Second annual meeting of the

Netherlands Society for Extracellular Vesicles (NLSEV)

Friday Nov 9th 2018, Amsterdam UMC

AMC, Collegezaal 4

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Program Second Meeting Netherlands Society for Extracellular Vesicles (NLSEV)

Friday November 9th 2018
Collegezaal 4, AMC (Meibergdreef 9, 1105 AZ Amsterdam Zuidoost)

9:00 – 9:50 **Registration**

9:50 – 10:00 **Welcome by Marca Wauben and Rienk Nieuwland**

Chair Michiel Pegtel

10:00 – 11:00 **Selected presentations**

Leonie de Rond (Amsterdam UMC - location AMC)
Refractive index to evaluate staining specificity during flow cytometric analysis of extracellular vesicles

Martin van Royen (Erasmus MC)
EV analysis of clinical urine samples from prostate cancer patients

Tom Keulers (Maastricht University)
GABARAPL1 is required for the secretion of pro-angiogenic extracellular vesicles during hypoxia

Onno Arntz (RadboudUMC)
Rheumatoid Arthritis Patients with Circulating Extracellular Vesicles Positive for IgM Rheumatoid Factor have Higher Disease Activity

11:00 – 11:10 **Sponsored talk (Distrilab/Particle Metrix)**

Sascha Raschke
Biomarker detection using Particle Metrix ZetaView® and Nanoparticle Tracking Analysis (NTA)

11:10 – 11:50 **Coffee break & Posters meet and greet**

Chair Guido Jenster

11:50 – 12:50 **Selected presentations**

Corina Ghebes (Sanquin)
Extracellular vesicles, a key mediator of mesenchymal stromal cell's ability to provide hematopoietic support and elicit immunosuppressive effects

Kyra Defourny (Utrecht University)
Encephalomyocarditis virus infection induces the release of phenotypically and functionally diverse extracellular vesicle subsets

Maarten Bebelman (VU University Medical Center)
Tools for live-monitoring of exosome release from single cells

Aleksandra Gasecka (University of Amsterdam)
Fully automated flow cytometry software reveals that concentrations of extracellular vesicles decrease after acute myocardial infarction

12:50 – 13:00 **Sponsored talk (KdBio)**

Antony Rutt
Efficient production of EVs using a continuous, high density cell culture system (FiberCell)

13:00 – 14:20 **Lunch & Posters meet and greet**

Programme

Chair Kasper Rouschop

14:20 – 15:00 **Keynote lecture**
Eva-Maria Krämer-Albers (Johannes Gutenberg University Mainz)
Extracellular vesicles in cell communication

15.00 – 15.10 **Sponsored talk (Izon)**
Camille Roesch
Combining Size Exclusion Chromatography (SEC) & Tunable Resistive Pulse Sensing (TRPS) technology for the advanced characterization of extracellular vesicles

15:10 – 15:40 **Selected presentation**
Maarten Ligtenberg (Erasmus MC)
Selection and validation of nanobodies against prostate cancer-derived extracellular vesicles
Marijke Zonneveld (Maastricht University)
Hypoxia-driven changes in EV composition and function

15:40 – 15:50 **Sponsored talk (Wyatt)**
Koen Hollebekkers
Wyatt Exosome Platform for Isolation, Identification, Quantitation, and Biophysical Characterization

15:50 – 16:20 **Coffee break & Posters meet and greet**

Chair Edwin van der Pol

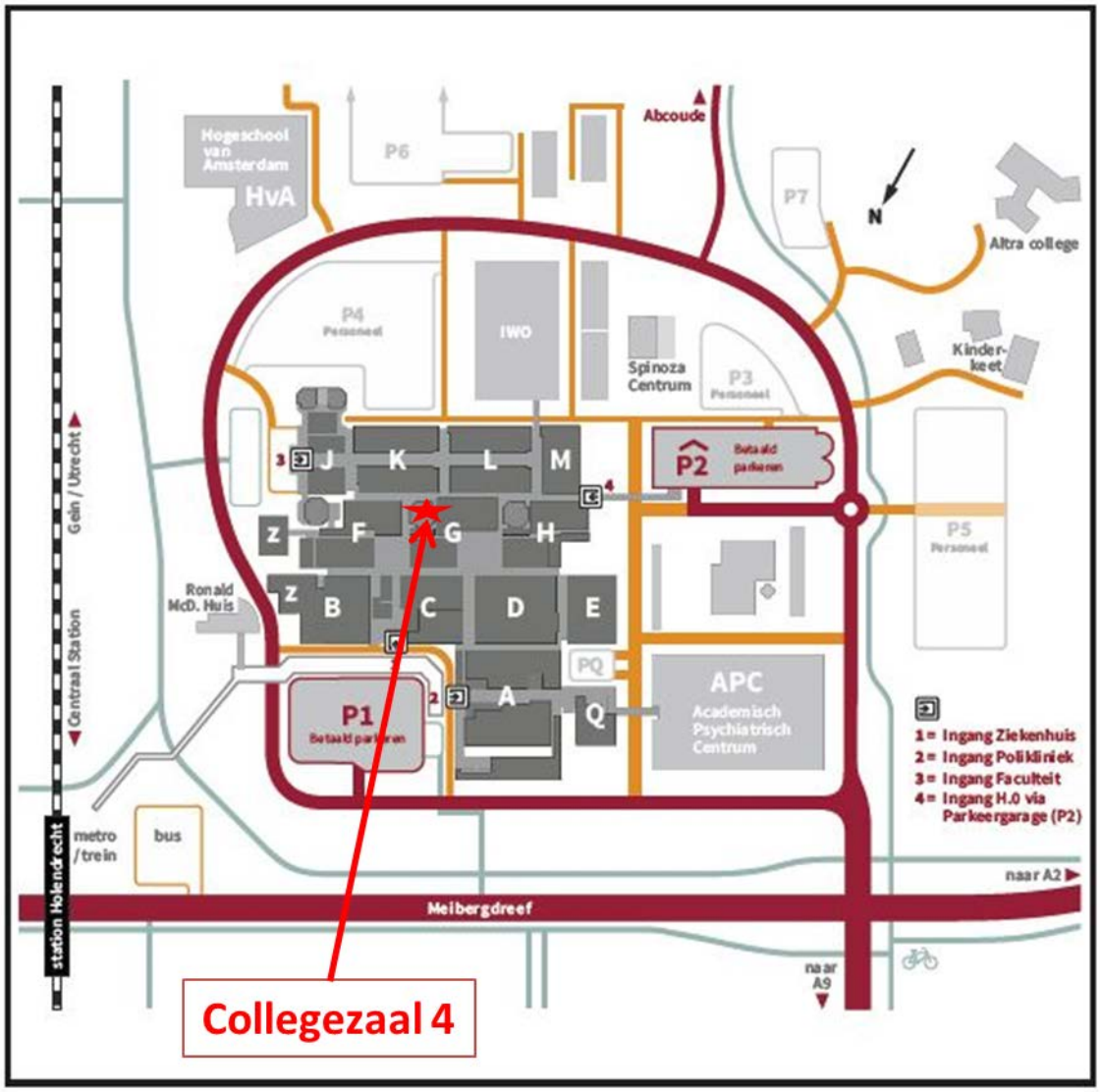
16:20 – 17:00 **Keynote lecture**
Tom Würdinger (VUmc, Amsterdam)
Extracellular vesicles and platelets as biomarkers

17:00 - 17:30 **NLSEV General Assembly and Awards**

17:30 – 20:00 **Drinks & Posters meet and greet**

Route

amc.nl/route



NLSEV2018-O1 Leonie de Rond

Refractive index to evaluate staining specificity during flow cytometric analysis of extracellular vesicles

de Rond, L.[1,2,3], Libregts, S.F.W.M. [4], van der Pol, E. [1,2,3], Wauben, M.H.M. [4], Coumans, F.A.W.[1,2,3], Nieuwland, R. [2,3] , van Leeuwen, T.G.[1,3]

*Presenting author. [1] Biomedical engineering and Physics; [2] Laboratory Experimental Clinical Chemistry, [3] Vesicle Observation Center, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands, [4] Department of Biochemistry & Cell Biology, Faculty of Vetinary Medicine, Utrecht University, Utrecht, the Netherlands.

Background: Antibodies and generic markers are commonly used to identify extracellular vesicles (EVs) in biofluids. However, whether these markers are specific for EVs is often unknown. Using Flow-SR we can derive the refractive index (RI) of single particles >200 nm from its flow cytometry scatter signal. In a previous study, we demonstrated that plasma contains two populations with different RI, one population with RI<1.42 which includes the EVs and the other with RI>1.42 which we hypothesised to be lipoproteins based on literature. If this hypothesis is true, the RI could enable direct evaluation of markers binding to lipoproteins in the sample of interest.

Methods: Density gradient centrifugation was applied to separate EVs and lipoproteins in plasma. The density fractions were analysed using transmission electron microscopy, western blot and flow cytometry, and the RI of the particles in each fraction was evaluated using Flow-SR. Binding of markers to lipoproteins was investigated by staining human plasma with several commonly used markers (calcein AM, calcein violet, di-8-ANEPPS, lactadherin and CD61-PE) and evaluating the EV specificity.

Results: On transmission electron microscopy and western blot only the lowest density fractions contained lipoproteins as well as the highest concentration of particles with RI>1.42. Using the RI to identify lipoproteins in the sample, 19% of the calcein violet-positive particles were found to be lipoproteins, 82% for di-8-ANEPPS, 15% for lactadherin and 7% for CD61-PE. Calcein AM stained neither lipoproteins nor EVs compared to other labels.

Conclusion: RI allows identification of lipoproteins using Flow-SR, and this information is useful to evaluate the specificity of antibodies and generic markers used to identify EVs in body fluids.

NLSEV2018-O2 Martin van Royen

EV analysis of clinical urine samples from prostate cancer patients

Hartjes T.A.[1,4], Leivo J.[3,4], Vredenbregt M.S.[2], Veldhoven-Zweistra J.[2], Bangma C.H.[2], Houtsmuller A.B.[1], Van Weerden W.M.[2], Jenster G.W.[2,5], Van Royen M.E.[1,5,#]

#Presenting author, 1 Erasmus MC, Dept. of Pathology/Erasmus Optical Imaging Centre (OIC), Rotterdam, The Netherlands, 2 Erasmus MC, Dept. of Urology, Rotterdam, The Netherlands, 3 University of Turku, Department of Biochemistry and Food Chemistry, Turku, Finland, 4. equal contribution, 5. equal contribution

Background: Urinary extracellular vesicles (EVs) are a promising source of biomarkers for urogenital cancers, but EV analysis in clinical samples remains challenging. We recently developed two assays that enable quantification and characterization EVs in urine (EVQuant and TR-FIA) and applied these on minimally processed urines of prostate cancer (PCa) patients.

Methods: Urinary EVs from patients with and without PCa, men after radical prostatectomy and females were compared. EVs were quantified and analyzed for CD9, CD63 and PSMA, and compared to clinical data.

Results: EV concentration and CD63 markers where higher in urines from men that received digital rectal examination (DRE), indicating an increase of prostate EV after DRE. EV concentration and CD9 and CD63 levels are lower in urines from man with PCa. Higher EV counts in men without PCa could be caused by more prostate fluid in urine due to an enlarged prostate in this control group. As marker for prostate fluid in urine, uPSA was indeed higher in men without PCa. When marker levels are corrected for uPSA prostate fluid levels in urine the EV concentration and CD9, CD63 and PSMA levels in where elevated in the presence of PCa.

Conclusion: EVQuant was able to quantify EVs in clinical urine samples, indicating that DRE increases the urinary EVs. In this cohort of men with and without PCa, the dominant difference was uPSA, indicating higher concentration of prostate fluid in urine after DRE in men without PCa, likely caused by enlarged prostates. The concentration of prostate fluid represented by uPSA fluctuates among men after DRE, affecting the numbers of prostate-derived EVs and is an important factor for future clinical assays. The ratio of EV numbers or TR-FIA signals divided by uPSA is higher in men with PCa and expected to be a more consistent indicator for the presence of PCa. Together, this indicates that both EVQuant and TR-FIA have diagnostic potential for PCa, but also shows the need for PCa-specific markers to enable direct detection of PCa-derived EVs in urine for clinical use. (funding: Dutch Cancer Society).

NLSEV2018-O3 Tom Keulers

GABARAPL1 is required for the secretion of pro-angiogenic extracellular vesicles during hypoxia

Tom G. Keulers1, Sten F. Libregts2, Marco B.E. Schaaf1, Hanneke J.M Peeters1, Kim G. Savelkouls1, Johan Bussink3, Hans Duimel4, Marijke I. Zonneveld1, Karel Bezstarosti5, Jeroen A. Demmers5, Marc Vooijs1, Marca Wauben2, Kasper M.A. Rouschop1

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Introduction: Hypoxia is a hallmark of solid tumours and is associated with tumour progression and therapy resistance. In response to hypoxia, tumour cells secrete pro-angiogenic factors to induce blood vessel formation and restore oxygen supply to the tumour. Extracellular vesicles (EVs) are emerging as mediators of intercellular communication in the tumour microenvironment and mediate intercellular communication by shuttling biological information such as miRNA's, mRNA, proteins and growth factors to recipient cells. Previously, we demonstrated that the expression of GABARAPL1, a member of the LC3/GABARAP protein family, is induced during hypoxia. Now, we demonstrate that GABARAPL1 is required for secretion of pro-angiogenic EVs during hypoxia.

Methods: HT29 and U87 doxycycline-inducible GABARAPL1 knockdown cell lines were exposed to hypoxia (16 hours, <0.02% O2). EVs were isolated by sucrose density gradient isolation, and analysed by western blot, qNANO or high-resolution flow cytometry. Angiogenic potential of cells was assessed by tube formation assays. Xenografts were implanted subcutaneously at the lateral flanks of NMRInu/nu mice and tumour size was measured by calliper.

Results: GABARAPL1 is expressed on the EV surface and can be targeted with antibodies. This results in blockade of pro-angiogenic responses in vitro. Silencing GABARAPL1 with inducible knockdown models perturbs EV secretion and results in decreased tumour growth due to decreased vascularisation and enhanced necrosis. Additionally, Targeting GABARAPL1 directly after tumour irradiation resulted in enhanced tumour regrowth delay. Furthermore, we demonstrate that GABARAPL1+ EVs are detectable and increased in blood of cancer patients.

Conclusion: Here we reveal that hypoxic tumour cells secrete a unique EV subset, marked by GABARAPL1 expression. These EVs control tumour progression, are targetable and are therefore interesting to pursue as biomarker and therapeutic target.

NLSEV2018-O4 Onno Arntz

Rheumatoid Arthritis Patients with Circulating Extracellular Vesicles Positive for IgM Rheumatoid Factor have Higher Disease Activity

Onno Arntz*, Bartijn Pieters, Rogier Thurlings, Peter van der Kraan, Fons van de Loo.* presenting author. Radboudumc, Nijmegen.

Background: Rheumatoid arthritis (RA) is an autoimmune inflammatory disease where B-cells play several critical roles. Most research on RA has focused on cytokines as main effectors in disease progression however, cell-cell communication involves a much broader scope of responses. Extracellular vesicles (EVs) has been postulated as important communicators between resident and inflammatory cells. B-cells release EVs that contain the B-cell receptor on their surface that binds and presents antigens to T-cells. The presence of IgM-RF in seropositive RA patients clearly points to a role of B-cells in the pathogenesis RA. In this study we investigate the presence of RF-IgM on circulating EVs and the relation to the severity of RA.

Methods: EVs were isolated from platelet-free plasma of 41 RA patients and 24 healthy controls (HC) by size exclusion chromatography. Particle and protein concentration were quantified by NTA and micro-BCA. IgM-RF, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were determined by standard laboratory blood tests. Disease activity score (DAS28) was calculated visual analogue score (VAS) was determined by the patient.

Results: RA and HC pEVs were not different in particle size, protein content and particle concentration (115nm, 49fg, 3.5x1010 and 108nm, 45fg, 3.8x1010, respectively). Also between seronegative (RA-) and seropositive (RA+) patients no statistical differences were observed. In plasma of 28 out of 41 RA patients IgM-RF was detectable, and in 13 out of these 28 RA+ IgM-RF was also detected on their isolated pEVs (RF+pEVs). When comparing disease parameters we found no differences between RA+ and RA- patients, except for increased ESR levels in RA+ patients. However, RA+ patients with RF+pEVs showed significant higher levels of CRP and ESR and also VAS and DAS28 were significant increased compared to RA+ patients without RF+pEVs.

Conclusions: This study shows for the first time the presence of IgM-RF on pEVs in a subset of RA+ patients. The higher disease activity in RA patients expressing IgM-RF on their EVs suggests that RF+ EVs are involved in RA pathogenesis

NLSEV2018-O5 Corina Ghebes

Extracellular vesicles, a key mediator of mesenchymal stromal cell's ability to provide hematopoietic support and elicit immunosuppressive effects

C.A. Ghebes* [1], J. Morhayim[2], M. Kleijer[1], A.S. Cornelissen[1], B.C. van der Eerden[3], J. van de Peppel[3], E. Braakman[2], C. Voermans[1].

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Background: Mesenchymal stromal cells (MSC) hold a great potential for clinical application, as these cells possess hematopoietic support, immunomodulatory properties and regenerative potential. In the bone marrow (BM) microenvironment, MSC interact with other cells by means of direct cell-cell interaction or soluble factor secretion. Growing evidence presents small vesicles as important carrier of soluble factors, such as bioactive lipids, nucleic acids and proteins, which are secreted in the extracellular space by donor cells and are taken up by recipient cells, contributing to their change in faith. In this study we aim to determine whether BM-MSC-secreted extracellular vesicles (EV) are involved in the ability of MSC to provide hematopoietic support, by activating hematopoietic stem and progenitor (HSPC) proliferation, and elicit immunosuppressive effects by inhibition of peripheral blood mononuclear cells (PBMC) proliferation.

Methods: Umbilical cord blood (UCB)-derived CD34+ HSPC were ex vivo cultured for ten days in serum-free medium supplemented with cytokines (SCF and Flt-3) with or without EV. EV were isolated from MSC condition medium by sequential centrifugation steps at low (300g-2000g) and high speed (20000g-100000g). We assessed the capacity of the HSPC to proliferate, to generate colony forming units and to differentiate towards the various hematopoietic lineages based on the expression of surface markers. Co-culture assay with MSC-derived EV and PBMC were performed to assess immunomodulatory capacities.

Results: We found that BM-MSC-derived EVs improve ex vivo expansion of UCB-derived HSPC compared to cytokines only. We observed an increase in proliferation of total nucleated cells, their CD34+ subset and their immature subset (CD34+CD90+CD38-CD45RA-) by two-fold. Analysis of surface marker expression and colony forming unit capacity show a skewing towards the myeloid lineage. Furthermore, BM-MSC-derived EV addition to PBMC cultures, stimulated with anti-CD3/anti-CD28 antibodies, significantly inhibited the proliferation of PBMC.

Conclusion: Currently, we are preparing next-generation sequencing- and protein sequencing library to elucidate the key regulatory components present in BM-MSC-secreted EV that promote ex vivo HSPC expansion but inhibit PBMC proliferation.

NLSEV2018-O6 Kyra Defourny

Encephalomyocarditis virus infection induces the release of phenotypically and functionally diverse extracellular vesicle subsets

K. Defourny*[1], S. van der Grein[1], G. Arkesteijn[2], S. Goerdayal[3], H. Rabouw[2], C. Galiveti[1], M. Altelaar[3], F. van Kuppeveld[2], E. Nolte-'t Hoen[1]

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Background: Traditionally, dissemination of non-enveloped viruses as well as antiviral responses have been studied under the premise that viral progeny is solely released as 'naked' virions upon lysis of infected cells. Importantly, we and others recently discovered that virus particles can also be sorted into extracellular vesicles (EV), enabling the release of virions cloaked by a host-derived lipid 'envelope' in the pre-lytic phase of infection. Mechanistic understanding of how these EV-enclosed viruses may facilitate either infection or antiviral immunity is still limited. In this light, an important yet unexplored issue is whether the dynamic nature of EV production is reflected in heterogeneity within populations of virus-carrying EV and EV carrying only host molecules.

Methods: Here, we performed molecular characterization of EV induced by the encephalomyocarditis virus (EMCV) to refine our understanding of the role of EV during infection. EV released by infected cells were isolated by centrifugation at 10,000xg (10K) and 100,000xg (100K) and purified using density gradient centrifugation. The resulting EV subpopulations were analyzed for protein content and infectivity by western blotting, mass spectrometry, and end-point titration. In addition, we employed a unique, in-house developed high-resolution flow cytometric sorting approach to further distinguish EV subsets, quantify EV release, and assess the infectious potential of EV at the single particle level.

Results: We report the timed release of virus-containing EV sedimenting at 10K and 100K during the pre-lytic phase of the infection. These EV subpopulations carry markers indicative of distinct biogenesis routes and differ in the packaging of host molecules. Moreover, we demonstrate that within both 10K and 100K vesicle populations, EV subsets with strong differences in infectious potential are present, indicating functional diversity among virus-induced EV.

Conclusions: Combined, our data shows that the EV released upon EMCV-infection are heterogeneous with regard to subcellular origin, molecular content, timing of release, and potential to spread infection. Awareness of heterogeneity in virus-induced EV is essential to further delineate their role in infection, as distinct pro-viral or pro-host functions may be ascribed to different EV subpopulations.

NLSEV2018-O7 Maarten Bebelman

Tools for live-monitoring of exosome release from single cells

Maarten P. Bebelman* [1,2], Frederik J. Verweij [3], Philippe Bun [4], Martine J. Smit [2], Guillaume van Niel [3], Dirk Michiel Pegtel [1].

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Endosome-derived exosomes are small extracellular vesicles (EVs) implicated in cell-cell communication and are secreted when multivesicular bodies (MVBs) fuse with the plasma membrane (PM). We recently developed pH-sensitive tetraspanin reporters, including CD63-pHluorin that was used to visualize MVB-PM fusion by live Total Internal Reflection Fluorescence (TIRF) microscopy. These reporters were successfully used to study various aspects of exosome biogenesis and release. Notably, we identified a SNARE fusion machinery that mediates MVB-PM fusion under control of a G protein-coupled receptor signaling pathway. Independent studies using CD63-pHluorin have uncovered additional aspects of exosome physiology. Thus optical reporters are considered powerful tools to delineate the mechanisms and kinetics of exosome release under variable experimental conditions in vitro and in vivo. However, interpretation of TIRF-imaging data using optical reporters can be challenging and may lead to mis- and/or overinterpretation of results.

Here I will discuss how to avoid the potential pitfalls that are associated with this live imaging approach. In addition, I will share our current efforts in the generation of an ImageJ plug-in to facilitate unbiased analysis of the imaging data to allow accurate quantitation of MVB-PM fusion. Finally, I will provide a perspective as to how optical reporters may change the way we think about exosome biology.

NLSEV2018-O8 Aleksandra Gasecka

Fully automated flow cytometry software reveals that concentrations of extracellular vesicles decrease after acute myocardial infarction

Aleksandra Gąsecka* (1,2,3), Edwin van der Pol (2,3,4), Frank A.W. Coumans (2,3,4), Kinga Pluta (1), Grzegorz Opolski (1), Krzysztof J. Filipiak (1), Rienk Nieuwland (2,3)

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Background: Acute myocardial infarction (AMI) is a major cause of human death and disability, but early biomarkers for AMI are lacking. AMI is due to atherothrombosis, i.e. formation of platelet aggregates (thrombi) on ruptured atherosclerotic plaques. Because platelets release extracellular vesicles (EVs) during thrombus formation, we hypothesized that EVs are a biomarker of atherothrombosis and an early biomarker of AMI.

Methods: Venous blood was collected 24 hours (acute phase), 72 hours (hospital discharge) and 6 months (late phase) after AMI from fasting patients (n=60, age 64.5±10.8 years, 68% male), and once from fasting healthy volunteers (n=30, mean age 57.7±6.6 years, 62% male). Flow cytometry (Apogee A60 Micro) was used to determine concentrations of plasma EVs labelled with markers for endothelial cells (CD146), leukocytes (CD45), phosphatidylserine (lactadherin), platelets (CD31, CD61, CD62p), and fibrinogen. Analysis of the 1,224 flow cytometry data files was fully automated with in-house developed software (MATLAB R2018a), enabling automatic flow rate stabilization, application of Rosetta Calibration (Exometry) and Flow-SR for diameter and refractive index determination, size distribution fitting, fluorescent gate determination, and statistics reporting. To differentiate between EVs and small platelets, only particles <700 nm were included. Populations for which an unpaired t-test or one-way ANOVA with Bonferroni correction resulted in $p<0.05$ were considered significant.

Results: EV concentrations from leukocytes and endothelial cells were lower in patients in the acute AMI phase, compared to healthy volunteers ($p<0.05$ for both), and increased to the level observed in healthy volunteers in the late phase ($p<0.05$ for both). Concentrations of PS-exposing EVs and platelet EVs (CD31, CD61) were decreased in the acute AMI phase, compared to the late phase ($p<0.05$ for all).

Conclusions: We identified decreased concentrations of EVs from leukocytes and endothelial cells as new candidate biomarkers to differentiate patients with atherothrombosis from healthy volunteers. Further, we identified decreased concentrations of PS-exposing EVs and EVs from platelets as new candidate biomarkers to differentiate patients in the acute and late AMI phase. Multicentre clinical trials are required to confirm the clinical utility of EVs as biomarkers of atherothrombosis.

NLSEV2018-09 Maarten Ligtenberg

Selection and validation of nanobodies against prostate cancer-derived extracellular vesicles

Maarten Ligtenberg*(1), Joke Veldhoven(1), Caitlin Jenster(1), Tanja van de Nadort(1), Wytske van Weerden(1), Eric Bindels(2), Martijn van Duijn(3), Janne Leivo(4), Guido Jenster(1)

Erasmus MC Rotterdam, Departments of Urology (1), Hematology (2), Neurology (3); University Turku Finand (4)

Background: Prostate cancer (PCa) is common in elderly men and early diagnosis and therapy can prevent PCa to become a life-threatening disease. Our aim is to utilize extracellular vesicles (EVs) from urine and blood for the diagnosis, prognosis and monitoring of PCa. For this purpose, we selected and classified antibodies using an adapted phage display technology to generate nanobodies that specifically recognize PCa-derived EVs.

Methods: A phage library was created by immunization of a Lama glama with 4 different PCa cell lines. Phage display selections were performed on (1) PSMA positive and negative cells, (2) a panel of 10 PCa cell lines versus 29 cancer cell lines of different origin, (3) pools of 9 PCa-cell lines versus 5 normal prostate cell lines, (4) EVs secreted by 5 PCa cell lines versus EVs from 7 cancer cell lines of different origin. Bound bacteriophages were analyzed by Next-Generation Sequencing (NGS) and computational methods applied to select nanobodies for DNA synthesis (Twist BioScience) based on specificity for PSMA or PCa cells and EVs. A Time-Resolved Fluorescence ImmunoAssay (TR-FIA) was used to validate specificity of the selected nanobodies.

Results: A comparison of the classical clone-picking phage display routine using multi-round negative/positive selections was compared to our novel approach of single-round binding and NGS. For the PSMA selections, we showed that our top PSMA nanobodies were identified using both methods and that the NGS provided novel nanobodies and more insight in nanobody families and extent of subclasses. From the PCa selections, we identified over 60 nanobody sequences targeting EVs that are highly specific for PCa as compared to normal prostate and other cancers. From the extensive NGS database, we could also identify nanobodies that specifically recognize other cancer types. For validation, 20 promising nanobodies were DNA synthesized and their specificity and selectivity for PCa-derived EVs confirmed in a TR-FIA using medium from cell lines.

Conclusions: Our adapted technology outperforms classical phage display and provides more information on families and diversity of selected nanobodies. We identified new PSMA nanobodies and a large collection of nanobodies directed against PCa cells and their EVs. This technology can be implemented for any cell type or EV comparison and we are looking forward to future collaborations.

NLSEV2018-O10 Marijke Zonneveld

Hypoxia-driven changes in EV composition and function

Marijke I. Zonneveld* [1], Tom G. Keulers [1], Kim Savelkouls [1], Hanneke J.M. Peeters [1], Kasper Derks [2], Sten F.W. Libregts [3], Marca H.M. Wauben [3], Kasper M.A. Rouschop [1].

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Background: Hypoxia is an important component of the tumor microenvironment (TME), associated with increased angiogenesis, migration and treatment resistance. Changes in TME can alter the cargo and secretion of tumor-derived extracellular vesicles (EV) and can therefore impact tumor communication with its environment. However, the effect of hypoxia on tumor-derived EV remains unclear. Here, we investigated the impact of hypoxia on EV secretion, composition and function.

Methods: HT-29 and MDA-MD-231 cells were exposed to moderate (0.2% O2) or severe (0.02% O2) hypoxia. EV were isolated using a density gradient or size-exclusion chromatography. EV were characterized by high resolution flow cytometry (HR-FC) and western blot. Hypoxia-naïve tumor cells were pre-conditioned with EV. Changes in transcriptome of pre-conditioned cells was assessed by Next Generation Sequencing and validated with qPCR. The effects of EV pre-conditioning was assessed through clonogenic survival assay. Additionally, endothelial cells were exposed to EV and monitored for tubule formation to assess differences in angiogenic potential.

Results: Using HR-FC, we observed that the total number of secreted EV was not altered during hypoxia. In addition, gross protein and RNA content did not seem to be affected. However, differences in light scattering were observed by HR-FC, while immunoblot analysis revealed distinct patterns for CD9, CD63 and Flotillin-1. These results are indicative of differences in EV subpopulations and compositional changes dependent on oxygen level. Pre-conditioning of hypoxia-naïve cells with EV from hypoxic cells resulted only in minor changes in the acceptor cell transcriptome, although cells showed a trend towards increased survival of subsequent hypoxic insults. In addition, EV from hypoxic cells increased endothelial tubule formation.

Conclusion: Collectively, these results indicate that hypoxia alters EV composition and influences EV-mediated communication with the TM

NLSEV2018-P1 Sander Kooijmans

Mapping heterogeneity of EV properties using a novel EV subpopulation isolation platform

Olivier G. de Jong [1], Mark Tielemans [2], Raymond M. Schiffelers [2], Pieter Vader [2], Sander A.A. Kooijmans* [2]

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Background: Extracellular vesicles (EVs) are heterogeneous in terms of size and molecular composition. Given that the surface of EVs drives interactions with their environment, it can be anticipated that EV properties vary among subpopulations with different surface signatures. Unfortunately, methods to separate EV subpopulations based on surface marker expression without affecting their functionality are currently not available. Here, we describe a novel approach to separate intact EVs based on their surface molecule signature and compare their properties.

Methods: EVs from MDA-MB-231 cells were isolated using size exclusion chromatography. Subsequently, EVs were separated based on surface marker expression, including tetraspanins and phospholipids, using a novel magnetic bead-based capture-and-release platform. EVs were characterized by Western blotting, Nanoparticle Tracking Analysis (NTA) and transmission electron microscopy (TEM). Uptake of fluorescently labelled EV subpopulations by various cell types was assessed using flow cytometry.

Results: EVs were separated into subpopulations based on their expression of specific surface molecules. NTA and TEM analysis showed that EV size and morphology were not affected by the separation protocol. Western blot analysis revealed that expression of canonical EV markers varied among subpopulations. Furthermore, the rate of cellular uptake of fluorescently labelled EVs differed between EV subpopulations and between recipient cell types.

Conclusions: We show that EVs can be separated into surface molecule-specific subpopulations with retained integrity using a novel capture-and-release platform. EV uptake rates by recipient cells varied among these subpopulations, suggesting that subpopulations of EVs exert different functional effects. Our novel separation platform can be used to further elucidate such effects and possibly improve therapeutic applicability of EVs through enrichment of functional EV subpopulations.

NLSEV2018-P2 Ayse Erozenci

Minimally-invasive detection of prostate cancer stage by identifying protein signatures in urinary extracellular vesicles

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Introduction: Prostate cancer (PCa) is the most common cancer and the second cause of cancer-related death in males. Although multiple PCa biomarkers are already in clinical use, they hold limited value on their own. To select the best therapy, a precise diagnostic and prognostic test is needed that specifies patient's PCa stage. PCa cells secrete extracellular vesicles (EVs), which reflect in part PCa stage. EVs are present in urine and can be a non-invasive source as biomarkers.

Aim: To examine PCa protein signatures in urinary-EVs by mass-spectrometry-based proteomics

Methods: We determined protein signatures in PCa-urinary-EVs isolated by ultracentrifugation from 3 control males (age-matched), 3 intermediate and 3 advanced PCa patients by label-free LC-MS/MS and comprehensive bioinformatics analysis.

Results: We identified 3950 proteins in urinary-EVs, of which 2166 constitute the core proteome (i.e. expressed in >75% of the patients). Total proteome had 75% overlap with 3 independent datasets, with 1055 unique proteins identified in our dataset, demonstrating that we achieved a great depth in our samples. Unsupervised hierarchical cluster analysis separated PCa stages. Selection of candidate signatures was based on abundant and significant ($p<0.05$) proteins, showing >1.5 fold change between either intermediate and control or advanced and control plus intermediate. This led to the identification of 40 upregulated and 17 downregulated proteins for intermediate, and 80 upregulated and 268 downregulated proteins for advanced patients. In a preliminary analysis, we focused on upregulated proteins. Hierarchical cluster analysis with further filtered candidates for presence in all 4 datasets (10 proteins for intermediate and 13 proteins for advanced) separated patient groups. Validation of the biomarkers using independent assays is ongoing.

Conclusion: We observed a distinct proteome profile in urinary-EVs between different PCa stages and identified a potential candidate protein signature that may ultimately improve PCa diagnosis and forecast disease prognosis and outcome.

Future: We will target selected candidates with antibodies to develop assays to capture and detect PCa-derived EVs in urine. ELISA-like and multiplex assays will be further adapted and pre-validated.

NLSEV2018-P3 Tom Driedonks

Technical approaches to reduce interference of Fetal Calf Serum derived RNA in the analysis of extracellular vesicle RNA from cultured cells

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Background: Fetal calf serum (FCS) is a common supplement of cell culture medium and a known source of contaminating extracellular vesicles (EV) that contain RNA. To prevent unwanted interference in (functional) characterization of cell culture EV, FCS-EV are commonly depleted by overnight ultracentrifugation. It was recently shown that only part of the FCS-RNA could be depleted by ultracentrifugation, and that remaining FCS-RNA may confound EV-RNA analyses. Since different methods to deplete bovine EV-RNA from FCS are being used, we compared their efficiency to deplete bovine RNA, and determined the contribution of remaining bovine RNA to EV-RNA purified from cell cultures.

Methods: We tested the effects of 1) FCS dilution factor and 2) decanting versus pipetting off EV-depleted supernatant on FCS-RNA depletion. The depletion efficiency of various miRNAs and other non-coding RNAs was determined by RT-qPCR. Cell lines releasing high or low numbers of EV were cultured in EV-depleted media, for which the contribution of FCS-derived RNA to density gradient purified EV-RNA isolates was assessed.

Results: Depletion of FCS-RNA after overnight ultracentrifugation was most efficient in diluted FCS and when avoiding decanting of supernatant. Dilution of FCS from 100% to 30% increased the percentage of pelleted RNA. Importantly, we observed large differences in depletion efficiency not only between classes of non-coding RNAs (miRNAs and mid-sized RNAs such as 7SL and Y-RNA) but also between different miRNAs. We additionally demonstrate that the use of optimized FCS-EV depletion protocols combined with methods for high-grade purification of EV allows reliable detection of cell-derived EV-RNA above background levels of residual EV-RNA in medium controls.

Conclusion: Optimization of FCS-EV depletion protocols reduces the levels of contaminating bovine RNAs in culture medium, but the depletion efficiencies for different RNA classes are variable. Therefore, parallel processing and analysis of medium control samples is essential to accurately assess the RNA content of cell culture EV. Importantly, accurate reporting of EV-depletion methods, and inclusion of medium control samples will increase experimental reproducibility in EV-RNA studies.

NLSEV2018-P4 Eric Barros

Renal tubular exosomes modulate extracellular levels of ATP in a nephron-segment specific fashion

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Background: ATP plays a role in the regulation of renal physiological processes. In the kidneys, exosomes are secreted by different nephron portions carrying specific proteins from each segment. Furthermore, exosomes released from the proximal tubules regulate collecting duct sodium channels, suggesting a role as intrarenal signaling vesicles. However, this role has been poorly studied. Additionally, renal epithelial cells release ATP in a polarized manner, which can affect electrolyte handling. Furthermore, exosomes from different cells types carry ectonucleotidase activity, which could modulate ATP-dependent signaling processes. However, it remains unknown whether renal tubular exosomes exert this regulatory function. The aim of this study is to investigate the role of exosomes derived from different nephron segments in the regulation of extracellular levels of ATP

Methods: Exosomes were isolated from human proximal tubule (HK-2) and collecting duct (HCD) epithelial cells culture medium by ultracentrifugation and characterized by electron microscopy, nanotracking-analysis and western blot. Cultured cells were incubated with their corresponding exosomes for different timepoints (15min, 6 and 24h), after which extracellular ATP was measured using the ATPlite-Luminescence Assay®. In parallel, HK-2, and HCD-derived exosomes were incubated with ATP (0.1uM) for 6h, after which ATP concentration was measured.

Results: Isolated exosomes display a round donut shape and have a modal size of 83.50±2.40 and 87.20±2.70nm for HK-2 and HCD-exosomes, respectively. Exosomes carry CD9, CD63 and TSG101. HK-2 cells exposed to HK-2 derived exosomes for 6h showed no effect on extracellular ATP, while HCD cells exposed to HCD-derived exosomes had a significant reduction of extracellular ATP (0.60±0.22 vs 1.00±0.11; $p<0.05$; n=3). HK-2 derived exosomes incubated with synthetic ATP for 4 hours did not affect ATP concentration, while HCD exosomes reduced ATP concentration (0.65±0.01 vs 1.00±0.02; $p<0.05$; n=3).

Conclusion: Collecting duct exosomes modulate extracellular ATP-levels, probably due to ectonucleotidases in their cargo. If this took place in vivo, could suggest a segment-specific potential to regulate ATP-dependent processes.

NLSEV2018-P5 Pascalle van Eijck

Respiratory exposures modulate the release of extracellular vesicles by various pulmonary cell types

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Background: Chronic exposure to respiratory stressors increases the risk for pulmonary and cardiovascular diseases. Previously, we have shown that cigarette smoke extract (CSE) triggers the release of CD63+CD81+ and tissue factor (TF)+ procoagulant extracellular vesicles (EVs) by bronchial epithelial cells (BEC) via depletion of cell surface thiols. Here, we hypothesized that this represents a universal response for different pulmonary cell types and respiratory exposures.

Methods: The bronchial epithelial cells (BEAS-2B), pulmonary fibroblasts (MRC-5 and HEL299 cells), pulmonary microvascular endothelial cells (HPMEC-ST1.6R), and macrophages (PMA-differentiated THP-1 cells) were exposed to CSE, silica nanoparticles, and particulate matter (PM) with or without NAC, or to bacitracin for 24 h. The exosomes were quantified using CD63+/CD81+ and TF+ bead-coupled flow cytometry, and cell surface thiols were analyzed using flow cytometry.

Results: We found that BEC and pulmonary fibroblasts, but not pulmonary microvascular endothelial cells or macrophages release CD63+CD81+ and TF+ EVs in response to CSE. Cell surface thiols decreased in all cell types upon CSE exposure, but targeted depletion of cell surface thiols using bacitracin only triggered EV release by BEC and fibroblasts. The thiol-antioxidant NAC prevented the EV induction by CSE in BEC and fibroblasts. Exposure of BEC to occupational silica nanoparticles and particulate matter (PM) from outdoor air pollution also enhanced EV release. Cell surface thiols were mildly decreased and NAC partly prevented the EV induction for PM10, but not for silica and PM2.5.

Conclusion: Induction of procoagulant EVs is a cell type specific response to CSE. Moreover, induction CD63+CD81+ and TF+ EVs in BEC appears to be a universal response to various respiratory stressors. TF+ may serve as biomarkers of exposure and/or risk in response to respiratory exposures. Inhibiting their induction could be a novel strategy to prevent respiratory exposure-associated morbidity.

NLSEV2018-P6 Nader Kameli

The role of Bacteroides fragilis- derived outer membrane vesicles in the pathogenesis of inflammatory bowel disease

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Introduction: The mechanisms underlying the pathogenesis of IBD are not completely understood; however, immune responses to the gut microbiota are considered important factors in IBD pathogenesis. Bacteroides fragilis (BF) is strongly associated with IBD, as it may also promote intestinal inflammation, in particular those strains containing the BF toxin. Outer membrane vesicles (OMVs) released by different Gram-negative bacteria show various functions for bacterial pathogenicity. Here, we aim to investigate the role of BF-derived OMVs in IBD thereby focusing on two possible mechanism: 1) stimulation of the inflammatory responses, and 2) indirect effects on intestinal epithelial cells (Caco-2).

Methods: Three strains of B.fragilis were used: 2 strains (1 toxin-positive (BFT+) and 1 toxin-negative strain (BFT-)) and a BFT- refrence strain (ATCC 25285). PCR was performed to confirm the origin of OMVs and presence of bft gene in BFT+ strain. OMVs were isolated from bacterial cultures by a combination of ultrafiltration and size exclusion chromatography, and thereafter quantified by tunable resistive pulse sensing. Prior to stimulation with OMVs, THP-1 cells were differentiated with 200nM PMA. TNFα and IL-1β were measured by ELISA. The OMVs-depleted conditioned medium from stimulated THP-1 cells was applied to the basal site of Caco-2 monolayers for Transepithelial electrical resistance (TEER) measurement to test intestinal barrier function.

Result and conclusion: All three B.fragilis strains released a significant amount of OMVs (~2-8*108/ml) during 24h of culturing. Also, we demonstrated the presence of BF DNA gene inside the OMVs. Interestingly, OMVs released by the BFT+ strain stimulated the release of TNFα and IL-1β by THP-1 more prominently than the other BFT- strains. Only conditioned medium from BTF+-stimulated THP-1 cells slightly decreased the integrity of the Caco-2 monolayer. To conclude, OMVs derived from the BFT+ strain stimulated the release of inflammatory cytokines in THP-1 cells which subsequently decreased the integrity of the intestinal epithelial barrier function. Their precise role in the pathogenesis of IBD needs to studied in more detail.

NLSEV2018-P7 Charles Blijdorp

Kidney Function determines Excretion of Urinary Extracellular Vesicles

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Background: Urinary extracellular vesicles (uEVs) are emerging as non-invasive biomarkers for various kidney diseases. We previously showed that the uEV-marker CD9 decreases with disease progression in autosomal dominant polycystic kidney disease (ADPKD; Salih et al., J Am Soc Nephrol 2016). Therefore, here, we hypothesize that nephron loss decreases uEV excretion.

Methods: We quantified uEVs in two different settings of nephron loss, including donor nephrectomy and ADPKD with progressive decline of estimated glomerular filtration rate (eGFR). To do so, we obtained urine samples from 20 kidney donors the day prior and 3 months after nephrectomy, and from 27 ADPKD patients at baseline and after a median follow-up time of 3 years. uEVs were quantified using nanoparticle tracking analysis (NTA), EVQuant (a novel technique which counts individual fluorescently labeled EVs after immobilization in a matrix), a time-resolved fluorescence immunoassay capturing CD9+ uEVs (CD9-TRFIA), and immunoblot analysis of uEV markers after ultracentrifugation.

Results: Baseline kidney function correlated with uEV excretion in both healthy donors (eGFR vs. NTA R2 0.35, P=0.01 and vs. EVQuant R2 0.41, P = 0.008) and ADPKD patients (measured GFR vs. NTA R2 0.32, P = 0.005, and vs. CD9-TRFIA: R2 0.23, P = 0.02). Donor nephrectomy reduced eGFR by 38%, and significantly decreased uEV excretion (decrease [95%CI]: NTA 21% [7-34%], EVQuant 18% [6-31%], CD9-EVQuant 37% [17-57%], CD9-TRFIA 30% [12-49%], immunoblot CD9 31% [13-49%], and TSG101 36% [18-55%]. In ADPKD, while the eGFR decline was 18%, CD9-TFIA decreased by 22% [9-36%], but no change was observed with NTA. Conclusions: In summary, kidney function correlates with uEV excretion, and nephron loss reduces uEV excretion both after donor nephrectomy and in progressive ADPKD. CD9+ uEVs showed a greater decrease compared with total uEV number. At present it is unclear whether this represents a true biological phenomenon or may be explained by differences in quantification techniques. Therefore, it is important to adjust uEV excretion for kidney function when performing analyses of uEVs in patients with reduced kidney function.

NLSEV2018-P8 Martijn van Herwijnen

Abundantly present miRNAs in milk-derived extracellular vesicles are conserved between mammals

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Mammalian milk is not only a source of nutrition for the newborn, but also contains various components that regulate further development. For instance, milk is an abundant source of microRNAs (miRNAs), which are evolutionary conserved small non-coding RNAs that are involved in post-transcriptional regulation of target mRNA. MiRNAs present in milk can occur in extracellular vesicles (EV), which are nanosized membrane vesicles released by many cell types as a means of intercellular communication. The membrane of EV protects enclosed miRNAs from degradation and harbors molecules that allow specific targeting to recipient cells.

Although several studies have investigated the miRNA content in milk EV from individual species, little is known about the evolutionary conserved nature of EV-associated miRNAs among different species. In this study, we profiled the miRNA content of purified EV from human and porcine milk. These data were compared to published studies on EV from human, cow, porcine and panda milk to assess the overlap in the top 20 most abundant miRNAs.

Interestingly, several abundant miRNAs were shared between species (e.g. let-7 family members let-7a, let-7b, let-7f, and miR-148a). Moreover, these miRNAs have been implicated in immune-related functions and regulation of cell growth and signal transduction.

NLSEV2018-P9 Sanne Mol

Differential neutrophil activation: The effect of various stimuli on neutrophil activation and extracellular vesicle release

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Background: Activated neutrophils are abundant in the synovial fluid of patients with inflammatory joint diseases and contribute to the pathogenesis of these diseases. Upon activation, neutrophils degranulate, release cytokines, form neutrophil extracellular traps and release extracellular vesicles (EVs). EVs are known to be important cell communicators. However, the characteristics and role of neutrophil-derived EVs in inflammatory joint diseases is yet unknown. In this study we investigate the influence of different activating stimuli on the release of EVs by neutrophils.

Methods: Neutrophils were isolated from peripheral blood of healthy donors and cultured in the absence or presence of activating stimuli or combinations, including GM-CSF, TNFα, LPS, and fMLP. After stimulation, cells were collected. Degranulation and survival were analyzed using flow cytometry. Cytokine release was determined using ELISA. EVs were isolated from supernatants by differential ultracentrifugation, followed by density ultracentrifugation and quantified by high-resolution flow cytometry.

Results: We found that GM-CSF does not activate neutrophils as measured by degranulation (CD16 decrease and CD63 increase) and cytokine release (IL-8 and elastase), but does induce moderate EV release. The combination of GM-CSF with fMLP, TNFα or LPS results in strong neutrophil activation and enhanced EV release compared to non-activated neutrophils.

Conclusion: These results indicate that specific pro-inflammatory stimuli induce differential effects in neutrophils and that this influences EV release by neutrophils. Given the abundance of pro-inflammatory stimuli and neutrophils in the inflamed joints of arthritis patients, this may indicate that neutrophil derived-EVs are present in greater abundance in inflamed tissue in autoimmune disorder patients than in healthy individuals and that these EVs may play a role in the pathogenesis of autoimmune disorders.

NLSEV2018-P10 Martin van Royen

Zebrabow as in vivo model system to monitor vesicles mediated transfer in cancer

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Background: Tumor cells influence their microenvironment, enhancing tumor progression and metastasis via extracellular vesicle (EV) mediated transfer of proteins and RNAs. Zebrafish are ideal in vivo model system for fluorescent imaging because of their simple manipulation and natural transparency. Using non-invasive imaging and the Cre-LoxP switch-reporter system we explored the potential of this model system to visualize in vivo spreading and uptake of cancer cell-derived EVs.

Methods: Vesicles were isolated from prostate cell lines stably expressing high levels of Cre-recombinase. 4 nL of vesicle isolate, or synthetic Cre-recombinase mRNA was injected in yolk of embryos in early development. The Zebrabow fish contains a Cre-LoxP -reporter that will switch fluorescence in cells expressing Cre-recombinase, mediated through injected mRNA or via uptake of EVs isolated from Cre-expressing cell lines. After 4-6 day of development, fish were immobilized for microscopic inspection using a high content screening system. EV Cre mRNA was quantified with qPCR. EV concentrations were determined using EVQuant.

Results: Injected synthetic Cre-recombinase mRNA was able to efficiently switch the Cre-LoxP reporter system in injected embryos, resulting in mosaic patterns of fluorescent cells distributed in complete zebrafish. In contrast, injected EVs derived from cells with high Cre expression were able to color switch cells in only 1 out of 60 zebrafish. The low efficiency in EV-mediated Cre-protein or RNA transfer is correlated with small quantities of Cre-mRNA present in 4 nL EV isolate that contained approximately 30x10E-14 pg compared to the 50 pg present in 4 nL synthetic Cre-mRNA solution.

Summary/Conclusion: The Zebrabow Cre-LoxP reporter system is an efficient reporter for Cre activity and could therefore be an ideal model system to study EV-transfer in vivo. However, the amount of EV-mediated transfer of Cre-mRNA is too low with a single injection of 4 nL of purified EVs from Cre-expressing cell lines. This very low efficacy can be explained by low Cre-mRNA quantities in EVs and the small volume that can maximally be injected in yolk of zebrafish embryos."

NLSEV2018-P11 Melissa Piontek

Mechanical properties of extracellular vesicles probed by atomic force microscopy

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One species of natural compartments enclosed by a membrane, which consist of intraluminal proteins and lipids, is called extracellular vesicles (EVs). Carrying different cargos, EVs are increasingly studied as potential drug delivery vehicles, but also to understand the interactions between cells and vesicles. In this context, also synthetic particles mimicking natural EVs are investigated (liposomes). Being present in many body fluids, e. g. urine, breast milk and plasma, the EV's role as potential biomarkers is of great interest, too. A possible difference in the mechanics of e.g. tumor derived EVs compared to EVs from healthy patients could provide a non-invasive method for the detection of cancer. However, little is known about their mechanical properties, partly because the mechanical characterization of EVs remains challenging. On one hand a low deformation of the particle during imaging must be ensured while at the same time the particle needs to be stably attached.

We observed a variation of the response of EVs to the indenting force, ranging from reversible to irreversible changes of the EVs. Here, we discuss this different behavior of EVs and liposomes in the context of the mechanical characterization of those particles as well as the method used for measurement.

NLSEV2018-P12 Estefanía Lozano-Andres

Optimization of the generic labelling of extracellular vesicles in human plasma for single particle analysis by flow cytometry

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Background: All body fluids contain cell-derived extracellular vesicles (EVs). EVs, comprised of selected proteins, lipids and nucleic acids that reflect the status and origin of cells, are interesting candidates for biomarkers. High resolution flow cytometry (FC) allows for the detection of single EVs in blood and enables high throughput and multi-parametric EV characterization. Besides many technical challenges due to the small size, low refractive index and heterogeneity of EVs, the presence of lipoprotein particles (LPPs) in blood plasma further hamper robust detection of single EVs due to their overlap in size and buoyant density. Therefore, we here evaluated whether the presence of LPPs could obscure the generic fluorescent labelling and FC-detection of plasma-EVs.

Methods: Blood samples from healthy human donors were collected in sodium citrate. To obtain platelet-poor plasma samples were centrifuged two times for 15 min. at 2680 x g. Plasma-EVs were subsequently isolated by differential ultracentrifugation, density gradient floatation or size-exclusion chromatography (SEC). Generic fluorescent staining of EV-plasma samples and commercial LPP preparations were performed by using PKH67. FC-analysis was performed with a BD Influx that was modified and optimized for detection of submicron-sized particles.

Results: We found that generic fluorescent labelling of EVs by PKH67 is affected when EVs are present in plasma samples that contain LPPs. Furthermore, we found that PKH67 has the capacity to also label various types of LPPs and these can be poorly discriminated from EVs based on fluorescent and light scatter signals, thus influencing the detection of plasma-EVs. However, these problems can be overcome by combining SEC and density gradient floatation in order to obtain a highly enriched plasma-EV sample.

Conclusions: In order to perform reliable and reproducible fluorescent-based FC-analysis of single EVs in human plasma, either EV-specific fluorescent labels should be used or plasma samples should be cleared from particles prone to incorporate the generic dye.

NLSEV2018-P13 Linda Rikkert

Centrifugation affects the purity of liquid biopsy-based tumor biomarkers

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Biomarkers in blood of cancer patients include circulating tumor cells (CTCs), tumor-educated platelets (TEPs), tumor-derived extracellular vesicles (tdEVs), EV-associated miRNA (EV-miRNA), and circulating cell-free DNA (ccfDNA). Because the size and density of biomarkers differ, blood is centrifuged to isolate or concentrate the biomarker of interest.

Here, we applied a model to estimate the effect of centrifugation on the purity of a biomarker according to published protocols. The model is based on the Stokes equation and was validated using polystyrene beads in buffer and plasma. Next, the model was applied to predict the biomarker behavior during centrifugation. The result was expressed as recovery of CTCs, TEPs, tdEVs in three size ranges (1-8 µm, 0.2-1 µm, and 0.05-0.2 µm), and ccfDNA. Bead recovery was predicted with errors <18%. None of the centrifugation protocols yields a pure biomarker. Most notable cofounders are the 22% contamination of 1-8 µm tdEVs for TEPs, and 8-82% of tdEVs <1 µm for ccfDNA. A Stokes model can predict biomarker behavior in blood. None of the evaluated protocols produces a pure biomarker.

Thus, care should be taken in interpretation of obtained results, as, for example, results from TEPs may originate from co-isolated large tdEVs, and ccfDNA may originate from DNA enclosed in tdEVs <1 µm.

NLSEV2018-P14 Marije Kuipers

Extracellular vesicles from Schistosoma mansoni larvae contain oligomannosyl and Lewis X glycan motifs and bind to DC-SIGN on human dendritic cells

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Extracellular vesicles (EV) are intercellular messengers and can transport various molecular cargo. Although it is known that the parasitic worm Schistosoma mansoni releases EV into the host, their exact composition and interaction with the host immune system are largely uncharacterised. One of the main classes of molecules involved in schistosome-host interaction are parasite glycans.

Here, we investigated the glycosylation of EV released by S. mansoni larvae (schistosomula) and studied glycan-mediated effects of the EV on human monocyte-derived dendritic cells (moDC), central immune cells bridging innate and adaptive immunity. Mass spectrometric glycosylation analysis, performed on EV obtained from 72 hours cultured schistosomula, revealed the presence of oligomannose glycans and complex N-glycans with Galβ1-4(Fucα1-3)GlcNAc (Lewis X) motifs, on the in- and outside of the EV, respectively. To investigate binding of EVs by host immune cells, moDCs were cultured with fluorescently labelled vesicles and maturation factors (MF) IL1β and TNFα for 48 hours. Subsequent flow cytometric analysis showed binding of the EV to moDCs, which was significantly reduced after preincubation with anti-DC-SIGN antibodies but not in the presence of anti-mannose receptor (MR) or isotype controls. In addition, EV-induced PD-L2 expression on moDC was partly reduced by blocking DC-SIGN, while the increased expression of PD-L1, CD80, and CD86 by EV stimulation seemed less affected by DC-SIGN inhibition. Furthermore, schistosomula EV induced (5 to 10-fold compared to MF alone) IL-12, IL-6, and IL-10 cytokine release by moDC. Altogether, these data show that schistosomula EV bind host immune cells via the C-type lectin receptor DC-SIGN and we hypothesize that fucose containing ligands on EV are involved in this interaction.

Currently, we are investigating the functional consequences on EV-DC-SIGN interaction in human DC function.

NLSEV2018-P15 Joëlle Klazen

Vitamin D alters bone metastatic prostate cancer cell extracellular vesicle number and mRNA content and their interaction with osteoblasts

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Background: Bone is a preferential site for cancer metastases, especially from prostate and breast cancer. Here, metastasized cells are resistant to current therapies and create a vicious cycle of negative influences on bone metabolism to stimulate cancer cell growth via complex interactions. Extracellular vesicles (EVs) may be important messengers in the creation of the metastatic niche in bone. Compounds that restore bone integrity, like vitamin D, are promising therapies in bone metastases. EVs may be part of the mechanism of the beneficial effects of vitamin D. The aim of our research is to unravel the role of vitamin D in prostate cancer cell EV interaction with the bone.

Methods: We investigated the effect of vitamin D on bone metastatic prostate cancer cell EV production, EV mRNA content and interaction with human osteoblasts. EVs from vitamin D treated and untreated GFP-expressing PC-3 cells were isolated by differential ultracentrifugation (20,000xg and 100,000xg fractions), and counted by high-resolution flowcytometry (BD influx) and microscopy-image-guided counting (EVQuant). mRNA content was analyzed by high throughput sequencing. EV uptake by human osteoblasts (SV-HFO) was measured by flowcytometry (BD Accuri C6).

Results: Vitamin D treatment of the PC-3 cells resulted in a decreased number of cells with a significantly higher number of EVs produced per cell in the 100,000xg fraction only. This 100,000xg EV fraction of the vitamin D treated PC-3 cells had a different mRNA profile compared to the 100.000xg EVs of the untreated PC-3 cells. Furthermore, vitamin D treated PC-3 EV uptake by osteoblasts was reduced compared to EV uptake from untreated PC-3 cells.

Conclusion: Vitamin D has an effect on the number and mRNA content of bone metastatic prostate cancer EVs and influences their interaction with osteoblasts. This may be of importance in preventing the creation of a metastatic niche in the bone. Further research will focus on exploring the changed EV content of cancer cell EVs after vitamin D treatment of the cells and the cross-talk with the bone environment.

NLSEV2018-P16 Silvia Oggero

Monocyte-platelet aggretates stimulation drives formation of microvesicle subsets to promote vascular inflammation

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Background: Released from the plasma membrane of virtually all cells, microvesicles (MV) are emerging as novel effectors of cell-to-cell communication in inflammation. Besides expressing common markers of the cell of origin, MV differ in biological composition in relation to the mode of cell activation. This heterogeneity raises the question as to whether MV subtypes might be new tools for diagnosing diseases. The aim of this study is to investigate human monocyte-derived MV heterogeneity and function in vitro thus mimicking vascular inflammation.

Methods: Monocytes isolated with Rosette Sep™ technology (previously treated with 2 µM PGI2 to inhibit platelet aggregation), were stimulated for 1h with 1 µM PAF or 50 ng/mL TNFα. Purity of monocyte was determined by flow cytometry while MV profile was studied with imaging flow cytometry. Human umbilical vein endothelial cells (HUVEC) or human vascular smooth muscle cells (hVSMC) were incubated with different MV subsets at a ratio of 10:1 MV:cell for 6h, and expression of adhesion molecules (ICAM-1, VCAM-1, CD62E) on HUVECs and calcification in hVSMCs was quantified.

Results: Comparing Rosette Sep™ technology isolation with density gradient PBMC isolation by flow cytometry, revealed high degrees of platelet-monocyte aggregation, which was not modulated by PGI2. MV analyses identified MV from platelets (CD41+/CD14-) and monocytes (CD41-/CD14+) as well as a subset bearing both markers (CD41+/CD14+). In the presence of PAF, PGI2 reduced the levels of double positive MV while it was ineffective on the MV response evoked by TNFα. Functional studies showed that TNFα-stimulated monocyte-derived MVs enhanced expression of VCAM-1, but not ICAM-1 or CD62E, in HUVEC. An activation effect was also demonstrated for calcification of hVSMC.

Conclusions: We have identified a CD41+/CD14+ MV subset generated following monocyte-platelet aggregation; PGI2 affects this subset indicating a potential dominating role for the platelet and platelet activation. Since these MV promote HUVEC and hVSMC activation, we propose that monocyte-platelet aggregates exposed to activators may perpetuate vascular inflammation.

NLSEV2018-P17 Xiaogang Zhang

Isolation of extracellular vesicles from human plasma using a novel three step protocol

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Introduction: Several methods have been applied to isolate extracellular vesicles (EVs) from human plasma, including differential (ultra)centrifugation, density gradient centrifugation, ultrafiltration, size exclusion chromatography (SEC), and polymer-based precipitation. In plasma, however, the abundance of extracellular vesicles is very low relative to other particulate constituents with comparable size and/or buoyant densities, including lipoprotein particles and protein complexes. Until now, EV isolation to homogeneity remains a problem. We here describe a novel three step isolation method to purify EVs from human plasma.

Methods: Fresh blood was collected using citrate carrying anticoagulant tubes. Cells, platelets and large microvesicles were removed from human blood by differential centrifugation. EVs were then precipitated using polyethylene glycol (PEG). Pelleted EVs were resuspended and separated from co-precipitated lipoprotein particles and protein complexes by upward displacement into a linear Nycodenz density gradient. Finally, EV carrying fractions were applied onto a Sepharose CL-2B column for SEC.

Results: As compared to ultracentrifugation, EVs were more efficiently precipitated from human plasma using PEG. However, PEG precipitated EVs were highly contaminated with low density lipoprotein particles (LDL), high density lipoprotein particles (HDL), and non-EV associated protein (complexes). EVs were efficiently separated from these contaminants by subsequent fractionation on Nycodenz density gradients. However, some HDL contaminants remained, which could be removed in the third step using SEC.

Conclusion: These data indicate that subsequent isolation steps are required to isolate EVs to homogeneity from plasma. Single step isolation methods may result in gross overestimation in the amount of EV associated protein or misinterpretation of EV molecular compositions.

NLSEV2018-P18 Ana Merino

Membrane particles from MSC for endothelial immune regulation and regeneration

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Background: Cardiovascular (CV) risk factors such as inflammation have been strongly linked to the dysfunction of endothelial cells (EC), and consequently to an increase in CV events. Mesenchymal stromal cells (MSC) are studied as immunomodulatory therapy. However, MSC get trapped in the capillary networks of the lungs after intravenous infusion, where they have a short survival time. Recent work demonstrated that dead MSC maintain their immunomodulatory capacity, suggesting that cell membrane dependent interactions with immune cells are responsible for the immune regulatory effects. We propose a new cell-free therapy based on the generation of small plasma Membrane Particles (MP) from MSC. We previously showed that MP were effective in reducing the inflammatory phenotype of monocytes. In the present study we investigated the effect of MP on EC.

Aim: Study the therapeutic potential of MP as immunomodulatory and regenerative therapy for dysfunctional EC.

Methods: MSC were treated with and without IFN-γ. MP from both type of cells were generated by hypotonic shock and extrusion. Three ratios of EC:MP were tested in an EC model of inflammation. The taking up of MP by EC was analyzed by confocal microscopy. Toxicity was examined at 24, and 48h by apoptosis assay. Monocyte adhesion to EC, angiogenesis, and scratch wound healing assays were examined by microscopy.

Results: Confocal microscopy analysis showed that within 24 hours >90% of EC have taken up MP, both under normal and TNF-α conditions. The toxicity experiment showed that even the highest concentration of MP does not induce apoptosis in EC (<5%). TNF-activated EC do not upregulate their adhesion surface markers after treatment with MP. This correlates with the finding that monocytes do not adhere more firmly to the EC after treating them with MP. With respect to the regenerative capacity of EC after MP treatment, we have observed an enhancement of angiogenesis by increasing the number of tubes, and branches formation compared to the negative control (non-treated EC). In the scratch wound healing assays, MP had a stimulating effect on EC to fill the scratch in a dose-dependent manner.

Conclusion: Under inflammatory conditions MP enhance the regenerative capacity of EC.

NLSEV2018-P19 Laura Bongiovanni

Developing a novel cancer biomarker blood test by measuring mRNA levels of cell cycle genes in circulating extracellular vesicles

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Background: Malignant cancer cells are highly proliferative. These cells actively release extracellular vesicles (EVs) in the tumor environment and into body fluids, such as blood. Cancer-derived EVs are enriched with cell cycle gene-related molecules, such as the target transcripts of the transcription factor family E2F. Quantification of cell cycle gene mRNA in blood-derived EVs of cancer patients can represent an indicator of this proliferation signature, which can be used to diagnose the presence of malignant tumors, predict the prognosis and efficacy of anti-cancer treatment and/or monitor disease progression, representing a potential new useful, minimally invasive, clinically applicable test.

Methods: Six highly expressed cell cycle gene transcripts were selected based on the analysis of transcriptome data of cancer tissues available in public databases. The expression levels of these transcripts were then evaluated by qPCR in EVs isolated from cultured canine melanoma cells. To investigate whether the mRNA levels in tumor cells is mirrored into their released EVs, cell proliferation was blocked using a CDK4/6 inhibitor. As a first step to detect the cell cycle gene transcripts in plasma-derived EV, total RNA was extracted from the plasma of canine cancer patients and analyzed for the presence of the selected cell cycle transcripts.

Results: The selected transcripts were detected in both large and small melanoma-derived EVs, but were most highly enriched in exosomes. CDK4/6 inhibitor treatment induced a decrease in cellular transcript levels, which were accompanied by a decrease of mRNA levels in EVs. In the plasma of canine cancer patients mRNA of some of the selected genes was detected with overall higher expression levels when compared to plasma of healthy controls.

Conclusions: In vitro, cell cycle gene mRNAs are present within cancer cell-derived EVs and their levels reflect the expression levels and proliferation status of donor cells. Furthermore, cell cycle gene transcripts are detectable in the plasma of canine cancer patients. Further investigation will be applied to validate their association to circulating EVs and the correlation with tumor proliferation rate, patient response to therapy, and follow-up data.

NLSEV2018-P20 Manon Mulders

Tumor-driven alterations of mesenchymal stem cell immunomodulatory properties as novel therapeutic targets for osteosarcoma

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Background: Osteosarcoma is a pediatric bone tumor characterized by high metastatic potential and poor prognosis. While the genetic heterogeneity of this cancer type challenges the development of targeted therapy, defining the interactions between osteosarcoma and the complex bone microenvironment can provide insights into novel therapeutic strategies. We previously showed in a xenograft mouse model that osteosarcoma cells release extracellular vesicles (EVs) that “educate” mesenchymal stem cells (MSC) to promote tumor growth and metastasis formation. The pro-tumorigenic effect of cancer EVs was dependent on membrane-bound TGFβ, inducing IL6 expression in MSCs. We hypothesized that, apart from having direct tumor-promoting activity, tumor-educated MSCs (TEMSC) modulate immune cell function.

Methods: To find evidence of immune cell infiltration in the osteosarcoma microenvironment, we performed multiplex immunofluorescence staining of human osteosarcoma tumors. The global gene expression changes in MSCs induced by osteosarcoma EVs were analyzed by RNA sequencing. Validation of the expression of immunomodulatory mediators was performed by qPCR.

Results: We found that osteosarcoma tumors are highly infiltrated with immune cells with suppressive phenotypes, such as CD163+ M2 macrophages, CD3+FoxP3+ T cells and CD8+PD1+ T cells. The functional profiling of the gene expression changes in tumor educated MSCs vs naïve MSCs revealed high representation of chemokine signaling-related pathways. Upregulation of a set of 6 chemokines involved in neutrophil, myeloid derived suppressor cells (MDSC) and T cells recruitment was validated by qPCR. Blockade of TGFβ signaling demonstrated that, differently from IL6, the EV-mediated chemokine induction is TGFβ-independent.

Conclusion: Our study suggests that tumor-educated MSCs might favor osteosarcoma progression by generating an immunosuppressive environment.

Future: The role of the tumor-MSC-immune cell crosstalk in osteosarcoma progression will be evaluated in vivo using immunocompetent mouse models.

NLSEV2018-P21 Jennifer Perez Boza

EV-derived microRNA profiles from patients with classical Hodgkin Lymphoma can discriminate between active disease and complete response

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Classical Hodgkin lymphoma (cHL) is a malignancy characterized by a low number of pathologically transformed cells (Hodgkin and Reed-Sternberg cells) surrounded by an inflammatory microenvironment mostly composed by other immune cells. The bidirectional communication between malignant cells and the tumor microenvironment (TME) is crucial both for the progression of the disease as well as in mediating response to treatment. Part of this communication is carried out via the exchange of small extracellular vesicles (EVs) loaded with various biomolecules, including microRNAs.

In the present study, we have analyzed the microRNA content of EVs from patients with cHL before and after treatment with chemotherapy with or without stem cell transplantation and/or radiotherapy. We have found that the expression profile of microRNAs can discriminate between patients not having a complete response following treatment (residual disease phenotype) vs. those in remission after treatment (responsive disease phenotype). The microRNA profiling allowed us also to uncover a secondary subset of patients that, while they still present an active phenotype, were considered as an independent group during the discovery of potential biomarkers. Differential expression analysis was performed comparing all three groups and a subset of microRNAs capable of stratifying patients according to their disease state were identified. Additionally, it was shown that the microRNA expression footprint can be grouped into two major clusters. Independent pathway enrichment analysis of the targets of the two microRNA clusters show that, even though the majority of pathways are regulated cooperatively by both clusters, some pathways involved in the regulation of the extracellular matrix, survival, and in cell-to-cell communication are differentially targeted by each microRNA cluster.

These results may suggest that EV-derived microRNA profiles can discriminate between patients with residual disease from those with complete response to treatment, thus proposing a new set of biomarkers of response.

NLSEV2018-P22 Agustin Enciso-Martinez

Optical tweezers and Raman spectroscopy of single extracellular vesicles (EVs)

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Background: Extracellular vesicles (EVs) are small lipid-membrane-bound vesicles released by cells for intercellular communication purposes, among other functions. EVs derived from cancer cells play a role in tumor cell proliferation, migration, invasion, and metastasis. Their presence in body fluids, such as blood, makes them potential biomarkers for cancer disease.

Methods: Optical tweezers have allowed us to study individual vesicles and detect the individual trapping events from Rayleigh scattering. Synchronous recording of Raman scattering enabled the acquisition of Raman spectra of both, individual and multiple EVs. Furthermore, Mie light scattering theory was used to relate the Rayleigh scattering intensity to the size of trapped beads, used for calibration.

Results: The light scattered of trapped vesicles gave rise to different time traces that can be used as a signature to distinguish individual trapping events from accumulative cluster events. Next, we confirmed the trapping of individual EVs derived from PC3 cells, red blood cells, platelets and blood plasma by acquiring both, Rayleigh and Raman scattering signals. While the step-wise trend in the Rayleigh scattering signal suggests trapping of single particles, the Raman scattering signal demonstrates single-EV trapping, as this signal resulted from the integration of a lipid-protein band in the Raman spectrum (2811-3023 cm-1). Through principal component analysis, the main spectral variations among the four type of EVs were identified. Furthermore, when comparing principal component scores, the PC3-derived EVs can be identified from the rest of the EVs.

Conclusions: We have developed an automated optical tweezer-confocal Raman – and light scattering (OT-Raman) setup to trap and release EVs over time. We demonstrated single-EV trapping by simultaneous acquisition of Rayleigh and Raman scattering. Multivariate analysis enabled the identification of single-EVs derived from a cancer cell line PC3. This discloses chemical information as a step towards the identification and characterization of tumor-derived EVs in blood.

NLSEV2018-P23 Esther Drees

Pre-analytical considerations for multicenter extracellular vesicle associated miRNA biomarker studies

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Previously, we showed that in classical Hodgkin lymphoma (cHL) patients a panel of cell-free circulating miRNAs reflects the presence of metabolically active tumor. These miRNAs were measured in the extracellular vesicle (EV) fractions of EDTA plasma and proved suitable markers for therapy response monitoring. In order to externally validate these, we wished to obtain material from cHL patients treated at peripheral hospitals. However, logistical constraints at these locations hamper EDTA blood collection. To overcome this, we investigated the suitability of blood collection tubes containing a preservative that prevents hemolysis (PAXgene ccfDNA and STRECK RNA/DNA tubes). So that the extracellular vesicles may be preserved and material can be shipped prior to isolation.

EV-associated extracellular RNA (exRNA) are isolated with size-exclusion chromatography (SEC) from healthy donor plasma in the various blood collection tubes. The tubes were incubated for 0 or 48 hours prior to plasma extraction. Pre- and post-treatment samples was obtained from a cHL patient. qRT-PCR is used to quantify exRNAs. Transmission electron microscopy is used to check the presence of extracellular vesicles. Comprehensive small RNA sequencing is performed on cHL patient and healthy donor EDTA and PAXgene plasma.

Our cHL biomarker panel can be detected in STRECK RNA and STRECK DNA and PAXgene ccfDNA tubes that were extracted after 0 and 48 hours. The levels between t=0 and t=48 are rather stable in the STRECK RNA tube and PAXgene ccfDNA tube, whilst increasing in the STRECK DNA and EDTA. In the STRECK RNA plasma, multiple of the our biomarkers are below the detection limit prior to treatment, making this tube unsuitable for monitoring with these specific markers. The presence of extracellular vesicles was confirmed with electron microscopy in the PAXgene ccfDNA plasma after 48 hours of incubation. Moreover we were able to confirm treatment related changes of our biomarker panel in PAXgene tubes.

It is feasible to perform EV-miRNA studies with blood collection in PAXgene ccfDNA tubes. Further validation of the biomarkers needs to performed before directly comparing cohorts with different type of collection tubes.

NLSEV2018-P24 Niels Eijkelkamp

Macrophage-derived vesicles transfer mitochondria to neurons to resolve inflammatory pain

Michiel van der Vlist[1]*, Ramin Raoof[1] , Hanneke Willemen[1], Judith Prado Sanchez[1], Sabine Versteeg[1], Martijn Vos[1], Linde Meyaard[1], Niels Eijkelkamp[1]*

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Pain is a consequence of inflammation, and the current paradigm is that this pain disappears when the inflammation resolves. However, in 30% of rheumatic patients pain does not resolve, whilst inflammation is minimal/absent.

We show that resolution of inflammatory pain is regulated remotely and distinctly from that of inflammation. During transient inflammatory pain CD11b+F4/80+ macrophages accumulated in the Dorsal root ganglia (DRGs) that contain the cell bodies of sensory neurons innervating the inflamed hind paw. Depletion of monocytes and tissue-resident macrophages prevented the resolution of inflammatory pain, but did not affect the duration or magnitude of the inflammatory response. Intrathecal injection of macrophages in monocyte/macrophage-depleted mice rescued the resolution of inflammatory pain, demonstrating that monocytes are required for the resolution of inflammation induced pain. Further analysis revealed that macrophages transfer mitochondria to sensory neurons through releasing extracellular vesicles containing mitochondria. The transfer of mitochondria requires CD200 receptor, because macrophages or vesicle deficient for CD200R were are unable to resolve pain. Finally, full resolution of inflammatory pain by macrophages required expression of the CD200R ligand iSec1.

These findings unveil a mechanism as to how immune cells control inflammatory pain resolution with extracellular vesicles, and may provide a complete novel direction for development of pain-resolving drugs.

Sponsor Presentations

NLSEV2018 Particle Metrix

Biomarker detection using Particle Metrix ZetaView® and Nanoparticle Tracking Analysis (NTA)

Sascha Raschke, Particle Metrix

By using the ZetaView® NTA instrument Extracellular Vesicles (EVs) and other biological nanoparticles in a heterogeneous sample can be individually analyzed in terms of their size, number and concentration. Furthermore, they can be phenotypically characterized using fluorescence-labeled antibodies or intercalating membrane dyes. Two different fluorescence channels of the ZetaView® machine allow analysis of two different fluorescent labels in rapid succession with just one sample, eliminating the time and effort required to produce a second fluorescently labeled sample.

NLSEV2018 KdBio

Efficient production of EVs using a continuous, high density cell culture system (FiberCell)

Antony Rutt, KdBio

A FiberCell hollow fibre bioreactor (F-HFBR) enables many types of mammalian cells and cell lines to be continuously cultured for long periods at densities equal to or exceeding 10e8 cells per ml. These high cell densities are made possible thanks to:

a) cartridge fibre-surface-area-to-volume ratio of 150 cm2/ml

b) continuous perfusion of media through porous fibres enabling cells to grow post-confluently

Up to 10e11 cells can be maintained for long periods using 20ml or 70ml F-HFBR cartridge modules. Secreted products that are larger than the fibre molecular weight cut-off will be retained in the cell compartment, or “extra-capillary space”, and can be periodically collected in small volumes using syringes. This is therefore a time-scalable process that can greatly boost overall productivity. Product concentrations in raw supernatant are typically 40 – 100X that of flask conditioned media (CM) which facilitates downstream processing.

Hollow fibre technology has been routinely employed for monoclonal antibody and recombinant protein production over many years. It is widely acknowledged as a more in vivo like way of maintaining cells in culture compared to growing them on impermeable plastic surfaces.

EV production is a relatively new application for F-HFBR and feedback from users indicates that it offers a significant production efficiency improvement for obtaining large amounts of EVs. One study with MSCs showed that a 20ml F-HFBR cartridge module produces enough EVs over 4 weeks to outperform 130 x T225 flasks by at least an order of magnitude. Bioreactor CM is highly enriched in EVs compared to flask CM. Also, whereas flask culture is a batch process involving a lot of flasks and a period of serum starvation, F-HFBR fits easily on one shelf in a standard incubator and offers a continuous culture process with no cell passaging. Another advantage of high density culture is that it becomes possible to employ a simplified, serum-free, protein-free media formulation.

This talk will cover the basic principles and practical aspects of operating the FiberCell hollow fibre bioreactor and will provide examples of results from customer laboratories.

NLSEV2018 Izon

Combining Size Exclusion Chromatography (SEC) & Tunable Resistive Pulse Sensing (TRPS) technology for the advanced characterization of extracellular vesicles

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Tunable Resistive Pulse Sensing (TRPS) is a particle-by-particle technology that enables measurements of nano-size particles with a very high-resolution. TRPS measures individual particle by analyzing the durations, the frequencies and magnitudes of the resistive pulses under varying driving forces across a pore-based sensor and by using reference particles calibrated for size, surface charge and concentration. For the extracellular vesicles (EVs) and viruses fields, the complete cycle of sample preparation, measurement, analysis of samples from biological fluids using both size exclusion chromatography and TRPS is now by far the fastest option as well as the most reliable and accurate. In this communication, will be described how TRPS offers significant and unique advantages over optics based approaches for characterizing Evs (e.g. size and concentration). Furthermore, our latest developments on particle-by-particle charge analysis and phenotyping EVs population will be outlined and discussed.

References:

A.N Boing et-al; “Single-step isolation of extracellular vesicles by size-exclusion chromatography”, Journal of Extracellular Vesicles 2014, 3: 23430.

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NLSEV2018 Wyatt

Wyatt Exosome Platform for Isolation, Identification, Quantitation, and Biophysical Characterization

Koen Hollebekkers, Wyatt Technology Europe

The Wyatt AF4-MALS-DLS platform is used for separation, purification and biophysical characterization of exosomes. It is using field flow fractionation (AF4), multi angle light scattering (MALS) and dynamic light scattering (DLS). Field flow fractionation is a uniquely versatile means of size-based separations, capable of fractionating and characterizing proteins and aggregates, liposomes, emulsions, viral particles, polysaccharides, nanoparticles, polymer latex particles, colloidal soil suspensions, chemical mechanical polishing slurries, and – exosomes.

Common solution-based methods for determining the size and size distributions of exosomes, such as dynamic light scattering and nanoparticle tracking analysis (NTA), cannot offer the resolution, statistical confidence and absolute accuracy of a true separation-based technique that incorporates MALS and DLS downstream detection. AF4 MALS DLS offers excellent resolution and accuracy in analysis of size distributions. The MALS detection is the established benchmarks for molar mass and radius determination in solution, cited in thousands of peer-reviewed publications.

In my presentation I will discuss results from the last AF4 MALS DLS publication from the Lyden group: Zhang, Haiying, et al. "Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation." Nature Cell Biology (2018): 1. doi:10.1038/s41556-018-0040-4

The Lyden lab is one of the leading groups in the exosome research field. The group used AF4-DLS as a highly reproducible and robust analytical tool for isolating and identifying the exosome subpopulations for future exosome research. They isolated and identified from conditioned cell media three subpopulations of particles, addressing the processing speed and sample heterogeneity problems with current isolation methods.