ASEMV2022/AAEV2022
Asilomar Conference Grounds
Pacific Beach, CA, USA
September 29-October3, 2022

Dear Colleagues,

Welcome to ASEMV2022/AAEV2022, the yearly meeting of the *American Society for Exosomes and Microvesicles*. In the next few days we will hear exciting presentations, gain new insights into extracellular vesicles and extracellular RNAs, meet new colleagues, and greet old friends. Please accept our thanks for your participation and support – this meeting would not be possible without your efforts.

Table of Contents

ASEMV/AAEV2022 was made possible by generous support from:

ASEMV2022/AAEV2022 Meeting Schedule – Merrill Hall

Thursday September 29	**Session I**	7:00-9:05PM
	Openings Reception	9:05-11:00PM
Friday September 30	**Session II**	8:30AM-11:00AM
	Coffee break	9:50AM-10:20AM
	Short Talks	11:00AM-12:00PM
	Lunch	12:00PM-2:00PM
	Session III	2:00PM-4:00PM
	Poster Session A	4:00PM-6:00PM
	Dinner	6:00PM-7:00PM
	Session IV	7:00PM-9:00PM
	Poster Reception A	9:00PM-11:00PM
Saturday October 1	**Session V**	8:30AM-11:00AM
	Coffee break	9:50AM-10:20AM
	Short Talks	11:00AM-12:00PM
	Lunch	12:00PM-2:00PM
	Dinner	6:00PM-7:00PM
	Session VI	7:00PM-9:00PM
	Poster Reception B	9:00PM-11:00PM
Sunday October 2	**Session VII**	8:30AM-11:00AM
	Coffee break	9:50AM-10:20AM
	Short Talks	11:00AM-12:00PM
	Lunch	12:00PM-2:00PM
	Session VIII	2:00PM-4:00PM
	Dinner	6:00PM-7:00PM
	Session IX	7:00PM-8:00PM
	Business meeting	8:00PM-9:00PM
	Bonfire	9:00PM-11:00PM
Monday October 3	Session X	8:30AM-11:00AM
	Short Talks	11:00AM-12:00PM
	Lunch	12:00PM-1:00PM
	Session XI	1:00PM-3:00PM

Crocker Dining Hall

Merrill Hall

Nautilus

LODGING

Afterglow	Rooms 1301-1312	F2
Breakers East	Rooms 821-832	C5
Breakers West	Rooms 833-840	C5
Cypress	Rooms 717-724	H5
Deer Lodge	Rooms 1121-1130	H3
Director's Cottage		C3
Embers	Rooms 1313-1324	F2
Engineer's Cottage		G3
Forest Lodge	Rooms 1202-1211	F1
Guest Inn	Rooms 901-903	F2
Hearth	Rooms 1325-1336	F1
Live Oak	Rooms 1101-1110	G3
Lodge	Rooms 201-218	D4
Long View North	Rooms 101-110	A3
Long View Middle	Rooms 111-120	A3
Long View South	Rooms 121-130	A3
Manzanita	Rooms 1001-1012	B4
Oak Knoll	Rooms 1013-1024	C4
Pirates' Den	Rooms 501-510	G5
Sand	Rooms 605-610	G6
Scripps	Rooms 301-323	D4
Shores	Rooms 709-716	H5
Spindrift North	Rooms 849-856	C5
Spindrift South	Rooms 841-848	C6
Stuck-up Inn	Rooms 401-414	F4
Surf	Rooms 601-604	H6
Tree Tops	Rooms 111-1120	H3
Whitecaps North		C5
Whitecaps South	Rooms 809-820	D5
Willow Inn	Rooms 801-808	B4
Windward	Rooms 1025-1036	H5
Woodside	Rooms 701-708	G1

MEETING ROOMS

Acacia		B4
Chapel Auditorium		D5
Curlew		C4
Dolphin		C5
Evergreen		F1
Fred Farr Forum		E2
Heather		C4
Kiln		E2
Madrone		G3
Manzanita I & II		B4
Marlin		D4
Merrill Hall		G4
Nautilus		H4
Oak Knoll I & II		C4
Oak Shelter		F1
Sanderling		C6
Scripps		D4
Surf & Sand		G5
Toyon		B4
Triton		H4
Willow I & II		B4
Whitehead		G3

OTHER

BBQ Area		E6
Crocker Dining Hall		F6
Fire Pits		E6/H5
Guest Check-in		E5
Hearst Social Hall		E5
Human Resources		F1
Meditation Space		A3
Mott Training Center		G2
Park Ranger Office		G1
Park Store		E5
Phoebe's Café		E5
Seascape		F6
Swimming Pool		A5
Group Sales		D4
Viewpoint		E4
Volleyball Court		E4
Woodlands		H5
Yoga Room		F5
		A3

PARKING LOTS

Parking Lot A		E5
Parking Lot B		G5
Parking Lot C		H4
Parking Lot D		F2
Parking Lot E		E3
Parking Lot F		D4
Parking Lot G		D3
Parking Lot H		B3
Parking Lot J		A4
Parking Lot K		B4
Parking Lot L		B5
Parking Lot M		E2
Parking Lot N		H2
Parking Lot P		I3

Session I	**7:00PM-9:05PM, Thursday September 29 2022**
Chair: Michael Graner; 7:00-7:05PM University of Colorado	
Nihal Altan-Bonnet; **7:05-7:35PM** NIH/NHLBI ***A new infectious unit: extracellular vesicles carrying virus populations***	
Stephen Gould; **7:35-8:05PM** Johns Hopkins University ***Mechanism-informed exosome engineering***	
Louise Laurent; **8:05-8:305PM** UCSD ***Development of an exRNA-based pregnancy clock***	
Raghu Kalluri; **8:35-9:05PM** MD Anderson Cancer Center ***Basic and applied biology and function of extracellular vesicles***	

Opening Reception **Sponsored by Codiak**	**9:05PM-11:00PM, Thursday September 29 2022**

Session II	**8:30AM-11:00PM, Friday September 30 2022**
Chair: Alissa Weaver *Vanderbilt University*	
Wei Guo; **8:30-8:50AM** U. Pennsylvania, Philadelphia, PA, USA ***Extracellular Vesicles in Immune Suppression and Tumor Progression***	
Kendall Jensen; **8:50-9:10AM** TGen; Phoenix, AZ, USA ***Brain-enriched extracellular RNAs in plasma, urine and CSF***	
Saumya Das; **9:10-9:30AM** Harvard/MGH; Boston, MA, USA ***Investigating a functional role for EVs in modulating disease signaling pathways in organ-on-chip models***	
Susanne Gabrielsson; **9:30-9:50AM** Karolinska Institute, Stockholm, Sweden ***Dendritic cell derived extracellular vesicles for cancer immunotherapy***	
COFFEE BREAK – 9:50-10:20AM **Sponsored by Beckman**	
Yongjie Yang; **10:20-10:40 AM** Tufts University; Boston, MA, USA ***Cell-type Specific Exosome Signaling in Neurodegenerative Diseases***	
Kianna Buttiens; **10:40-11:00AM** KU Leuven, Leuven, Belgium ***Ultrasensitive in vivo BLI tool for non-invasive detection of tumor cells receiving genetic information via EVs.***	
Short talks (6); 5 min presentation, 5 min discussion **1**. *A universal platform for rapid production of exosome-based vaccines and therapeutics*; **Chenxu Guo**, Johns Hopkins University, Baltimore, USA **2**. *Development and preclinical testing of a novel extracellular vesicle-based vaccine platform*; **Xin Luo**, MD Anderson Cancer Center, Houston, USA **3**. *The immunomodulatory role of Urinary Bladder Cancer (UBC) EVs and their use as novel and early biomarkers*; **Loïc Steiner**, Karolinska Institute, Stockholm, Sweden **4**. *Proteomic Profiling of neuronal and microglial derived extracellular vesicles reveals differential profiles of Alzheimer's disease*; **Charisse Winston**, UCSD, San Diego, USA **5**. *Oncolytic exosomes for cancer immunotherapy*; **Seong Kim**, KIST, Seoul, Korea **6**. *Systematic Humanization of Yeast Extracellular Vesicles*; **Joseph Trani**, Concordia U., Montreal, Canada	**11:00AM-12:00PM**

Session III	**2:00PM-4:00PM, Friday September 30 2022**
Chair: Frederik Verweij *Utrecht University*	
Alissa Weaver; **2:00-2:20PM** Vanderbilt University, Nashville, TN, USA ***Biogenesis of RNA-containing extracellular vesicles at ER membrane contact sites***	
Michelle Pleet; **2:20-2:40PM** NIH/NINDS, Bethesda, MD, USA ***Analysis of Viral Immune Signatures in Chronic Neurological Diseases from Extracellular Vesicles in Cerebrospinal Fluid***	
Chulhee Choi; **2:40-3:00PM** Ilias Biologics, Daejeon, Korea ***Exosome-based delivery of protein therapeutics: from the placenta to the brain***	
Olivier de Jong; **3:00-3:20PM** Utrecht University, Utrecht, The Netherlands ***Extracellular-vesicle mediated delivery of CRISPR/Cas9 by aptamer-based loading and inducible cargo release strategies***	
Ashok Shetty; 3:20-3:40PM Texas A&M University, College Station, USA Intran***asal hMSC-EV Treatment after TBI Inhibits NLRP3-p38/MAPK Signaling and Prevents Chronic Brain Dysfunction***	
John Nolan; **3:40-4:00PM** Scintillon Institute, San Diego, CA, USA ***Quantitative analysis of molecular cargo transfer from tumor cells to EVs***	

Poster session A	**4:00PM-6:00PM, Friday September 30 2022**

Session IV	**7:00PM-9:00PM, Friday September 30 2022**
Chair: Nihal Altan-Bonnet NIH/NHLBI	
Michael Graner; **7:00-7:20PM** University of Colorado, Denver, USA ***Extracellular Vesicles from Rare Cancers -- Can They Tell Us Anything?***	
Serena Lucotti; **7:20-7:40PM** Weill Cornell Medical College, New York, USA ***Extracellular vesicles from the lung pro-thrombotic niche drive cancer-associated thromboembolism via integrin beta 2***	
Dolores Di Vizio; **7:40-8:000PM** Cedars Sinai Medical Center, Los Angeles, CA, USA ***Functional heterogeneity of cancer-derived extracellular vesicles***	
Huiping Liu; **8:00-8:20PM** Northwestern University, Chicago, USA ***Cancer stemness regulation and anti-viral functions of exosomes***	
Jack Bui; **8:20-8:40PM** UCSD, Sand Diego, USA ***MEK1 within extracellular vesicles inhibits tumor growth by promoting anti-tumor immunity***	
Samir El Andaloussi; **8:40-9:00PM** Karolinska Institute ***Advanced engineering of extracellular vesicles for targeted delivery of biotherapeutics***	

Poster session A with evening refreshments **Sponsored by IZON**	**9:00PM-11:00PM, Friday September 30 2022**

Session V	**8:30AM-11:00PM, Saturday October 1 2022**
Chair: Raghu Kalluri MD Anderson Cancer Center	
Dirk Dittmer; **8:30-8:50AM** UNC Chapel Hill, Raleigh, USA ***Imaging individual Extracellular Vesicles and their protein components by superresolution microscopy***	
Michiel Pegtel; **8:50-9:10AM** VU Amsterdam, Amsterdam, The Netherlands ***CD63-nanoluc sensors reveal novel EV biogenesis mediators and drug targets***	
In-San Kim; **9:10-9:30AM** Korean Institute of Science and Technology (KIST), Seoul, Korea ***Molecule transplantation on membrane and intracellular delivery of biomolecules by fusogenic EVs for cancer immunotherapy***	
Ke Cheng; **9:30-9:50AM** NC State University, Raleigh, USA ***Engineering extracellular vesicles to combat lung diseases and COVID-19***	
BREAK 9:50-10:20AM **Sponsored by Chip Diagnostics**	
Gagan Deep; **10:20-10:40AM** Wake Forest University, Raleigh, USA ***A novel liquid biopsy-based approach to isolate and characterize adipose tissue-derived extracellular vesicles from blood***	
Qiana Matthews; **10:40-11:00AM** Alabama State University, Birmingham, USA ***Impact of Coronavirus Infection on Biogenesis and Trafficking of the Cell Derived-Extracellular Vesicles***	
Short talks (6); 5 min presentation, 5 min discussion **1**. *Single-molecule assay for the characterization of extracellular vesicles from patient biofluid*; **Andras Saftics**, COH, LA, USA **2**. *Circulating ACE2-expressing extracellular vesicles block broad strains of SARS-CoV-2*; **Lamia El-Shannawy**, Northwestern University, Chicago, USA **3**. *Understanding the Role of J-Domain Protein Chaperones in EVs*; **Janice Braun**, U. Calgary, Calgary, Canada **4**. *Advantage of extracellular vesicles in hindering the CD47 signal for cancer immunotherapy*; **Yeji Lee**, KIST, Seoul, Korea **5**. *Exosome biogenesis in the absence of CD9, CD63, & CD81*; **Yiwei Ai**, Johns Hopkins University, Baltimore, USA **6**. *Morphological diversity of extracellular vesicles revealed by cryo-electron microscopy*; **Kshipra Kapoor**, MD Anderson Cancer Center, Houston, USA	**11:00AM-12:00PM**

Session VI	**7:00PM-9:00PM, Saturday October 1 2022**
Chair: Louise Laurent UCSD	
Emily Wang; **7:00-7:20PM** UCSD, San Diego, USA ***Cancer-cell-secreted extracellular vesicles impair systemic glucose homeostasis by suppressing insulin secretion***	
Natacha Carnel; **7:20-7:40PM** MGH/Harvard, Boston, USA ***Identification of tissue specific Extracellular Vesicles in a transgenic mice model***	
Rubina Baglio; **7:40-8:00PM** VU, Amsterdam, The Netherlands ***Blood cell-derived extracellular vesicles induce a pro-inflammatory phenotype in tubular epithelial cells of proliferative Lupus Nephritis patients***	
Kathleen McAndrews; **8:00-8:20PM** MD Anderson Cancer Center, Houston, USA ***Early transfer of cancer cell derived CD9+ extracellular vesicles in pancreatic canceres***	
Yukiya Sako; **8:20-8:40PM** UCSD ***Identification of a Novel Small Molecule that Enhances the Release of Extracellular Vesicles with Immunostimulatory Potency via Intracellular Calcium Induction***	
Sven Kreutel; **8:40-9:00PM** Particle Metrix ***Current advances in nanoparticle tracking analysis***	

Poster session B with evening refreshments **Sponsored by Particle Metrix**	**9:00PM-11:00PM, Saturday October 1 2022**

Session VII	**8:30AM-11:00PM, Sunday October 2 2022**
Chair: Susanne Gabrielsson Karolinska Institute	
Lane Christenson; **8:30-8:50AM** University of Kansas ***Mapping tissue EV release and uptake using multiple Cre promoters in mice***	
Yong Woo Cho; **9:10AM-9:30AM** Hangyang University ***Taking exosome therapeutics from bench to bedside***	
Jared Lynch; **9:30-9:50AM** IZON ***Paving the Way to Automation and Standardization for Scalable Isolation of EVs***	
BREAK 9:50-10:20 **Sponsored by Brexogen**	
Sushrut Kamerkar; 10:20-10:40AM Codiak, Boston, USA ***Exosome-mediated delivery of antisense oligonucleotides reprograms tumor-associated macrophages and induces anti-tumor responses***	
Frederik Verweij; **10:40-11:00AM** Utrecht University, Utreecht, Netherlands ***Exploiting CD63-Based Reporter Systems to Image EV Release in Vitro and in Vivo***	
Trainee short talks (6) 5 min presentation, 5 min discussion **1**. *Identification of global EV reference mRNA transcripts for EV-associated mRNA expression analysis*; **Antje Zickler**, Karolinska Institute, Stockholm, Sweden **2.** *Development of single exosome membrane protein quantification assay using TIRF microscopy*; **Jiyoung Goo**, KIST, Seoul, Korea **3**. *B cell targeting of extracellular vesicles by a novel fusion protein*; **Loes Teeuwen**, Karolinska Institute, Stockholm, Sweden **4**. *Contribution of Cigarette Smoke to the Pathogenicty of Neutrophil Derived Extracellular Vesicles*; **Yixel Soto-Vasquez**, University of Alabama, Birmingham, USA	**11:00AM-12:00PM**

Session VIII	**2:00PM-4:00PM, Sunday October 2 2022**
Chair: Michiel Pegtel VU Medical Center	
Prashanth Vallabhajosyula; **2:00-2:20PM** Yale University, New Haven, USA ***Circulating Tissue Specific Extracellular Microvesicles for Noninvasive Monitoring of Transplant Organ Rejection***	
Muller Fabbri; **2:00-2:40PM** Children's National Research Institute; Washington DC, USA ***MicroRNAs in Extracellular Vesicles orchestrate the biology of the Tumor Microenvironment***	
Bong Hwang Sung; **2:40-3:00PM** Vanderbilt University, Nashville, USA ***Spatiotemporal imaging tools for studying the role of exosomes in migrating cancer cells***	

Ge Jin; **3:00-3:20PM** Case Western Reserve University ***HIV-associated extracellular vesicles: from cancer to co-infection in people with HIV***
Janos Zempleni; **3:20-3:40PM** University of Nebraska-Lincoln, USA ***Bovine mammary alveolar MAC-T cells afford a tool for designing milk exosomes for drug delivery***
Charisse Winston; **3:40-4:00PM** University of Montreal, Montreal, Canada ***Towards consensus harmonization of brain-secreted extracellular vesicle (BEV) protocols for blood biomarker work in age-related dementias: An international overview***

Session IX	**7:00PM-8:00PM, Sunday October 2 2022**
Chair: Samir El Andaloussi Karolinska Institute	
Norman Haughey; **7:00-7:20PM** Johns Hopkins University ***Clearance of amyloid-beta into peripheral circulation evokes an innate immune response and leukocyte transmigration into brain that is regulated by circulating EVs***	
Robert Raffai; **7:20-7:40PM** UCSF/VA ***Macrophage Exosomes in Cardiometabolic Diseases***	
Dong-Gyu Jo; **7:40-8:00PM** Sungkyunkwan University, Seoul, Korea ***Cellular reprogramming with extracellular vesicles derived from differentiating stem cells***	

AAEV/ASEMV business meeting	**8:10PM-9:00PM, Sunday October 2 2022**

Bonfire & Refreshments	**9:00PM-11:00PM, Sunday October 2 2022**

Session X	**8:30AM-11:00PM, Monday October 3 2022**
Chair: Emily Wang UCSD	
Shinichi Kano; **8:30-8:50AM** University of Alabama, Birmingham, USA ***Blood EVs and associated molecules in brain function and behavior***	
Bing Sun; **8:50-9:10AM** UCSF/VA, San Francisco, USA ***Biomarker Discovery for Cognitive Decline via Neuronal Extracellular Vesicles using Multiplexed Assays***	
Takahisa Nakamura; **9:10-9:30AM** Cincinnatti Children's Hospital, Cincinnati, USA ***Role of hepatocyte-derived extracellular vesicles in the regulation of immunometabolism***	
Shi-He Liu; **9:30-9:50AM** University of Toledo, Toledo, USA ***Smart exosome-enhanced tumor targeting drug delivery for efficient PDAC therapies***	
COFFEE BREAK 9:50-10:20AM **Sponsored by**	
Jeffrey Savas; **10:20-10:40PM** Northwestern University, Chicago, USA ***Exosomes mediate local neuronal communication through Notch activation***	
Xiaohua Huang; **10:40-11:00AM** University of Memphis, Memphis, USA ***Dual Imaging Single Vesicle Technology for Exosome Characterizations and Cancer Detection***	
Short talks (6); 5 min presentation, 5 min discussion **1**. Immunocapturing Cardiac Enriched Extracellular Vesicles (CEEVs); **Michail Spanos**, MGH/Harvard, Boston, USA **2**. *Machine Learning Identifies Exosome Protein Signatures to Distinguish Multiple Human Cancer*s; **Bingrui Li**, MD Anderson Cancer Center, Houston, USA **3**. *Cardiac Inflammation & Heart Failure Control with IL-4 Polarized Human Macrophage Exosomes*; **Alex Gao**, UCSF/VA, San Francisco, USA **4**. *Construction of tumor-targeted fusogenic extracellular vesicles for cytosolic delivery of drugs*; **Yuan Wan**, Binghamton University, Binghamton, USA **5**. *Delineation of CD9 engineering on Exosomes for PDAC-Specific Targeting*; **Jonathan Sevier**, U. of Toledo, USA **6**. *Novel smoke-derived EV mediated in vivo model of emphysema*; **Sari Ezgi**, University of Alabama, Birmingham, USA	**11:00AM-12:00PM**

Session XI	**1:00PM-3:00PM, Monday October 3 2022**
Chair: Stephen Gould Johns Hopkins University	
Kristopher Genschmer; **1:00-1:20PM** University of Alabama, Birmingham **Neutrophil derived extracellular vesicles in a mouse emphysema model**	
Ionita Ghiran; **1:20-1:40PM**	

Harvard/Beth Israel Deaconess Medical Center ***Diurnal changes in circulating small RNAs***
Ramkumar Menon; **1:40-2:00PM** University of Texas, Galveston ***Mechanistic Roles and Therapeutic Applications for Extracellular Vesicles in Preterm Parturition***
Je-Hyun Yoon; **2:00-2:20PM** Medical University of South Carolina ***Mature microRNA-binding proteins and extracellular release***
Masako Harada; **2:20-2:40PM** Michigan State University, East Lansing, USA ***Exploring Conditions for Developing Engineered Extracellular Vesicles as Targeted Delivery Vehicles***
Maneesh Bhomia; **2:40-3:00PM** Uniformed Services University of the Health Sciences, Bethesda, USA ***Exosomal biomarkers of traumatic brain injury and sports concussion***

ASEMV2022/AAEV ABSTRACTS

Note: The abstract number corresponds to the poster number.

001

Clearance of amyloid-beta into peripheral circulation evokes an innate immune response and leukocyte transmigration intobrain that is regulated by circulating EVs.

Zhigang Li1, Lauren DeVine2, Robert N. Cole2, Barbara S. Slusher3,
Kenneth W. Witwer5, Norman J. Haughey1,*

1Department sof Neurology, 2DBiological Chemistry, Mass Spectrometry and Proteomics Facility, Hopkins Drug Discovery, 4Molecular and Comparative Pathobiology, the Johns Hopkins University School of Medicine, Baltimore, MD, USA. 21205

Abstract:
It is now well established that neuroinflammation is associated with Alzheimer's disease (AD) and that inflammatory processes contribute to AD pathogenesis. Recent evidence indicates that extracellular vesicles (EVs) play important roles in regulating inflammation. However, the extent to which EVs may mediate cross-talk of inflammatory processes in the central nervous system and the periphery remains largely unknown. In this study we used the APP/PS1 transgenic mouse model to examine EV-mediated communication between the brain and periphery. We found that plasma EV numbers were increased APP/PS1 mice. However, this increase was not explained by enhanced release of neuron- or astrocyte-origin EVs into peripheral blood. Instead, this increase in circulating EVs appeared to be stimulated by A peptides, and the vast majority of this A was not associated with EVs. The adoptive transfer of EVs from APP/PS1 mice triggered a peripheral acute phase cytokine response in donor mice, and primed neutrophils that infiltrated into brain. EVs themselves could also enter the brain parenchyma without any apparent damage to the blood-brain barrier, where they prompted microglial intracellular ROS formation and increased the expression of CXCL1 that presumably acted as a chemoattractant for activated neutrophils. The catalytic unit of the Na+/K+-ATPase ATP1A1 that was enriched and exposed on the surface of EVs isolated from the plasma of APP/PS1 mice, was required for EVs to increase brain ROS and CXCL1 expression. This study identifies a mechanism whereby peripheral EVs respond to a pathological form of a A cleared from brain by increasing the circulating content of EVs that serve to communicate a peripheral immune response back to the brain.

002

Exosomal miRNA as biomarkers of traumatic brain injury and sports concussion..

Manish Bhomia 1, Yanru Feng 1, Linda Papa 2 and Barbara Knollmann-Ritshcel 2
1. Uniformed Services University of the Health Sciences, Bethesda, MD
2. Orlando Regional Medical Center, Orlando, Florida

1. Uniformed Services University of the Health Sciences
2. Orlando Regional Medical Center, Orlando, Florida.

Abstract:

Background: Traumatic brain injury (TBI) is one of the leading causes of morbidity worldwide. In United States alone, nearly 2.4 million cases of TBI are reported every year out of which approximately 52,000 patients bear fatal injuries according to the CDC. Currently, FDA has approved a diagnostic test for TBI diagnosis however this test is not yet tested in clinical setting due to some limitations. Therefore, there the pursuit of novel biomarkers in needed. MicroRNAs (miRNAs) are a family of small non-coding RNA molecules that act as post-transcriptional regulators of gene expression. Emerging evidence emphasize the key role of miRNAs in regulating neuroinflammation as commonly observed in neurodegenerative diseases and CNS injuries. It has been shown that miRNAs are released from cells into the extracellular vesicles called exosomes, which are of approximately 30-200 nm in size. Methods: We have studied the blood and brain samples from pre-clinical brain injury studies and clinical samples of individuals after TBI and sports concussions. These studies identified a panel of miRNA that can detect mild TBI/concussions. From these studies we have identified miR-9-3p as a neuronal marker and miR-195 as an astrocytic injury marker. Further, we investigated the origins of these miRNA biomarkers using various in vitro assays using primary human astrocytes and neuronal cells. Our results show that these miRNA biomarkers are released from astrocytes and neuronal via exosomes which is then possibly released in the peripheral circulation. Conclusion: Exosomal miRNAs released from astrocytes and neurons can be potentially used to detect mild TBI as well as other neurodegenerative disorders.

Disclaimer: The opinions expressed herein are those of authors and are not necessarily representative of those of the Uniformed Services University of the Health Sciences, Department of Defense or, the United States Army, Navy, or Air Force or DMRDP and NIH.

003

Proteomic Profiling of neuronal and microglial derived extracellular vesicles reveals differential profiles of Alzheimer's disease.

Charisse N. Winston1, Saranya Canchi1, Sonia Podvin2, James J. Moresco 3, Carolina Fernández-Costa3, Jennifer Ngolab1, Eliezer Masliah4, Vivian Hook,2 John R. Yates III3, Robert A. Rissman*1,5

1 University of California San Diego, La Jolla, CA, USA, 2 The Scripps Research Institute, La Jolla, California, USA, 3Skaggs School of Pharmacy and Pharmaceutical Sciences, 4National Institute on Aging/NIH, Bethesda, Maryland, USA, 5VA San Diego Healthcare System, La Jolla, CA, USA,

Abstract:

Introduction: Recent studies suggest neuronal (NEVs) and astrocyte-derived extracellular vesicles (AEVs) may serve as the source of biomarkers for many neurodegenerative diseases, include Alzheimer's Disease (AD). However very few studies have characterized the biomarker potential of microglial derived EVs (MEVs). Here, we employed the use of unbiased mass spectrometry (MS) to characterize the molecular composition of NEVs and MEVs derived from healthy controls, prodromal AD, and pathologically confirmed AD patients.

Method: Plasma exosomes were extracted, precipitated, and enriched against a neuronal (L1CAM) and microglial source (TMEM119) using magnetic immunocapture and fluorescence-activated cell sorting (FACS) sorting. NEVs and MEVs were characterized by size (Nanosight) and exosome marker profiling was done by western blot. Unbiased proteomic profiling was conducted for novel biomarker identification using LC-MS/MS Mass Spectrometry.

Result: Bioinformatic analyses revealed that out of the 100 unique proteins, four NEV cargo proteins (KNG, S100A9, CYS, PRDX1) were unique to the prodromal AD group. Out of 143 total proteins identified by MS, only 1 protein, thrombospondin, was unique to the control group as compared to the AD group. NEV cargo proteins from AD patients are associated with a spectrum of molecular processes including inflammation, metabolic processing, stress response regulation and autophagy. MEV cargo proteins are associated with lipid metabolism (APOE), the complement cascade (Clusterin, C3, C5), and DNA translation and repair (CDK12).

Conclusion: NEV cargo may serve as potential plasma biomarkers for delineating the stages of dementia with PRDX1 and CSY demonstrating the highest degree of protein change, albeit in opposing directions, in progression from MCIS to AD. Further characterization is required to determine the biomarker potential of MEV cargo in AD.

004

EVery miRNome Profiler for Human Serum and Plasma reveals distinct miRNA signature in extracellular vesicles (EVs) from normal and breast cancer serum.

Wei Zheng, Suvarna Sathe, Fangting Wu

System Biosciences (SBI), Palo Alto, CA 94303

Abstract:

Introduction: MicroRNAs (miRNAs) are small noncoding RNAs function as post-transcriptional gene regulators. Dysregulation of miRNAs is usually associated with disease implication. It has been well demonstrated that miRNAs are often transported between cells via extracellular vesicles (EVs). Therefore, EV-associated miRNAs are implicated in cancer progression, as well as neurogenerative, infectious, and autoimmune disorders. Due to these features, identification of miRNA-based biomarkers from circulating EVs can open up new avenues for early diagnosis, prognosis and treatment monitoring.

Objective: To accelerate the discovery of EV-associated miRNA biomarkers, SBI developed a qPCR-based miRNA profiling panel, EVery miRNome Profiler for Human Serum and Plasma, which consists of 182 detection primers for a comprehensive collection of publication-validated miRNAs from serum and plasma EVs.

Methods: Starting with 250 µL of serum sample from normal or breast cancer patient, EV RNA was isolated using SmartSEC and EVery EV RNA Isolation Kit, followed by cDNA synthesized with EVery cDNA Synthesis Kit. Expression of the miRNA in EVs from normal and breast cancer serum samples were analyzed by qPCR using EVery miRNome Profiler for Human Serum and Plasma.

Results: Breast cancer serum showed distinct EV miRNA expression pattern compared to normal serum. Significant upregulation of a group of breast cancer associated miRNA markers were observed, including miR-451a, miR-21-5p, miR-16-5p and miR-103a, whereas the tumor suppressor miR-124-3p was significantly downregulated in breast cancer compared to normal serum. Additional differences in EV miRNA expression between normal and breast cancer serum were identified, opening up potential for new biomarker discovery.

Conclusions: Our study demonstrated that EVery miRNome Profiler and associated EVery family products can serve as powerful tools for new biomarker discovery.

005

A novel liquid biopsy-based approach to isolate and characterize adipose tissue-derived extracellular vesicles from blood.

Shalini Mishra, Ashish Kumar, Susy Kim, Yixin Su, Sangeeta Singh, Mitu Sharma, Heetanshi Jain, Jingyun Lee, Cristina Furdui, Chia-Chi Chuang Key, Martin Wabitsch, Stephen B. Kritchevsky, Tom Register, Gagan Deep

Departments of Cancer Biology, Internal Medicine, Pathology, J Paul Sticht Center for Healthy Aging and Alzheimer's Prevention, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA; Department of Pediatrics and Adolescent Medicine, Ulm University Medical Centre, Ulm, Germany

Abstract:
Obesity is associated with an increased risk for multiple metabolic disorders and diseases. Therefore, numerous studies have focused on understanding the role of adipose tissue in obesity-related comorbidities. However, currently anthropometric and imaging approaches are primarily used to assess adiposity, and there is a dearth of techniques to determine the changes in adipose tissue at the molecular level, especially for the hard-to-access visceral adipose tissue (VAT), despite its critical role in obesity-associated pathologies. Lately, extracellular vesicles (EVs) have emerged as a novel and less-invasive source of biomarkers for various pathologies. Furthermore, the possibility of enriching cell or tissue-specific EVs from the biofluids based on their unique surface markers has led to classifying these vesicles as 'liquid biopsies', offering valuable molecular information of inaccessible tissues. However, currently, we lack specific surface biomarkers for adipose tissue-derived EVs for their isolation or enrichment from biofluids. In the present study, we isolated and characterized small EVs (sEV) from the subcutaneous adipose tissue (SAT) and VAT of lean and diet-induced obese (DIO) mice. Next, we identified several unique proteins present on the surface of these sEV by mass spectrometry (MS), and subsequently validated those 'hits' by immunogold labeling-transmission electron microscopy and flow cytometry. Based on these studies, we have identified a signature of 5 unique proteins (CA3, STEAP4, FABP4, GGT5, and CAMKIIα) presented on the surface of sEV secreted by adipose tissues (sEV-AT), including both VAT and SAT. Next, using this unique signature, we pulled out sEV-AT from the blood of lean and DIO mice using a cocktail of 5 biotin-tagged antibodies and streptavidin-coated beads. Next, we validated the specificity of isolated sEV-AT by measuring the expression of adiponectin, 38 adipokines on an array, and several adipose tissue-related miRNAs (miR27b, miR34a, miR146b, miR145a, miR130a, and miR107). Furthermore, in-depth proteome characterization of sEV-AT, isolated from the blood of lean and DIO mice utilizing these signatures, identified unique molecular pathways associated with obesity. Additionally, analysis of 20 cytokines on an array showed more loading of pro-inflammatory cytokines (e.g., IL-21, TNF-α, IFN-γ, IL-1α, Il-2, etc.) in sEVAT from DIO compared to sEV-AT of lean mice. In the functional assay, sEV-AT from DIO mice showed a stronger pro-inflammatory effect on THP1 monocytes compared to sEV-AT from the blood of lean mice. Altogether, we have identified novel surface markers to isolate sEV-AT from blood, which could provide valuable molecular information for identifying novel biomarkers and therapeutic targets for obesity-associated disease conditions.

006

Immunocapturing Cardiac Enriched Extracellular Vesicles (CEEVs) from human plasma and saliva.

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Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA, Beckman Research Institute, City of Hope, Duarte, CA, Division of Allergy and Inflammation Beth Israel Deaconess Medical Center, Boston, MA, Translational Genomics Research Institute, Phoenix, AZ, USA

Abstract:

Introduction

Cardiac cells release Extracellular Vesicles (EVs) that could be potentially detected in biofluids to serve as novel biomarkers. EVs carry specific membrane antigens of the cell of origin that may act as antigenic anchors for capture, detection, and characterization of cardiac enriched EVs (CEEVs) in human plasma and saliva.

Objective

We sought to discover heart-specific antigens on EV membrane of CEEV populations and to further develop and implement immunoaffinity-based methods to capture and characterize CEEVs in plasma and saliva.

Methods

We used Liquid Chromatography- Mass Spectrometry (LC-MS) methods to define the membrane proteome of cardiomyocyte-derived EVs and bioinformatic analysis to retrieve the cardiac-enriched EV markers. We performed immunoaffinity-based pull-down method to capture CEEVs from human plasma and saliva using biotinylated antibodies against cardiac-specific EV surface markers. The contents of CEEVs were characterized by immunoblotting, RNA sequencing, and microscopy.

Results

EV membrane proteins CHRNE, POPDC2, and HIR2.3 had a plasma membrane subcellular localization score > 3 and showed enrichment in cardiomyocytes. CHRNE was selected for CEEVs immunocapturing. CEEVs isolated from plasma were enriched in CHRNE, troponin T, and EV-specific protein markers CD81, ALIX, and TSG101. RNAseq from CHRNE pulldown showed enrichment of cardiac transcripts. CEEVs could also be isolated from saliva and showed protein enrichment for EV markers CD81, TSG101, and CHRNE.

Conclusion

CEEVs isolated from plasma have potential as a novel biomarker for cardiovascular disease and are enriched in cardiac troponin and other cardiac-enriched proteins and RNAs. Interestingly, CEEVs are also readily detectable in the saliva, although correlations with plasma EVs and changes with disease are not yet clear. The ease of salivary measurements may allow for a novel avenue for rapid diagnostics in the cardiovascular field.

Disclosure of conflict of interest
The authors declare no conflict of interest

007

Blood from portal vein tributaries of colorectal cancer excised specimens has different protein profiles with peripheral veins.

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Geriatric Cancer Center, Huadong Hospital Affiliated to Fudan University, Shanghai 200040, China

Abstract:

Objective: The surgical excised specimens have invaluable information for clinical use. But nowadays, almost pathology examination is carried out after formalin fixation. The vein blood, especially portal vein blood of colorectal surgery specimens, whether could be collected after surgery and have diagnostic as well as prognostic information or not should be explored.

Methods: Standard radical surgeries were performed in 4 resectable colon cancer patients. Soon after the specimens were removed intraoperatively, the blood from the tributary of portal vein (specimen derived portal vein blood, spBlood). spBlood were collected in EDTA-containing tube for anti-coagulation. The specimens were 8ml of patient peripheral venous blood before surgery were collected as control. The plasmas were collected after 10000xg centification to remove the cells. The exosomes in the plasma were then extracted with an exosome extraction kit.

ELISA kit was used to detect the expression of tumor markers in spBlood and peripheral venous blood. LC-MS/MS analysis were used to analyze extracted exosomes lysates. Bioinformatics was used to examine the relationship between colorectal cancer and exosome proteins. SPSS 27.0 and GraphPad Prism 9 statistical software were used for data analyzation.

Results: 8-16ml spBlood could be drew from specimen veins. The ELISA kit showed that the expression of CEA in spBlood was significantly higher than that in peripheral venous blood ($p<0.001$). Three exosomal proteins, RPL12, PRDX1, and H2AC20, which were analyzed by LC-MS/MS, were highly expressed in spBlood. After bioinformatics analysis, these three proteins were all correlated with tumor metastasis.

Conclusion: Our team proposed for the first time that blood samples can be collected from ex vivo colon cancer specimens after surgery. Subsequent studies confirmed that spBlood may reflect tumor metastasis and prognosis more accurately than peripheral venous blood. Future analysis of the spBlood may provide additional information for colon cancer specimen pathology, which could provide diagnostic and prognostic value.

008

Functional heterogeneity of cancer-derived extracellular vesicles.

Dolores Di Vizio

Cedars-Sinai Medical Center

Abstract:
Cancer cells release a wide variety of EV populations that contain different cargo and all together make up for the cargo of the originating cell. Large oncosomes represent a type of EV released by highly metastatic cancer cells. We are focused on investigating their role in cancer trying to dissect specific pathways that might be elicited by specific classes of EVs. In this unpublished study, we demonstrate that large oncosomes activate an innate immune response that results in altering the phenotype of neutrophils to an immune-suppressive phenotype. We are investigating the mechanism underlying this phenomenon and the functional consequences in tumor metastasis and resistance to therapy.

009

Exosomes in Immune Suppression.

Wei Guo

University of Pennsylvania
Department of Biology

Abstract:
Tumor cells evade immune surveillance by up-regulating surface expression of PD-L1, which interacts with PD-1 on T cells to elicit the immune checkpoint response. Antibodies that block the PD-1/PD-L1 interaction have shown remarkable promise in treating tumors, including metastatic melanoma. However, a better understanding of PD-L1-mediated immune evasion is needed to improve treatment efficacy. Metastatic melanoma releases a high level of extracellular vesicles, mostly in the form of exosomes, that carry PD-L1 on their surface. Exosomal PD-L1 interacts with PD1 on the surface of CD8 T cells and suppresses the function of CD8 T cells and facilitates tumor growth. The loading of PD-L1 to the exosomes is controlled by the ESCRT machinery. Oncogenic phosphorylation of HRS, a pivotal component of the ESCRT, up-regulates PD-L1 loading to exosomes and promotes their immunosuppressive capacity. Immunohistochemistry study of melanoma tissues demonstrated a negative correlation between HRS phosphorylation and CD8 T cell infiltration to the tumor sites. Our studies suggest a mechanism by which tumor cells systemically suppress the immune system and provides a rationale for targeting exosomes as a strategy in immune checkpoint blockade-based therapies.

010

iExosomes targeting oncogenic Kras suppresses pancreatic cancer by inducing CD8+ T cell mediated Fas-dependent apoptosis.

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2. Feinberg School of Medicine & Kellogg School of Management, Northwestern University, IL
3. Department of Epigenetics and Molecular, MD Anderson, TX
4. Department of Immunology, MD Anderson, TX
5. James P. Allison Institute, MD Anderson, TX

Abstract:

Introduction: Immune checkpoint blockade therapy (iCBT) has revolutionized the treatment of cancers such as melanoma, bladder cancer and lymphomas (1,2). However, pancreatic ductal adenocarcinoma (PDAC) patients remain refractory to iCBT, with no survival benefit observed in clinical trials to date (2, 3). Oncogenic KrasG12D (Kras*) is critical for the initiation and maintenance of pancreatic ductal adenocarcinoma (PDAC), and a known repressor of tumor immunity. However, the precise role of Kras* in shaping the immune microenvironment and understanding the nodes of regulation governing immune infiltration in PDAC is critical to the development of effective therapeutic strategies.

Objectives: To determine the impact of Kras* on the PDAC immune microenvironment using exosomes engineered to carry siRNA (iExosomes) that targets Kras* (4).

Methods: We used orthotopic tumors and genetically engineered mouse models that spontaneously develop PDAC to assess the tumor infiltrating lymphocytes following Kras* inhibition in iExosomes treated and control mice. Immunostains, real time quantitative PCR and chromatin immunoprecipitation were used to demonstrate Kras* mediated suppression of T cell response through epigenetic regulation of Fas.

Results: Kras* inhibition in PDAC models using siRNA containing engineered exosomes led to the reactivation of Fas and CD8+ T cell mediated apoptosis, and suppressed tumor progression. Kras* inhibition recruits CD4+ and CD8+ T cells and suppresses CD11b+ myeloid cell infiltration. Mechanistically, Kras* mediated immune evasion involves epigenetic repression of the death receptor Fas in cancer cells. Furthermore, CD8+ T cell depletion reverses the tumor inhibition response in Kras* suppressed PDAC mice, highlighting the role of CD8+ T cells.

Conclusion: iExosome mediated targeting of Kras* results in the CD8+ T cell mediated, Fas dependent apoptosis of cancer cells and suppression of PDAC.
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011

Nanoplasmonic Sensing Technologies for Extracellular Vesicle Analysis.

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Abstract:
Exosomes, or more broadly extracellular vesicles (EVs) have emerged as promising cancer biomarkers and present new opportunities for cancer diagnoses and treatment monitoring through liquid biopsies. These cell-derived membrane-bound vesicles are abundantly present and easily accessible in biological fluids (e.g. more than billions of vesicles per mL of blood). More importantly, they carry cell-specific biomaterials (proteins, lipids, genetic materials), which reflect their originating cells and thus can be used as a minimally invasive means to probe the molecular status of tumors. Despite the clinical potential, routine, reliable, and sensitive EV analyses are still challenging due to their small sizes under micron and the low sensitivity of conventional analytical methods such as western blotting and enzyme-linked immunosorbent assay (ELISA) for EV proteins. To address these unmet needs, we developed nanoplasmonic sensing technologies to sensitively detect EVs directly from clinical samples. Termed "nPLEX" (nano-plasmonic exosomes), it harnesses surface plasmon resonances excited by periodic gold nanohole arrays tuned to have matched sensing range to the size of EVs, maximizing the sensitivity while simplifying the assay procedures. We demonstrated that nPLEX could sensitively detect and profile EVs directly from clinical samples. Using the nPLEX sensors, we showed that i) tumor-derived EVs contain protein markers reflective of primary tumor cells; ii) nPLEX sensors can rapidly and sensitively detect tumor EVs directly from clinical samples; iii) EV analyses offer the great clinical potential for cancer diagnostics and longitudinal monitoring of tumor response to therapy. With further clinical validations with large cohorts in prospective trials, the technology could become powerful clinical tools to enable early diagnosis and improve treatment outcomes.

012

Early transfer of cancer cell derived CD9+ extracellular vesicles in pancreatic cancer.

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James P. Allison Institute, University of Texas MD Anderson Cancer Center
Department of Bioengineering, Rice University
Department of Molecular and Cellular Biology, Baylor College of Medicine

Abstract:

Introduction

Intercellular signaling between cancer cells and stromal cells within the tumor microenvironment (TME) and distant metastatic sites is known to be critical for regulating the progression of cancer. A number of recent studies have indicated a role for ex vivo isolated cancer cell extracellular vesicles (EVs) in cancer progression and metastasis; however, the precise role of endogenous EV transfer in tumorigenesis is unknown.

Objective

We developed novel mouse models to fluorescently track the endogenous exchange of CD9+ EVs in the development and progression of pancreatic cancer.

Methods

A loxP-STOP-loxP followed by mCherry-CD9 (LSL-mCherry-CD9) allele was generated and crossed to Pdx1-Cre; LSL-KrasG12D (KC), and Pdx1-Cre; LSL-KrasG12D; LSL-Trp53R172H (KPC) mice expressing a cytoplasmic YFP, which was used to identify pancreatic epithelial or cancer cells. Transfer of mCherry CD9+ EVs was evaluated by confocal microscopy.

Results

Exchange of epithelial cell derived CD9+ EVs (EC EVs) with cells in the microenvironment, including fibroblasts and immune cells, of KPC pancreata occurs prior to formation of precancerous lesions and increases throughout cancer progression. Analysis of the metastatic organs lung and liver revealed an accumulation of EC EVs with cancer progression and metastasis in KPC mice. EC EVs were also detected in the lung and liver of KC mice in which metastasis does not occur. Transfer of EC EVs was detected in organs lacking metastasis; however, such transfer was less common in non-metastatic organs compared to metastatic organs.

Conclusions

These data suggest that EC EVs are endogenously transferred prior to tumor formation, suggesting that EV transfer may play a role in intercellular communication in the context of normal physiology. EC EV accumulation in both non-metastatic and metastatic organs indicates that EVs may act to promote metastasis and exert other yet to be uncovered functions.

Disclosures

MD Anderson Cancer Center and R.K. hold patents in the area of exosome biology that are licensed to Codiak Biosciences, Inc. MD Anderson Cancer Center and R.K. are stock equity holders in Codiak Biosciences, Inc. R.K. is a consultant and scientific adviser for Codiak Biosciences, Inc.

013

Delineation of CD9 engineering on Exosomes for PDAC-Specific Targeting.

Jonathan Sevier, Christian Harris, Jing-Ting Zhang, Yakov Lapitsky, Charles Brunicardi and Shi-He Liu

1. University of Toledo College of Medicine & Life Sciences;
2. SUNY Downstate Health Sciences University

Abstract:
Introduction: CD9 is an exosome biomarker that has been demonstrated to successfully display RGD peptide on exosomes through E174 engineering. We hypothesized that CD9 can be engineered to accommodate multiple peptides simultaneously through multiple engineering sites on its large extracellular loop (LEL), thereby displaying multiple pancreatic ductal adenocarcinoma (PDAC) targeted peptides on exosomes.
Methods: exosomes were obtained from culture medium of HEK 293 cells that were transfected with CD9 expressing vectors. Engineered exosomes were evaluated by western blotting, ELISA, flow cytometry, and dynamic light scattering (DLS), pulldown assays and cellular uptake assays. CellTiter-Glo was used to evaluate cell viability.
Results: we screened the engineering sites on CD9 LEL by incorporation of a HA tag into R140, C152, G157, F162, C167, K170, E174 and V178 of CD9. We found over-expression of CD9-C167HA, CD9-K170HA, E174HA and V178HA in both cellular protein and exosome protein levels by western blot analysis of HA expression. The binding capability of Exo-CD9K170HA, E174HA, V178HA and CD9-C167HA was further confirmed by ELISA and HA pulldown assays. To test the feasibility of displaying multiple peptides on exosomes through one CD9 protein, we engineered CD9 through incorporation of His, Her2P, a peptide binding to Her2 and, CD47p110-130, a minimal self-peptide mediation of inhibition of macrophage phagocytosis, into K170, E174 and V178 of CD9, respectively. We demonstrated an exosome displaying His, Her2P, and CD47p110-130(ExoHHSP) enhanced cellular uptake by pancreatic cancer cells in an Her2 expression dependent manner in vitro. ExoHHSP highly bound to SIRP in ELISA assay and significantly reduced phagocytosis by THP1 cells in phagocytic assay. Furthermore, ExoHHSPencapsulated gemcitabine/paclitaxel (ExoHHSP -GEM/PTX) caused significant cytotoxicity to Capan 2 as compared to ExoCtrl -GEM/PTX.
Conclusions: These data demonstrate a successful display of multiple peptides on exosomes through multiple insertion sites engineering at CD9 LEL. This suggests the potential of displaying multiple PDAC-specific targeted peptides on exosomes for multifunctional applications.

014

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015

Dual Imaging Single Vesicle Technology for Exosome Characterizations and Cancer Detection.

Xiaohua Huang

The University of Memphis

Abstract:

Single exosome profiling of surface proteins would provide unprecedented insight into biological events and invaluable information for biomarker discovery. It can probe tumor-derived exosomes in the presence of abundant non-tumor exosomes, providing sensitive, precise, and quantitative information superior to bulk methods. However, single exosome protein profiling is challenging due to the small size and low abundance of antigens on individual exosomes as well as the difficulties in isolation of pure exosomes for downstream analysis. Here, we present a facile single vesicle technology for surface protein detection and profiling of individual exosomes based on dual imagine in conjunction with unique properties of plasmonic nanoparticles. Using breast cancer as a disease model, we demonstrate that this single vesicle technology, but not the classic bulk enzyme-linked immunosorbent assay, detected breast cancer at early-stage, raising exciting possibilities of single exosome surface protein profiling for early cancer detection.

016

The immunomodulatory role of Urinary Bladder Cancer (UBC) EVs and their use as novel and early biomarkers.

Loïc Steiner, Rosanne Veerman, Maria Eldh, Anouk van den Brink, Amir Sherif and Susanne Gabrielsson

Division of Immunology and Allergy, Department of Medicine Solna, Karolinska Institutet
Department of Surgical and Perioperative Sciences, Urology and Andrology, Umeå University

Abstract:

Introduction

Urinary Bladder Cancer (UBC) is the 4th most common cancer type in men and associated with a poor survival rate. At time of diagnosis, 1/3 of detected cancers are already muscle-invasive with the risk of invading other organs. Due to their abundance in urine, Extracellular Vesicles (EVs) make ideal biomarker candidates for early diagnosis of UBC and informative tools to predict the development of the disease.

Objective

To investigate if it is possible to identify a specific EV protein signature of bladder cancer and if it correlates with relevant clinical features. As differentially expressed proteins were involved in immunological processes, we also wanted to investigate the role of UBC-EVs in immune modulation.

Methods

Urine was collected from 40 bladder cancer patients at different stages and 5 age-matched healthy controls. EVs were enriched from urine by centrifugation followed by tangential flow filtration and a final ultracentrifugation step. The vesicles were lysed and their protein content was analyzed by proximity elongation assay.

Results

The proteomic analysis highlighted important changes in the protein composition of EVs. Firstly, urinary EVs from UBC patients greatly differed from healthy controls establishing an EV protein signature of urinary bladder cancer. We also compared matched samples of urinary EVs and whole urine (not EV-enriched) from UBC patients and discovered a set of proteins exclusively found on purified EVs. Additionally, the protein profile was correlated with clinical data such as invasiveness or response to chemotherapy and sets of proteins were identified as predictors of such features.

Additionally, we investigated the effect of EVs on PBMCs and isolated monocytes and identified an immune-suppressive effect of muscle-invasive-derived EVs compared to non-invasive.

Conclusions

Taken together, these results suggest a potential of using EVs as biomarkers and prognostic tools for UBC. Moreover, their immuno-modulatory effect highlights a potential mechanism of immune evasion in aggressive cancers.

Conflict of interest: Susanne Gabrielsson has a patent on B cell derived exosomes in immune therapy and is part of the Scientific Advisory Board of Anjarium Biosciences.

017

SMART EXOSOME-ENHANCED TUMOR TARGETING DRUG DELIVERY FOR EFFICIENT PDAC THERAPIES.

Shi-He Liu, Justin Creeden, Jing-Ting Zhang and F Charles Brunicardi

University of Toledo College of Medicine & Life Sciences;
Center S U N Y Downstate Health Sciences University

Abstract:

Not available in English

018

MEK1 within extracellular vesicles inhibits tumor growth by promoting anti-tumor immunity.

Stephen C. Searles, Jarrod D. Yee, Preston Lee, Calvin K. Lee, Christine Caron, Irina Matei, David Lyden, and Jack D. Bui

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2. Weill Cornell Medical College, New York, NY, USA

Abstract:
Extracellular vesicles (ECVs) mediate intercellular communication in many physiologic processes and can modulate immune responses in individuals with cancer. Using a model of highly immunogenic regressor versus poorly immunogenic progressor tumor cells, we determined that secreted factors produced by regressors could activate anti-tumor immunity. Focusing on ECVs, we found that the signaling molecule MAP2K1 (MEK1) is enriched in ECVs secreted by regressor relative to progressor cells. Progressor ECVs engineered to have levels of MEK1 similar to regressor ECVs could inhibit tumor growth by indirectly promoting adaptive immunity in both syngeneic and 3rd party tumors. This effect required MEK1 protein within the ECVs and could occur by activating macrophages to promote adaptive immune responses against the tumor via the cytokine interferon-gamma. These findings suggest that, in some instances, MEK inhibition may be deleterious to cancer treatment, since MEK1 plays an important cell-extrinsic, tumor-suppressive role within ECVs. Moreover, the delivery of MEK1 to tumor-associated macrophages, either by ECVs, nanoparticles, or some other means, could be a useful strategy to treat cancer via the activation of anti-tumor immunity.

019

AKT isoforms have discrete expression in triple negative breast cancers and roles in cisplatin sensitivity due to tutor microenvironment..

Bhumika Wadhwa, Masroor Paddar, Sameer Khan, Anna M. Grabowska, Devanahalli G. Vijay and Fayaz Malik

Department of Biochemistry, The Maharaja Sayajirao University, Vadodara-390002, India

Abstract:

Breast cancer metastasis is a serious cause of morbidity and mortality in breast cancer patients. Metastatic cells shows immune evasion, drug resistance and relapse and frequently differ from the preceding primary breast cancer in properties such as receptor status.

Tumor microenvironment of solid tumors including breast cancer includes tumor cells, cancer stem cells and their cross talk determine the progression of breast cancers. TME represents a key factor for tumor heterogeneity maintenance, tumor progression, and drug resistance. Due to its heterogeneity and adaptability, the TME has been associated with the maintenance of the malignant behavior of cancer cells throughout different tumoral stages. Additionally, TME plays an important role in protecting cancer cells from drug therapy via crosstalk between cancer cells and surrounding cells. Recent studies have shown that exosomes play a vital role in cancer metastasis contributing in the formation of pre-metastatic niche, influencing tumor cells and microenvironment and determining specific organotrophic metastasis. Exosomes in TME have been shown to participate in multiple stages of cancer progression, including metastasis initiation.

Similarly, AKT, a serine threonine kinase, exists in three different isoforms and is known for regulating several biological processes including tumorigenesis. In this study, we investigated the expression and net effect of the individual isoforms in triple negative breast cancers and response to cisplatin treatment using cellular, mice models and clinical samples. Interestingly, analysis of the expressions of AKT isoforms in clinical samples showed relatively higher expression of AKT1 in primary tissues; whereas lung and liver metastatic samples showed elevated expression of AKT2. Similarly, triple-negative breast cancer cell lines, BT-549 and MDA-MB-231, with high proliferative and invasive properties, displayed higher expression levels of AKT1/2. By modulating AKT isoform expression in MCF-10A and BT-549 cell lines, we found that presence of AKT2 was associated with invasiveness, stemness and sensitivity to drug treatment. It was observed that the silencing of AKT2 suppressed the cancer stem cell populations (CD44high CD24low, ALDH1), invasive and migratory potential in MCF-10A and BT-549 cells. It was further demonstrated that loss of function of AKT1 isoform is associated with reduced sensitivity towards cisplatin treatment in triple-negative breast cancers cellular and syngeneic mice models. The decrease in cisplatin treatment response in shAKT1 cells was allied with the upregulation in the expression of transporter protein ABCG2, whereas silencing of ABCG2 restored cisplatin sensitivity in these cells. In conclusion, our study demonstrated the varied expression of AKT isoforms in triple-negative breast cancers and also confirmed differential role of isoforms in stemness, invasiveness and response towards the cisplatin treatment due to TME.

020

Ultrasensitive in vivo BLI tool for non-invasive detection of tumor cells receiving genetic information via EVs..

Kiana Buttiens, Bella B. Manshian, Stefaan J. Soenen

Kiana Buttiens (1), Bella B. Manshian (2), Stefaan J. Soenen (1),
1 NanoHealth and Optical Imaging Group, Department of Imaging and Pathology, KULeuven, Belgium
2 Translational Cell and Tissue Research Unit, Department of Imaging and Pathology, KULeuven, Belgium

Abstract:
Introduction: Recent studies from our end have shown that uncontrolled NM-mediated inflammation can have significant effects on tumor malignancy. Also, different NMs can have distinct effects on tumor cell migration and metastasis formation, based on the chemical nature of NMs used and on the specific tumor type. For a further understanding on how clinically studied NMs may affect the tumor microenvironment, many questions currently remain unanswered, mainly related to the delivery of these NMs to the tumor site and their precise intratumoral localization.
Objective: In this study we aimed to look into differences in cellular behavior elicited by different types of biomedically relevant NMs and the extent thereof on tumor-associated signaling, more specifically, on intercellular communication via extracellular vesicles (EVs).
Methods: First, we have studied the effect of different biomedically relevant NMs (gold, silver, silica and iron oxide) on EV generation in different cancer cell types in vitro, using image-based flow cytometry. Furthermore, we have developed a novel non-invasive preclinical in vivo imaging method based on a custom-designed lentiviral vector system, using ultrasensitive bioluminescence imaging that enables us to detect low numbers of tumor cells receiving genetic information (e.g. via EVs).
Results: From our in vitro studies, we found significant upregulation of EV generation upon Au NP administration in MDA-MB-231 cells. Furthermore, we're presenting the validation of our newly developed tool in a human breast cancer (MDA-MB-231) subcutaneous tumor model in female NSG mice, where we confirm the extracellular-vesicle mediated transfer of genetic information in MDA-MB-231 tumor reporter cells.
Conclusion: This newly developed imaging method confirms the uptake of EV-mediated genetic information in tumor cells in a non-invasive way.

021

Quantitative analysis of molecular cargo transfer from tumor cells to EVs.

John P Nolan, Erika Duggan

Scintillon Institute, San Diego, CA 92121

Abstract:

Introduction. EVs carry molecular cargo from their cell of origin, and are attractive as potential liquid biopsy targets, but the mechanisms of cargo selection and loading into EVs are not well understood. We used quantitative single cell and vesicle flow cytometry to measure membrane protein expression on cells and EVs with the aim of better understanding how tumor cell cargo is released via EVs.

Methods. PC3 prostate cancer cells were cultured, the media collected, and EVs concentrated using ultrafiltration (UF-100K MWCO). Cell surface markers were measured by flow cytometry (FC). EV concentration, size, and cargo were measured by single vesicle flow cytometry (vFC). Instruments were calibrated and intensity reported in units of antibodies per cell or EV.

Results. PC3 cells express surface markers at high (>250K median copies/cell: CD71, CD29, CD44, CD54), medium (50K-250K copies: CD9, CD63, CD49f) and low (<50K copies: CD81, EPCAM, EGFR, STEAP-1) abundances. EVs expressed detectable (>~10 PE MESF) CD9, CD63, CD81 and CD29, with a fraction (~50%) also staining with AnnV. Expression was proportional to EV surface area, with surface densities ranging from a background of ~10 molecules/um2 to >1000 molecules/um2 for high abundance targets. Several high abundance markers (CD71, CD44, CD54) were not detectable on EVs, suggesting differential packaging of cell surface cargo into released EVs. CD63 was expressed at low abundance overall, but a subset of smaller EVs (<100 nm) expressed CD63 at high surface density (~1000 um2).

Conclusions. We find that the abundance and surface density of cargo on vesicles can be higher or lower than on the cell of origin. Some abundant cell surface molecules (CD71, ICAM, CD44) were undetectable on EVs, while others (CD9, CD81, CD29) were present at surface density similar to cells. CD63 was present at high density on smaller EVs, consistent with enrichment of CD63 on small exosomes formed inside the cell.

022

Biogenesis of RNA-containing extracellular vesicles at ER-membrane contact sites.

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Abstract:

Extracellular RNA is a novel mechanism for cell-to-cell communication and drives many physiological and pathological processes including cancer. Extracellular vesicles (EVs) are a major vehicle for transmitting these RNAs between cells. However, the underlying mechanisms by which RNA-containing EVs are generated are poorly understood. Due to the association of many RNA processing granules with the endoplasmic reticulum, we investigated the role of endoplasmic reticulum membrane contact sites (ER MCS) as key subcellular locations for the biogenesis of RNA-containing EVs. We found that inhibition or overexpression of key molecules that control ER MCS (VAP-A-KD, VAP-A OE) and ceramide transport at ER MCS (CERT-KD) greatly affected the small RNA content of both small and large EVs. Density gradient sub-fractionation of EV pellets revealed that VAP-A regulates a select subpopulation of small EVs that are enriched with RNA. Confocal microscopy data revealed that key RNA and RNA binding proteins are altered in multivesicular endosomes in VAP-A KD cells. Experiments testing EV function indicated that this VAP-A-controlled small EV population is critical for both in vitro transfer of miR-100 to recipient cells and for in vivo growth of xenograft mouse tumors. Altogether, these and additional data suggest a model in which ceramide transfer at ER MCS drives biogenesis of a select subpopulation of EVs containing RNA-RBP complexes. We are now actively investigating regulatory mechanisms that control this key sorting event, as well as the functional consequences.

023

Assessment of the Biogenesis and Inhibition of Lung Cell-Derived Extracellular Vesicles in Human Adenovirus type-3 Infection.

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Abstract:

Respiratory human adenovirus (HAdV) has been closely linked with acute respiratory infections such as pharyngitis and coryza (common cold). While some HAdV serotypes antagonize the immune system leading to meningitis, gastroenteritis, and acute hemorrhagic cystitis among others, HAdV type 3 (HAdV3) predominantly infects the upper respiratory tract. Studies have shown that the release of small, membrane-derived extracellular vesicles (EVs), termed exosomes, may offer a mechanism by which certain viruses, enter cells via receptor-independent entry. EVs play a major role in cellular homeostasis and intercellular communication under both healthy and pathological conditions. Recently, we demonstrated that EVs isolated from HAdV3 infected A549 cells showed a significant increase in particle mean sizes, concentrations, and total EV content relative to uninfected with similar topographical structures. Infected cell-derived EV cargo protein detection also revealed upregulated levels of EV-associated classical markers, stress, and apoptotic responses mostly at the highest multiplicities of infection. Subsequent selective inhibition of exosomes using pharmacological inhibitory agents (ketoconazole, climbazole, and heparin) altered particle sizes, and significantly reduced total exosome released. Climbazole and heparin undermined membrane-bound tetraspanin CD63 expression and significantly disrupted the ESCRT pathway by inhibiting ALIX protein ($p \leq 0.0001$) and TSG101 ($p \leq 0.001$). We also observed disruption of transmembrane trafficking associated-Ras binding protein signaling ($p \leq 0.001$). Our findings suggest that HAdV3 modulates EV biogenesis and trafficking which could impact the progression of viral infection and specific responses in vitro and that pharmacological inhibition of exosome regulates the endocytic pathway and expression of ESCRT mediators.

024

Exploiting CD63-Based Reporter Systems to Image EV Release in Vitro and in Vivo.

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Abstract:

Exosomes are endosome-derived Extracellular Vesicles (EV) involved in intercellular communication. They are generated as intraluminal vesicles within endosomal compartments that fuse with the plasma membrane (PM). The molecular events that generate secretory endosomes and lead to the release of exosomes are not well understood.

We developed a comprehensive toolset to study exosome and other sEV secretion at high patio-temporal resolution, using a combination of pH-sensitive, bioluminescent and optogenetically controlled CD63-based reporters in vitro and in vivo. This allowed us to 1) identify the compartment of origin of CD63 positive exosomes as a subclass of non-proteolytic endosomes at prelysosomal stage. These compartments undergo a Rab7a/Arl8b/Rab27a GTPase cascade to fuse with the plasma membrane (PM). Dynamic ER-LE Membrane Contact Sites MCS via ORP1L have the distinct capacity to modulate this process by affecting LE motility, maturation state and small GTPase association. These novel insights open up new avenues for optogenetic modulation of this secretory process. 2) CRISPR mediated integration of the NanoLuc reporter to the endogenous locus of CD63 enables high-throughput analysis of EV secretion using a kinase inhibitor library, and revealed novel mediators of EV secretion in a SNAP23 dependent and -independent fashion. 3) Exploiting pH-sensitive CD63-based reporters in vivo using a transparent zebrafish embryo model allows a tissue-specific interrogation of inter-organ communication pathways in vivo.

025

Impact of Coronavirus Infection on Biogenesis and Trafficking of the Cell Derived-Extracellular Vesicles.

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Abstract:

Introduction

Coronavirus (CoV) has persistently become a major global health concern causing respiratory, gastrointestinal, and neurological diseases in a wide variety of hosts including humans, and companion animals. However, the virus-mediated responses on these hosts have not been studied extensively due to pathogenesis complexity and severe disease developments. Extracellular vesicles (EVs), particularly exosomes, derived from virus-infected cells are widely explored in several viral infections for their intercellular communications, nano-carriers, and immunomodulatory properties.

Objective

Our objective is to evaluate the impact of coronavirus on EVs production, biogenesis, composition and trafficking in different animal host. We proposed that coronavirus hijack the host exosomal pathway accomplishing receptor-independent entry and alter the EVs production, biogenesis, composition and trafficking in the host cells.

Methods

In the present study, Crandell-Rees Feline Kidney cells (CRFK) were infected with canine coronavirus (CCoV) in an exosome-free media at 0.0008 multiplicity of infection for different time points. The cell viability was assessed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay and the EVs were purified via series of ultracentrifugation method. The purified EVs were characterized via various molecular techniques such as transmission electron microscopy (TEM), NanoSight tracking analysis (NTA), SDS-PAGE, Western blot, Dot blot and so on.

Results

The CRFK cell viability was significantly decreased over time and TEM showed the presence of exosomes in purified EVs after CCoV infection. The NTA showed substantial increase in EVs concentration over time, however, the mean size of the CRFK-derived infected EVs were significantly decreased at 48h but relatively increased at 72h when compared to the uninfected control group. Expression of several proteins such as coronavirus host receptor (ACE-2), Syncytin-1, Annexin-V, Flotillin-1, TLR-7, Hsp-100, LAMP, TNF-alpha, Caspase-8, and so on was altered in EVs after virus infection.

Conclusion

Our study suggests that coronavirus modulate exosome production, biogenesis, composition and trafficking in the host. Further investigation with different CoVs and host cells will provide a detailed understanding of infection pathogenesis and disease progression. This study could also be extended to study interspecies cross and adaptation of animal CoVs in humans, which could be further explored in other viral infections and EV-based therapeutics.

026

Synthetic Substrates for Interrogating EV Biogenesis.

Lucia Morales (UG), Allison Tsai (UG), Steven Santana

Harvey Mudd College

Abstract:
Extracellular vesicles (EVs) play a crucial role in mediating cell communication in a range of contexts including development, maintaining homeostasis, and in pathological states. Furthermore, it is well-documented that cellular production and dissemination of EVs is responsive to microenvironmental cues. To better understand EV biogenesis in response to outside-in signals, we investigate the role played by cell-matrix interactions on vesicle production. We utilize synthetic poly(ethylene glycol) hydrogels—formed via a photoinitiated thiol-ene click-chemistry polymerization reaction—to present EV-producing cells with intentionally-engineered substrate-associated signals to elucidate the signals that material-bound signals that initiate EV production.

027

Imaging Extracellular vesicles and their protein components in 3D.

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Abstract:
EVs, such as exosomes, are between 30-200 nm in diameter. Their diameter is below the diffraction limit of light microscopy. We developed direct stochastic optical reconstruction microscopy (dSTORM) to visualize Tetraspanin (CD81, CD63, CD9) complexes on the surface of an individual EV in 3D under physiological conditions. The dSTORM observations, together with Cryo-EM, support the existence of (i) membrane domains akin to the fluid mosaic model and (ii) tetraspanin-enriched microdomains (TEM) containing multiple tetraspanin complexes on EVs.

028

Paving the Way to Automation and Standardization for Scalable Isolation of EVs.

Jared Lynch, Ph.D

Izon Science Ltd.

Abstract:
As the potential for EV-based diagnostics and therapeutics continues to grow, so too does the need for scalable isolation and precise characterization. In this presentation, I will present how technologies and services offered by Izon Science fill this gap. I will introduce Izon's newly released Gen 2 qEV SEC isolation product line as well as our recent work on scaling up EV isolation and purification using larger sized qEV columns with automated flow systems. Finally, you will be taken through an overview of Izon platforms and offerings, to gain a clear understanding of how Izon technologies can fit into a larger workflow.

029

Morphological diversity of extracellular vesicles revealed by cryo-electron microscopy.

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Abstract:
Introduction: Exosomes are extracellular vesicles 80-150 nm in diameter, containing proteins, mRNAs, microRNAs, and lipids reflecting the parent cell. While there has been an extensive characterization of the cargo incorporated in exosomes, a detailed morphological analysis of exosomes purified by various isolation techniques has not been performed.
Objective: We aimed to determine the heterogeneity of exosomes morphology and if such morphological features are conserved across sample types.
Methods: Our study used Cryogenic Electron Microscopy (Cryo-EM) to examine exosome size and morphology.
Results: Our results revealed significant diversity in extracellular vesicle morphology independent of the isolation method, suggesting that morphological subpopulations of these vesicles exist. Based on their shape, our analysis classified exosomes into seven categories. In addition, we developed a semi-automatic image analysis framework to accurately characterize exosome attributes and distribution to facilitate reliable quantification of specific bio-nanoparticle features in Cryo-EM micrographs.
Conclusions: Morphological features of exosomes inform their biophysical properties, which influence both biodistribution and biological activity in vivo. Our data demonstrating the innate morphological diversity of exosomes may have implications for improving the specificity and precision of exosome-delivered therapeutics.
Conflict of interest: R.K. and MD Anderson Cancer Center hold patents in exosome biology and are stock equity holders in Codiak Biosciences Inc. R.K. is a consultant and a scientific advisor of Codiak Biosciences Inc.

030

Impact of Storage Temperature Conditions on the Cargo and Function of EVs from hiPSC-derived Neural Stem Cells.

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Abstract:

Introduction: Extracellular vesicles (EVs) from human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) have potent antiinflammatory properties. They are also proficient in permeating the entire brain and incorporating into different neural cell types after an intranasal (IN) administration. Moreover, proteomics and small RNA sequencing studies have revealed multiple therapeutic proteins and miRNAs in their cargo, making them an attractive off-the-shelf product for treating neurodegenerative diseases. However, whether long-term storage of EVs in different temperature conditions would affect the stability of EV cargo and function remains to be addressed.

Objective: To determine the better storage temperature conditions for the short- and long-term storage of EVs derived from hiPSC-NSCs and to quantify changes in EV cargo and function after short- and long-term storage.

Methods: Fresh hiPSC-NSC-EVs were isolated through anion-exchange followed by size-exclusion chromatography (SEC). A portion of fresh EVs was immediately quantified for total RNA and protein contents. Another portion of EVs from the same prep underwent similar measurements following their storage in SEC buffer at 4o C or -20o C for 7 or 30days. Moreover, the amounts of most enriched miRNAs (miR-320a, 103a-3p, 21-5p, 26a-5p, 320b, 30a-3p, 181a-5p, 191-5p) and proteins (pentraxin 3, agrin, galectin-3-binding protein, hemopexin, and nidogen-1) typically found in hiPSC-NSC-EVs were compared between fresh EVs and stored EVs. Furthermore, the antiinflammatory activity of stored EVs was assessed using lipopolysaccharide-challenged mouse macrophages in vitro. Finally, the ability of stored EVs to permeate the brain and incorporate into neural cells following an IN administration was determined in adult mice.

Results: The total RNA and total protein contents of the NSC-EVs were stable with 7 or 30 days of storage at 4o C or -20o C. However, the number of EVs was significantly reduced after 30 days of storage at 4o C. The miRNAs measured were also stable at -20o C for 7 or 30 days, but there was a significant decline in miRNA content within EVs stored at 4o C. Similarly, the individual proteins were stable in EVs stored at -20o C but not at 4o C. The EVs stored for 30 days at -20o C retained their antiinflammatory property, evident from the reduced IL-6 secretion by LPS-stimulated mouse macrophages in the presence of such EVs. Moreover, these EVs also retained their ability to permeate the brain and incorporate into neural cells in the mouse brain following IN administration.

Conclusion: Storage of NSC-EVs at -20o C for up to 30 days does not alter their cargo, antiinflammatory activity, or ability to incorporate into neural cells in the brain following IN administration. However, storage of hiPSC-NSC-EVs at 4o C for 30 days is associated with reductions in their number and the amount of highly enriched miRNAs and proteins.

Funding: R01NS106907 and RF1AG074256 to A.K.S

031

Effect of blood collection tubes and specimen processing on extracellular vesicle (EV) surface protein detection.

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Abstract:

Introduction: Proteomic analysis of circulating extracellular vesicles (EVs) is being explored for diagnostic and prognostic applications. EV surface proteins may be affected by blood collection methods, sample processing, and platelet depletion. The purpose of this study was to compare the effects of blood collection tube type, platelet depletion methods, and freeze-thaw on EV detection.

Methods: We collected blood using four tube types: serum separation clot activator tube (SSCAT, Greiner), plasma EDTA tube (BD), Streck RNA Complete BCT, and Streck DNA BCT. SSCAT samples were centrifuged (1300g, 10min) and serum collected. Plasma EDTA tubes were centrifuged (250g, 15min); supernatants were retained as platelet-rich plasma (PRP) or centrifuged (5000g, 20min) to produce platelet-depleted plasma. Streck RNA Complete BCT and DNA BCT samples were processed per manufacturer protocols. Aliquots were retained for immediate analysis and matched samples were frozen for freeze-thaw analysis. Samples of frozen/thawed PRP were platelet-depleted to assess the effect of platelet-depletion after freezing. We characterized samples by resistive pulse sensing (RPS) and nanoparticle tracking analysis (NTA). Samples were analyzed with MACSPlex Exosome Kits (Miltenyi).

Results: EV concentrations were similar across sample types and freeze-thaw conditions. In PRP samples, fewer EV-binding beads were recovered, suggesting platelet:bead aggregation. In Streck DNA samples and in PRP that were platelet-depleted after thawing, platelet-associated protein detection increased, consistent with residual platelet fragments. EV protein detection was consistent before and after freezing for samples collected in SSCAT and Streck RNA BCT.

Conclusion: PRP is not effective for use with MACSPlex EV surface marker analysis. Platelet fragments in routinely frozen PRP subjected to platelet depletion after thawing and in Streck DNA BCT samples may also interfere with bead-based EV assays. SSCAT and Streck RNA Complete BCT both appear to have minimal residual platelet fragments and maintained EV protein integrity.

Disclosure of conflict of interest:
We declare no conflicts of interest.

032

Identification of tissue specific Extracellular Vesicles in a transgenic mice model.

Natacha Carnel, Sara Abu-Elreich, Andras Saftics, Michail Spanos, Emeli Chatterjee, John Tigges, Ionita Ghiran, Kendall Jensen, Robert Raffai, Tijana Jovanovic-Talisman , Stephen Gould, Saumya Das

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Abstract:

Introduction: Extracellular vesicles (EVs), collected from accessible biofluids, hold diagnostic promise. Healthy and diseased cells continuously shed EVs of different sizes and contents from diverse biogenesis pathways. In this study we propose to apply high resolution tools on a novel transgenic mice model (exoMap) to probe specific EV populations arising specifically from immune cells and characterize EV heterogeneity at the single EV level. This mouse model provides a platform to probe the content and markers of tissue-specific EV subpopulations to understand the complex heterogeneity of EVs with the ultimate goal of measuring disease-specific markers in a highly specific manner.

Method: Tissue-specific cre-expression in the ExoMap mouse model (tD-tomato-HsCD81-mNeonGreen) leads to tdTomato ORF excision and expression of humanized CD81/membrane mNeonGreen on the cell membranes as well as exosomes derived from them. Using Size Exclusion Column (SEC) and validated anti-human CD81 Abs for Western Blot and Flow Cytometry, we isolated EVs from mice plasma to evaluate the expression of HsCD81 and mneon green in double transgenic, Exomap and Wild Type mice (used as control mice). We used immunoprecipitation method to evaluate the expression of HsCD81 in those mice. Confocal microscopy and quantitative single molecule localization microscopy (qSMLM) were used to show the presence of mNeonGreen EVs and HsCD81+ EVs.

Results: In the double transgenic vav-cre/ExoMap mice hsCD81 and mNeonGreen expression is noted only in the hematopoietic system (consistent with vav-cre expression), particularly in the spleen and thymus, as shown by western blotting and bone marrow cells as shown by FACS analysis. Notably, expression of hsCD81 and mNeonGreen is specific and not seen in other tissues. Confocal microscopy confirmed the presence of mNeonGreen EVs only in the double transgenic mice, but not in control ExoMap mice. Immuno-pulldown of the Evs using the HsCD81 antibody demonstrated the presence of this marker only in the double TG mice and not in the controls. Importantly, the pull-down EVs from the double TG mice showed expression of CD169 and CD68, two markers we have found to be specific for macrophage sub-populations. Lastly, using the qSMLM we were able to visualize a large number of HsCD81+ EVs in the double transgenic mice plasma, but not in the control mice.

Conclusion: These results validate for the first time a new model to visualize tissue specific EVs and characterize them at their cargo as well as image them at the single EV level. This platform has the potential to provide a novel pathway to discovery of tissue-specific EV markers and cargo.

033

Development of single exosome membrane protein quantification assay using TIRF microscopy.

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3. Division of Life Sciences, Korea University, Seoul, South Korea

Abstract:

Exosomes are a type of extracellular vesicles (EVs) that are made in multi-vesicular bodies (MVBs) and then released by fusion of MVBs with the plasma membrane. Despite originating from the same cell, there are numerous evidence that exosomes are heterogenous. However, shedding a light on such heterogeneity of exosomes has shown to be difficult, due to the fact that current tools are not yet equipped to verify small sized vesicles. In particular, exosomes typically scatter > 105 fold less light, have > 104 fold less electric resistance, and have 103 fold less surface area to expose antigens. Therefore, we developed a single exosome membrane protein quantification assay based on single molecule biophysics. We used a TIRF microscopy to measure the presence of tetraspanin family proteins on exosome membrane surfaces by fluorescent antibody in real time.

034

Identification of global EV reference mRNA transcripts for EV-associated mRNA expression analysis.

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Abstract:
Introduction and Objective:
The identification of EV-associated reference mRNA transcripts is absolute key for accurate analysis and valid normalization of gene expression data from EV preparations of any kind. However, the field currently lacks comprehensive studies reporting robust EV-associated mRNA expression datasets.
Methods:
Here, we isolated EVs from 12 different human cell sources and isolated full RNA by RNA precipitation. We prepared 50 bp single-ended oligo-dT primed cDNA libraries and performed Smart-seq2 RNA sequencing using the Illumina® platform. After mapping the reads to the human transcriptome (GRCh37), we analyzed the acquired dataset to identify highly abundant mRNA transcripts with low variation between samples to potentially serve as universal EV reference genes. Next, we validated our results by RT-qPCR as a more commonly used methodology.
Results:
Within the RNAseq data set, we found 7 transcripts with highly abundant expression and low variation across all samples. For validation, we normalized the expression of two highly variable transcripts to the 7 putative reference transcripts to test for their robustness as reference mRNA, using both RNAseq and RT-qPCR data. Here, we successfully identified 4 protein-coding and one lncRNA-coding transcripts that could be used as global EV reference mRNA transcripts.
Conclusion:
Our study is the first to provide a comprehensive and unbiased RNAseq dataset from EV-associated mRNA from a vast range of human cell sources. These data are crucial for robust normalization and valid reporting of EV-associated mRNA expression data and can be an invaluable leap forward to a deeper understanding of EV-associated mRNA cargo.

035

Single-molecule assay for the characterization of extracellular vesicles from patient biofluid.

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3 Cardiology Division and Corrigan Minehan Heart Center, MGH, Harvard Medical School;
4 Neurogenomics Division, TGen

Abstract:
Introduction. Extracellular vesicles (EVs) are excellent source of biomarkers with potential in diagnostics. These nanoscale particles can provide molecular information about the disease, deliver a rapid readout on changes to health status, and can be sampled noninvasively and frequently. However, due to the challenges in robust isolation and characterization of select EV populations, the clinical implementation of EV-based diagnostic platforms is still not fully established.
Objective. To address some of these challenges, we combine affinity isolation with quantitative single-molecule localization microscopy (qSMLM), a powerful method that can simultaneously assess EV size, molecular content, and heterogeneity. Here, we present a novel qSMLM-EV assay for the characterization of EV subpopulations from human plasma samples.
Methods. We optimized the analytical protocols (chemical functionalization of microscope coverslips, the affinity pulldown of EVs, and fluorescent labeling of EVs). Next, we isolated and imaged tetraspanin (CD9, CD63, and CD81) enriched EVs and analyzed images with new algorithm tools developed in our laboratory.
Results. For size exclusion chromatography (SEC) isolated EVs, the number of detected EVs positively correlated with sample dilution in a 64-fold dilution range. Moreover, we have shown that the assay can be directly applied to crude plasma samples; the number of detected EVs positively correlated with sample dilution in a 50-fold dilution range. We assessed how SEC isolation affected EV size, molecular content of tetraspanins, heterogeneity, and EV yield.
Conclusion. Using our novel qSMLM-EV method, we were able to characterize specific EV subpopulations from patient biofluid. Our ultimate goal is to further develop this assay into a platform for performing personalized and molecular-level diagnoses.
Disclosure of conflict of interest. KVJ is a member of the Scientific Advisory Board for HTG and Dyrnamix, neither of which have played a role in this study. SD is a founding member and holds equity in LQTT and Switch Therapeutics, and has consulted for Renovacor, none of which played any role in this study.

036

Macrophage Exosomes in Cardiometabolic Diseases.

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Abstract:
Extracellular vesicles (EVs) including exosomes are recognized as sources of intercellular communication in cardiovascular disease. Exosomes produced by inflammatory M1-like macrophages have recently been shown to drive cardiovascular inflammation through the delivery of microRNA cargo. In contrast, exosomes produced by alternatively activated M2-like macrophages are increasingly recognized to control inflammation. Recent findings from our laboratory uncovered that exosomes produced by macrophages cultured in the presence of elevated glucose levels are enriched in microRNA cargo including miR-486-5p that downregulates the expression of the lipid efflux pump Abca1 in recipient macrophages, contributing to foam cell formation. Furthermore, such "high-glucose" macrophage exosomes were found to potently drive cellular glycolytic metabolism and proliferative signaling that enhanced hematopoiesis and accelerated atherosclerosis when infused into Apoe-/- mice. In contrast, our studies of exosomes produced by IL-4 exposed, alternatively activated macrophages were found to be enriched with miR-99a/146b/378a. Such IL4-exosomes robustly increased mitochondrial respiration, oxidative phosphorylation and suppressed inflammatory signaling by communicating this cluster of microRNA to recipient cells that reprogrammed bioenergetic metabolism. When infused into hyperlipidemic Apoe-/- mice, IL4-exosomes potently suppressed hematopoiesis, systemic inflammation and favorably remodeled atherosclerotic lesions. Our findings also revealed a capacity for IL4-exosomes to improve mitochondrial function in white adipose tissue that improved insulin sensitivity and glucose tolerance in obese diabetic mice. Taken together, these findings support the need for future studies of macrophage exosomes to gain a better appreciation for their involvement in cardiometabolic disease onset and progression. More importantly, our findings support the use of IL4-exosomes as novel therapeutics to control cardiometabolic diseases.

037

Mature microRNA-binding proteins and extracellular release.

Je-Hyun Yoon

Medical University of South Carolina

Abstract:
Although Argonaute (AGO) proteins have been the focus of microRNA(miRNA) studies, we observed AGO-free mature miRNAs directly interacting with RNA-binding proteins. To investigate microRNA-binding proteins (miRBPs) globally, we used high-density protein arrays to identify RBP quaking (QKI) as a novel miRBP for let-7b. We have shown that QKI regulates miRNA-mediated gene silencing at multiple steps, and controls the release of a subset of miRNAs into the extracellular environment via exosomes. Depletion of QKI decreases interaction of AGO2 with let-7b and target mRNA, consequently controlling target mRNA decay. This finding indicates that QKI is a complementary factor in miRNA-mediated mRNA decay. QKI, however, also suppresses the dissociation of let-7b from AGO2, and slows assembly of AGO2/miRNA/target mRNA complexes at the single-molecule level. Furthermore, QKI inhibits exosomal release of let-7b likely by controlling the availability of let-7b for loading into exosomes, via additional miRBPs such as AU-rich element RNA-binding protein 1 (AUF1) and heterogeneous nuclear ribonucleoprotein K (hnRNPK). The accelerated exosomal release of let-7b after QKI depletion activates the Toll-like Receptor 7 (TLR7), and promotes the production of proinflammatory cytokines in recipient cells leading to brain inflammation in mouse cortex. Our data show that QKI is a new type of RBP implicated in the versatile regulation of miRNA metabolism.

038

Mimicking physiological ways to generate Red Blood Cells-Extracellular Vesicles.

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Abstract:

RBCs are distinctive among all body cells, surviving in circulation for over 100 days while having no organelles, active cytoskeleton, transcription, translation machinery or endo/exo-cytic pathways. Activation of the complement system follows circadian cycles and culminates with the formation and insertion of the terminal complement components C5b-9, (membrane attack complex or MAC) into nearby plasma membranes, leading to an ungated Ca++ influx and generation of EVs. Due to their overall abundance in blood, and enrichment of their membrane glycoproteins in hydroxyl groups, circulating RBCs represent the main target for the C5b-9/MAC pore complex.

The goal of the current project was to investigate if RBCs of different circulating ages, following complement activation, release EVs with unique characteristics and whether the targets of the RBC-EVs are different depending on the age of the parent RBC.

RBCs of new, intermediate and old circulatory ages were separated using a self-forming Percol gradient and then each treated with PIPLC to cleave CD55 and CD59. Complement was activated by successive incubation with C5-6, C7, C8 and C9 fragments. RBCs-EVs were isolated and purified by SEC and characterized for morphology and size by nan-flow cytometry, TRPS, dark field microscopy and for the RNA content using a custom Fireplex kit. The miRNA were quantified in both large and small EVs using fireplex assay in which we identified that hsamiR-126-3p, hsamiR-195-5p, hsamiR-93-5p, hsamiR-92a-3p, hsamiR-26a-5p were higher in large EVs whereas hsamiR-342-3p, hsamiR-328-3p hsamiR-320b, hsamiR-210-3p, hsamiR-28-5p and hsamiR-423-5p were higher in small EVs. The miRNA profile shows the difference between small and large RBCs-EVs; hence can be used as a signature candidate to study RBCs-EVs in circulation. The cellular communication ability of generated EVs was validated by incubating them with human T cells. Our data suggest that complement-mediated generated RBCs-EVs contain miRNAs, and these EVs can be uptake by T cells in vitro.

Keywords: Red blood Cells derived Extracellular Vesicles, Small and large EVs, T cells, miRNA

039

Human placenta-derived exosomes stimulate chondrocyte activities through multiple signaling pathways.

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Abstract:

Introduction: Placenta exosomes are known to play roles in regulating fetus development and immune tolerance between mother and fetus1. We developed a process to isolate exosomes from full-term human placenta (pExo). pExo contains cytokines including PDGF-BB, HGF, IL-8 and FGF2 and shows pro-regenerative activity in vivo2,3,4. Osteoarthritis (OA) is a degenerative disease characterized by impairment of cartilage and loss of chondrocytes. Promoting the growth and activity of chondrocytes can improve the repairment of cartilage5. Here we investigated the activities of pExo on the proliferation and migration of human chondrocytes and analyzed the signaling pathways in chondrocytes activated by pExo.

Methods: pExo was isolated from full-term human placenta by ultracentrifugation. Chondrocyte proliferation was measured and quantified using Water Soluble Tetrazolium-1 (WST-1) solution-based assay (Roche). Chondrocyte migration was evaluated using 8-um transwell by seeding chondrocytes on top chamber and pExo the bottom chamber well for 24 hrs. Signaling pathways were assessed by Multi-Pathway Magnetic Bead 9-Plex-Cell Signaling Assay kit (Millipore). For flow cytometry analysis, chondrocytes were stained with antibodies (BD Biosciences) and analyzed by BD canto II Flow Cytometer (BD).

Results: Flow cytometry analysis showed human chondrocytes expressed receptors of pExo-enriched cytokines, including PDGFR, HGFR, and IL-1R. pExo demonstrated dose-dependent activity in promoting chondrocyte proliferation and migration. Among those cytokines abundantly expressed in pExo, PDGF-BB significantly promoted chondrocyte proliferation and FGF2 stimulated chondrocyte migration. The inhibitors of SRC, AKT, and PLC signaling pathways suppressed pExo-stimulatory effect on chondrocyte proliferation. In addition, signaling pathway analysis revealed pExo significantly activated ERK and CREB pathways, which were known to be activated by growth factors in chondrocytes6.

Conclusion: pExo promotes proliferation and migration of chondrocytes through its growth factors and downstream signaling pathways. This study provides rationale for developing pExo as a therapeutic for OA.

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040

3D spheroid culture alters the secretion and uptake of extracellular vesicles.

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2 Department of Electrical and Computer Engineering, Rice University
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Abstract:

Introduction: Extracellular vesicles (EVs) are lipid bilayer-delimited particles secreted by all cell types that mediate local or systemic cellular communication and behavior. To date, most of the EV related studies have been performed in conventional two-dimensional (2D) cell culture systems, which does not recapitulate in vivo extracellular environments.

Objective and methods: Here, we employed a three-dimensional (3D) culture system to model in vivo organ specific architecture and evaluate EV secretion and entry into recipient cells using mammary epithelial cell-derived cell lines, including MCF-7, MCF-10A and HMLE cells.

Results: We showed that all cell line-derived EVs obtained from 2D and 3D cultures had similar average particle diameter and were enriched in the tetraspanins CD9, CD63 and CD81. A significant increase in EV secretion was observed in all cell lines cultured in 3D compared to 2D. Modulation of cell polarity, a critical feature provided by 3D environments, did not impact EV secretion. Additionally, the entry of EVs into recipient cells cultured in 2D and 3D models revealed that EV uptake was consistently lower in 3D compared to 2D over a broad range of EV dosage and is likely independently of the EV cell source.

Conclusion: Overall, this work shows distinct variation in EV production and uptake dependent on cues from the extracellular environment, which may provide insight into the biology of EVs in vivo.

Disclosure of conflict of interest: MD Anderson Cancer Center and R.K. hold patents in the area of exosome biology that are licensed to Codiak Biosciences, Inc. MD Anderson Cancer Center and R.K. are stock equity holders in Codiak Biosciences, Inc. R.K. is a consultant and scientific adviser for Codiak Biosciences, Inc.

041

P2X7R signaling controls exosome release by donor cells after allotransplantation in mice.

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Abstract:
Purpose: Exosomes released by cells of transplanted organs play an important role in the initiation of the T cell alloimmune response leading to their rejection. In this study, we investigated the role of innate immunity triggered by extracellular ATP-mediated P2X7R signaling in exosome release by macrophages activated in-vitro and by donor cells in allotransplanted mice.
*Methods:
First, mouse macrophages were generated by culturing bone marrow cells with macrophage-colony stimulating factor for 7 days. The purity of bone marrow-derived macrophages (BMDM) was > 98%. Next, BMDM were stimulated with lipopolysaccharide (LPS, 1 ug/mL) with or without a P2X7R inhibitor (A-438079) for 24h and 5mM of adenosine triphosphate (ATP) during the last 30 min of culture. The release of exosomes by activated macrophages was assessed by nanoparticle tracking analysis. Next, BALB/c mice were transplanted with an allogeneic B6-GFP skin graft or a B6-GFP heart graft and treated or not with a P2X7R inhibitor, A-438079 (150 μmol/kg), given intraperitoneally for 7-10 days. The presence of donor GFP+ exosomes was assessed in the blood of recipient mice by NanoFacs.

We detected 160 donor exosomes per ul of plasma.
*Results: LPS + ATP induced a massive release of exosomes by macrophages in vitro. Exosome release by macrophages activated in vitro was abolished by A-438079.

042

Cancer-cell-secreted extracellular vesicles impair systemic glucose homeostasis by suppressing insulin secretion.

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Abstract:
Epidemiological studies demonstrate an association between breast cancer and systemic dysregulation of glucose metabolism. However, how breast cancer influences glucose homeostasis remains unknown. We show that breast cancer cell-derived extracellular vesicles (EVs) suppress pancreatic insulin secretion to impair glucose homeostasis. EV-encapsulated miR-122 targets PKM in β-cells to suppress glycolysis and ATP-dependent insulin exocytosis. Mice receiving high-miR-122 EVs or bearing breast tumors exhibit suppressed insulin secretion, enhanced endogenous glucose production, impaired glucose tolerance, and fasting hyperglycemia. These effects contribute to tumor growth and are abolished by inhibiting EV secretion or miR-122, restoring PKM in β-cells, or insulin supplementation. Compared to non-cancer controls, breast cancer patients have higher levels of circulating EV-encapsulated miR-122 and fasting glucose concentrations but lower fasting insulin; miR-122 levels are positively associated with glucose and negatively associated with insulin. Therefore, EV-mediated impairment of whole-body glycemic control may contribute to tumor progression and incidence of type 2 diabetes in some breast cancer patients.

043

Alleviation of atopic dermatitis by exosomes derived from interferon-gamma-stimulated mesenchymal stem cells.

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Graduate School of International Agricultural Technology, Seoul National University
Institutes of Green-Bio Science and Technology, Seoul National University

Abstract:

Exosomes from mesenchymal stem cells are now being recognized a novel therapeutics for various diseases because of their immune-modulatory and anti-inflammatory potential. Atopic dermatitis (AD) is one of the most incurable skin disease manifested by pruritus, impaired immunity, and tissue destruction. In this study, we tested whether exosomes from interferon-gamma-primed induced MSCs (IFN-gamma-iMSC-exo) can reduce AD progression using a mice model of AD induced by 2,4-dinitrochlorobenzene (DNCB). IFN-gamma-iMSC-exo was enriched with proteins responsible for regulating interferon activity and inflammatory signaling, as shown by proteome profiling followed by detailed bioinformatic study. Together with the diminished expression of major Th2 receptors (IL-4a/13Ra1/31Ra), the their corresponding intercellular signaling molecules also became less active. IFN-gamma-iMSC-exo alleviated clinical symptoms including itching, and blocked infiltration of inflammatory and mast cells in AD skin. In addition, IFN-gamma-iMSC-exo led to a reduction in the expression of of thymic stromal lymphopoietin (TSLP), NF-kB activation, and IgE receptors. Further, disintegrated skin barrier by AD progression was reversed by IFN-gamma-iMSC-exo, which was supported by the upregulation of key genes responsible for epidermal differentiation and lipid synthesis. In conclusion, we demonstrated that IFN-gamma-iMSC-exo has potential to become a novel exosome-based therapeutics for AD via blocking inflammation/Th2 response as well as promoting skin restoration.

044

Spatiotemporal Imaging Tools for Studying The Role of Exosomes in Migrating Cancer Cells.

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2 The Vanderbilt Center for Extracellular Vesicle Research, Vanderbilt University School of Medicine.
3 Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center.

Abstract:

Introduction: Exosomes are a type of small extracellular vesicle (EV) that are actively secreted from cells and originate from multivesicular bodies (MVBs). Recently, the mechanisms by which exosomes mediate cell-cell communication and regulate cellular physiology and behavior have been investigated using live imaging techniques visualizing the spatial and temporal dynamics of exosome secretion and interactions with cells.

Objective: Here, we introduce several spatiotemporal imaging tools for studying the function and secretion of exosomes, and demonstrate their utility in the visualization of secreted exosomes in 3D culture and in vivo using pH-sensitive live cell reporters for MVB trafficking and exosome secretion and uptake.

Methods: A stabilizing mutation, M153R is incorporated in the pHluorin moiety of pHluorin-CD63 and now exhibits stable expression in cells and superior monitoring of exosome secretion. A dual-tag reporter was created by incorporating a further pH-insensitive red fluorescent protein, mScarlet to the C-terminus of pHluo_M153R-CD63. Cancer cells stably expressing the constructs were imaged using a variety of microscopy techniques in vitro as well as in vivo. Purified small EVs labeled with pHuo_M153R-CD63 were imaged using immunogold transmission electron microscopy (TEM) and quantitated for the half-life in the blood circulation using flow cytometry.

Results: Using a pH-sensitive, stable live cell reporter of exosome secretion, pHluorin_M153R-CD63, we identify a key role for exosomes in promoting leader-follower behavior during cancer cell migration in 2D and 3D environments. These findings are complemented by experiments showing that exosome secretion drives directionally persistent migration of cancer cells in a topologically defined electrospun fiber environment. Finally, using a combination of pH-sensitive and non-pH-sensitive fluorescent tags, we visualize multiple steps of exosome secretion and uptake in migrating cancer cells.

Conclusion: Overall, we find that dynamic exosome secretion drives directionally persistent migration and quorum sensing behavior.

Disclosure of conflict of interest
We have no conflict of interest to declare.

045

Neutrophil derived extracellular vesicles in a mouse emphysema model.

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Lung Health Center and Gregory Fleming James CF Center, UAB, Birmingham, AL, USA

Program in Protease and Matrix Biology, UAB, Birmingham, AL, USA

Abstract:

Chronic Obstructive Pulmonary Disease (COPD) is a chronic lung disease characterized by inflammation due to airway neutrophilia. Extracellular vesicles derived from neutrophils that have undergone activation/degranulation have active neutrophil elastase on their surface in a conformation that protects it from its endogenous anti-protease, alpha-1 antitrypsin. These EVs have been found in the lungs of COPD patients, and are able to transfer an emphysema like phenotype in mice when transferred via the intratracheal route. While these human-mouse transfer model is useful for examining the effects of these EVs on extracellular matrix damage in the lung, we sought to develop an in vivo preclinical mouse model. Through LPS-induced inflammation in a mouse lung, we were able to isolate the NE+, alpha-1 antitrypsin resistant EVs and transfer the emphysema-like phenotype to a naïve mouse. This new in vivo mouse-mouse transfer model of neutrophil derived proteolytic EVs will allow us to further elucidate their pathogenic role in inflammatory lung disease

046

Receptor Interactome Discovery Using Recombinant Extracellular Vesicles.

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Genentech Inc.

Abstract:

Membrane proteins and their interactions are major drug targets because of their central roles in regulating cellular communication. Despite this, membrane receptors remain underrepresented in our knowledge base of protein–protein interactions because of the limitations of most available techniques. To overcome these challenges, we developed an extracellular vesicle-based method for membrane protein display that enables purification-free and high-throughput detection of receptor–ligand interactions in membranes. We demonstrate that this platform is broadly applicable to a variety of membrane proteins, enabling enhanced detection of extracellular interactions over a wide range of binding affinities. We were able to recapitulate and expand the interactome for prominent members of the B7 family of immunoregulatory proteins such as PD-L1/CD274 and B7-H3/CD276. Moreover, when applied to the orphan cancer-associated fibroblast protein, LRRC15, we identified a membrane-dependent interaction with the tumor stroma.

047

Circulating ACE2-expressing extracellular vesicles block broad strains of SARS-CoV-2.

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Abstract:

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the pandemic of the coronavirus induced disease 2019 (COVID-19) with evolving variants of concern. It remains urgent to identify novel approaches against broad strains of SARS-CoV-2, which infect host cells via the entry receptor angiotensin-converting enzyme 2 (ACE2). Herein, we report an increase in circulating extracellular vesicles (EVs) that express ACE2 (evACE2) in plasma of COVID-19 patients, which levels are associated with severe pathogenesis. Importantly, evACE2 isolated from human plasma or cells neutralizes SARS-CoV-2 infection by competing with cellular ACE2. Compared to vesicle-free recombinant human ACE2 (rhACE2), evACE2 shows a 135-fold higher potency in blocking the binding of the viral spike protein RBD, and a 60- to 80-fold higher efficacy in preventing infections by both pseudotyped and authentic SARS-CoV-2. Consistently, evACE2 protects the hACE2 transgenic mice from SARS-CoV-2-induced lung injury and mortality. Furthermore, evACE2 inhibits the infection of SARS-CoV-2 variants (α, β, and δ) with equal or higher potency than for the wildtype strain, supporting a broad-spectrum antiviral mechanism of evACE2 for therapeutic development to block the infection of existing and future coronaviruses that use the ACE2 receptor.

048

Intranasally Administered EVs from hiPSC-derived Neural Stem Cells Can Target Different Cell Types in a Mouse Model of Familial Alzheimer's Disease.

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Abstract:

Introduction: Extracellular vesicles shed by human-induced pluripotent stem cell (hiPSC)-derived neural stem cells (NSCs) have robust antiinflammatory and neurogenic properties due to therapeutic miRNAs and proteins in their cargo (Upadhya et al., Journal of Extracell Vesicles, 2020). Hence, hiPSC-NSC-EVs are an excellent biologic for treating neurodegenerative disorders, including Alzheimer's disease (AD). However, it is unknown whether such EVs can be targeted to different neural cell types in the AD brain through following intranasal (IN) administration.

Objective: To investigate whether IN administration of hiPSC-NSC-EVs would lead to their incorporation into neurons, microglia, astrocytes, interneurons, and oligodendrocytes in the entire AD brain.

Methods: Small EVs from passage 11 hiPSC-NSC culture spent media were isolated through anion-exchange and size-exclusion chromatographic methods. Following assessment of EV number using Nanosight and EV marker expression through CD63 ELISA, we labeled EVs with PKH26 and performed IN administration of EVs into three-month-old 5XFAD mice, a model of early-onset AD. Different cohorts of AD animals were euthanized at 45 minutes or 6 hours following IN administration of EVs to determine the ability of the IN-administered EVs to incorporate into neurons, microglia, astrocytes, interneurons, and oligodendrocytes in the AD brain. The incorporation of EVs into different cell types was assessed through immunofluorescence methods and Z-section analysis in a confocal microscope.

Results: At 45 minutes after IN administration, hiPSC-NSC-EVs permeated the entire brain and incorporated into all NeuN+ neurons, IBA-1+ microglia, and PV/NPY-positive interneurons in different regions of the forebrain, midbrain, and hindbrain. There was also sizable incorporation of hiPSC-NSC-EVs into GFAP/S-100b+ astrocytes and CNPase+ oligodendrocytes. At 6 hours after IN administration, EVs were still present in neurons, microglia, interneurons, astrocytes, and oligodendrocytes. Immunofluorescence studies using antibodies against CD63 and CD81 with neuronal/microglial markers revealed that the PKH26+ structures within brain cells were indeed the IN-administered EVs.

Conclusion: Extracellular vesicles (EVs) are a promising drug delivery platform as they compartmentalize bioactive payloads prior to delivery, have low immunogenicity, and are capable of tissue-specific targeting. EV-based thera

049

Blood EVs and associated molecules in brain function and behavior.

Shinichi Kano

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Abstract:
Extracellular vesicles (EVs) are small membrane vesicles secreted by multiple cell types in the body . EVs contain various cellular components, including RNAs, proteins, lipids, and carbohydrates, and transfer them between cells in a paracrine and endocrine manner. As EVs are readily detected in various body fluids, such as the blood and cerebrospinal fluid, extensive efforts have been made to explore them as possible biomarkers for brain disorders. In contrast, it remains unclear whether and how circulating EVs and associated molecules play any roles in brain function and dysfunction. In this talk, I present our recent findings on the role of blood EV-associated molecules, particularly microRNAs (miRNAs), in rodent social interaction behaviors using two established mouse models for sociability deficits: chronic social stress and immunodeficiency. Our data suggest that EV-associated miRNAs affect gene expression in neurons and microglia. I will further discuss the potential underlying mechanisms.

050

Understanding the Role of J-Domain Protein Chaperones in EVs.

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Abstract:
Molecular chaperones and their client proteins enter the extracellular spaces through the classic secretory pathway, extracellular vesicles as well as cell lysis. In the human genome, over 300 genes encode for molecular chaperones and a subset of these chaperones are exported extracellularly. Proteins residing in or passing through extracellular spaces are monitored by molecular chaperones to prevent the formation of protein aggregates. Mutations in extracellular chaperones have been linked to a number of human diseases. In addition, local fluctuations in the expression of subsets of extracellular chaperones, in terms of transient up-regulation, within the various extracellular spaces is widely recognized.

Work by several laboratories has shown that cells rid themselves of α-synuclein, TDP-43, tau, SOD-1, Aβ, and polyglutamine expanded huntingtin through a pathway involving the molecular chaperone, CSPα/DnaJC5. ~70 entries identifying EVs containing DnaJC5/CSPα or are listed in Vesiclepedia. We have found that DnaJC5/CSPα exports disease-causing proteins from cells in 180-240nm and 10-30□m EVs, but it is not known whether export of DnaJC5/CSPα in complex with misfolded substrates is protective or if it is a mechanism of distributing toxic proteins during disease progression. In addition to DnaJC5/CSPα, 24 other related J-domain protein (JDPs) chaperones are reported to be exported in EVs. Here we investigate the roles of JDPs in EVs focusing on EV cargo loading.

051

Analysis of Viral Immune Signatures in Chronic Neurological Diseases from Extracellular Vesicles in Cerebrospinal Fluid.

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Abstract:

Extracellular vesicles (EVs) are released from virtually all cell types, and may package many inflammatory factors and, in the case of infection, viral components. As such, EVs can play not only a direct role in the development and progression of disease, but they can also be used as biomarkers. We analyzed the EVs from the cerebrospinal fluid (CSF) of healthy volunteers (HVs) and patients with a variety of chronic neurologic diseases of both known viral and non-viral etiologies including Multiple Sclerosis (MS), HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), HTLV-1-infected asymptomatic carriers (ACs), and other neurological diseases (ONDs), to investigate the surface repertoires of CSF EVs during disease. Significant increases in CD8+ and CD2+ EVs were found in HAM/TSP patient CSF samples compared to other clinical groups, consistent with the immunopathologically-mediated disease associated with CD8+ cells in the CNS of HAM/TSP patients. Furthermore, CD8+, CD2+, CD44+, and CD40+ EVs were significantly increased in the CSF from patients with viral infections compared to those without. These data suggest that CD8+ and CD2+ CSF EVs may be important as: 1) potential biomarkers for viral-mediated neurological diseases, and 2) as possible meditators of areas of the disease process in infected individuals.

052

Intranasal hMSC-EV Treatment after TBI Inhibits NLRP3-p38/MAPK Signaling and Prevents Chronic Brain Dysfunction.

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Abstract:

Not Available in English

053

Towards consensus harmonization of brain-secreted extracellular vesicle (BEV) protocols for blood biomarker work in age-related dementias: An international overview.

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Abstract:

INTRODUCTION AND AIMS: Brain-secreted extracellular vesicles (BEVs) in blood provide a novel, minimally invasive way to serve as a biomarker discovery platform and treatment tool for Alzheimer's disease (AD) and other age-related dementias. While some consensus on extracellular vesicles (EV) nomenclature and validation tests have recently emerged, largely driven by the "minimal information for studies of extracellular vesicles (MISEV)" initiative, enrichment protocols for BEVs from blood still vary from center to center. Given the growing popularity of BEVs enriched from blood in age-related dementia research, our study aims were to: 1) systematically assess immunoprecipitation (IP)-based BEV enrichment protocols from blood (plasma or serum) ; and 2) assess CNS-specificity and extracellular accessibility of proteins used for BEV enrichment and/or detected in BEV enriched isolates.

METHODS: PubMed searches (50 keyword combinations) were performed to identify original studies investigating age-related dementia(s) in human and employing IP-based protocols to enrich BEVs from blood. Identified studies were used to: (1) compare IP-based BEV enrichment protocols, and (2) compile a list of proteins used for BEV enrichment and/or investigated using ELISA-based assay post enrichment. Proteins in our list were categorized by CNS specificity and abundance, extracellular accessibility, and EV presence, using the following databases, respectively: "RNA consensus tissue gene data" from The Human Protein Atlas (version 21.0 and Ensembl version 103.38); UniProt human database (version 2022_01); Exocarta and Vesiclepedia.

RESULTS: 39 studies were included. Assessment of IP-based BEV enrichment protocols found that 74% of these studies used plasma to perform enrichment. 54% plasma studies reported a defibrination step prior to EV enrichment. Exosomes, or more broadly extracellular vesicles (EVs) have emerged as promising cancer biomarkers and present new opportunities for cancer diagnoses and treatment monitoring through liquid biopsies. These cell-derived membrane-bound vesicles are abundantly present and easily accessible in biological fluids (e.g. more than billions of

054

Exosomes mediate local neuronal communication through Notch activation.

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Abstract:

INTRODUCTION

Neuron-to-neuron communication has historically been thought to be mediated by neurotransmitters and electrical signals. However, exosomes are emerging as facilitators of intercellular communication in the central nervous system and beyond. Despite this fact, some skepticism regarding the importance of neuron derived exomes has persisted since many aspects of their biology remain elusive.

OBJECTIVE

The goal of this research is to investigate how synaptic activity modulates neural cell-derived exosome release, characterize their protein cargo, and determine their functional impact on recipient cells.

METHODS

We examined N-methyl-d-aspartate (NMDA) mediated synaptic strengthening using a glycine-based chemical stimulation paradigm and electrophysiology to confirm enhanced synaptic transmission. Cultured rodent neurons and knock out mice were used as model systems. Exosomes were isolated using size exclusion chromatography and analyzed with negative staining electron microscopy, antibodies, and mass spectrometry. Individual exosome particles were characterized using antibody chips with interferometric and fluorescent microscopy.

RESULTS

Activation of synaptic NMDA receptors led to Alix phosphorylation at Serine 717 (S717) by protein kinase A, which facilitated neuronal exosome release from dendritic spines. Biochemical analysis revealed high levels of Notch1 and Notch2 proteins in activity-induced exosomes. Deletion of Pdcd6ip gene, which codes for the Alix protein, abolished synaptic activity-induced exosome release. Overexpression of wild type Alix or Alix S717D but not Alix S717A restored synaptic activity-induced exosome release in Pdcd6ip-/- neurons. Furthermore, we found that synaptic activity-induced exosomes were selectively internalized by neuronal somadendritic membranes. In the recipient neurons, Notch became activated and translocated into the nucleus. In the hippocampal CA1 and CA3 regions of Pdcd6ip-/- neonates, nuclear-localized Notch1 was significantly reduced compared to wild

type littermates. Furthermore, there was nearly no evidence of activated Notch2 in Pdcd6ip-/- neonates at P14.

CONCLUSION
Our results indicate that synaptic activity-induced exosomes facilitate dendrite-dendrite and dendrite-soma communication and regulate Notch activation in local neuronal circuits.

DISCLOSURE of COI
The authors declare that they have no conflicts of interest.

055

Cell-type Specific Exosome Signaling in Neurodegenerative Diseases.

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Abstract:
Altered glia to neuron signaling significantly impact pathological development in neurodegenerative diseases. Intercellular exosome signaling emerges as a new pathway that has been implicated in different neurodegenerative diseases. In this talk, I will be talking about our efforts in understanding exosome-dependent neuron to glia signaling and how cell-type specific exosome signaling is differentially involved in neurodegenerative diseases ALS and AD. In ALS study, we investigated how astroglia-derived exosomes, affect (motor) neuron survival especially axon properties in ALS. By optimizing a filtration and size exclusion chromatography (SEC)-based purification of astroglial exosomes, we are able to minimize contamination of known astroglial proteins with exosomes. Interestingly, astroglial exosomes from non-transgenic (NTg) control astroglia ACM are able to strongly stimulate neuronal axon growth and protect neurons from glutamate-induced excitotoxity. Expression of SOD1G93A mutant and cytokine treatment in astroglia significantly reduced astroglial exosomes' stimulatory and protective effect on neuronal axons. Unlike exosomes prepared from ultracentrifugation method, astroglial exosomes prepared from SEC method have no evident association with mutant SOD1G93A or other misfolded SOD1. We further found that NTg astroglial exosomes activates neuronal focal adhesion kinase (FAK) by increasing its tyrosine (Tyr) 397 phosphorylation. Subsequent proteomic analysis of NTg and SOD1G93A astroglial exosomes with cytokine treatment further found that HepaCAM, a glia-specific cell adhesion molecule (CAM), is abundantly and selectively expressed on NTg astroglial exosomes that is reduced by the expression of SOD1G93A and cytokine treatment. HepaCAM deficiency from HepaCAM KO mice significantly reduce NTg astroglial exosomes' stimulatory effect on neuronal axons. By employing our newly generated cell-type specific exosome reporter mice (CD63-GFPf/f) and focal AAV-Gfap-Cre injections, we also found that astrocyte-derived exosomes travel significantly less in the spinal cord in SOD1G93A mice compared to littermate NTg mice. At the end of the talk, I will also touch on the role of neuronal exosomes in AD pathogenesis, especially a sex-specific effect on □-amyloid pathology and cognitive deficits in a humanized APPNL-F/NL-F AD mouse model.

056

Exploring Conditions for Developing Engineered Extracellular Vesicles as Targeted Delivery Vehicles.

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* Presenting Author

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Abstract:

Extracellular vesicles (EVs) hold tremendous potential for drug delivery applications due to their natural capacity in intercellular communication to transport molecules in the body. In addition to its outstanding characteristics, EVs avoid many shortcomings associated with current nanocarrier-mediated therapeutic delivery systems, such as inadequate efficacy, toxicity, and failure to target specific organs or diseased tissues. Thus, we have established and are further developing cell-derived engineered EVs (eEVs) for targeted therapeutic delivery using an EV-surface display technique. Due to the variability in the eEV quality for each experiment, we explored variable conditions focusing on the capacity of EVs to sustain the quality in size and packaging capacity. We investigated eEVs' ability to protect plasmid DNA (pDNA) under variable conditions using our method for generating eEVs encapsulating pDNA. These results uncovered the pitfalls of the current methodology but will bring eEV therapy to the next level by striving to establish reproducible eEV therapeutic delivery applications.

057

Blood cell-derived extracellular vesicles induce a pro-inflammatory phenotype in tubular epithelial cells of proliferative Lupus Nephritis patients.

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Abstract:
Introduction:

Proliferative lupus nephritis (LN) is a severe condition causing impaired kidney function that occurs in 40-60% of systemic lupus erythematosus (SLE) patients. LN is characterized by a marked interferon (IFN) response in tubular epithelial cells (TEC), but the biological trigger has remained unclear. We previously showed that extracellular vesicles (EVs) released by B cells are enriched in polymerase III (Pol III) transcripts of endogenous and viral (EBV) origin. These transcripts carry pathogen-associated molecular patterns (PAMPs) that can be recognized by innate RNA sensors. We hypothesize that in LN, due to glomerular damage, these inflammatory EVs disproportionately reach the tubular epithelium inducing an IFN response that worsens the disease.
Method:
Gene set enrichment analysis was applied to identify key pathways and cell signatures in proliferative LN. RNA-seq was used to define the pol-III trascriptome of B cell EVs. In situ hybridization was employed to reveal the presence of EBV transcripts in human tissues. In vitro assays were used to unravel the mechanism of internalization and the functional effect of B cell EVs on TEC cells.
Results:
scRNA-seq analysis of proliferative LN identified enrichment of blood cell, including (EBV-infected) B lymphocyte, transcripts in TEC cells. Notably, the same blood cell signatures were enriched in plasma EVs. SLE patient plasma and purified EVs contained the EBV-encoded pol III-transcript EBER1, which selectively accumulated in the cytoplasm of TEC from Lupus but not IgA nephritis biopsies. Functional assays showed uptake of B cell EVs by primary TECs in a phosphatidylserine (PtdSer)-dependent manner. Crucially, B cell EVs deliver EBERs into endosomes of primary TECs driving IFN gamma, TNF alpha and IL6 production through TLR3 activation.

Conclusions:
In SLE patients, blood cell-secreted, PtDSer+, inflammatory EVs target TEC cells via KIM-1 inducing an IFN-mediated inflammatory response.

058

Machine Learning Identifies Exosome Protein Signatures to Distinguish Multiple Human Cancers.

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James P. Allison Institute at MD Anderson, Houston, TX
Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX
Department of Bioengineering, Rice University, Houston, TX

Abstract:

Introduction: Non-invasive early cancer diagnosis remains challenging due to the low sensitivity and specificity of current diagnostic approaches. Exosomes are membrane bound nanovesicles secreted by all cells. They contain biological cargo in the form of DNA, RNA, and proteins that are representative of the parent cell. This property, along with the abundance of exosomes in biological fluids makes exosomes compelling biomarker candidates. However, a robust and flexible exosome-based diagnostic method to distinguish human cancers across cancer types in diverse biological fluids is yet to be defined.

Objective: The primary objective of this study was to develop a novel computational method to distinguish cancers using a panel of exosome biomarkers.

Methods: Using exosome data generated by our lab, along with several public datasets of exosome proteins detected in human plasma, serum, and urine from a variety of cancers, we defined three panels of pan-cancer exosome proteins in plasma, serum, and urine and built machine learning models to distinguish patient samples from normal samples using those protein panels.

Results: All the models yielded AUROC scores higher than 0.95 using plasma, serum, or urine-derived exosome samples.

Conclusion: Our data provided three reliable biomarker panels and scalable machine learning models for sensitive and specific non-invasive cancer diagnosis.

059

B cell targeting of extracellular vesicles by a novel fusion protein.

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2 Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Sweden.
3 CDL Research, University Medical Center Utrecht, The Netherlands.

Abstract:

Introduction

Allogeneic extracellular vesicles (EVs) loaded with ovalbumin (Ova) can induce an anti-tumor immune response in mice with Ova-expressing melanoma tumors. This anti-tumor immunity of Ova-loaded EVs relies on the activation of T cells, a response that is dependent on B cells. Additionally, previous research showed that EBV-derived GP350-containing EVs target human CD21 present on B cells. We therefore hypothesized that EVs targeting B cells would induce stronger T cell responses to EVs.

Objective

To examine if decorating Ova-loaded allogeneic EVs with a fusion protein binding CD21 target the EVs to B cells and thereby improve antigen-specific immune responses.

Methods

A fusion protein containing the phosphatidylserine-binding domain (C1C2) of lactadherin and CD21-binding domain (D123) of GP350 was designed. Ultracentrifugation-isolated macrophage-like RAW264.7 EVs were dyed with cell tracker deep red, washed by size exclusion and incubated with fusion protein C1C2-D123 or free mutated C1C2-D123 control. Fusion protein-decorated EVs were incubated with splenocytes of human CD21+ mice or human PBMCs. In vitro targeting was assessed by flow cytometry.

Results

The results show that, depending on the amount of EVs used, the percentage of B cells that are positive for C1C2-D123 fusion protein-decorated EVs increased by more than 50% in mouse splenocytes and by more than 90% in human PBMCs compared to EVs in absence of the fusion protein.

Conclusion

In conclusion, C1C2-D123 fusion protein-decorated EVs improves B cell targeting in vitro. Future in vivo experiments will assess if B cell targeted EVs improve antigen-specific immune responses as compared to non-targeted EVs.

060

Role of hepatocyte-derived extracellular vesicles in the regulation of immunometabolism.

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Department of Metabolic Bioregulation, Institute of Development, Aging and Cancer, Tohoku University

Abstract:

Inflammatory processes, particularly those resulting in chronic inflammation, have been implicated in a number of obesity-associated chronic diseases. However, the mechanisms by which obesity provokes aberrant inflammation have yet to be clearly defined. Here, we show that extracellular vesicles (EVs), particularly hepatocyte-derived EVs (H-EVs), cause aberrant inflammation through mechanisms involving the RNA cargo of H-EVs and an RNA-binding protein in the recipient immune cells.

Our study analyzing EVs from obese adolescents undergoing bariatric surgery and healthy lean subjects suggests that in circulation, liver-derived EVs are increased in obesity and are decreased post-bariatric surgery. Then, we focused on the role of liver-derived EVs and generated novel research tools that enable us to track H-EVs in vivo selectively. Analysis of the novel mouse models monitoring H-EVs in vivo indicates that in obesity, H-EVs behave like a pathogen recognized by macrophages and induce inflammation. Mechanistically, we found that in hepatocytes, metabolically-driven stresses enhance the generation of inflammatory H-EVs that are uptaken by macrophages and induce inflammation. In addition, we discovered that a specific RNA-binding in macrophages is an unexpected mediator of pro-inflammatory EVs and is required for EV-induced macrophage activation. Consistently, myeloid cell-specific deletion of the RNA-binding protein in mice becomes resistant to obesity-induced inflammation and insulin resistance.

This study suggests that specific RNA cargo of H-EVs and their receptor in myeloid cells are critical regulators of obesity-associated inflammation and chronic diseases.

061

Displaying and Delivering Viral Membrane Antigens via WW domain-Activated Extracellular Vesicles (WAEVs).

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Abstract:
Membrane proteins expressed on the surface of enveloped viruses are conformational antigens readily recognized by B cells of the immune system. An effective vaccine would include the synthesis and delivery of such native-conformational antigens in lipid membranes that preserve specific epitope structures. We have created an extracellular vesicle (EV)-based technology that allows viral membrane antigens to be selectively recruited onto the surface of novel WW domain-activated extracellular vesicles (WAEVs). Budding of WAEVs requires SCAMP3 (secretory carrier-associated membrane protein 3), which through its PPAY motif interacts with WW domains to recruit fused viral membrane antigens onto WAEVs. Immunization with influenza and HIV viral membrane proteins displayed on WAEVs elicits production of virus-specific neutralizing antibodies and, in the case of influenza antigens, protects mice from the lethal flu infection. WAEVs thus represent a versatile platform for presenting and delivering membrane antigens as vaccines against influenza, HIV and potentially many other viral pathogens.

062

A novel smoke-derived EV mediated in vivo model of emphysema.

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Abstract:
Abstract

Rationale: Extracellular vesicles play essential roles in different lung diseases. Our recent work showed that human-derived activated neutrophil (PMN) exosomes cause emphysema when transferred into naïve mice, a hallmark of chronic obstructive pulmonary disease (COPD) (Genschmer, Cell, 2019). Further, PMN-derived exosomes show increased neutrophil elastase (NE) expression on their surface and surprising resistance to the pulmonary antiprotease barrier, therefore promoting the degradation of the extracellular matrix and subsequent alveolar enlargement (emphysema). Given the transfer of emphysema mediated by PMN-derived exosomes from human to mouse, we investigated whether these extracellular vesicles (EVs) were generated in a mouse model of smoke-induced COPD and if they could transfer disease phenotype to naïve recipients.

Methods: In this study, 8-week-old A/J or C57Bl/6 wild type or NE knockout (KO) mice were smoked for 5 days per week for up to 6 months. EVs were isolated from bronchoalveolar lavage fluid (BAL) by sequential ultracentrifugation at 1 week, 2 weeks, 1 month, 3 months, and 6 months. After purification, EV size and concentration was measured by using Nanosight (Malvern). EVs were transferred to naïve mice intratracheally, and the presence of emphysema was determined after one week by the measure of median linear intercept on H&E staining. The presence of NE on the surface of EVs was measured by flow cytometry on CD63+ EVs isolated by a bead-based pull-down method (Genschmer, Cell, 2019).
Results: EVs isolated from wild-type smoked mice showed somewhat lower numbers in the BAL compared to air control mice. However, transfer of EVs to naïve mice induced emphysema only from the smoke, but not air, derived EV group. EV-mediated pathology correlated with a longer duration of smoke exposure within the EV donor mice. Further, smoke-derived EVs showed markedly increased presence of NE on their surface compared to controls. Lastly, smoke-exposed EVs derived from NE KO mice showed a reduced ability to cause emphysema compared to those isolated from wild-type mice.

Conclusions: In this study, we show that smoke induces the production of NE+ EVs in the lung. Further, the transfer of these pathogenic EVs into naïve recipients induced the

development of emphysema in a NE-dependent manner, showcasing a novel preclinical model of smoke-induced COPD.

Acknowledgments: This study was supported by the NIH NHLBI 5R35HL135710 to JEB, VA Merit Review 1I01CX001969 and NHLBI RO1 HL153113 to AG, NIGMS K12 GM088010 to MCM, 1R01HL162705 to KRG; Cystic Fibrosis Foundation: Postdoc-to-Faculty Award MARGAR21F5 to CM

063

Contribution of Cigarette Smoke to the Pathogenicty of Neutrophil Derived Extracellular Vesicles.

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Abstract:

Rationale: Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory lung disease that is caused primarily by cigarette smoke. Extracellular vesicles (EVs) isolated from bronchoalveolar lavage fluid (BALF) of patients with COPD were found to have increased neutrophil elastase (NE) on their surface as well as the ability to cause alveolar enlargement when transferred intratracheally to naïve mice. The NE on these EVs was found to be resistant to the inhibitor alpha-1 antitrypsin, leading to a robust NE activity on these vesicles. Given that EVs tested previously were from patients who already had COPD, we investigated what role cigarette smoke, in absence of a COPD diagnosis, had on EV phenotype observed in human BALF.

Methods: In this study, purified peripheral blood neutrophils were isolated and subjected to 0.5% cigarette smoke extract (CSE) for 30 mins at 37C. The EVs produced were isolated via differential ultracentrifugation. These EVs were tested for NE activity, as well as transferred into 10-week-old A/J mice over a 2-week period where the presence of alveolar enlargement was measured via lung histology and mean linear intercept (Lm) calculations. Subsequently, BALF procured from human subjects (current smokers without COPD, former smokers without COPD, or healthy never smokers) were collected and EVs were subsequently isolated. These EVs were administered intratracheally to naïve 10-week-old A/J mice over a 2-week period and Lms measured.

Results: EVs isolated from neutrophils exposed to CSE had increased NE proteolytic activity as well as the ability to cause alveolar enlargement in mice. EVs isolated from human BALF of smokers and former smokers without COPD caused a significant increase in alveolar enlargement in a mouse model of emphysema when compared to BALF EVs of healthy never smoker controls.

Conclusions: In this study, we have shown that PMNs activated with 0.5% CSE produce EVs that have proteolytic NE activity and can cause increased alveolar enlargement when administered intratracheally to naïve mice. Similarly, EVs isolated from BALF of human subjects who are current and former smokers, but do not have COPD, show the ability to cause alveolar damage in mice when compared to EVs isolated from BALF of healthy, never smoker control subjects. These data illustrate the contribution cigarette smoke has on PMN-derived proteolytic EVs and their effect in a mouse model of emphysema.

Acknowledgments: This study was supported by the NHLBI 5R35HL135710 to JEB, VA Merit Review 1I01CX001969 and NHLBI RO1 HL153113 to AG, and NHLBI1R01HL162705 to KRG.

064

Mechanistic Roles and Therapeutic Applications for Extracellular Vesicles in Preterm Parturition.

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Abstract:
Spontaneous preterm birth impacts 15 million pregnancies annually and causes 1 million neonatal deaths. The mechanistic mediation of labor and delivery at term is by cellular derangements in the fetal tissues (the fetal membranes and the placenta) that generate inflammatory cargo enriched EVs. These fetal-derived EVs propagate to maternal tissues, causing inflammatory imbalance and disrupting tissue homeostasis, transitioning the quiescent uterus to an active labor and delivery process, a physiologic process. However, premature activation of these events in response to various risk exposures (e.g. infection) by the mother can activate fetal inflammatory cargo enriched EVs reaching maternal tissues prematurely leading to preterm birth. Reduction of preterm birth requires regulation of fetal inflammatory response (FIR) characterized by the influx of fetal immune cells and inflammatory cargo enriched EVs reaching the maternal tissues. Current drugs do not cross the placental barrier to treat FIR or, if they cross, are often teratogenic. We have electroporetically engineered HEK293-derived EVs to overcome these limitations to contain the anti-inflammatory cytokine IL-10 (eIL-10). These EVs can cross placental barriers and are not cytotoxic. After testing the functional properties of eIL-10 using various cellular biological approaches, we determined that eIL-10 can delay preterm birth induced by an ascending infection by E. coli in mouse models of pregnancy, developed in our laboratory. We demonstrate that eIL-10 can cross the placental barriers, reach the fetal tissues. The delay in preterm birth and deliver live pups is associated with a reduction in inflammation (NF-kB activation and cytokine production), histological chorioamnionitis (neutrophil infiltration into the placenta, a detrimental factor for neonatal morbidity) and minimize fetal immune cell migration towards maternal uterine tissues. Most importantly, pups delivered showed no immuno suppressiveness during neonatal period. Thus, we demonstrate a safe and specific approach to deliver antiinflammatory mediators using EVs to treat the fetus as a patient to reduce the risk of preterm birth induced by infection.

065

Extracellular-vesicle mediated delivery of CRISPR/Cas9 by aptamer-based loading and inducible cargo release strategies.

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Abstract:

Background: CRISPR/Cas9 is a prokaryotic endonuclease capable of targeting and editing genomic sequences with high specificity and efficiency. As such, CRISPR/Cas9 holds tremendous therapeutic potential for the treatment of genetic pathologies. One of the major hurdles for the development of CRISPR/Cas9-based therapeutics is the intracellular delivery of the Cas9-sgRNA ribonucleoprotein (RNP) complex because of its large size, negative charge, and immunogenicity. Extracellular vesicles (EVs) hold the potential to overcome this hurdle due to their biocompatibility and intrinsic capability of highly efficient intercellular transfer of RNA molecules and proteins.

Methods: To facilitate targeted loading of the RNP complex, sgRNAs with high-affinity MS2 coat protein-interacting aptamers were generated and expressed alongside Cas9 and EV-enriched proteins fused to the MS2 coat protein. The MS2 coat protein, lacking the Fg loop to prevent capsid formation, was cloned in tandem on the N-terminus of CD9, CD63, CD81 and ARRDC1 or the C-terminus of Δ687-PTGFRN or a myristoylation sequence, linked by a UV-sensitive photocleavable protein (PhoCl). Cas9 loading and UV-mediated PhoCl cleavage were measured by Western Blot analysis. To study Cas9 delivery, we used a previously published fluorescent stoplight reporter system which is activated by Cas9 activity (De Jong et al, Nat Commun. 2020).

Results: EV loading of Cas9, as well as UV-mediated cleavage of the PhoCl fusion proteins, was confirmed by Western Blot analysis. Using EVs with MS2-PhoCl-CD63 fusion proteins we observed efficient Cas9 delivery (14.5%), but only after UV-treatment of EVs and co-expression of the VSV-G glycoprotein. Comparing RNP delivery efficiency using various EV-targeted fusion proteins revealed that CD9 and the myristoylation sequence showed notably high delivery of Cas9, followed by CD63, Δ687-PTGFRN, CD81, and lastly ARRDC1. Western Blot analysis revealed that these results strongly correlated to Cas9 loading in EVs. Further analysis showed Cas9 delivery in a dose-dependent manner using both CD9 and CD63 fusion proteins.

Conclusions: Here, we describe a novel modular platform for EV-mediated loading and delivery of Cas9 RNPs. Our results demonstrate that EVs are indeed capable of functional Cas9-RNP delivery and that Cas9 loading and delivery was strongly dependent on the targeted loading protein that was employed.

066

Molecule transplantation and xenogenization of cancer cells for cancer immunotherapy.

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Abstract:

A number of major human diseases are related to defects in membrane proteins. Despite several efforts aimed at delivering membrane proteins to the defective cell membranes, currently workable approaches to treat such membrane defects have been elusive. Here, we investigated an unprecedented exosome-based nano-platform for delivering membrane proteins directly into recipient cell membranes. Based on the features of exosomes that could be engineered by nature, we developed a fusogenic exosome platform in which expression of the viral fusogen, vascular stomatitis virus (VSV)-G protein, enables the exosomal membrane to fuse with recipient cell membranes. Our results revealed that the fusogenic exosomes could efficiently deliver GFP fused CD63 (CD63-GFP) or glucose transporter-4 (GLUT4-GFP) to recipient cell membrane. Fusogenic exosomes mediated transfer of biologically active GLUT4 to mouse muscle membranes both in vitro and in vivo, allowing the increased glucose uptake of recipient cells. This highlights the potential of our fusogenic exosome platform for delivering membrane proteins. Many cancer patients not responding to current immunotherapies fail to produce tumor-specific T cells for various reasons, such as a lack of recognition of cancer cells as foreign. Here, we suggest a novel method for xenogenizing (turning self to non-self) tumors by using fusogenic exosomes to introduce fusogenic viral antigens (VSV-G) onto the tumor cell surface. We found that xenogenized tumor cells were readily recognized and engulfed by dendritic cells, thereby tumor antigens were efficiently presented to T lymphocytes. Moreover, exosome-VSV-G itself acts as a TLR4 agonist and stimulates the maturation of dendritic cells, leading to CD8+ T cell cross-priming. The administration of these exosomes in multiple tumor mouse models xenogenized tumor cells, resulted in tumor growth inhibition. The combinatorial treatment with anti-PD-L1 exhibited complete tumor regression (30%) and better long-term overall survival. These results suggest that tumor xenogenization by fusogenic exosomes provides a novel strategy for cancer immunotherapy.

067

Exosome-mediated systemic delivery of antisense oligonucleotides reprograms tumor-associated macrophages and induces strong anti-tumor responses as a monotherapy.

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Codiak BioSciences

Abstract:

Introduction:

Tumor-associated macrophages (TAMs) regulate the tumor microenvironment (TME) by promoting an immunosuppressive milieu through inhibition of T-cell activation and recruitment, leading to poor clinical outcomes. STAT6 and C/EBPβ are critical transcription factors that regulate the immunosuppressive phenotype of TAMs.

Objective:

We have developed a new class of therapeutics, exoASO™, consisting of engineered exosomes loaded with antisense oligonucleotides (ASOs) directed against STAT6 or C/EBPβ, that selectively deliver ASOs to TAMs, resulting in their reprogramming to an anti-tumoral, immunostimulatory ('M1') phenotype

Methods:

exoASO-STAT6 and exoASO-C/EBPβ were extensively studied in-vitro and in-vivo to characterize their selective uptake, target-engagement, and mechanism of action in TAMs and the TME. Several efficacy studies were performed in tumor-bearing mice to elucidate the translational significance of exoASOs.

Results:

In-vitro assays using primary human M2-macrophages demonstrated dose-dependent target-gene knockdown (KD) with a higher potency vs free ASO. exoASO-mediated KD resulted in profound changes in gene expression and cytokine secretion profile, consistent with reprogramming to an immunostimulatory phenotype. Systemic administration of exoASO in tumor bearing mice demonstrated up-to 11-fold improvement in selective ASO delivery to immunosuppressive myeloid cell populations, compared to free ASO. Utilizing intratumoral dosing of exoASO as a proof-of-concept, single-cell RNA sequencing revealed profound changes in macrophage populations towards a pro-inflammatory phenotype. Intravenous treatment with both exoASOs in an orthotopic hepatocellular carcinoma model demonstrated complete remission of tumor lesions in 50% of treated mice, while gene expression and histological analysis demonstrated effective reprogramming of the TME by showing a significant increase in interferon and cytotoxic T-cell gene signatures. Finally, systemic administration of exoASO-C/EBPβ in a lung metastasis model resulted in a significant attenuation of tumor growth as a single-agent, thereby also demonstrating the potential for targeting of extra-hepatic tumors.

Conclusion:

exoASO-STAT6 and exoASO-C/EBPβ are novel, systemically administered exosome therapeutic agents that selectively target and attenuate key transcription factors in TAMs, resulting in their reprogramming and potent single-agent anti-tumor activity.

068

Cardiac Inflammation & Heart Failure Control with IL-4 Polarized Human Macrophage Exosomes.

Alex Gao, Martin Ng, Anh Phu, Ngan Vu, Robert L. Raffai

Department of Veterans Affairs, Surgical Service (112G), San Francisco VA Medical Center, San Francisco, CA 94121, USA; Northern California Institute for Research and Education, San Francisco, CA 94121, USA;

Abstract:

Introduction

Coronary artery disease often results in cardiac inflammation and ischemia, which in turn can lead to chronic heart failure (CHF). We previously reported that the anti-inflammatory compound FTY720 is effective in controlling CHF by suppressing cardiac inflammation following myocardial infarction (MI). Unfortunately, FTY720 is contraindicated for patients with CHF since it causes bradycardia. To this end, we tested exosomes as an alternative treatment option. Our previous studies reported that THP-1 macrophage polarized to an M2-like state via exposure to interleukin-4 (IL4), produce exosomes (THP1-IL4-exo) that exert anti-inflammatory properties. In this study, we tested if THP1-IL4-exo could serve to improve cardiac function and extend longevity through the reduction of cardiac and systemic inflammation in a mouse model of diet-induced occlusive coronary atherosclerosis.

Methods/Preliminary results:

We used Hypomorphic ApoE mice deficient in scavenger receptor Type-B1 expression (HypoE/SR-B1−/−) to model CHF that develops in response to MI caused by diet-induced coronary atherosclerosis. Our findings show that tri-weekly intraperitoneal injections of THP1-IL4-exo into HypoE/SRB1 mice fed a high-fat diet (HFD) extended their lifespan. Our data show that THP1-IL4-exos exerted this effect by attenuating inflammation. They did so by reducing the number of neutrophils in the circulation as well as the expression levels of M1 macrophage-related genes (Tnfa, Il6) and increasing the expression levels of M2 macrophage-related genes (Il10, Chil3) in cardiac tissue. They also attenuated the expression of Matrix Metalloproteinases, a family of proteins that is upregulated in response to cardiac injury and participate in driving left ventricular dysfunction. Such control of systemic and cardiac inflammation by THP1-exo led to functional improvements in cardiac function that included an increase in fractional shortening and left ventricular ejection fraction.

Conclusion

Our findings support the use of THP1-IL4-exo as a therapeutic agent to control cardiac injury in response to occlusive coronary atherosclerosis.

Disclosure

The authors report no conflict of interests.

069

Systematic Humanization of Yeast Extracellular Vesicles.

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Abstract:
Cells communicate by releasing macromolecules encapsulated within lipid membrane vesicles into surrounding fluids. After recognition and uptake by specific recipient cells, bioactive cargo within these extracellular vesicles (EV) elicit a variety of physiological responses. Despite their importance, the molecular basis of most EV functions remains largely unknown. This study aims to characterize bioactivities of individual human proteins expressed on or within inert EVs released from Saccharomyces cerevisiae (baker's yeast). For unbiased screening experiments, we expressed > 15,000 human genes each in yeast using a modular genetic approach called ExoClo which accommodates EV targeting and tags proteins with fluorescent proteins (GFP) for monitoring. This involved segregating a collection of cDNAs with near complete human genome coverage into 18 distinct pools (960 genes each) and developing a new en-masse cloning method to engineer humanized yeast strains. To validate this procedure, we conducted Oxford nanopore sequencing to track cloning efficiency and proteomic analysis by immunopurifcation and mass spectrometry to confirm human protein expression in yeast cells and EVs. Preliminary sequencing results suggest a cloning success rate of 70%, and Western blot analysis shows that a wide range of GFP-tagged human proteins are expressed. Only a small fraction (~4%) of human proteins are expected to be sorted into yeast EVs. Thus, to place all proteins into EVs, we are engineering a second collection of humanized yeast stains that fuses human proteins to an EV targeting peptide. Comparing EV protein expression profiles from both approaches will help reveal evolutionary conserved mechanisms underlying EV cargo selection and biogenesis. Future bioactivity screening experiments will identify proteins sufficient for EV stability, targeting, uptake and cargo delivery within mammalian cells and tissues. Additionally, this yeast–based platform to produce engineered EVs may be used to help develop new EV-based therapeutics.

070

A Single Intranasal Administration of EVs from hiPSC-derived NSCs Can Reduce TBI-induced NLRP3 Inflammasome Signaling in the Hippocampus.

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Abstract:

Introduction: Moderate to severe traumatic brain injury (TBI) results in significant acute neuroinflammation, which can accelerate tissue loss and evolve into a state of chronic neuroinflammation, contributing to long-term cognitive and mood impairments. Numerous drugs have shown efficacy in suppressing acute neuroinflammation following a TBI, but none could be translated into the clinic due to their inadequate long-term functional efficacy or undesirable side effects. In this context, extracellular vesicles (EVs) secreted by stem cells are appealing for treating TBI due to their potential for modulating both acute as well as chronic neuroinflammation. EVs can also be quickly delivered into neurons and microglia after TBI through intranasal (IN) administration.

Objective: To investigate whether a single IN administration of EVs from human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) after a moderate TBI can reduce NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome signaling in the hippocampus.

Methods: Nine-week-old mice were first subjected to a unilateral controlled cortical impact injury using a velocity of 5m/s, dwell time of 300ms, and an injury depth of 0.8 mm. Ninety minutes after the induction of TBI, mice received IN administration of ~25 billion hiPSC-NSC-EVs or the vehicle. The hippocampi ipsilateral to the CCI were harvested 48 hours after EV/VEH administration for analysis of neuroinflammation, particularly NLRP3 inflammasome activation using immunofluorescence and biochemical analyses.

Results: Moderate TBI resulted in NLRP3 inflammasome activation in the hippocampus, which was evident from the upregulation of nuclear factor kappa B (NF-kB), NLRP3, its adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), and activated caspase-1. Moreover, the concentrations of the end products of NLRP3 inflammasomes, interleukin-1 beta (IL-1β), and IL-18 were also increased. Remarkably, hiPSC-NSC-EV treatment restored the various components involved in NLRP3 inflammasome activation to naïve control levels. Analysis of microglia using IBA-1, NLRP3, and ASC triple immunofluorescence revealed the occurrence of NLRP3-ASC complex in a significant percentage of microglia after TBI. However, hiPSC-NSC-EV treatment after TBI reduced the incidence of NLRP3-ASC complex within microglia, implying the efficacy of hiPSC-NSC-EVs for reducing NLRP3 inflammasome activation in activated microglia after TBI.

Conclusion: A single IN administration of hiPSC-NSC-EVs shortly after a moderate TBI can substantially reduce neuroinflammation by inhibiting the activation of NLRP3 inflammasomes.

Disclosure of conflict of interest: The authors report no conflicts of interest.

071

Engineered S. cerevisiae EVs for lysosomal enzyme replacement.

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Abstract:
Extracellular vesicles (EVs) are a promising drug delivery platform as they compartmentalize bioactive payloads prior to delivery, have low immunogenicity, and are capable of tissue-specific targeting. EV-based therapeutics in most research pipelines are produced using cultured human mesenchymal stem cells. This approach is limiting as these cells are not amenable to complex genetic engineering, are expensive to grow and maintain, produce heterogenous EVs that are challenging to purify, and cannot be easily upscaled. Baker's yeast (Saccharomyces cerevisiae) is an excellent candidate for EV research, as it exhibits similar fundamental EV biology and may overcome these and other limitations. Using S. cerevisiae as a model, this study aims to determine if yeast EVs can deliver bioactive protein cargos that reverse the cellular effects associated with two rare metabolic disorders linked to loss-of-function mutations in lysosomal proteases: galactosialidosis and CTSA, and CLN10 disease and CTSD. To achieve this, we genetically engineered yeast that express GFP-tagged versions of these human lysosomal enzymes (or their yeast orthologs) within EVs. After confirming expression by live-cell fluorescence microscopy, we will collect EVs by ultracentrifugation and characterize them by measuring protein content (fluorimetry, protein quantification), size (quasielastic light scattering, nanoparticle tracking analysis), and charge (zeta potential measurement). Yeast strains deficient in lysosomal enzyme activity will be treated with engineered EVs and phenotypic rescue will be assessed by measuring lysosomal protein cleavage activity and sensitivity to metabolic stress (nitrogen starvation). Anticipated results from this proof-of-concept study will validate this approach as a potential treatment strategy for these and related metabolic disorders.

072

Extracellular Vesicles Derived from hiPSC-NSCs Protect Human Neurons Against Amyloid β-Induced Toxicity.

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Abstract:

Introduction: Alzheimer's disease (AD) is the primary cause of dementia in the aged population. AD is typified by progressive cognitive impairments associated with amyloid-beta (Aβ) plaques, neurofibrillary tangles, synapse loss, and neurodegeneration. Aβ accumulation in the extracellular space induces toxicity, oxidative stress, and neuroinflammation, thereby contributing significantly to AD pathogenesis. Hence, preventing Aβ-induced toxicity via appropriate therapeutic compounds could be one of the avenues to slow down AD progression. Since extracellular vesicles (EVs) shed by human induced pluripotent stem cell (hiPSC)-derived neural stem cells (NSCs) carry a cargo of therapeutic proteins and miRNAs, hiPSC-NSC-derived EVs have the promise to provide neuroprotection against Aβ-induced toxicity.

Objective: Analyze the neuroprotective properties of EVs secreted by hiPSC-NSCs against Aβ-induced toxicity in human neuronal cultures.

Methods: The EVs secreted by hiPSC-NSCs (hNSC-EVs) were isolated from the cell culture spent media by anion exchange followed by size exclusion chromatography. Next, mature human neurons were expanded from hiPSC and exposed to 1.0μM Aβ oligomers with different concentrations (10, 20, or 40 x 109 B) of hNSC-EVs. The protective effects of hNSC-EVs on neurons exposed to Aβ were examined using MTT, live/dead cell assays, and mitochondrial membrane potential analysis via JC-1 staining. Moreover, genes such as BAD, BCL2, BCL2L10, PTEN, and FOXO3A were measured to determine the effects of hNSC-EVs against the Aβ-induced neuronal apoptosis.

Results: The cultured hNSCs expressed nestin and Sox2, and Nanosight analysis of EVs revealed a mean size of 157 ± 2.3 nm. Thirty-day-old human neurons derived from hiPSCs expressed NeuN and MAP-2. Exposure of such mature human neurons to 1-μM Aβ resulted in cytotoxicity, evidenced by significant neuronal death. However, adding EVs to neuronal cultures exposed to Aβ reduced neuronal death. Moreover, JC1 staining revealed a reduced fluorescence ratio implying depolarization of mitochondrial membrane potential (Δψm) in Aβ-exposed human neurons. EV addition to human neurons exposed to Aβ rescued their Δψm, which was apparent from an increased fluorescence ratio. Additionally, neurons exposed to Aβ displayed upregulation of pro-apoptotic genes (AD, FOXO3a, and PTEN) and downregulation of an anti-apoptotic gene (BCL-2), indicating that Aβ-exposure can induce neuronal apoptosis. However, the expression of these genes remained closer to control levels when EVs were added to Aβ-treated neuronal cultures.

Conclusion: hNSC-EV treatment can substantially reduce Aβ-induced toxicity in human neurons. Such a conclusion is supported by observations of higher cell viability, standard

mitochondrial membrane potential, downregulation of apoptotic genes, and upregulation of an anti-apoptotic gene in Aβ-exposed neuronal cultures treated with hNSC-EVs.
Funding: NIH-AD grant: RF1AG074256 to A.K.S.

073

Preclinical biodistribution and toxicology assessment of GMP MSC exosomes.

Michelle Kirtley, Kathleen M. McAndrews, Sujuan Yang, Rick Finch, Mihai Gagea, Stephen Ludtke, Adam Fluty, Valerie S. LeBleu and Raghu Kalluri

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Abstract:
Preclinical biodistribution and toxicology assessment of GMP MSC exosomes

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Introduction: Exosomes are small extracellular vesicles secreted by all cells. The role of exosomes in intercellular communication, and their ability to negotiate a variety of biological barriers and efficiently enter cells or delivery their cargo, has made them excellent candidates for targeted delivery of therapeutics for diverse diseases. Here, we describe a comprehensive preclinical toxicology analysis of clinical grade exosomes engineered for siRNA therapeutic payload delivery (iExosomes) in mice and rhesus macaques to inform on their utility as therapeutic agents in patients.
Objectives: Ascertain the comprehensive toxicology profile and biodistribution of exosomes in mice and rhesus macaques.
Methods: Human bone marrow mesenchymal stromal cells (MSCs) were cultured and exosomes purified from conditioned culture medium and engineered to encapsulate therapeutic siRNA (iExosomes) using standardized Good Manufacturing Practices (GMP). Graduated doses of GMP iExosomes were administered i.v. or i.p. to adult wild-type C57BL/6 albino mice and rhesus macaques along with vehicle control. A total of nine doses were administered to over the course of six weeks. The animals were euthanized one week after the final dose and tissue and serum studied using a comprehensive blood chemistry and hematological panels, gross tissue necropsy, and histopathological examination of tissues. To study the biodistribution of exosomes, DiR and PKH-67 labeled GMP iExosomes were administered i.v. or i.p. to adult rhesus macaques, and the tissues were analyzed 24 hours following administration using IVIS and confocal microscopy imaging.
Results: In both species studied, serum chemistry and hematological studies showed values within the normal ranges for all administered concentrations of iExosomes. There were no significant changes in body weight or organ weights; and no abnormalities were found on gross necropsy examination. Histopathological studies of tissues reveal no evidence of parenchymal damage following GMP-iExosomes administration. Confocal microscopy imaging of tissue sections revealed that iExosomes accumulated in the brain, pancreas, liver, and lung.

Conclusions: Data from testing in mice and rhesus macaques demonstrate the safety and lack of toxicity of human MSCs-derived and engineered exosomes at the dose and route studied. This study provides support for the development and use of exosomes for clinical therapies.
Disclosures: UT MD Anderson Cancer Center and R.K. hold patents in the area of exosome biology and are licensed to Codiak Biosciences, Inc. MD Anderson Cancer Center and R.K. are stock equity holders in Codiak Biosciences, Inc. R.K. is a consultant and scientific adviser for Codiak Biosciences, Inc.

074

Development and preclinical testing of a novel extracellular vesicle-based vaccine platform.

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Abstract:

Introduction: The emergence of SARS-CoV-2, the causative agent of COVID-19, has highlighted the importance of novel vaccination strategies and the need for development of platforms which can be rapidly adapted to address emerging viruses and variants. Current vaccines have some limitations in their effectiveness and durability in inducing immune protection, along with post vaccination side effects. Vaccines can be protein-based or messenger RNA (mRNA)- based and each type has specific advantages. Extracellular vesicles are naturally generated by cells and can efficiently deliver their cargo to recipient cells. Extracellular vesicles have low intrinsic immunogenicity and can be readily engineered to carry custom biological cargos. These properties contribute to their suitability as a vaccine delivery system.

Objective: We combined the advantages of mRNA and protein vaccines and designed a novel COVID-19 vaccine candidate (ExoVAX) that included both the stabilized viral spike protein and the mRNA for the spike protein in engineered extracellular vesicles.

Methods: We first assessed the physical and delivery properties of our vaccine and then evaluated the effectiveness of this vaccine platform in defined preclinical models, mainly for the post-vaccination immune response.

Results: Our results confirmed that ExoVAX effectively delivers spike protein and spike mRNA to cells in vitro. Further, vaccination of mice with ExoVAX confirmed the successful delivery of the protein and induction of a robust immune response in vivo.

Conclusion: This work describes a novel vaccine approach for COVID-19 and confirms the potential for rapid development of vaccines using an extracellular vesicle-based vaccine platform.

Disclosures: UT MD Anderson Cancer Center and R.K. hold patents in the area of exosome biology and are licensed to Codiak Biosciences, Inc. MD Anderson Cancer Center and R.K. are stock equity holders in Codiak Biosciences, Inc. R.K. is a consultant and scientific adviser for Codiak Biosciences, Inc.

075

Identification of a Novel Small Molecule that Enhances the Release of Extracellular Vesicles with Immunostimulatory Potency via Intracellular Calcium Induction.

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2 Department of Rheumatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Tokyo, Japan

Abstract:

Introduction

As extracellular vesicles (EVs) transfer the antigens and immunomodulatory molecules in the immunologic synapses, EVs equipped with immunostimulatory functions have been utilized for vaccine formulation. To identify small molecules that enhance immunostimulatory EVs released by antigen-presenting cells, we performed a high-throughput screening on a 28K compound library using three reporter cell lines for CD63, NFκB, and interferon-sensitive response element (ISRE) and have identified 80 hit compounds (Shukla.et al.,Front Pharmacol.2022 (13),869649). Since intracellular Ca2+ elevation enhances EV biogenesis/secretion, we screened compounds for Ca2+ influx.

Objective

We aim to identify small molecules that enhance immunostimulatory EV release by antigen-presenting cells via elevation of intracellular Ca2+ levels.

Methods

Intracellular Ca2+ levels were measured by ratiometric Ca2+ indicators, Fura-2 and Fura-8. EVs were isolated from the supernatant of mBMDCs treated with compounds using differential ultracentrifugation and validated by western blot, microfluidic resistive pulse sensing, transmission electron microscopy, and vesicle flow cytometry. EV-induced ovalbumin TCR transgenic DO11.10 T-cell proliferation was quantified by the proliferation measured by CFSE dilution. Structure-activity relationship studies were performed in our laboratory.

Results

We found that compound 634 induced Ca2+ influx and enhanced EV release by 45% in mBMDCs. In addition to EV release, 634 increased costimulatory molecule expression on the surface of EVs and the parent cells. EVs from 634-treated mBMDCs induced T-cell proliferation in the presence of antigenic peptides. The structure-activity relationship analogs that retained the ability to induce Ca2+ influx induced a higher number of EVs with immunostimulatory properties from mBMDC compared to those analogs that lost the ability to induce Ca2+ influx.

Conclusion. Our study shows that a small molecule, 634, enhances immunostimulatory EV release via Ca2+ induction, leading to discovery of a novel tool for EV-based vaccine development.

Disclosure of Conflict of Interest

The authors have no conflicts of interest to declare.

076

Advantage of extracellular vesicles in hindering the CD47 signal for cancer immunotherapy.

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Abstract:
The cluster of differentiation 47 (CD47) protein is abundantly expressed on various malignant cells and suppresses the phagocytic function of macrophages and dendritic cells. High CD47 levels are correlated with poor cancer survival. Antagonizing CD47 antibodies with potent antitumor effects have been developed in clinical trials, but have critical side effects, inducing anemia and thrombocytopenia. To develop a safe and potent CD47 blockade, we designed extracellular vesicles (EVs) harboring signal regulatory protein alpha (SIPRα)—EV-SIRPα (EVs that express SIPRα). EV-SIRPα showed minimal toxic effects on hematologic parameters and utilized RBCs as delivery vehicles to tumors rather than inducing anemia. EV-SIRPα inhibited residual CD47 ligation, which attribute to the EV-endocytosis-mediated CD47 depletion and steric hindrance of EV. In an immunologically cold tumor model, EV-SIRPα induced tumor-specific T-cell-mediated antitumor effects. When directly administered to the accessible lesions, EV-SIRPα monotherapy elicited an abscopal effect in the B16F10 tumor model by increasing immune cell infiltration and CD8+-mediated immunity against non-treated tumors. The combinational approach by loading doxorubicin into the EV-SIRPα dramatically reduced the tumor burden and led to 80 % complete remission rate. Thus, a potent EV-based CD47 blockade that is hematologically safe, has efficient signaling blocking efficacy, and has systemic antitumor immunity against cancer is recommended.

077

Oncolytic exosomes for cancer immunotherapy.

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Abstract:
Immunotherapy is a rising mode of cancer treatment, but only a few patients respond, largely due to the fact that most non-responders fail to produce T cells recognizing neoantigens. To facilitate such tumor-specific T cell production, antigen-presenting cells should be able to efficiently recognize and engulf tumor cells followed by cross-presentation and priming. We have engineered exosomes (ORSVF-Exo) to express a viral fusion protein, respiratory syncytial virus F protein (RSVF), and loaded them with unfolded proteins by treating their parental cells with a vacuolar-Type-H+-ATPase (vATPase) inhibitor, bafilomycin A1 (bafA1). We found that ORSVF-Exo preferentially fused with tumor cells and transplanted non-self-antigens on the tumor cell membrane in a nucleolin-dependent manner. These xenogenized tumor cells were readily engulfed by phagocytic cells. Furthermore, ORSVF-Exo induced endoplasmic reticulum stress (ER stress)-mediated immunogenic cell death (ICD) of tumor cells by dumping unfolded proteins into the tumor cell cytoplasm. Intratumorally injected ORSVF-Exo showed remarkable tumor regression effects through immune activation in CT26.CL25 tumor-bearing mice, and a combinational therapy with PD-1 antibody elicited synergistic anti-tumor effects and long-term survival, with 22% showing complete remission and rejection of rechallenged tumor growth. Collectively, these oncolytic fusogenic exosomes would be a cutting-edge strategy for non-responders to current immunotherapy.

078

Taking exosome therapeutics from bench to bedside.

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Abstract:
Loss or damage of tissues that result from traumatic injury, tumor resection, and virus infection need reconstructive approaches, such as cell/tissue transplantation or tissue engineering. Although stem cell-based therapies have clear beneficial effects on tissue regeneration, there are still a number of concerns, such as limited survival and the reduced regenerative capacity of engrafted stem cells, as well as immune-mediated rejection. Stem cells secrete a relatively large amount of extracellular vesicles (EV) containing various proteins and genetic materials, which could act as critical signals of cell-to-cell communication for tissue regeneration. Stem cell EVs provide a cell-free therapeutic approach for the regeneration of various tissues. Small EVs, commonly called exosomes, were isolated from conditioned media of stem cells. The tissue regeneration potency of exosomes was analyzed in different animal models. Exosomes contained various cytokines and microRNAs related to each tissue development. New tissue formations were observed in the exosome injection sites of animal models. We are now developing several exosome products; 1) naive exosomes for treatment of osteoarthritis and liver fibrosis, 2) priming exosomes for treatment of lung fibrosis and obesity/diabetes, 3) protein/miRNA delivery systems for treatment of Parkinson's disease and tendinitis. We plan the future hopefully for entry to clinical trials of some exosome products. In this presentation, we hope to discuss several important issues for entry to clinical trials of exosome therapeutics such as physical/chemical/biological characterization, mass production in GMP, quality control, CMC documentation, pre-clinical experiments, clinical protocols, etc.

079

Stem Cell Extracellular Vesicles as Anti-SARS-CoV-2 Immunomodulatory Therapeutics: A Systematic Review of Clinical and Preclinical Studies.

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Abstract:

Background: COVID-19 rapidly escalated into a worldwide pandemic with elevated infectivity even from asymptomatic infected patients. The disease can result in severe pneumonia and acute respiratory distress syndrome (ARDS), which are the main contributors to death. Stem cells and their secretomes are deemed among the most effective therapeutic approaches for lung regeneration and survival rate improvement in these patients. Stem cell-derived extracellular vesicles (EVs) serve as acellular-based therapy with regenerative and immunomodulatory capacities against severe pulmonary conditions associated with COVID-19. Herein, we aimed to evaluate the safety and efficacy of stem cell EVs in curing COVID-19, pneumonia, acute lung injury, and ARDS. We also cover relevant preclinical studies to recapitulate the current progress in stem cell-derived EVs-based therapy. Methods: Using PubMed, Cochrane Central Register of Controlled Trials, Scopus, and Web of Science, we searched for all English-published studies (2000-2022) that used stem cell EVs as a therapy for COVID-19, ARDS, or pneumonia. The risk of bias (ROB) was assessed for all studies. Results: 45 studies matched our inclusion criteria. Various sized EVs derived from different types of stem cells were reported as a potentially safe and effective therapy to attenuate the cytokine storm induced by COVID-19. EVs alleviated inflammation and regenerated alveolar epithelium by decreasing apoptosis, pro-inflammatory cytokines, neutrophil infiltration, and M2 macrophage polarization. They also prevented fibrin production and promoted the production of anti-inflammatory cytokines and endothelial cell junction proteins. Conclusion: Stem cell EVs mediate lung tissue regeneration via targeting multiple pathways, similar to their parental cells. EVs thus hold promise in promoting the recovery of COVID-19 patients and improving the survival rate of severely affected patients.

080

Cellular reprogramming with extracellular vesicles derived from differentiating stem cells.

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Abstract:
Stem cell–derived extracellular vesicles (EVs) offer alternative approaches to stem cell–based therapy for regenerative medicine. In this study, stem cell EVs derived during differentiation are developed to use as cell-free therapeutic systems by inducing tissue-specific differentiation. EVs are isolated from human adipose-derived stem cells (HASCs) during white and beige adipogenic differentiation (D-EV and BD-EV, respectively) via tangential flow filtration. D-EV and BD-EV can successfully differentiate HASCs into white and beige adipocytes, respectively. D-EV are transplanted with collagen/methylcellulose hydrogels on the backs of BALB/c mice, and they produce numerous lipid droplets in injected sites. Treatments of BD-EV attenuate diet-induced obesity through browning of adipose tissue in mice. Furthermore, high-fat diet–induced hepatic steatosis and glucose tolerance are improved by BD-EV treatment. miRNAs are responsible for the observed effects of BD-EV. These results reveal that secreted EVs during stem cell differentiation into white adipocytes or beige adipocytes can promote cell reprogramming.

081

THE THERAPEUTIC APPLICATION OF ENGINEERED EXOSOMES FOR CNS DISEASES.

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Abstract:

Not available in English

082

Bovine mammary alveolar MAC-T cells afford a tool for designing milk exosomes optimized for drug delivery.

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Abstract:

Introduction:

Milk exosomes (MEs) have properties that make them suitable for delivering drugs to diseased tissues. MEs resist degradation in the gastrointestinal tract, are bioavailable following oral administration, cross the blood brain barrier to deliver functional cargo, and they do not elicit strong immune reactions. The production of MEs is scalable. As is the case for all nanoparticles, MEs are cleared by macrophages upon absorption, which limits their therapeutic potential. MEs are produced and secreted by alveolar cells in the mammary gland, and bovine mammary alveolar MAC-T cell secrete exosomes with properties nearly identical to MEs. The objective of this study was to genetically engineer MAC-T cells to secrete MEs with decreased affinity for macrophages and increased homing to brain tumors (glioblastoma multiforme, GBM).

Methods:

Lentiviral vectors were used to engineer MAC-T cells that secrete MEs featuring proprietary surface proteins (denoted UNL1 and UNL2) assumed to decrease elimination by murine bone marrow-derived macrophages (BMDMs) and a protein (UNL3) assumed to enhance accumulation in brain tumors. MEs were isolated from MAC-T cell culture media supernatant and labeled using a carbonyl-reactive fluorescent. Primary BMDMs isolated from C57BL/6J mouse hind legs for assessing ME uptake at a physiological concentration (10exp10 MEs/mL). GBM cells (IDH1R132H mutants) were obtained from ATCC (HTB141G). GBM mice were prepared by intracranial transplant of cells from GBM patients.

Results:

The uptake of both ME UNL1 and ME UNL2 by BMDMs was reduced by 50% compared to unmodified BMEs in culture dishes ($p < 0.01$; $n = 5$). UNL2 increased the accumulation of MEs in brain tumors from “not detectable” to “substantial” in GBM mice. The uptake of ME UNL3 was 70% greater than control MEs in GBM cell cultures ($p < 0.05$; $n = 3$).

Conclusion:

ME modifications enhance the therapeutic potential of MEs.

Disclosures:

Supported by NIH P20GM104320, NIFA (2016-67001-25301 and 2022-67021-36407), USDA Hatch and W-4002, and the SynGAP Research Fund (all to J. Z.). J.Z. is a consultant for PureTech Health, Inc.

083

Construction of tumor-targeted fusogenic extracellular vesicles for cytosolic delivery of drugs.

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Abstract:

Introduction

Cells derived small extracellular vesicles (sEV) have been exploited for drug delivery. Compared with micelles, liposomes, and polymeric nanoparticles, autologous sEVs act as a natural delivery system have received considerable attention due in part to their innate biocompatibility over artificial nanoparticles. Moreover, sEVs can fuse with the cell membrane and deliver drugs directly into the cytoplasm. By evading engulfment by lysosomes, EVs remarkably enhance delivery efficiency of vulnerable molecules. It is noteworthy that endocytosis is still the major way of sEV uptake. Therefore, engineered EVs that can specifically and efficiently trigger membrane fusion for cytosolic delivery of drugs are highly desired.

Objective

The aim of this study is to construct Glypican 3 (GPC3)-targeted fusogenic extracellular vesicles.

Methods

Intrinsic major histocompatibility complex I (MHC-1) and GPC3 of hepatocellular carcinoma cell line HepG2 cells were knocked out (KO) with CRISPR/Cas9. Subsequently, anti-GPC3 single-chain scFv antibodies and reengineered fusogenic proteins were co-expressed on the surface of double-KO HepG2 cells. The generated monoclonal cell line was expanded for sEV harvest.

Results

Sanger sequencing confirmed that intrinsic MHC-1 and GPC3 was successfully knocked out with CRISPR/Cas9. Western blot analysis did not detect beta-2 microglobulin (B2M) or GPC3 in cellular lysates. Flow cytometry analysis demonstrated that anti-GPC3 scFv and fusogenic proteins were co-expressed on double-KO HepG2 cells. Membrane fusion was observed when we co-culture the constructed HepG2KO/Ab+F cells and wild-type GPC3-overexpressing HepG2 cells. Approximately, 237,736 anti-GPC3 scFv and 1,867 fusogenic proteins were expressed on double-KO HepG2 cell surface. The harvested sEVs derived from HepG2KO/Ab+F exhibited the characteristic saucer-shaped morphology under the TEM. Immunogold staining indicated average 7 fusogenic proteins on a sEV surface. The average size of sEVs derived from HepG2KO/Ab+F was 127.8 nm. Western blot identified EV classical markers, including CD9, CD63, and CD81.

Conclusion

GPC3-targeted fusogenic sEVs were successfully prepared.

084

Role of HIV-associated extracellular vesicles in comorbidities of people living with HIV.

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Abstract:

People living with HIV/AIDS on antiretroviral therapy have an increased risk of non-AIDS defining cancers, such as those in the head neck and lung, and co-infection with the Kaposi sarcoma-associated herpesvirus (KSHV) compared with those in the general population. However, the underlying mechanism for the development and progression of the comorbidities in those with HIV remains obscure. We have demonstrated the presence of HIV-associated extracellular vesicles (EVs) in the blood and saliva of people with HIV and uncompromised immune systems. We have also identified HIV-associated EVs in plasma of HIV-positive oral and lung cancer patients. HIV-associated EVs from the plasma of HIV-positive donors and those from culture supernatants of HIV-infected T cells significantly increased proliferation and invasion of oral and lung cancer cells. However, EV-depletion abolished the stimulatory effect. The HIV trans-activation response (TAR) element RNA in HIV-infected T-cell EVs is responsible for promoting cancer cell proliferation and inducing expression of proto-oncogenes and Toll-like receptor 3 (TLR3)-inducible genes. These effects depend on the loop/bulge region of the double-stranded RNA (dsRNA) structure of the molecule. HIV-infected T-cell EVs rapidly enter recipient cells and retain HIV components through epidermal growth factor receptor (EGFR) of recipient cancer cells; they also stimulate ERK1/2 phosphorylation via the EGFR/TLR3 axis. The growth and metastasis of allograft tumors from murine non-small cell lung cancer (NSCLC) cells were significantly accelerated in chimeric mice of HIV-positive bone marrow transplant animals relative to those in HIV-negative counterparts, correlating with enrichment of the viral dsRNA in the tumor. HIV-associated T-cell exosomes stimulated phosphorylation of several receptor tyrosine kinases (RTKs), including EphA2 and ROR1, in lung cancer cells based on the RTK antibody array experiment. Our results indicate that HIV-associated EVs in the body fluids contribute to comorbidities, particularly cancer progression and co-infection of KSHV, in people living with HIV.

085

Abstract:

086

Abstract:

087

Abstract: