamse Liga tegen Kanker (PI: prof. Johan Swinnen), Kom op Tegen Kanker (PI: An Hendrix) and Marie Curie ITN action with additional 11 European partners.

In this presentation, we will briefly describe these different technologies and highlight their potential both as life science research tools for studying EVs as well as diagnostic tools.

Keywords:

Biosensors, microfluidics, DNA nanotechnology, optical sensors, point-of-care diagnostics

URL:

www.biosensors.be

Abstract 37

Ingrid Struman

Molecular Angiogenesis Laboratory, GIGA research center, ULiège, Liège, Belgium

Abstract:

As a PI of the Laboratory of Molecular Angiogenesis, I have a longstanding experience in the field of angiogenesis. Ten years ago we initiated projects aimed at identifying and characterizing microRNAs involved in angiogenesis. Two of our studies (i.e. miR146a in PPCM (Halkein, JCI 2013) and miR-503 in tumoral angiogenesis (Bovy, Oncotarget 2015) highlighted a novel mechanism of cell communication via EV exchange of microRNAs. The PPCM paper was pioneer in the field since it was one of the first demonstration that this mechanism is involved in the etiology of a disease.

Over the years we acquired experience in RNA (small and long RNA) profiling in EVs. We are interested in deciphering cell communication mechanisms occurring via the exchange small/long RNA of EVs.

For example, by the use of next generation sequencing we have characterized in depth the RNA composition of healthy endothelial cells and EVs and provided an accurate profile of the different coding and non-coding RNA species found per compartment. We have also discovered a set of unique genes preferentially included (or excluded) into vesicles. In the same topic, we recently decipher a mechanism of specific export of microRNA in EVs. Several years ago we initiated a project aiming to determine the impact of microRNAs secreted by the endothelium on tumor development and found a microRNA, miR-503, for which functional studies revealed protumoral activities in vitro. In this study we highlighted an EV-dependent transfer of microRNAs from endothelial to tumor cells. To determine how microRNAs are specifically exported into EVs is currently a challenging question in the field of EV research. We thus decided to decipher this mechanism and a novel mechanism involving several RNA binding proteins.

In parallel with basic research, we are also involved in several projects in collaboration with the clinics aiming to identify RNA/EV biomarkers in breast cancer, pancreas cancer, pulmonary fibrosis...

Key words

microrna, noncoding RNA, angiogenesis, cancer, EV

URL

www.giga.uliege.be/lam

Abstract 38

Roosmarijn Vandenbroucke

Barriers in Inflammation lab, VIB Center for Inflammation Research, Ghent, Belgium & Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

Abstract:

The research of the 'Barriers in Inflammation' lab focusses on the importance of brain and gut barriers in (neuro)inflammatory disorders. While the intestinal barrier prevents leakage of gut luminal components into the periphery, tight epithelial and endothelial barriers form the major protection for the brain against external insults such as toxins, infectious agents, and peripheral blood fluctuations. One important research line studies the biological role of extracellular vesicles at these barriers in response to gut inflammation (e.g. inflammatory bowel disease (IBD)), systemic inflammation (e.g. sepsis), and neuroinflammation (e.g. Alzheimers and Parkinsons disease). Additionally, the obtained knowledge on the mechanisms of extracellular vesicle production and transport at the brain barriers is used to develop innovative delivery systems for brain targeting of drugs.

Keywords:

Inflammation, neuroinflammation, gut-brain axis, barriers

Abstract 39

Pascale Zimmermann

University of Leuven, Leuven, Belgium & Cancer Research Center, Marseille, France

Abstract:

Extracellular vesicles are lipid bilayer-limited organelles supporting cell communication over short or long distance, controlling tissue homeostasis as well as disease progression. Extracellular vesicles in general and exosomes (nanovesicles) in particular carry complex messages and are secreted by any type of cell. They are currently under intensive study, as sources of biomarkers, as alternative therapeutic targets and as efficient vehicles for drug delivery.

In an effort to support the rational design of exosome-based medicine, our laboratory attempts to decipher molecular mechanisms supporting the biogenesis, composition, functional diversity, and turnover of exosomes. We will illustrate how membrane scaffolds like syndecan heparan sulfate proteoglycans, adaptor PDZ proteins like syntenin, lipid-modifying enzymes like phospholipase D2 and oncogenes like SRC tyrosine kinase cooperate and control exosome-based cell signaling.

For selected publications see Baietti et al., Nat Cell Biol 2012; Ghossoub et al., Nat Commun 2014 and Imjeti et al., PNAS 2017.

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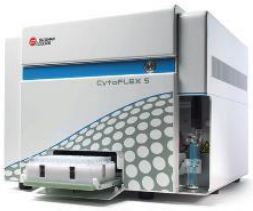
Complete solutions for your specific exosome workflow from preparation to analysis

Analis provides solutions customized for each step in your specific exosome research workflow – from sample preparation to sample analysis – and can also help improve processes and assist with related challenges.

This chart provides a sample workflow and the solution for each step.

Sample Preparation	Specific Need	Solution
Prep Cell Culture Media	Easily centrifuge fetal bovine serum (FBS) at high speeds (typically overnight) to eliminate bovine exosomes from media.	Ultracentrifuge
Cell Culture	Quickly determine cell concentration and viability, eliminating variability inherent in manual counting methods.	Vi-Cell
Exosome Isolation	Use differential centrifugation to remove cells, debris and large aggregates prior to density gradient isolation.	Tabletop centrifuge
Automated Density Gradient Layering	Minimize user to user variability and save time by automating density gradient set-up.	Biomek workstation
Exosome Purification by Density Gradient	Isolate exosomes from co-purified proteins and other vesicles.	Ultracentrifuge & Biomek workstation
Exosome Concentration	Use ultracentrifugation to remove density gradient material and to concentrate exosomes.	Tabletop ultracentrifuge
Exosome Sizing	Measure size and zeta potential of purified exosomes.	DelsaMax Pro/CORE
NGS Library Construction and Sequencing	Convert exosomal RNA into libraries with a NEBNext Small RNA Library Preparation Kit or Illumina TruSeq Stranded mRNA kit automated on a Biomek workstation.	Biomek workstation & automated NGS library preparation methods
Population Characterization	Characterize and sort exosome-specific populations using scatter or fluorescence.	CytoFlex Flow Cytometer (Analysis) Astrios EQ (Sorting)
Size Characterization	Use analytical ultracentrifugation (AUC) to get better size resolution and other properties of your exosomes.	Optima AUC



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Info: FR - Vincent Jadin - vjn@analis.be NL - Ann Desplanques - ads@analis.be

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Phenotyping of EVs by Multi-Wavelength Fluorescence Nanoparticle Tracking Analysis

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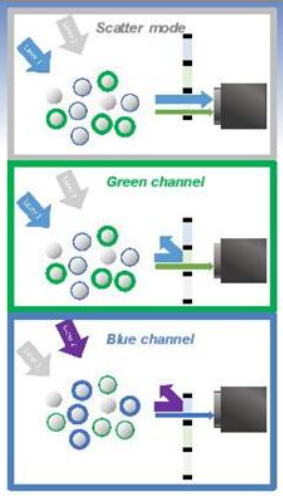
[2]) University Hospital Essen, Institut for Transfusion Medicine, Virchowstr. 179, D-45147 Essen, Germany
[3]) HansaBioMed Life Science LTD, Akadeemia tee 15A, 12618 Tallinn, Estonia
Corresponding author: helmbrecht@particle-metrix.de

Introduction



Nanoparticle Tracking Analysis of fluorescent labelled bionanoparticles, such as vesicles or liposomes, is an efficient technique for quantification of size and total concentration. Analysis with single wavelength requires measurement of samples in sequence.

For the first time, we present an NTA instrument equipped with two lasers, allowing analysis of at least two different biomarkers on the same sample.

An NTA with the combination of 405 and 488 nm was evaluated with fluorescent standards as well as antibody-labelled EVs.

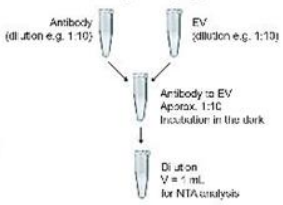
Material and Methods

NTA: ZetaView® TWIN Laser NTA with 405 and 488 nm Laser and long pass filters

HEK293-EVs (Hansabiomed LTD) were reconstitued in water by 30 s of vortexing.

MSC-EVs (AG Giebel) were purified by Ultracentrifugation and PEG precipitation.

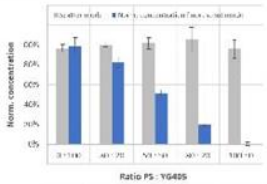
Antibody conjugation



Standards – Fluorescent PS

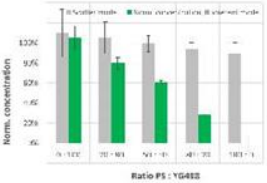
405 **488**

Mixture of unlabeled polystyrene and fluorescent particles YG405 (440|490)



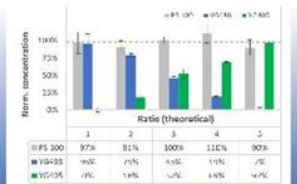
405 **488**

Mixture of unlabeled and fluorescent particles YG488 (505|515)

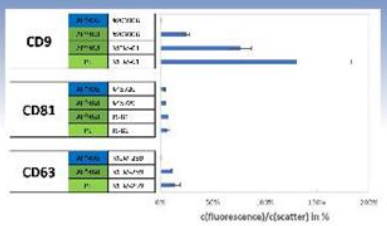


405 **488**

Mixture of YG405 and YG488 analyzed byTWIN laser NTA (spill-over correction = 20%)

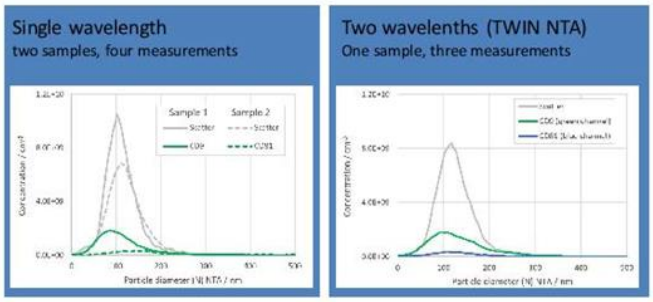


Immortalized MSC derived EVs

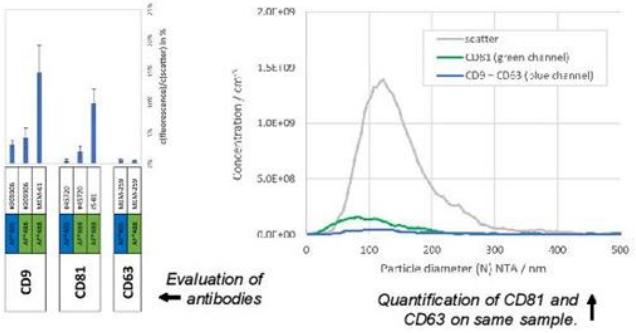


Evaluation of direcly labeled antibodies, influence of clone and fluorophore.

Comparison of tetraspanin detec-tion procedures: in sequence (left) or by two wavelengths on same sample (right).



Lyophilized EVs from HEK293



Evaluation of antibodies

Quantification of CD81 and CD63 on same sample.

Conclusion

- **Antibodyquality** has significant impact on the result. Parameters for quantifying antibody quality need to be determined.
- **Labeling protocol** still needs optimization and standardization.
- **Improvement of reproducibility**, reduction of **sample amount**, more **information per sample**.

Acknowledgement

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Characterization of RNA from extracellular vesicles

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[3]QIAGEN GmbH, Hilden, Germany. [4]QIAGEN, Maryland, USA.

Introduction

Extracellular vesicles (EVs or "vesicles") are a rich source of high-quality RNA, released by all virtually all cells and present in all biofluids. Many current protocols to isolate vesicles use ultracentrifugation; however, development of faster, more convenient methods with high specificity for vesicles and good RNA quality is an area of active research. Here we present a characterization of vesicle RNA isolated from plasma and serum by several different methods, including ultracentrifugation and a novel spin-column-based method. The RNA was subjected to electrophoresis, RT-qPCR arrays and NextGen Sequencing to demonstrate the utility of vesicle RNA as a source for blood-based biomarkers.

Figure 1. The new exoRNeasy Serum/Plasma Maxi Kit. Disposable spin column to purify vesicles and workflow of the exoRNeasy Serum/Plasma Maxi Kit.

Small and large RNAs in extracellular vesicles

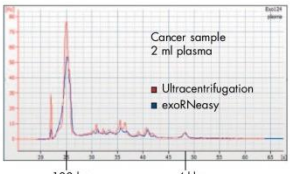

Figure 2. exoRNeasy and ultracentrifugation give similar RNA yields from vesicles present in blood plasma. Bioanalyzer sizing of vesicle-derived RNA purified by two methods. The plasma was pre-filtered (0.8 µm) to exclude larger particles and subjected to either ultracentrifugation, the current gold standard of vesicle isolation, or the exoRNeasy procedure. Both methods purify RNA of similar size and yield.

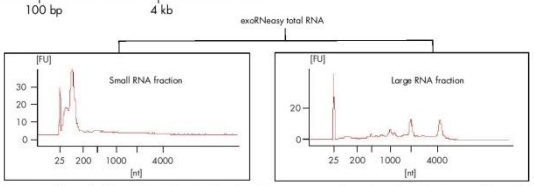

Figure 3. EVs contain both, small and large RNAs. RNA from 2 ml of pre-filtered plasma was separated into a large and small fraction using different ethanol concentrations for binding to the RNeasy purification column. Subsequently, both fractions where analyzed using a Bioanalyzer Pico assay. The presence of sharp ribosomal RNA peaks demonstrates the purification of large, intact, non-degraded RNAs from EVs.

Integrity of mRNA in vesicles and detection of oncogenes in different sample volumes

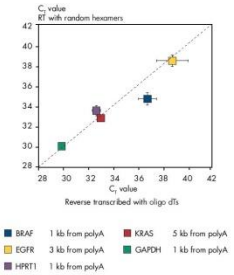

Figure 4. Extracellular vesicles contain full-length mRNAs with intact poly A tails. RNA from 2 ml of pre-filtered plasma was extracted with exoRNeasy and subjected to reverse transcription with the superscript III enzyme using either random hexamers or oligo-dTs and 6 replicate reactions for each. Both methods yield comparable C_ts in RT-qPCR, demonstrating that the quantified mRNA transcripts are not degraded.

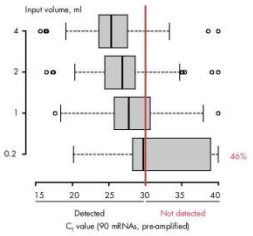

Figure 5. mRNAs from known oncogenes are readily detected in high volumes of plasma. RNA from various volumes of plasma were isolated with exoRNeasy, reverse transcribed and pre-amplified using the RT² PCR system and detected using the Human Cancer Pathway Finder PCR Array. At 0.2 ml only 46% of the mRNAs are robustly detected (C_t<30) but the isolation with exoRNeasy can be scaled up to 4 ml with linear increase in C_t signal and mRNA detection rate.

Separation of vesicular from non-vesicular RNA

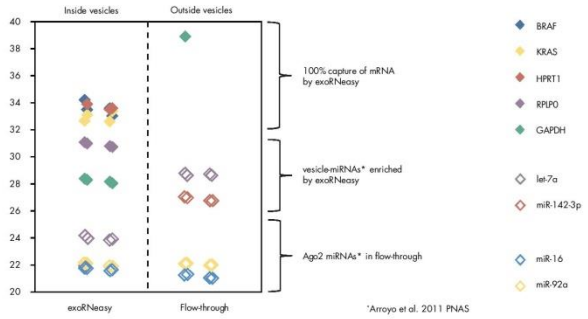

Figure 6. exoRNeasy captures all mRNA and vesicle-specific miRNAs in plasma. RNA from 0.2 ml of pre-filtered plasma was isolated with exoRNeasy and the flow through of the exoEasy column was used in direct lysis. Shown are raw C_t values from RT-qPCRs with rows as replicate isolations and colored diamonds as replicate qPCRs.

RNA recovery with different methods and detection of oncogenic mutations

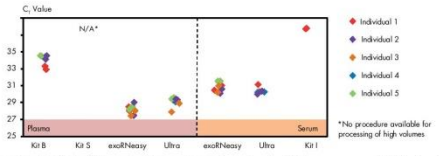

Figure 7. exoRNeasy enables extraction from high volumes of plasma and serum. Vesicles from 4 ml of plasma or serum were isolated with exoRNeasy, ultracentrifugation (Ultra) and three commercially available methods based on filtration (Kit B) or precipitation (Kit S, Kit I). Only exoRNeasy and ultracentrifugation efficiently recover RNA from high sample volumes.

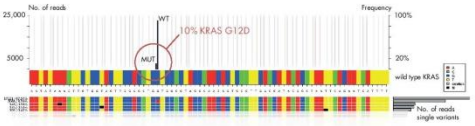

Figure 8. NextGen Sequencing detects somatic mutations from CRC in vesicle RNA. A 2 ml sample of pre-filtered plasma was drawn from a patient with KRAS G12D positive colorectal cancer (CRC). RNA from vesicles was isolated using exoRNeasy and analyzed with targeted re-sequencing on an Illumina® MiSeq®. Over 10% of all reads that matched to the KRAS gene carry the c.35 G>A/p.G12D mutation previously identified in the primary tumor.

Conclusions

- The exoRNeasy Serum/Plasma Maxi Kit can be used to extract high-quality RNA from plasma EVs using a fast and convenient spin-column procedure.
- EVs contain small and large non-degraded mRNAs.
- EVs contain plasma mRNA and a specific fraction of miRNA.
- EVs enable detection of somatic mutations and oncogene expression.
- Sensitivity of oncogenic transcript detection is highly dependent on sufficient sample volume

This work was supported partly by the m4 spitzencluster initiative.

The applications presented here are for molecular biology applications. They are not intended for the diagnosis, prevention or treatment of a disease.

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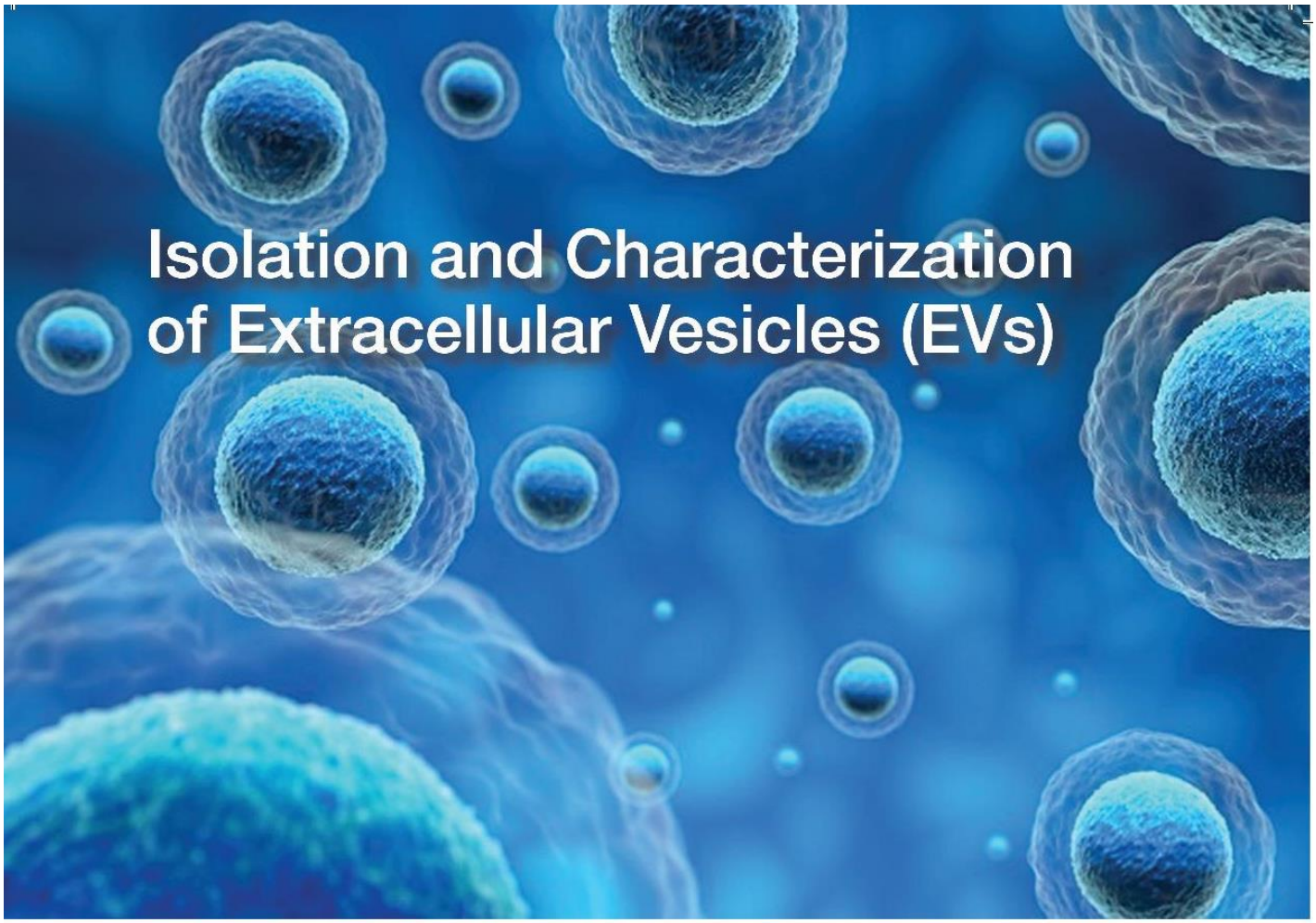

The unique combination of FFF-MALS-DLS

Field-flow fractionation combined with multi-angle and dynamic light scattering (FFF-MALS-DLS) has increasingly received recognition as a key tool in extracellular vesicle research, especially for exosomes. It is evident that the differences in size, surface molecules and chemistry, and mechanical properties among exosome subpopulations all collectively contribute to their biodistribution and systemic functions *in vivo*. FFF-MALS-DLS combines true size-based separation using an Eclipse® FFF system with independent determination of size and structure by a DAWN® HELEOS® II light scattering instrument. It can determine highly accurate size distributions and particle concentrations in native solution and the size fractions may be isolated and automatically collected for further off-line investigation using a standard fraction collector.

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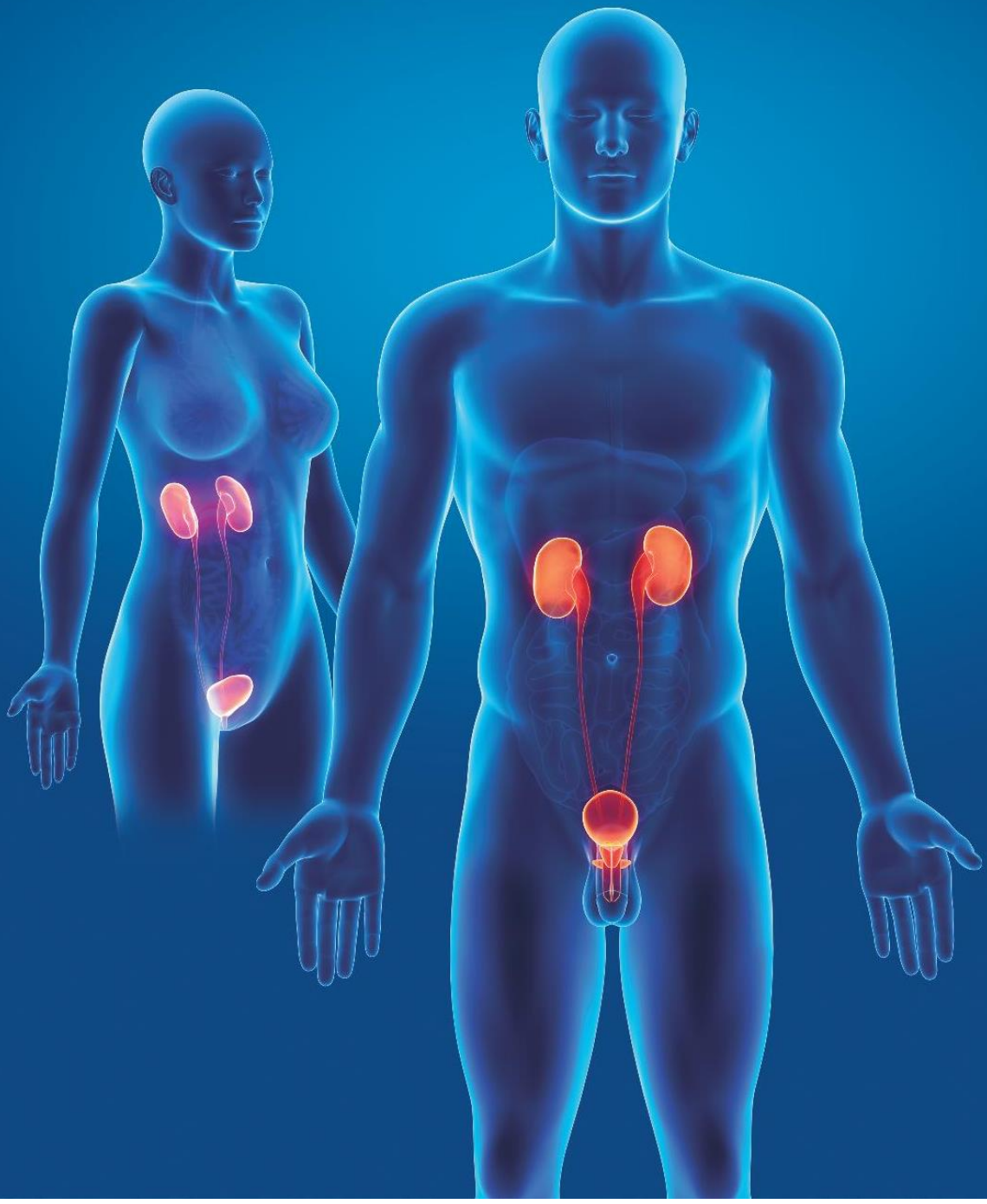

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