EXOSOMES AND MICROVESICLES: APPLICATIONS FOR TRANSLATIONAL RESEARCH FROM BIOMARKERS TO THERAPEUTIC APPLICATIONS"

2013 ASMEV Meeting Report

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Abstract

Extracellular vesicles, comprised of exosomes derived from the multi-vesicular bodies and ectosomes or microvesicles, released by shedding from the plasma membrane, have been implicated in mediating intracellular communication. These extracellular vesicles not only carrying proteins derived from the parental cells, but also contain subsets of mRNA, miRNAs and other non-coding RNAs. Since vesicles are found in all bodily fluids, they also are a source of disease markers. This new field of extracellular vesicles, including exosomes and microvesicles, represents a rapidly expanding area of biomedical research relevant to the development of disease-specific, non-invasive methods for clinical diagnostics as well as to the development of new therapeutic approaches. The annual meeting of the American Society for Exosomes and Microvesicles (ASEMV) recently was held in Orlando, Florida, USA (September 7-9, 2013). At the meeting, novel findings and ideas related to the role of microvesicles in a variety of biological processes and as well as diagnostics were presented. The meeting also facilitated networking and the inception of collaborative projects among researchers working on extracellular vesicles. A summary of the meeting is presented.

The annual meeting of the *American Society for Exosomes and Microvesicles (ASEMV)* took place in Orlando, Florida from September 7-9, 2013. The goal of this annual meeting was to serve as a forum for interdisciplinary exchange between researchers from academia and industry, working on the identification, characterization and utilization of extracellular vesicles including exosomes and microvesicles. The main topics of the 2013 ASEMV Conference were the diverse roles of exosomes and microvesicles in cell signaling and their potential use as diagnostic and prognostic biomarkers. Almost all cell types release extracellular vesicles, which appear to be important for cell-to-cell communication both locally and at a distance. These extracellular vesicles are characterized predominantly by their size, composition and mechanism of generation. Microvesicles or ectosomes are released from the plasma membrane by shedding or budding, are usually larger than 0.2 μM size. In contrast, exosomes are nanovesicles between 30-100 nm in diameter, derived by inverse budding of multivesicular bodies (MVBs) or late endosomes. The protein content of these different types of extracellular vesicles reflects those of the parental cells. Moreover, these extracellular vesicles contain miRNAs, mRNAs, ncRNAS and even extra-chromosomal DNA. Thus extracellular vesicles are an excellent source of disease specific biomarkers.

The first session began with a presentation by Douglas Taylor ("Comparison of technologies for isolation of specific extracellular vesicles populations"), in which he presented a general overview of exosomes and microvesicles research. In the next presentation, Dolores Di Vizio ("Functional and quantitative proteomic analysis of large oncosomes reveals novel effects of extracellular vesicles on the tumor environment") demonstrated a role for extracellular vesicles in tumor pathogenesis. In particular, she presented evidence that microvesicles can activate transcription factors including hnRNP-K, a multifunctional regulator of transcription and translation that enhances cancer cell proliferation, affecting the tumor microenvironment. Michael W Graner ("Brain Tumor exosomes stress allegiance in recipient cell signaling: tumor cells under stress and T

cells under fire yield to the tumor's bidding") presented data suggesting a role for exosomes in proximal and distal signaling in brain tumors. Leonora Balaj ("Heparin affinity purification of extracelular vesicles") presented a new method for the isolation of exosomes, based on heparin affinity. In the next presentation, Kevin L. Schey ("Quantitative Proteomic Analysis of Human Urine Exosomes: Effect of Dietary Salt") examined the protein content of isolated urine exosomes. The results indicate that urine exosomes are a potential source of disease biomarkers. In fact, a reoccurring theme of the meeting is that microvesicles, which are found in all bodily fluids including blood, semen, bronchial lavage fluid, saliva, cerebral spinal fluid and urine, carrying disease associated RNA and proteins that could be used as prognostic and diagnostic markers. The final presentation of the first session was given by Davide Zocco ("EXOTEST: an immune-capturing assay for accurate quantitative and qualitative analysis of exosomes from unfractionated plasma samples: A candidate platform for cancer immunodiagnostics"). EXOTEST™ is a platform for isolation, enrichment and analysis of exosomes, which can be used for molecular profiling in cancer diagnostics and other diseases. However, despite the new ways presented to isolate, characterize and profile extracellular vesicles molecular, there is still no clear consensus on the best approaches.

In the second session, Meta Kuehn ("Elucidating the mechanical basis for vesicle production by Gram-negative bacteria") presented evidence that even bacteria produce membrane vesicles. In particular, gram-negative bacteria can produce outer membrane vesicles able to facilitate interaction with their environment including other bacteria. This is in addition to observations regarding the role of extracellular vesicles released from cells infected with mycobacterium. Stephen Gould ("Exploring the genetics of exosome biogenesis") showed that plasma membrane is the major site of budding for vesicles containing CD63, an exosomal marker. Natalia Luhtala ("Evidence for exosomal secretion of Ras proteins and beta-catenin in glioblastomas") continued the session by demonstrating that extracellular vesicles from brain tumors can carry oncoproteins such as Ras. Thus, vesicles containing activated oncoproteins can affect cell growth within the tumor microenvironment. Jolene Read ("The reovirus Fusion-Associated Small transmembrane (FAST) proteins usurp host exosome biogenesis to enhance cell-cell fusion") reported the first example of a non-enveloped virus protein that is targeted to exosomes. Pierre-Yves Mantel ("Mechanism of cellular communication in Malaria by parasite-derived microvesicles) provided compelling results supporting a role for exosomes in communication by parasites. In the final presentation of this session, Johan Skog ("Microvesicle RNA profiling from clinical samples") reported on the progress made towards developing a clinical test using exosomes as biomarkers.

The third session began with a presentation by Prashanth Vallabhajosyula ("Donor organ specific exosome platform for monitoring transplant organ rejection/injury") on using exosomes as a diagnostic marker for the tissue state for monitoring transplant rejection/injury. Depending upon the transplanted tissue this analysis could be performed on vesicles in blood, urine or lavage fluid. Masato Mitsuhashi ("Aging enhances release of exosomal cytokine mRNAs by Amyloid b1-42-stimulated macrophages") presented the possible use of exosomes as markers in neurodegenerative diseases. Analysis of a subset of mRNAs, including mRNAs encoding for inflammatory proteins, contained with circulating vesicles showed changes with age. Similarly, Cicek Gercel-Taylor ("Cargoes of circulating vesicles as diagnostic biomarkers") identified exosomes as carrying RNAs and proteins that could be biomarkers for ovarian cancer. Taku Murakami ("Urine exosomes capture device to quantify glomerulus-, tubule-, and collecting duct specific mRNA as a novel platform for the assessment of kidney function") presented a method to use exosomes and microvesicles as promising biomarkers in renal diseases, including the use of profiling specific mRNAs contained in circulating vesicles. Elena V. Batrakova ("Blood-borne macrophages hitchhike endosome networks for brain delivery of antioxidant, catalase") continued with a presentation of efforts to design a therapy to treat inflammatory and neurodegenerative diseases. She presented evidence that macrophage were able to cross the blood-brain barrier where they could then release vesicles containing catalase, able to protect neurons from apoptosis. In the final presentation of the third session Travis Antes ("What's in the box? Tools and methods to discover RNA and protein factors in exosomes") explored strategies for new research tools for RNA-seq next-generation sequencing and protein mass spectrometry in exosome research.

In the fourth session Margaret Petroff ("Placenta-derived exosomes and microvesicles as carriers of fetal antigens") showed that adaptive immune

response to paternally-inherited and placenta-specific antigens is potently modulated by placental-derived vesicles. Similarly, Yoel Sadovsky ("Unique function for trophomiRs in placental exosomes") showed that exosome-mediate transfer of placental derived miRNAs and possibly other factors confer resistance to viral infection during pregnancy. Lane Christenson ("Folliculosomes: exosomes and microvesicles within ovarian follicles") provided evidence that exosomes and microvesicles change within the periovulatory follicle. Antonio de Maio ("Insertion of Hsp70 into membranes is the initial step in the release of the protein within extracellular vesicles") presented evidence that insertion of Hsp70 in the membrane of exosomes is part of the surveillance mechanism of cellular communication after injury. Mikhail Skliar ("Comparison of hydrodynamic and geometric size distribution of exosomes") presented approaches for analyzing the size and shape of extracellular vesicles. Safinur Atay ("GIST exosomes mediated transformation: Role in tumor spread") explained that exosomes from GI stromal tumors (GIST) could be use as a new potential therapeutic strategy to block GIST progression and metastatic spread. The final presentation of the session was given by Emily Zeringer ("Total exosome workflow solution: From simplified isolation to complete analysis") on different protocols for the isolation and characterization of exosomes.

The fifth session started with a presentation by Sai Kiang Lim ("MSC exosome ameliorates tissue injury and enhance repair through proteomic complementation") on the importance of exosome-derived from mesenchymal stem cells (MSC) in cell homeostasis. Here MSC derived exosomes were demonstrated to treat cardiac ischemic injury as or more effectively than the parental MSC. Similarly, Winstom Kao ("Transplantation of Human Umbilical Mesenchymal Stem cell cures the corneal defects of Mucopolysaccharidosus VII mice") results suggested that exosome-derived from MSC could be used as a therapy for corneal defects. Theresa Whiteside ("Tumor-derived exosomes (TEX) induce changes of the mRNA profile in human regulatory T cells") showed that TEX are potentially involved in Treg differentiation. Subsequently, Paul Robbins ("Immune regulation by antigen-presenting cell (APC) and tumor derived exosomes") reported results demonstrating that APC derived exosomes in blood were able are capable to suppress antigen-specific immune responses, including immune responses against tumor antigens. Vilmar R. Martins ("Neurotrophic properties of the prion protein ligand the co-chaperone STI1 that is secreted in extracellular vesicles (EVs) by astrocytes") presented evidence that STI1 is released from astrocytes in exosomes. Finally, Anne Sanstrom ("Tunable Resistive Pulse Sensors (TRPS) for High Resolution Characterization of NanNo to Micro-Scale Vesicle") introduced a new tool for microvesicle characterization.

In the final session, Stefano Fais ("Exosomes: the prototype of biomimetism in Nanomedicine and the future of theranostic") defined exosomes as nanovectors for therapy and diagnostic. Ken Witwer ("Extracellular vesicles of the cervicovaginal compartment during health and HIV infection") results suggested a role for exosomes in HIV infection. Ionita Ghira ("The role of circadian rhythm in microparticle formation") showed that the level of exosomes and microvesicles in circulation varied during the day with a significant increase at the end of the day. Emanuele Cocucci ("Visualization of extracellular vesicles membrane traffic in real time") introduced a new method for monitoring exosomes in real time by labeling exosomes using a CD63-eGFP fusion protein. The last presentation of the meeting was given by Meghan Burke ("Exosomes shed from myeloid-derived suppressor cells carry biologically active proteins"), who characterized exosomes released from myeloid-derived suppressor cells. Consistent with other presentations, her results suggested that exosomes have a role in modulating the tumor microenvironment.

From all the presentations as the 2013 ASEMV, it is clear that the field of extracellular vesicle research is expanding with vesicles implicated in a variety of biological processes in both eucaryotes and procaryotes and serving as carriers of disease specific markers including proteins and RNAs. Thus, this meeting on extracellular vesicles was important since the field is still developing and refining approaches to isolated and characterize vesicles as well as examine their physiological roles. Overall, the 2013 ASEMV meeting fulfilled its goal of providing a forum for researchers from both academics and industry to present and discuss novel ideas related to exosome and microvesicles as well as facilitating collaborative projects. All attendees of the 2013 meeting are now looking forward to new advances in the field that will be presented at the 2014 ASMEV meeting at Asilomar in California, USA.