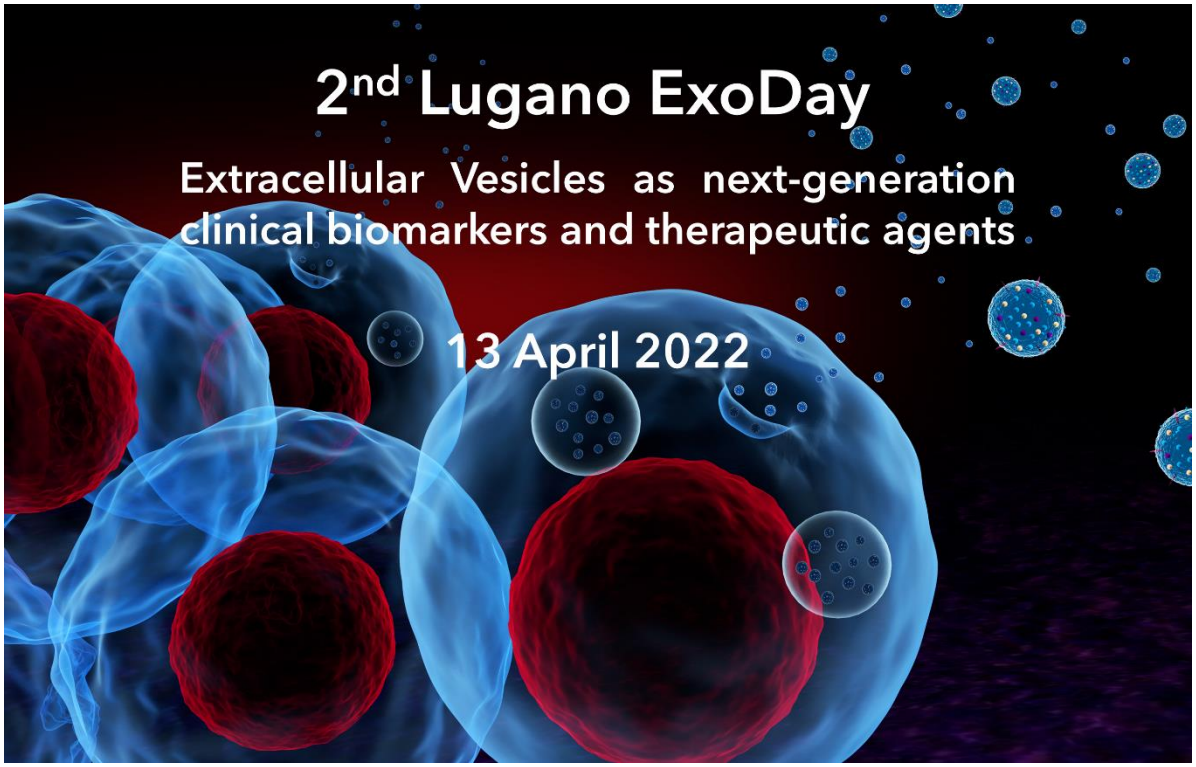

Abstract Book

Contents

Signature EV miRNAs associated with melanoma mutation status

Ala'a Al Hrout[1], Mitch Levesque[2], Richard Chahwan[1]

[1] Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland. [2] Department of Dermatology, University of Zurich Hospital, Zurich, Switzerland.

The tumor microenvironment (TME) is being increasingly implicated in promoting tumor survival and progression. The TME is composed of different cellular and non-cellular components that are involved in a bi-directional interaction with the tumor. One critical component of the TME are immune cells that have been reported to possess tumor-promoting or tumor-suppressing functions. Cancer cells crosstalk with immune cells to recruit them to sustain the growth and metastasis of the tumor. The release and uptake of extracellular vesicles (EVs) is one form of this immune-tumor cell communication. EVs have been implicated in cancer progression by promoting proliferation, angiogenesis, and immune-suppression. Melanoma-derived EVs have been associated with immune suppression and escape, promoting tumor progression. Understanding the nature of EV-based communication is critical in creating effective therapies.

Patient-derived cell lines with different mutation statuses (NRAS, BRAF, double mutants) were cultured as spheroids to mimic physiological architecture. RNA was isolated from each spheroid culture and their respective EVs. Based on RNASeq analysis, EV miRNA but not cellular RNA was able to segregate cell lines based on their mutation status. However, long-non-coding RNA, neither EV nor cellular, was not able to distinguish between cell lines. We were also able to identify candidate signature EV miRNAs that are uniquely associated with certain mutation statuses over others. To understand the contribution of these EV miRNAs in the context of TME interplay, we aim to validate their role in a patient-derived ex-vivo organoid model that includes different TME immune-tumor components.

Exploiting EVs for efficient chemotherapeutics delivery

Alessia Brancolin[i1] and Riccardo Vago[1,2]

[1]Università Vita-Salute San Raffaele, Milano, Italy

[2]Urological Research Institute, Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, Milano, Italy

Extracellular vesicles (EVs) are nanosized membrane delimited vesicles secreted by different cell types to mediate intercellular communication in a variety of cellular processes. Thanks to their intrinsic biological features, EVs are suitable tools for diverse therapeutical applications, such as drug delivery systems. A number of EVs loading strategies have been developed for this purpose, but all of them are endowed with intrinsic limitations, such as the poor loading efficacy or the disruption of EVs integrity.

The aim of the study is the setup and optimization of a strategy leading for EV loading with chemotherapeutics which combines method simplicity, EVs structure maintenance and efficient cargo loading. Three different clinically used chemotherapeutics have been used as EVs payloads, characterized by different chemical properties, but all efficacious in the treatment of different solid cancers. At first, we defined a linear correlation between chemotherapeutics' absorbance and their concentration at drug-specific wavelength. Such correlation was exploited to define a system for the quantification of drug loading efficacy upon EVs-coincubation. Bladder cancer cells were treated with chemotherapeutics loaded-EVs and their viability was then evaluated upon treatment. Finally, the IC50 values of either drug-loaded EVs and free administered chemotherapeutics were compared to claim the validity of EVs use as drug delivery system and the efficacy of the EVs co-incubation protocol exploited in the study.

With this study, we can conclude that: drug intrinsic physical and chemical properties may affect loading efficacy, the efficacy of EVs loading by co-incubation is drug dependent and it may be sufficient to decrease drug IC50 values with respect to free chemotherapeutics administration.

Supervised and unsupervised learning to define the cardiovascular risk of patients according to an extracellular vesicle molecular signature

Jacopo Burrello[1,2], Alessio Burrello[3], Elena Vacchi[4,5], Giovanni Bianco[6], Elena Caporali[1], Lorenzo Grazioli[1], Martina Amongero[7], Sara Bolis[1], Giuseppe Vassalli[5,8], Carlo Cereda[6], Paolo Mulatero[2], Benedetta Bussolati[9], Giorgia Melli[4,5], Giovanni G Camici[10], Silvia Monticone[2], Lucio Barile[1,5].

[1]Laboratory for Cardiovascular Theranostics, Cardiocentro Ticino Institute, Lugano, Switzerland. [2]Division of Internal Medicine and Hypertension Unit, Department of Medical Sciences, University of Torino, Italy. [3]Department of Electrical, Electronic and Information Engineering (DEI), University of Bologna, Italy. [4]Laboratory for Biomedical Neurosciences, Neurocenter of Southern Switzerland, Ente Ospedaliero Cantonale, Lugano, Switzerland. [5]Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano, Switzerland. [6]Neurology Clinic, Stroke Center, Neurocenter of Southern Switzerland, Lugano, Switzerland. [7]Department of Mathematical Sciences G. L. Lagrange, Polytechnic University of Torino, Italy. [8]Cellular and Molecular Cardiology Laboratory, Cardiocentro Ticino Institute, Lugano, Switzerland. [9]Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy. [10]Center for Molecular Cardiology, University of Zürich, Switzerland.

Secreted extracellular vesicles (EVs) are membrane-bound nanoparticles released from cells. Since their content reflects the specific signatures of cellular activation and injury, EVs display a strong potential as biomarkers in the cardiovascular (CV) field. We aimed at dissecting a specific EV signature able to stratify patients according to their CV risk and likelihood to develop fatal CV events.

A total of 404 patients were included in the analysis. For each subject, we evaluated several CV risk indicators (age, sex, BMI, hypertension, hyperlipidemia, diabetes, coronary artery disease, chronic heart failure, chronic kidney disease, smoking habit, organ damage) and the likelihood of fatal CV events at 10 years, according to the SCORE charts of the European Society of Cardiology. Serum EVs were isolated by immuno-capture and analyzed for the expression of 37 EV surface antigens by flow cytometry. Unsupervised and supervised learning algorithms were applied for clustering patients according to CV risk.

Based on expression levels of EV antigens, unsupervised learning classified patients into three clusters (cluster I, 288 patients; cluster II, 86 patients; cluster III, 30 patients). Prevalence of hypertension, diabetes, chronic heart failure, and organ damage (defined as left ventricular hypertrophy and/or microalbuminuria) progressively increases from cluster I to cluster III, with an average 6.9-fold increase. Several EV antigens, including markers from platelets (CD41b-CD42a-CD62P), leukocytes (CD1c-CD2-CD3-CD4-CD8-CD14-CD19-CD20-CD25-CD40-CD45-CD69-CD86), and endothelium (CD31-CD105) were independently associated to the CV risk indicators and correlated to age, blood pressure, glucometabolic profile, renal function, and SCORE risk. EV specific signature obtained by supervised learning allowed the accurate classification of patients according to their 10-year risk of future CV events, as estimated with the SCORE risk charts. EV profiling, obtainable from minimally-invasive blood sampling, may be integrated into CV risk stratification, displaying a potential role in the tailored management of these patients.

Exosomal mediated signal transduction through artificial microRNA (amiRNA): an opinion for target inhibition of mutant SARS-CoV-2

Chithravel Vadivalagana[1,2]

[1]Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei-110, Taiwan. [2]Department of Biochemistry, AKFA Medical School, AKFA University, Tashkent, Uzbekistan.

Exosome trans-membrane signals provide cellular communication between the cells through transport and/or receiving the signal by molecule and change the functional metabolism and stimulate and/or inhibit receptor signal complexes. COVID19 genetic transformations are varied in different geographic positions and single nucleotide polymorphic lineages were reported in the second waves, due to fast mutational rate and adaptation. Several vaccines were developed and are in treatment practice but effective control is yet to reach in cent present, it's originally a narrow immune-modulating protein target. Controlling these diverse viral strains, may inhibit their transuding mechanisms specially to target RNA genes that are responsible for COVID19 transcription. Exosomal miRNAs are chief sources of trans-membrane signals and trans-located miRNAs can directly target mRNA transcription of COVID19. These findings were discussed about targeted viral transcription by delivering the artificial miRNA (AmiR) mediated exosomes in the infected cells and major resources of exosome and their efficacy.

Extracellular Vesicles as targetable drug delivery vehicles

Maria Chiara Ciferri[1], Daniele Reverberi[2], Silvia Bruno[1], Cansu Gorgun[1], Chiara Gentili[1], Rodolfo Quarto[1,2], Roberta Tasso[1]

[1] Department of Experimental Medicine (DIMES), University of Genova, Genova, Italy. [2] IRCCS Ospedale Policlinico San Martino, Genova, Italy.

Thanks to their activity as short and long-distance mediators of intercellular communication, EVs have been recently considered a new class of nanocarriers. Their ability to transport functional cargoes to target cells makes them a valid delivery vehicle. EV surface can be modified to incorporate ligands for targeted therapy making natural nanoparticles valuable tools for the target-therapy of various diseases. Besides their delivering properties, the possibility of drug loading and the substantially successful preclinical results have encouraged researchers to study EVs for the delivery of therapeutic molecules.

The aim of this work is to: (i) load a medicinal cargo in plasma-derived EVs, and (ii) functionalize their surface to create a membrane anchoring tool to be used as a platform to bind molecules, thus obtaining a tissue-specific drug delivery system. We have carried out an exogenous method of membrane functionalization based on the most common copper-free click chemistry reaction: the alkyne-azide Huisgen 1,3-dipolar cycloaddition. Azide-modified molecules react with the terminal alkynes tied to the aminic groups on human plasma-derived EV surface giving rise to the surface functionalization to be used as a targeting tool. As a "proof of concept", we employed a fluorescent-azide and analyzed the presence of the fluorescent signal by flow cytometry and fluorescent NTA (Nanoparticles Tracking Analysis) to confirm the functionality of the system. Moreover, we examined in vitro the uptake of engineered EVs by responder cells (MDA-MB-231, a human breast cancer cell line) by flow cytometry and confocal microscopy. The chemotherapy drug Paclitaxel has been loaded into functionalized EVs through sonication.

We evaluated the achievement of EV-loading and the operation of our sonication method by flow cytometry using a fluorescent Pacific Blue-Paclitaxel (PB-PTX). The in vitro MTT and Annexin/PI assays have been employed to compare the cytotoxic effects of PTX loaded-EVs and free-PTX on MDA-MB-231. Our EV membrane functionalization system can be used to bind to the EV surface azido-modified antibodies/molecules, making the engineered EVs a valuable natural drug nanocarrier able to be directed to a specific antigen/target and so potentially applied in various pathological conditions. Oncology could be a field of application since it has been demonstrated a selected tropism of circulating cancer-derived EVs toward the tumor. Thus, the autologous transplantation of EVs (3) could be applied for a natural cancer-specific targeting enhanced by the acquired targeting capacity. Moreover, the loading with an anti-cancer molecule could execute a focused therapeutic effect avoiding the systemic side effects characterizing chemotherapy drugs.

Isolation of EV subpopulations from the nanoscale to the microscale with asymmetrical flow field-flow fractionation (A4F): an innovative approach for the EV-diagnostic field

Daniele D'Arrigo [1,2], Arianna Gennari[3], Chiara Arrigoni[1], Marco Vanoni[2], Marco Delcogliano[4], Christian Candrian[4,5], Nicola Tirelli[3], Matteo Moretti[1,5,6]

[1]Regenerative Medicine Technologies Lab, Service of Orthopaedics and Traumatology, Department of Surgery, EOC, via Federico Chiesa 5, 6500 Bellinzona, Switzerland (CH). [2]Department of Biotechnology and Bioscience, Università degli studi di Milano-Bicocca, 20126 Milan (IT). [3]Laboratory of Polymers Biomaterials, Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genoa, Italy (IT). [4]Service of Orthopaedics and Traumatology, Department of Surgery, EOC, Via Tesserete 46, 6900 Lugano, Switzerland (CH). [5]Euler Institute, Biomedical Sciences Faculty, Università della Svizzera Italiana (USI), via Buffi 13, 6900 Lugano, Switzerland (CH). [6]Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Via Riccardo Galeazzi 4, 20161 Milan, Italy (IT).

Extracellular vesicles (EVs) are generally described as lipid particles with a relatively defined and limited size distribution. However, increasing experimental evidence demonstrated that several EV subpopulations exist and have different and important biological roles. For instance, large EVs are involved in the inflammatory response, a key process in joint diseases like osteoarthritis (OA). However, most of the studies looking for new OA biomarkers are focused only on small EVs. Thus, our aim is to develop a protocol based on the asymmetrical flow field-flow fractionation (A4F) technique to separate different sized EV subpopulations from the synovial fluid (SF), also applicable to other biofluids.

We collected SF from the shoulder and the knee of arthritic and non-arthritic donors. We also got the plasma from a healthy donor and the supernatant of *in vitro* cultured synovial fibroblasts that were isolated from the synovial membrane of 4 arthritic patients. Firstly, we optimized the flow rates in the A4F to obtain the whole EV profile from the different samples. Then, we separated different sized EV subpopulations, evaluating the EV average size, their relative abundance, the Z potential, and the concentration of proteins and nucleic acids. We performed transmission and immune electron microscopy (EM) to confirm particle size and to assess EV-specific surface markers and the presence of lipoproteins.

With our protocol, we were able to isolate EVs with a radius ranging from 20 up to 700 nm that were divided into 4 subpopulations according to their size. We also obtained the whole EV profile of the different samples that resulted different in each biofluid, as well as the EV size, as confirmed by EM, and the relative percentage of each subpopulation. The results showed that EVs isolated from the arthritic or inflamed SF were larger than those isolated from the healthy SF. Moreover, the larger EVs showed a decreasing trend of the Z potential and the protein concentration, but not of the nucleic acid content. We demonstrated, also by immune EM, the presence of EV-specific markers in the eluted fractions and, as expected from a size-based separation approach, lipoproteins were found in the smaller EV subpopulations, especially in the plasma sample.

Our A4F-based protocol allows both the isolation of EVs with a radius ranging from 20 up to 700 nm and the separation of different size EV subpopulations. It also provides the whole EV

profile of different samples and we showed that it differs between healthy and inflamed SF. Importantly, this difference lies in the micrometric EVs, and therefore it is detectable only with this A4F-based approach, as the classic isolation techniques are not able to isolate EVs with this size range. Therefore, the experimental approach that we developed, allowing not only the isolation but also the separation and the successive characterization of different EV subpopulations in biofluids, would give intriguing new opportunities to find clinical biomarkers for OA and also for other diseases.

Engineering extracellular vesicles as refined vehicles for the delivery of recombinant proteins

Carlotta Emili[1], Alessia Brancolini[1], Stefania Zuppone[2], Natasa Zarovni[3] and Riccardo Vago[1,2]

[1] Università Vita-Salute San Raffaele, Milano, Italy. [2] Urological Research Institute, Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, Milano, Italy. [3] HansaBioMed Life Sciences, Tallin, Estonia.

Extracellular vesicles (EVs) are naturally secreted nanosized vesicles that recently emerged as suitable vehicles for the delivery of therapeutic molecules in cancer treatment. They have several advantages compared to current synthetic nanoparticles systems, which comprise their natural origin, controlled immunogenicity, and absence of cytotoxicity. However, successful EVs exploitation as a drug carrier system still requires further investigation.

Healthy and tumor cells were used for EV production. EV isolation was performed by sequential centrifugations and specific EV markers were detected by western blot. Genetically engineered EVs were obtained by transfecting cells with a construct encoding tetraspanins fused to a reporter gene through a protease-specific cleavage site.

We engineered donor cells to express a fluorescent reporter gene fused to tetraspanins resulting in a massive incorporation of fusion proteins in EVs and structural preservation. To induce the selective release of EV-carried, tetraspanin-fused recombinant proteins in target tumor cells, we inserted a cleavage site, which was selectively processed by proteases over-expressed in model cancer cells. The constructs were stable, but the sorting was dependent on cell lines and tetraspanins.

We found genetic engineering as the most promising approach to produce EVs carrying recombinant proteins, due to structural preservation and increased encapsulation efficiency. Furthermore, the introduction of a protease-specific cleavage site is likely to confer target selectivity to these nanocarriers.

Comparing Digital Detection Platforms in High Sensitivity Immune-phenotyping of Extracellular Vesicles

Roberto Frigerio[1], Angelo Musicò[1], Alessandro Strada[1], Greta Bergamaschi[1], Gabriella Andriolo2, Lucio Barile[2], Marcella Chiari[1], Alessandro Gori[1] and Marina Cretich[1]

[1]Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" (SCITEC) - Consiglio Nazionale delle Ricerche, Milano, Italy. [2]Istituto Cardiocentro Ticino, Ente Ospedaliero Cantonale, Lugano, Switzerland.

Extracellular Vesicles (EVs) represent one of the widest biomarker spaces in the liquid biopsy paradigm, given their ubiquitous distribution within biofluids. Despite their clinical potential, EVs struggle to take the scene as a preeminent source of biomarkers in liquid biopsy. EVs represent analytes of unprecedented complexity given the heterogeneity of EV sub-populations in terms of cellular origin, biogenesis, molecular composition, structure, and function. Limitations in the use of EVs originate from their inherent complexity and heterogeneity and from the sensitivity demand in detecting low to very low abundant disease-specific sub-populations. Such need can be met by digital detection, namely capable to reach the single-molecule sensitivity.

We compared, side by side, two digital detection platforms, both commercially available, that have recently gained increasing importance in the field of EVs. The ExoView® Analyzer (https://www.nanoviewbio.com) is based on the principle of Single Particle Interferometric Reflectance Imaging Sensing (SP-IRIS) and the Quanterix Simoa® Technology (https://www.quanterix.com/simoa-technology) based on the Single-Molecule Array technology (SiMoA).

Sensitivity in immune-phenotyping of a well-characterized EV sample has been studied, and possible applicative implications and rationales for alternative or complementary use of the two platforms in biomarker discovery or validation.

Comparative Analysis of Extracellular Vesicles from Adipose Tissue- And Bone Marrow-Mesenchymal Stromal Cells in Endothelial Proliferation and Chondrogenesis

Cansu Gorgun[1,2], Maria Elisabetta Federica Palamà[1], Daniele Reverberi[3], Maria Cristina Gagliani[1], Katia Cortese[1], Roberta Tasso[1], Chiara Gentili[1]

[1]Department of Experimental Medicine (DIMES), University of Genova, Genova, Italy. [2]U.O. Cellular Oncology, IRCCS Ospedale Policlinico San Martino, Genova, Italy. [3]U.O. Molecular Pathology, IRCCS Ospedale Policlinico San Martino, Genoa, Italy.

The secretome of mesenchymal stromal cells (MSCs) derived from different tissue sources is considered an innovative therapeutic tool for regenerative medicine. Although adipose tissue- and bone marrow-derived MSCs (ADSCs and BMSCs, respectively) share many biological features, the different tissue origins can be mirrored by variations in their secretory profile, and in particular in the secreted extracellular vesicles (EVs). In this study, we carried out a detailed and comparative characterization of middle- and small-sized EVs (mEVs and sEVs, respectively) released by either ADSCs or BMSCs. Their involvement in an endochondral ossification setting was investigated using ex vivo metatarsal culture models that allowed to explore both blood vessel sprouting and bone growth plate dynamics. Although EVs separated from both cell sources presented similar characteristics in terms of size, concentration, and marker expression, they exhibited different characteristics in terms of protein content and functional effects. ADSC-EVs over-expressed pro-angiogenic factors in comparison to the BMSC-counterpart, and, consequently, they were able to induce a significant increase in endothelial cord outgrowth. On the other hand, BMSC-EVs contained a higher amount of pro-differentiation and chemotactic proteins, and they were able to prompt growth plate organization. This work explores the role of MSC-EVs during the differentiation and maturation of cartilage and points out how EVs from different origins can modulate the early endochondral bone formation process in a different manner. Therefore, despite apparently few differences were found between EVs derived from ADSCs and BMSCs, a deep investigation of their biological effects is crucial, before selecting the optimal EV-cell source.

Optimizing isolation and purification of EV-like Nanoparticles from Ginger Rhizome (Zingiber officinale)

Francesca Loria[1], Andres Lõhmus[1], Danilo Mladenović[1], Paolo Guazzi[1], Jekaterina Kazantseva[2], Raivo Vilu[2,3], Natasa Zarovni[1]

[1]HansaBioMed Life Sciences Ltd, Mäealuse 2/1, 12618 Tallinn, Estonia. [2]Center of Food and Fermentation Technologies, Akadeemia tee 15A, 12618 Tallinn, Estonia. [3]Department of Chemistry and Biotechnology, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia

Extracellular Vesicles (EVs) have been highlighted as an important pathway of cellular communication, as well as a source of biomarkers and unprecedented biomedical applications across different kingdoms. Plant-derived EVs are among the most appealing next-generation biological and industrial agents, still facing a low readiness for underlying production and processing. EV-like nanoparticles have been recently recognized as bioactive components of ginger (Zingiber officinale) and have been proven to hold health-protecting and/or health-enhancing properties in pre-clinical settings, thus prospecting novel tools for delivering effector molecules with encouraging efficacy and safety profiles. To overcome the challenges in isolation and purification of ginger-derived nanoparticles (GDNs), we have investigated an enzyme-assisted ginger rhizome cell wall digestion to promote GDN extraction from the apoplast.

Ginger rhizome was washed, peeled, sliced, and ground using a centrifugal juicer to extract the juice and dry residues. Three concentrations of a commercially available multi-enzyme cocktail containing a wide spectrum of carbohydrases [0%, 0.1%, and 0.5% (v/w)] were used to perform overnight enzymatic pre-treatment at 37oC and pH 5.5, under continuous shaking. After incubation, GDNs were isolated and purified by a combination of differential centrifugation, (tangential flow and dead-end) filtration and size exclusion chromatography. GDN characterization was performed by nanoparticle tracking analysis, protein quantification assay, enzymatic activity assays, and fluorometric microplate-based nucleic acid and lectin assays.

We have piloted our GDN isolation method starting from 20 g of ginger. Digestion with 0.1% multi-enzyme enabled a 47% increase in particle recovery, correlated with particle size and protein content preservation. Isolated and purified GDNs were enzymatically active, displaying only minor changes in nucleic acid content and lectin-associated glycan expression, compared with undigested controls. Conversely, 0.5% multi-enzyme cocktail pre-treatment caused substantial GDN degradation.

Enzyme-assisted ginger rhizome cell wall digestion resulted in optimization of our original GDN isolation and purification protocol, as demonstrated by the observed process yield improvement in association with maintenance of tested biocomponents and enzymatic activity. Ad-hoc implemented multiparametric quantity and quality assessment supports high GDN translational potential and proceedings into proof-of-concept studies for clinical and/or industrial application.

Development of breakthrough liquid biopsy diagnostic via novel exosomal biomarkers for patient stratification in prostate cancer

Alekhya Mazumdar[1], Robert Lutz Guzman[1], Christopher Millan[2], Daniel Eberli[1]

[1]Laboratory for Urologic Oncology and Stem Cell Therapy, University Hospital of Zürich, Zürich, Switzerland. [2]Beken Bio Inc, San Diego, California, USA.

Approximately 1 man in 36 will die from prostate cancer. In most of these cases, cancer cells from the prostate metastasize to distal tissues, at which point the prognosis is particularly poor. To better identify patients at high risk of metastasis, and therefore enable earlier therapeutic intervention, there is a significant clinical need for robust clinical diagnostics that target the earliest molecular actors of this process. Recently, tumor-derived extracellular vesicles (EVs, of which exosomes are a subtype), have been implicated in the establishment of the pre-metastatic niche. In contrast with cell-free tumor DNA (cfDNA) or circulating tumor cells (CTCs), EVs are secreted by cancer cells in significantly higher concentrations and at earlier stages during disease progression. In preliminary biomarker discovery work performed by our lab, we identified a panel of proteins enriched in prostate cancer EVs. In EVs isolated from the plasma of prostate cancer patients, expression of these biomarker proteins was found to correlate with disease stage and therapeutic response, while eliminating false positives often associated with prostate-specific antigen (PSA), which is the current clinical standard blood test for prostate cancer.

We then are due to evaluate our target biomarkers in a larger prospective study involving 500 patients in collaboration between the Urology Clinic of USZ and Ludwig-Maximilians-Universität München (LMU) (ethics approvals already granted for both sites). If successful, this project will break new ground as the first to demonstrate the clinical utility of protein EV biomarkers as analytes to target for cancer diagnostics. This approach has the potential to open new avenues in prostate cancer management, increase the standard of living for patients, and combat metastasis-mediated patient mortality.

Characterizing EV population heterogeneity using label-free optofluidic platforms

Jaime Ortega Arroyo[1], Alexia Stollmann[1], Jose Garcia Guirado[1], Romain Quidant[1]

[1]Nanophotonic Systems Laboratory, Dept. of Mechanical and Process Engineering, ETH Zurich, Zurich, Switzerland.

The highly heterogeneous nature of samples containing EVs, and within the EV populations themselves, poses a significant challenge in terms of isolation, detection, and ultimately determining their functionality. In this talk, I will present a set of platforms developed in our lab that integrate three main tools: digital holography, microfluidics, and surface chemistry, with the aim of characterizing the underlying heterogeneity of EVs by achieving single EV sensitivity in a label-free manner and with high-throughput. I will specifically focus on describing the working principle and then showcase some example applications.

SPRi biosensor to explore extracellular vesicles role in neurological diseases

Silvia Picciolini[1], Alice Gualerzi[1], Francesca Rodà[1,2], Valentina Mangolini[1], Luana Forleo[1], Marzia Bedoni[1]

[1]IRCCS Fondazione Don Carlo Gnocchi, Laboratory of Nanomedicine and Clinical Biophotonics (LABION) [2]Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia.

Extracellular Vesicles (EVs) have been proposed as potential biomarkers of multiple diseases, as their composition changes according to the physio-pathological condition of the tissue of origin, mirroring their direct involvement in the pathogenesis of multiple human disorders, including neurological diseases. Despite the increasing scientific and commercial interest in EV application in diagnostics, traditional biomolecular techniques usually require consistent sample amount, rely on operator-dependent and time-consuming procedures, and cannot cope with the nano-size range of EVs, limiting both sensitivity and reproducibility of results. Surface Plasmon Resonance imaging (SPRi) is a well-known photonic-based technology that is experiencing increasing application in biomedical research, thanks to its sensitivity and high-throughput features. Herein, we propose SPRi as a promising and valuable tool for the characterization of circulating EVs with the specific aim of discovering new easily accessible biomarkers of neurodegenerative diseases, such as Alzheimer's disease, and cerebrovascular diseases. We developed an antibody SPRi-array to detect multiple EVs present in human plasma or serum, that was successfully applied for analysis of EVs derived from neurons, oligodendrocytes, astrocytes, microglia, and endothelium with good sensitivity and specificity. Then, the optimized biosensor was used for the analysis of the expression levels of potential proteins and lipids present on EV membranes related to the pathological processes of Alzheimer's disease, demonstrating the heterogeneous composition of each EV population and identifying the involvement of EVs in the disease progression. The SPRi-based biosensor was also used for the characterization of blood EVs from stroke patients, before and after rehabilitation. After the successful SPRi detection of EVs of different cellular origin, the quantification of specific surface molecules related to pathological or regeneration processes was accomplished. Our results demonstrated the ability of the SPRi biosensor to reveal differences in the relative amount of specific cell-derived EV subpopulations and in their cargo during disease progression and rehabilitation-induced recovery, providing support to the proposed role of EVs in neurological diseases, thus demonstrating great potentialities for their clinical translations.

Patient-derived Fibroblasts and induced Neurons (iNS) for the Study of pathogenic cellular Pathways involved in Neurodegenerative Disorders and Lysosomal Storage Disorders

Ester Piovesana[1,2], Ilaria Fregno[3], Stéphanie Papin[1], Maurizio Molinari[3], Paolo Paganetti[1,2]

[1]Laboratory for Aging Disorders, Laboratory for Translational Research, EOC Bellinzona. [2]Biomedical Sciences, University of Southern Switzerland (USI), Lugano. [3]Laboratory Protein Folding and Quality Control, IRB, Bios+, Bellinzona.

Tauopathies are a group of progressive neurodegenerative diseases characterized by intracellular fibrillar Tau protein species ultimately deposited in neurofibrillary tangles (NFTs). NFTs appear first in disease-specific brain regions and then gradually spread transneuronally along the brain connectivity to invade the whole brain, possibly explaining a lethal disease progression. Transneuronal Tau spreading involves a prion-like course where pathogenic Tau species self-propagate through an unclarified protein-to-protein mechanism requiring cell-to-cell transport. Among the different means of transcellular transport of macromolecules, extracellular vesicles (EVs) represent paracrine vectors, which are known to contribute to disease. Recent data from our laboratory demonstrated that acidic endolysosomal organelles represent a subcellular site where a profibrillogenic Tau fragment transported by EVs interacts with, and may accelerate the accumulation of, an intracellular Tau pool. Importantly, lysosomal dysfunction is found in a group of diseases called lysosomal storage diseases (LSDs), which are inherited metabolic disorders caused by enzyme deficiencies within the lysosome resulting in the accumulation of undegraded substrates. Abnormal substrate accumulation causes a broad spectrum of clinical manifestations depending on the specific substrate and on the affected organ. Increasing evidence shows that autophagy/lysosomal dysfunction and protein aggregation are functionally interconnected and act in synergy during neurodegenerative processes. Notably, mutated proteins causing LSDs in children may affect the prion-like transmission of neurodegeneration-associated proteins. In fact, mutations in glucocerebrosidase (GBA), a lysosomal enzyme associated with recessively inherited Gaucher disease, represent the most common risk factor to develop Parkinson's disease. My project consists in addressing if lysosomal dysfunction linked to GBA mutation has an impact on Tau prion-like transmission in the endolysosomal compartment of human post-mitotic neurons. The project aims at investigating pathogenic cellular processes linked to the progression of human disease, in particular neurodegenerative disorders. It is anticipated that the data obtained will advance our understanding of the role of degradative pathways of the cell in human disease.

Exosomes for therapeutic applications: GMP-grade large scale manufacturing method, product characterization, and stability

Elena Provasi[2], Gabriella Andriolo[1], Viviana Lo Cicero[1], Andrea Brambilla[1], Sabrina Soncin[1], Giuseppina Milano[2], Vanessa Biemmi[2], Maximilian Y. Emmert[3], Giuseppe Vassalli[2], Lucia Turchetto[1], Lucio Barile[2], Marina Radrizzani[1]

[1]Lugano Cell Factory and [2]Laboratory for Translational Research, Istituto Cardiocentro Ticino - Ente Ospedaliero Cantonale, Lugano, Switzerland. [3]University of Zurich, Zurich, Switzerland.

Exosomes (Exo) are a subset of cell-derived extracellular vesicles (EV) of 50-150 nm in diameter with potential therapeutic and diagnostic applications. Human cardiac progenitor cells (CPC) release Exo that prevents early decline in cardiac function in rat models of myocardial infarction. In view of the upcoming clinical translation, we set up a Good Manufacturing Practice (GMP)-compliant method for the large-scale culture of CPC and the production and isolation of their exosomes (Exo-CPC).

We prepared a Master Cell Bank (MCB) of CPC cultured in xeno-free conditions and frozen at passage 2 (p2). For Exo-CPC manufacturing, MCB aliquots were thawed, CPC expanded up to p4 (2.5 m2 culture surface, about 8x108 cells), and induced to release exosomes by 14 days starvation in basal medium, leading to the collection of about 8L of exosome-containing conditioned medium (CM). Exo-CPC were isolated through a closed system, encompassing CM clarification, extracellular vesicle concentration, and diafiltration by tangential flow filtration, and final sterilizing filtration. Quality control tests were performed to validate the identity, potency, and safety of both the cell source and the Exo-CPC preparations.

Three Exo-CPC lots produced so far resulted sterile and negative for bacterial endotoxins; they contained typical exosomes (121-143 nm diameter) expressing CD9/CD63/CD81 and CPC markers (CD44/CD105). These Exo-CPC showed anti-apoptotic and pro-angiogenic activity in vitro and therapeutic potential in vivo in rat and pig models of acute myocardial infarction. The stability study indicated no loss of in vitro anti-apoptotic activity after 24 months of storage at -80°C.

In essence, our standardized, large-scale Exo-CPC production method guarantees high yield (up to 3.6x1013 total EV particles/run, with an average concentration of ~1x1011 particles/ml in the final product) and efficient removal of contaminant proteins (>97%). Moreover, our purification process can be applied to different EV sources for several therapeutic purposes. Presently, we are upgrading our established manufacturing method thanks to the MARVEL EU collaborative project (FETPROACT-EIC-06-2019, grant agreement 951768, coordinated by CNR, Milano, Italy) by integrating an affinity step in the current workflow to improve product purity by minimizing the co-isolation of contaminants.

Fast quantification of extracellular vesicles levels in early breast cancer patients by Single-Molecule Detection Assay (SiMoA)

Alessandra Ricciardi[1], Carlo Morasso[1], Daisy Sproviero[2], Marta Truffi[1], Sara Albasini[3], Francesca Piccotti[1], Federico Sottotetti[4], Ludovica Mollica[4], Cristina Cereda[2], Luca Sorrentino[5], Fabio Corsi[3,6]

[1]Laboratory of Nanomedicine, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy. [2]Genomic and postGenomic Center, IRCCS Mondino Foundation, Pavia, Italy. [3]Breast Unit, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy. [4]Medical Oncology Unit, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy. [5]Colorectal Surgery Unit, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan,Italy. [6]Dipartimento di Scienze Biomediche e Cliniche "L. Sacco", Università di Milano, 20157Milano, Italy.

Breast cancer (BC) is the most commonly diagnosed cancer worldwide and the identification of new biomarkers remains a priority for biomedical research. In this regard, extracellular vesicles (EVs), lipid bilayer membrane vesicles, nanometer-sized secreted from many cell types, have been recently considered as a promising biomarker in BC. The predictive role of EVs is supported by the higher concentration of tetraspanin CD9, CD63 and CD24 receptors in EVs originated by cancer cells than those derived from normal epithelial mammary cells. However, there is a lack of clinical data supporting this value due to the difficulty of their quantification in the biological fluids through the traditional approaches. To solve this problem, we have developed an innovative ultrasensitive assay based on Single Molecule Array (SiMoA) Technology using a couple of antibodies targeting CD63 and CD9.

Thanks to this innovative technique, it is possible to analyze EVs without any previous purification process using only 4 µl of plasma. SiMoA assay intrinsically includes an isolation step for EVs based on the use of magnetic beads conjugated with an anti CD63 antibody and mixed with the samples to form an immunocomplex with the target and with the anti CD9 detection antibody. Each immunocomplex is individually distributed by the SiMoA system in a microwell with a volume of about 40 femtoliters which allows the amplification of the local concentration of the target obtaining a LOQ of 1.03 ng/ml.

Using this assay, we determined the plasma EV concentration of 95 early BC patients (stage I-III) and 86 healthy controls (HC). The obtained results showed that EVs levels resulted significantly higher in BC (median values 1179.1 ng/µl vs 613.0 ng/µl, $p < 0.0001$). ROC curve was calculated obtaining an AUC of 0.75 (95% CI 0.68- 0.82) with a sensitivity of 68% and specificity of 75% (PPV:75.6% and NPV: 68.4%). ROC curve was also used to define the optimal cut-off level of the test at 1034.5 ng/µl. In addition, we compared EVs levels before and after surgery for a subgroup of 45 patients, determining that post-surgery levels were lower than baseline ($p=0.014$) and not statistically different from HC.

Our results prove the efficacy of the SiMoA Technology for the determination of EVs levels in the biological fluid in a fast and reproducible way and the idea that EVs may be a promising biomarker for BC disease. Besides, considering the low amount of plasma used for the analysis, this new technology offers the possibility to study rare subpopulations of cancer-released EVs to increase the accuracy of EVs as a BC biomarker.

Raman spectroscopy for the biomolecular characterization of extracellular vesicles in Parkinson's disease

Francesca Rodà[1,2], Alice Gualerzi[2], Silvia Picciolini[2], Valentina Mangolini[2], Luana Forleo[2], Marzia Bedoni[2]

[1]Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Modena, Italy [2]IRCCS Fondazione Don Carlo Gnocchi, Laboratory of Nanomedicine and Clinical Biophotonics (LABION), Milan, Italy.

Parkinson's disease (PD) is chronic neurodegenerative pathology characterized by progressive movement disorders and by the intraneuronal accumulation of misfolded alpha-synuclein. As for most neurodegenerative diseases the early diagnosis, disease monitoring, and rehabilitation outcomes are difficult to be objectively assessed but mainly rely on clinical scaling and cognitive tests. Extracellular vesicles (EVs) are nanosized particles naturally secreted by all body cells and loaded with multiple bioactive molecules. Hence, they provide a mirror of the patient's health status and of the disease progression that might be correlated to the prognosis and the rehabilitation outcome. In this study, we propose the use of Raman Spectroscopy (RS), a light-scattering-based technique, to investigate the overall biochemical variation in the EV cargo related to PD progression and to the rehabilitation outcome.

We obtained serum samples from PD patients before and after the rehabilitation program and from healthy subjects. EVs were isolated by size exclusion chromatography and concentrated by ultracentrifugation. Then, the Raman analysis was performed in order to obtain an overview of the EV biochemical profile. Following the label-free protocol previously optimized in our laboratory, spectra were acquired taking advantage of a Raman microspectroscope (Aramis, Horiba) operating with a 532 nm laser beam in the spectral ranges 600-1800 cm-1 and 2600-3200 cm-1. Multivariate statistical analysis was performed for the comparison of the EV Raman spectra from healthy subjects and PD patients.

The results of our study demonstrated the ability of the proposed approach to highlight differences in the biochemical composition of EVs from human serum. In particular, we demonstrated the presence in the serum of PD patients of EVs associated or loaded with atypical cargoes compared to age and sex-matched healthy controls. The spectral modification of blood EVs in PD subjects was correlated with the most common clinical scales used to describe the PD stage, demonstrating a positive correlation with both Hoehn&Yahr and UPDRS III scales. Preliminary data demonstrated also the possibility to use the Raman fingerprint of blood EVs to monitor the rehabilitation effect.

Although a small cohort of patients, our data provide support to the already proposed prion hypothesis of PD pathogenesis. Moreover, our results suggest the possibility to evaluate the spectrum of circulating blood-derived EVs as a whole using the Raman fingerprint as a biomarker for PD to monitor the rate of progression and the effectiveness of rehabilitation and pharmacological therapy.

FTIR spectroscopy for cancer-derived Extracellular Vesicles discrimination: a promising liquid biopsy tool for cancer diagnosis

Sabrina Romanò[1], Riccardo Di Santo[2], Maria Vaccaro[2], Flavio Di Giacinto[1,2], Ewa Krasnowska[3], Marco De Spirito[1,2], Gabriele Ciasca[1,2]

[1]Dipartimento di Neuroscienze, Sezione di Fisica, Università Cattolica Del Sacro Cuore, Roma, Italy. [2]Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy [3]National Research Council of Italy, Institute of Translational Pharmacology (IFT-CNR), Rome, Italy.

Extracellular Vesicles (EVs) are recently getting much attention in personalized medicine. Oncology is probably the branch where the research is more active, with the perspective of their use both for diagnostic and clinical applications. In this scenario, some issues are still hindering the EVs application as cancer biomarkers in liquid biopsies; these include the need for standardized and low-cost extraction methods and the development of a cost-effective and robust platform for their characterization, which would allow the detection of EVs secreted by cancer cells. Since Fourier Transform Infrared Spectroscopy (FTIR) allows for direct access to the characteristic absorption bands of biomolecules, it has the concrete potential to provide a versatile platform for EVs characterization for diagnostic and classification purposes. Here, we used FTIR in the mid-IR range to investigate the composition of EVs and discriminate their origin from different cellular phenotypes and serum-derived EVs from patients diagnosed with hepatocellular carcinoma (HCC) and healthy subjects.

The efficacy of the technique was first tested on an in vitro model using the human colorectal adenocarcinoma intestinal cell line Caco-2 as a model system to discriminate EVs from two different cell states through an induced Epithelial-Mesenchymal reverse Transition (EMT). As EVs released into the tumor microenvironment play an important role in the progression of HCC, because they influence several biological pathways including the local regulation of the EMT, FTIR spectroscopy was applied to EVs obtained from the serum of patients diagnosed with HCC of metabolic origin and healthy donors. Statistical and machine learning methods were applied to both models to highlight possible spectral biomarkers of the disease, as well as for testing methods for automated classification. Our results show that EVs can be classified with high accuracy, precision, specificity, and sensitivity using logistic regression and PCA based on characteristic mid-IR bands. Our studies may have a positive impact on the development of FTIR spectroscopy as a tool for Extracellular vesicles-based liquid biopsy for cancer diagnosis.

Ischemic stroke risk assessment based on the detection of miRNA-638 derived from EVs in serum by a new molecular lateral flow assay

Ana Rubio-Monterde[1,2], Ana Luque[3], Marc Gallegos[1], Josep M. Aran[3], Arben Merkoçi[1,2,4,5], Daniel Quesada-González[1]

[1]Paperdrop Diagnostics S.L., MRB, Campus UAB, 08193 Bellaterra, Spain. [2]Nanobioelectronics and Biosensors Group, Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC, Barcelona, Spain. [3]Immune-inflammatory Processes and Gene Therapeutics Group, IDIBELL, L'Hospitalet de Llobregat, 08908 Barcelona, Spain. [4]The Barcelona Institute of Science and Technology (BIST), Campus UAB, 08036 Bellaterra, Barcelona, Spain. [5]ICREA, Institució Catalana de Recerca i Estudis Avançats, Pg. Lluis Companys 23, 08010, Barcelona, Spain.

According to a preliminary study, low levels of miRNA-638 are related to the risk of suffering an ischemic stroke. This miRNA, highly expressed in vascular smooth muscle cells and implicated in proliferative vascular diseases, can be found in EV in serum. Current preferred miRNA detection methods rely on various PCR-based approaches which, although highly sensitive, rely on qualified personnel and expensive equipment.

As an alternative method to detect miRNA-638, we present FASTROKE: A fast diagnostic test to evaluate the Ischemic stroke risk. The test consists of an isothermal amplification followed by a read-out in a rapid diagnostic test (RDT).

The isothermal amplification method used needs minimal sample preparation and works optimally at a temperature of around 37-42˚C. After 35 min of reaction time, the amplified product is applied over a test strip with a buffer, where the result of the test can be read after 5 min, giving a turnaround time of 40 min.

With these conditions, 1 pg/mL of miRNA-638 was detected and specificity was tested against another miRNA. Then, clinical samples previously tested positive by RT-qPCR were tested with the assay, obtaining positive results.

Development of blood-brain barrier-on-chip for EV permeability assessment

Hoang Tuan Nguyen[1], Prateek Singh[1]
[1]Finnadvance, Aapistie 1, Oulu, Finland.

Accurate modeling of the blood-brain barrier (BBB) is crucial in maintaining brain homeostasis and is the key barrier in understanding drug pharmacokinetics. However, it also exhibits a hurdle for EV delivery and development. One reason for poor success to target EVs to the brain is the lack of good in vitro models that could recapitulate the BBB function. Our microfluidic in-vitro model of the BBB organ-on-a-chip platform provides a high throughput assay for EV permeability.

Our microfluidic BBB-on-chip platform design includes static conditions of the brain-side, as well as the dynamic conditions of the brain microvasculature within the same platform, mimicking in-vivo conditions. The BBB-on-chip is established using a co-culture of endothelial cells (ECs) and astrocytes, cultured on opposing sides of a semipermeable membrane.

First, we compared mouse primary brain-derived-ECs (MBECs) to human immortalized cell line, HCMEC/D3, and found that MBECs are forming a better barrier much faster in our BBB-on-chip. Secondly, we tested the effect of different pore sizes on barrier formation and morphology. We concluded that 3 µm pore size supported the growth of ECs the best. Finally, a co-culture of MBECs with astrocytes showed a marked improvement in permeability compared to monoculture. In-vitro BBB generated with either mono or cultured brain endothelial cells and astrocytes showed marked improvement in Papp (apparent permeabilities) when cultured under flow. Mouse BBB-on-chip showed a Papp for 70 kDa Dextran to be 1.7E-7 cm/s, which is astonishingly close to in-vivo observed Papp of 1.5E-7 cm/s. Our microfluidic BBB model accurately reflects the in vivo conditions of the BBB by modeling both the static conditions of the brain-side of the BBB, as well as the dynamic conditions of the brain microvasculature within the same platform. As the highest-throughput platform currently in the field, our BBB model enables the development and screening of new therapeutics for neurological disorders still lacking effective treatments, such as Alzheimer's and Parkinson's disease.

In-silico model of Membrane-sensing peptides

Alessandro Strada[1,3], Gautieri Alfonso[2], Roberto Frigerio[3], Alessandro Gori[3], Marina Cretich[3]
[1]Politecnico di Milano - Department of Chemistry, Materials, and Chemical Engineering ''Giulio Natta''. [2]Biomolecular Engineering Lab, Dept. Electronics, Information and Bioengineering, Politecnico di Milano. [3]National Research Council, Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" (SCITEC-CNR), Milano.

Peptides derived from membrane-sensing proteins have emerged as convenient, easy to synthesize novel molecular probes for targeting highly curved membranes. Our recent work has identified a class of membrane-sensing peptides (RPPGFSPFR) derived from Bradykinin protein as a novel class of molecular ligands for integrated sEV isolation and analysis. The mechanism of binding is based on the intrinsic cooperation between an electrostatic interaction and a hydrophobic contribution.
Investigation of the mechanisms and dynamics of peptides is essential to studying how to generate new peptide designs. For this purpose, a microscopic-level view into the movements and interactions of individual atoms is invaluable.
In this work, we presented an atomic-scale Molecular Dynamics (MD) simulation in order to provide an enhanced understanding of the interactions involved in membrane sensing.
Using an ''atom's eye view'' of the system peptide/membrane, we have carefully designed a series of simulations, which follow the peptide and the membrane in atomic detail.
Starting from the Bradykinin peptide/membrane model, we have generated a series of in-silico model in which the hydrophobic moieties of the peptide sequence was modified by replacing the phenylalanine with residues with different degree of hydrophobicity. Different interactions were analyzed such as hydrogen bonds, hydrophobic contacts, and cation-pi interactions. In addition, the in-silico model was used to determine the free-energy profile of these peptides in order to provide a more in-depth structural and energetics insight into the peptide interaction with the phospholipid bilayer.
In this study, we showed how the computational approach can be considered a useful tool to fully understand these systems and their dynamic processes.

MicroRNAs in small extracellular vesicles (sEV) from Wharton's jelly mesenchymal stromal cells and their potential neuro-regenerative role

Vera Tscherrig[1,2], Sophie Cottagnoud[1], Valérie Haesler[1], Patricia Renz[1,2], Daniel Surbek[1], Andreina Schoeberlein[1], Marianne Jörger-Messerli[1]

[1]Department of Obstetrics and Feto-maternal Medicine, University Women's Hospital, Inselspital, Bern University Hospital, Bern, Switzerland, and Department for BioMedical Research (DBMR), University of Bern, Switzerland.

[2]Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland.

It has proved very promising to use small extracellular vesicles derived from mesenchymal stromal cells (MSC-sEV) as a therapeutic approach for neuronal injury. The cargo of MSC-sEV includes small non-coding RNAs such as microRNAs (miRNAs), which are predicted to target mRNAs encoding for proteins that are involved in premature birth-related white matter injury (WMI). Thus, we hypothesize that miRNAs, released by sEV upon uptake in their target cells play a significant role in the observed beneficial effects of MSC-sEV.

We isolated MSC from the connective tissue of human umbilical cords, the so-called Wharton's jelly. The cells were stained for typical MSC-markers by immunohistochemistry. Small EV were purified from the conditioned cell medium by serial ultracentrifugation, followed by size exclusion chromatography (SEC). The protein and RNA contents of each SEC fraction were measured with a NanoVue Plus™. The fractions with the highest protein content, in our case fractions 5-7, were characterized by Proteomics, Western blot, ImageStream, and ZetaView analyses. Quantitative PCR was used to measure the miRNA content of the sEV. Pathway enrichment of the miRNAs was analyzed following Next Generation Sequencing (NGS). A miRNA luciferase reporter assay was established in order to evaluate the regulatory activity of sEV miRNA.

The SEC fractions 5-7 were positive for the sEV markers CD81, CD63, and Syntenin-1, and the MSC markers CD73, CD90, and CD105. Moreover, they contained high amounts of miRNAs, such as miR-21-5p, miR-22-5p, miR-27b-3p, and members of the let-7 family. TP53 was identified as a target of 11 of the 32 most abundant MSC-sEV miRNA by NGS. Therefore, the 3'UTR sequence of TP53 was cloned into the pMirTarget 3'UTR assay vector downstream of a firefly luciferase sequence and co-transfected with MSC-sEV into the luciferase reporter cell line HEK293T. MSC-sEV significantly reduced the luciferase signal, strongly indicating an inhibitory effect of MSC-sEV miRNAs.

Our data identify miRNAs as a functional unit in the therapeutic effect of MSC-sEV in preclinical WMI models. The regulatory potential of MSC-sEV miRNAs in disease-related gene expression is currently being further analyzed using agomir / antagomir assays in an *in vitro* model of WMI.

On the surface-to-bulk partition of proteins in extracellular vesicles

Andrea Zendrini[1,2], Krizia Sagini[3,4], Giorgia Guerra[3], Tatyana Vagner[3], Dolores Di Vizio[3], Paolo Bergese[1,2,5,6]

[1]Department of Molecular and Translational Medicine, Università degli Studi di Brescia, Viale Europa 11, 25123, Brescia, Italy. [2]Center for Colloid and Surface Science (CSGI), Viale della Lastruccia 3, 50019 Sesto Fiorentino, Italy. [3]Department of Surgery, Division of Cancer Biology and Therapeutics, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA. [4]Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway. [5]National Inter-university Consortium of Materials Science and Technology (INSTM), Via Giuseppe Giusti 9, 50121 Firenze, Italy.[6]Institute for Research and Biomedical Innovation (IRIB-CNR), Consiglio Nazionale delle Ricerche, Via Ugo la Malfa 153, 90146 Palermo, Italy.

Nanomaterials are characterized by an astonishing surface-to-mass ratio. Extracellular Vesicles (EVs) – which have been recently recognized as the universal agent of intercellular communication, being involved in many physiological and pathological processes and interkingdom biochemical communication – are nanoparticles, but this key aspect has never been rationally addressed. We report on the first attempt to quantify the surface-to-bulk partition of proteins in EVs. A semi-quantitative model based on available well-established compositional and microstructural data is formulated. The model allows for estimation of the overall protein content of an EV as well as of the partition between membrane (surface) associated and lumen (bulk) contained proteins as a function of the EV size. Calculations identify a switch diameter at 180 nm, below which EVs result to carry more membrane than luminal proteins. At larger diameters the partition is reversed, reaching predominance of luminal proteins (> 80 %) in larger EVs (diameter > 800 nm). The model was successfully tested to analyze and describe a real preparation composed of subpopulations of small EVs and Large Oncosomes from human prostate cancer cells. These findings provide a tool for a better colloidal description of EV samples and will allow for improved design and interpretation of experiments, including EV engineering and (dosing in) in-vitro and in-vivo investigations.