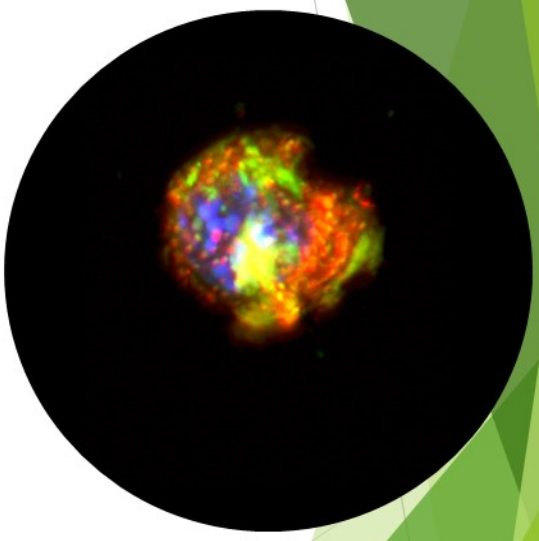
COBRE Center for SCA Stem Cells and Aging

Biologic Ramifications and the Therapeutic Potential of Extracellular Vesicles



Extracellular Vesicle Symposium

MARCH 28, 2019

Hotel Providence

139 Mathewson Street
Providence, RI 02903

Sponsored by

The Stem Cells and Aging Centers of Biomedical Research Excellence (COBRE)

Rhode Island Hospital Department of Hematology Oncology Research

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2019 Extracellular Vesicle Symposium
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GENERAL INFORMATION

The COBRE Center for Stem Cells and Aging is established in the Department of Hematology/Oncology Research, Rhode Island Hospital.

Principal Investigator: Peter J. Quesenberry MD
Associate Director: John Sedivy PhD

The Stem Cells and Aging COBRE program consists of research focused on stem cells and various aspects of aging, fibrosis and cellular senescence. Projects focus on hematopoietic stem cells, their microenvironment and the impact of aging on the fibrotic component of that microenvironment, while other projects deal with neural stem cells and their regulation with aging. Promising applications for the research include regeneration and repair for the treatment of leukemia, lymphomas, various neurodegenerative disorders and different aspects of aging.

Website: https://www.lifespan.org/centers-services/cobre-center-stem-cells-and-aging

LEARNING OBJECTIVES
At the conclusion of this activity, participants should be able to

- Understand the basic biology of extracellular vesicles.
- Understand the initial separative and characterization approaches in extracellular vesicle research.
- Understand the functional effects of vesicles and possible applications to injury and disease states.

TARGET AUDIENCE
Faculty, researchers, and affiliated staff at Lifespan, Brown, and URI who are interested in the biological and clinical role and potential of extracellular vesicles. Physician scientists and junior faculty are encouraged to attend.

SCHEDULE OVERVIEW

Continental Breakfast	8:00 AM - 9:00 AM
Morning Session	8:30 AM - 11:45 AM in the *Tilden Ballroom,* First Floor
Lunch	11:45 AM - 12:45 PM in the *Main Dining Room*, First Floor
Afternoon Session	12:45 PM - 4:40 PM in the *Tilden Ballroom,* First Floor
Reception	4:40 PM - 5:40 PM in the *Main Dining Room,* First Floor

HOTEL Wi-Fi Password: **Hotel139**

2019 Extracellular Vesicle Symposium
PROGRAM

8:00-8:30 AM **REGISTRATION & CONTINENTAL BREAKFAST**
Upon arrival, please check in at the registration table to pick up your program booklets and name badges. A continental breakfast will be available.

Presentations will be in the Tilden Thurber Ballroom

8:30 - 9:00 AM *Opening Remarks by Peter J. Quesenberry MD*
Paul Calabresi Professor of Oncology, Professor of Medicine, Warren Alpert Medical School of Brown University, Director, Research Division of Hematology/Oncology, Rhode Island Hospital, Director/Principal Investigator, COBRE Center for Stem Cells and Aging

9:00 – 9:45 AM **Keynote:** *Extracellular vesicles as stem cell paracrine mediators*
Giovanni Camussi MD
Professor Emeritus, Department of Medical Sciences, Scientific Coordinator of Stem Cell Projects at 2i3T Incubator and Technology Transfer, Molecular Biotechnology Center, University of Torino

9:45 – 10:00 AM Q&A

10:00 – 10:15 AM **BREAK**

James Padbury MD (Moderator)
Chief of Pediatrics, Women & Infants Hospital
Oh-Zopfi Professor of Pediatrics and Perinatal Research, Warren Alpert Medical School of Brown University

10:15 – 10:40 AM *Understanding Preeclampsia using Alzheimer's Etiology and Exosomal Cargo*
Surendra Sharma MD PhD
Professor of Pediatrics, Warren Alpert Medical School of Brown University; Director, COBRE for Reproductive Health, Women & Infants Hospital

10:40-10:45 AM Q&A

10:45 – 11:10 AM *Extracellular Vesicles and Reproductive Health*
Jessica S. Schuster PhD
Instructor in Pediatrics, Warren Alpert Medical School of Brown University; Department of Pediatrics, Women & Infants Hospital

11:10-11:15 AM Q&A

11:15 – 11:40 AM *Mechanical stretch differentially regulates release of miRNA EV in MLE12*
Juan Sanchez-Esteban MD
Associate Professor of Pediatrics (Exosomes and lung development), Warren Alpert Medical School of Brown University; Neonatal-Perinatal Medicine, Pediatrics, Women & Infants Hospital

11:40-11:45 AM Q&A

11:45 AM – 12: 45 PM **LUNCH** (Main Dining Room)

Bharat Ramratnam MD (Moderator)
Vice Chair of Research, Brown Medicine, Warren Alpert Medical School of Brown University
Chief Science Officer, Lifespan

12:45 – 1:10 PM *Daily rhythm modulates the ability of pulmonary-derived extracellular vesicles to alter target marrow cell phenotype*
Laura Goldberg MD PhD
Assistant Professor of Medicine, Warren Alpert Medical School of Brown University, Division of Hematology/Oncology, Rhode Island Hospital

1:10-1:15 PM Q&A

1: 15 – 1:40 PM *Prevention and reversal of pulmonary hypertension by MSC-EVs*
Olin Liang PhD
Assistant Professor of Medicine, Division of Hematology/Oncology, Warren Alpert Medical School of Brown University, Rhode Island Hospital

1:40-1:45 PM Q&A

1:45 – 2:10 PM *Prevention and reversal of pulmonary hypertension by MSC-EVs*
James R. Klinger MD
Professor of Medicine, Warren Alpert Medical School Brown University; Division of Pulmonary, Sleep and Critical Care Medicine, Rhode Island Hospital

2:10-2:15 PM Q&A

2:15 – 2:40 PM *Mesenchymal stem cell derived vesicles therapy for mitigation of radiation damage*
Sicheng Wen MD PhD
Assistant Professor of Medicine, Division of Hematology/Oncology, Warren Alpert Medical School of Brown University, Rhode Island Hospital

2:40 – 2:45 PM Q&A

2:45 – 3:00 PM **BREAK**

Peter Quesenberry MD (Moderator)
Paul Calabresi Professor of Oncology, Professor of Medicine, Warren Alpert Medical School of Brown University
Director, Research Division of Hematology/Oncology, Rhode Island Hospital
Director/Principal Investigator, COBRE Center for Stem Cells and Aging

3:00 – 3:20 PM *Extracellular Vesicles Play Multiple Roles in Leukemic Initiation, Identification and Treatment*
Theodor Borgovan MD PhD
Division of Hematology/Oncology, Rhode Island Hospital

3:20 – 3:25 PM Q&A

3:25 – 3:45 PM *Producing Functional Mesenchymal Stem Cell Extracellular Vesicles Using a Hollow Fiber Bioreactor*
Michael Del Tatto
Division of Hematology/Oncology, Rhode Island Hospital

3:45 – 3:50 PM Q&A

3:50 – 4:10 PM *Inflammation genetic profile in head trauma patient salivary extracellular vesicles*
Mandy Pereira
Division of Hematology/Oncology, Rhode Island Hospital

Yan Cheng PhD
Division of Hematology/Oncology, Rhode Island Hospital

4:10 – 4:15 PM Q&A

4:15 – 4:35 PM *Eliminating HIV/AIDS with novel biologics*
Xiaoli Tang, PhD
Assistant Professor of Medicine, Division of Infectious Diseases, Rhode Island Hospital, Warren Alpert Medical School of Brown University

4:35 – 4:40 PM Q&A

4:40 - 5:40 PM **RECEPTION** (Main Dining Room, First Floor)

KEYNOTE SPEAKER

Giovanni Camussi MD, is Professor Emeritus, Department of Medical Sciences, University of Torino, Italy. He has been Research Associate Professor in Microbiology and Pathology at the University at Buffalo, State University of New York, USA, and subsequently Full Professor of Nephrology at the University of Naples, at the University of Pavia and finally at University of Torino. He has been President of School of Medical Biotechnology, Director PhD program in Medical Pathophysiology, Director of the Laboratory of Renal and Vascular Pathophysiology and of the Research Center for Experimental Medicine and of the Immunopathology Laboratory of the Department of Medical Sciences. He has been also Director of the Department of Internal Medicine at the University of Torino. He is currently Scientific Coordinator of Stem Cell Projects at 2i3T Incubator and Technology Transfer and of the Stem Cell Laboratory at the Molecular Biotechnology Center University of Torino. He has over 500 publications in PubMed-indexed journals, dealing with inflammatory mediators, renal, liver and lung immunopathology, neo-angiogenesis, transplantation, stem cell biology and extracellular vesicles (https://www.ncbi.nlm.nih.gov/pubmed/?term=camussi+g).

Presentation Title: *Extracellular vesicles as stem cell paracrine mediators*

Extracellular vesicles (EVs) have recently emerged as a mechanism of cell-to-cell communication. They act as vehicles that deliver biologically active molecules from originator to recipient cells therefore influencing the phenotype and function of the latter. Stem cell-derived EVs act as paracrine mediators of stem cell action as they may activate regenerative programs in injured cells. We investigated the ability of EVs released by stem cells from different origins to modulate relevant cellular processes involved in tissue regeneration both at molecular and functional levels. Comparative studies have shown that these EVs derived from different stem cell types have both distinct and convergent mechanisms of action that differentially activate regenerative process such as cell proliferation, angiogenesis, oxidative stress, inflammation, immuno-tolerance and fibrosis. Through this analysis, we will review the potential application of stem cell-derive EVs in various preclinical models of human diseases.

PRESENTER ABSTRACTS

Theodor Borgovan MD, PhD
Division of Hematology/Oncology, Rhode Island Hospital

Extracellular Vesicles Play Multiple Roles in Leukemic Initiation, Identification and Treatment

T.Borgovan, MD1, P. Quesenberry, MD1, C. Nwizu2, L. Crawford, PhD2
1. Brown University Oncology Department
2. Warren Alpert Medical School

We have shown that extracellular vesicles (EVs) from explant prostate cancer induce a neoplastic phenotype in normal prostate cell lines. We have also shown EVs from mesenchymal stem cells (MSC) can have a healing effect in multiple disease states. The role of EVs in leukemia may provide insight into the pathophysiology of nascent disease, diagnosis and for therapeutic advances.

Mandy Pereira and **Yan Cheng PhD**
Division of Hematology/Oncology, Rhode Island Hospital

Inflammation genetic profile in head trauma patient salivary extracellular vesicles

Yan Cheng, Mandy Pereira, Neha Raukar, John Reagan, Mathew Quesenberry, Laura Goldberg, Bharat Ramratnam, Theodor Borgovan, Jeffrey Rogg, W Curt LaFrance Jr., Mark Dooner, Maria Deregibus, Giovanni Camussi, Peter Quesenberry

Mild Traumatic brain injury (mTBI) currently does not have reliable markers for diagnosis. Extracellular vesicles (EVs) released by damaged cells into biological fluids could be used to isolate potential biomarkers for diagnosis and progression of disease or injured state. EVs are known to traffic from the brain to the oral cavity and can be detected in saliva. We hypothesize the genetic profile of salivary EVs in patients who have suffered head trauma will differ from normal healthy controls, thus constituting a unique expression signature for mTBI. We enrolled a total of 19 subjects including for saliva sampling, 7 controls with no history of head traumas, 6 patients enrolled from an outpatient concussion clinic, and 6 patients from the emergency department who had sustained a head trauma within 24 hours. We performed real time PCR of the salivary EVs of the 19 subjects profiling 96 genes from the TaqMan Human Inflammation array. Real time PCR analysis revealed nine ($p<0.05$) upregulated genes in emergency department patients and 13 ($p<0.05$) upregulated genes in concussion clinic patients, when compared to controls. Each patient group had a unique profile, when compared to each other with 15 genes statistically significantly altered ($p<0.05$). Our results demonstrate that salivary EVs gene expression can serve as a viable source of biomarkers for mTBI.

Michael Del Tatto
Division of Hematology/Oncology, Rhode Island Hospital

Producing Functional Mesenchymal Stem Cell Extracellular Vesicles Using a Hollow Fiber Bioreactor

Michael Del Tatto, Sicheng Wen, Theodor Borgovon, Mark Dooner, and Peter Quesenberry, The Warren Alpert School of Medicine at Brown University; Rhode Island Hospital, Department of Medicine, Division of Hematology/ Oncology. Providence, Rhode Island.

For years, Mesenchymal Stem Cells (MSCs) have been used in treating cardiovascular diseases, stroke, spinal cord injury, kidney injury, lung injury and graft-versus-host disease. More recently Extracellular Vesicles (EVs) from MSCs have been discovered to produce similar effects. This creates a need for large numbers of EVs produced by MSCs. Traditional cell culture technique involves growing the MSCs in large numbers of tissue culture flasks. This is a very time and space consuming and an expensive endeavor. To address this problem an alternative method of culturing the cells has been developed. In this study we utilized a Hollow Fiber Bioreactor (HFBR) by FiberCell Systems. The FiberCell System HFBR is a high-density, continuous perfusion culture system that closely approximates the environment in which cells grow *in vivo.*
The HFBR was tested using MSCs under various culture conditions such as; cell number, media type and HFBR cartridge size. MSCs derived from adipose as well as Bone marrow were grown in the HFBR. The EVs produced by the HFBR were isolated in the same fashion as our laboratory isolates Flask derived EVs. The conditioned media is spun 100,000XG for 70 minutes and the pellet resuspend in 1% DMSO and frozen at -85^{0}C.
The function of the MSC EVs were tested by using several different assays; FDCP proliferation, FDCP radiation recovery and 3T3 fibroblast scratch assay. The HFBR produced EVs that were as functional as EVs from traditional flask cultures based on our functional assays.
The HFBR does not produce more EVs than traditional flask culture but it has many benefits. Compared to flask cultures the HFBR requires only a fraction of cell culture time, less incubator space and uses less culture media, making it a viable method for producing functional MSC EVs.

Laura Goldberg MD, PhD
Assistant Professor of Medicine, Division of Hematology/Oncology, Rhode Island Hospital, Warren Alpert Medical School of Brown University

Daily rhythm modulates the ability of pulmonary-derived extracellular vesicles to alter target marrow cell phenotype

Laura Goldberg, Yan Cheng, Shannon Johnson, Mandy Pereira, Connor Stewart, Sicheng Wen, Michael Del Tatto, Elaine Papa, Yanhui Deng, Jason Aliotta, Mark Dooner, Peter Quesenberry

Introduction: Extracellular vesicles (EVs) have tremendous therapeutic potential and it is becoming increasingly important to fully delineate the factors influencing their biogenesis and function in order to help standardize isolation techniques and establish normative controls. Numerous factors are thought to influence EVs, but little is known about how circadian oscillations alter EV function. We report here our studies exploring the effects of circadian rhythm on EV-mediated intercellular communication.

Method: We used a well-established *in vitro* model in which lung-derived EVs co-cultured with whole bone marrow (WBM) alter the target WBM transcriptome with an increase in pulmonary specific epithelial mRNAs. For these studies, C57BL/6 mice were entrained in 12-hour light/12-hour dark boxes, and at discrete Zeitgeber times (ZTs), lungs were isolated, minced and co-cultured across a cell-impermeable membrane with murine whole bone marrow (WBM) harvested at a constant ZT. Alternatively, WBM was harvested at discrete ZTs and co-cultured with lung-derived EVs harvested at a constant ZT. After 24hours of co-culture, WBM was collected, RNA isolated, and the amount of pulmonary specific mRNA levels was quantified by RT-PCR.
Results: There were clear time of day dependent changes in the ability of lung-derived EVs to modulate the transcriptome of WBM cells. These influences were seen when either the time of day of the EV originator tissue or the target WBM cells was altered. We found that peak pulmonary-specific mRNA levels were seen when WBM was exposed to lung harvested at 11am or 11pm. In turn, WBM was most susceptible to lung-derived EV communication when the marrow cells themselves were harvested at 3pm-7pm.

Conclusion: Based on our data, we conclude that circadian rhythm is likely an important modulator of EV-mediated intercellular communication and warrants further attention in EV research.

James R. Klinger MD
Professor of Medicine, Warren Alpert Medical School Brown University; Division of Pulmonary, Sleep and Critical Care Medicine

Prevention and reversal of pulmonary hypertension by MSC-EVs
J. R. Klinger, M. Pereira, S. Wen, M. Del Tatto, M. Dooner, T. Borgovan, L. Goldberg, J. M. Aliotta, C. E. Ventetuolo, P. J. Quesenberry, O. D. Liang

Division of Pulmonary, Sleep and Critical Care Medicine, and Division of Hematology/Oncology, Rhode Island Hospital and the Alpert Medical School of Brown University, Providence, RI, USA.

Rationale: In previous studies, we found that extracellular vesicles isolated from mesenchymal stem cells (MSC-EVs) prevented and reversed pulmonary hypertension (PH) in mice treated with monocrotaline. In the present study, we aimed to determine if MSC-EVs could blunt the development of more severe PH, right ventricular (RV) hypertrophy, and pulmonary vascular remodeling in a larger animal model of PH that more closely resembles human disease.

Methods: Males Sprague Dawley rats weighing approx. 200 g were injected with the VEGF receptor antagonist Sugen5416 at a dose of 25 mg/kg s.c. and placed in hypoxic chambers (10.5% O2) for 3 weeks, followed by 1 week of normoxic recovery (Su/Hx/Nx). Control rats were injected with an equal volume of DMSO vehicle and kept under normoxic conditions during the same time period (Nx Ctrl). EVs were isolated from adult human MSCs (Lonza, Switzerland) and administered by tail vein injection at a dose of 100 □g/kg in 500 □l of phosphate buffered saline (PBS) following different prevention and reversal protocols after SuHx treatment began. Treatment controls were given an equal volume of PBS alone. At the end point of the experiments, rats were anesthetized with isoflurane and right ventricular systolic pressure (RVSP) was measured by inserting a Millar® catheter into the right ventricle via the internal jugular vein. Rats were then sacrificed by exsanguination and the heart and lungs removed. RV hypertrophy was assessed by using Fulton's index [right ventricle to left ventricle + septum wet weight ratio (RV/LV+S)]. Lungs were fixed for histologic analysis. Pulmonary vascular muscularization was assessed by immunohistochemical (IHC) staining of □-smooth muscle actin. CD68+ macrophages and CD206+ M2-polarized macrophages were quantified following IHC staining. Total numbers of distal pulmonary vessels ≤ 50 □m were determined following vWF IHC staining.

Results and Conclusions: Compared to Nx Ctrl, Su/Hx/Nx treated rats had greater RVSP, RV hypertrophy and pulmonary vascular remodeling. In various prevention and reversal protocols, PH-rats treated with MSC-EVs showed significant reduction in RVSP, RV hypertrophy and pulmonary vascular muscularization. A marked increase in the presence of CD206+ M2-polarized macrophages, CD206+/CD68+ ratio, as well as total numbers of distal pulmonary vessels in MSC-EV treated animals suggest that alternative macrophage polarization and a likely M2-induced angiogenesis in the lung contributed to the normalization of the PH-rats. These results confirm the findings of our earlier studies showing that MSC-EVs prevent and reverse PH in mice treated with monocrotaline and suggest that MSC-EVs may represent a new therapy for the treatment of PH.

Olin Liang PhD
Assistant Professor of Medicine, Division of Hematology/Oncology, Rhode Island Hospital, Warren Alpert Medical School of Brown University

Prevention and reversal of pulmonary hypertension by MSC-EVs

J. R. Klinger, M. Pereira, S. Wen, M. Del Tatto, M. Dooner, T. Borgovan, L. Goldberg, J. M. Aliotta, C. E. Ventetuolo, P. J. Quesenberry, O. D. Liang

Division of Pulmonary, Sleep and Critical Care Medicine, and Division of Hematology/Oncology, Rhode Island Hospital and the Alpert Medical School of Brown University, Providence, RI, USA.

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Juan Sanchez-Esteban MD

Associate Professor of Pediatrics (Exosomes and lung development), Warren Alpert Medical School of Brown University; Neonatal-Perinatal Medicine, Pediatrics, Women & Infants Hospital

Mechanical stretch differentially regulates release of miRNA EV in MLE12

T. Najrana, L. Goldberg*, C. Schorl**, P. Quesenberry,* A. Uzun, and J. Sanchez-Esteban

Dept. of Pediatrics, Women & Infants Hospital, Prov. RI *Dept. of Medicine, Rhode Island Hospital, Prov., RI; **Dept. of Molecular and Cellular Biology and Biochemistry, Prov. RI

Introduction: Incomplete development of the lung can cause severe neonatal morbidity and mortality. Lung morphogenesis depends on mechanical signals. However, the mechanism by which mechanical forces promote lung development is not well-characterized. Extracellular vesicles (EVs) are membrane-bound particles released by cells. They are rich in miRNA and other molecules. MiRNAs have been shown to play critical roles in cell signaling during fetal lung development. The role of EVs in fetal lung development is unexplored.
Hypothesis: We hypothesized that stretch-induced release of EV-miRNA are important for fetal lung development.

Methods: MLE-12 cells, a murine cell line of alveolar type II epithelial cells, were exposed to continuous and cyclic stretch to mimic mechanical signals in fetal lung development. Cells under static conditions were used as control. Condition medium was collected and EVs were isolated using differential centrifugation. Size and quantification of EVs were determined by NanoSight technology. Total RNAs isolated from EVs were used to study miRNA profile by micro-array assay.
Results: Several miRNAs important for cell differentiation, proliferation, apoptosis and growth regulation were differentially regulated in the EVs after mechanical stretch.
Conclusions: Mechanical signals release specific EV/miRNAs in MLE12. We speculate that stretch-mediated release of miRNA is important for cell-to-cell communication during fetal lung development.

Jessica S. Schuster PhD
Instructor in Pediatrics, Warren Alpert Medical School of Brown University; Department of Pediatrics, Women & Infants Hospital

Extracellular Vesicles and Reproductive Health

Jessica Schuster, Valeria Zarate, Anthony Agudelo, Cherly Felber, Alper Uzun, James Padbury

Extracellular vesicles (EVs) are small lipid enclosed complexes secreted from cells under both physiological and pathological conditions. They contain proteins, messenger RNA, microRNA, and lipids and are involved in cell to cell communication and signaling. All biological fluids contain EVs and they are produced from all cells. There is mounting evidence for the role of EVs throughout the course of human reproduction. Changes within EV populations have been identified in various reproductive disorders. Despite decades of research, there still remain many unknowns regarding the mechanistic onset of labor, the placental involvement in fetal programming, and many reproductive disorders remain idiopathic. We are examining the microRNA and mRNA cargo profiles of exosomes in late stage of pregnancy vs postpartum and in women diagnosed with severe preeclampsia both for mechanistic insight and markers for early detection. Additionally, in order to gain insight into fetal programming and fetal/placental crosstalk, we are comparing the RNA profiles from exosomes in the umbilical vein versus the umbilical artery. We have isolated exosomes and sequenced both the mRNA and microRNA cargo of paired umbilical artery/vein from 10 full term women undergoing elective c-section. Sequence analysis reveals differential expression in microRNA leaving the placenta entering the fetus compared to that leaving the fetus returning to the placenta. MicroRNAs from the mir125 family are the most differentially expressed. Alterations in the expression of this miRNA family have previously been shown to be related to adverse pregnancy outcomes and target several genes known to be involved in the maintenance of pregnancy.

Surendra Sharma MD PhD
Professor of Pediatrics, Warren Alpert Medical School of Brown University; Director, COBRE for Reproductive Health, Women & Infants Hospital

Understanding Preeclampsia using Alzheimer's Etiology and Exosomal Cargo

Surendra Sharma, Shibin Cheng, Zheping Huang, Sukanta Jash, Sayani Banerjee, Warren J Huber, Paula Krueger, Xiao Zhou, Kun Ping Lu, Yoel Sadovsky, Akitoshi Nakashima, Shigeru Saito

Supported by the NIH grants 1P20 GM121298-01 (COBRE for Reproductive Health) and P30 GM114750 (COBRE for Perinatal Biology) and a Brown University DEANS Award

Pregnancy has been characterized as a stress factor in woman's life and recognized as a window to woman's future health. During pregnancy, the mother faces trauma/physiological stress because of the demands of the developing fetus. Sequelae of health risk factors can predispose pregnant women to severe pregnancy complications and long-term health risks. Preeclampsia (PE), new onset of hypertension and proteinuria with severe features after 20th week of gestation, is a multifactorial syndrome and affects 5-8% of all pregnant women

with a myriad of manifestations for both mother and offspring. PE has been linked to a higher incidence of future chronic health risks such as cardiovascular disease and diabetes in mothers and obesity in the offspring and entails the sequelae of health risk factors discussed above. A similar set of trauma-associated events are prevalent in chronic traumatic encephalopathy/traumatic brain injury (CTE/TBI) and Alzheimer's disease (AD). Our group has had long term interest in probing the question whether PE and diseases such as CTE and AD are programmed by similar mechanistic underpinnings. We have recently published novel findings suggesting that PE is etiologically associated with protein misfolding and aggregation, a common mechanism that underlies the onset of AD-like symptoms. In addition, our recent collaborative work has identified a unique role of *cis P-tau* in PE. *Cis* P-tau is prone to protein aggregation and induces tau pathology and neurodegeneration in TBI and AD. Our preliminary data are intriguing in that *cis* P-tau is significantly detected in the PE placenta and hypoxia-treated human primary trophoblasts with concomitant inhibition of physiological *trans P-tau*. Our published data also provide evidence for the presence of protein aggregates in the placental exosomal cargo from PE deliveries. We will discuss therapeutic options for inhibiting *cis* P-tau and for blocking onset of PE-like features in a humanized mouse model using small molecular drugs. Based on our results, we hypothesize that protein aggregation and toxic deposition in the PE placenta are common etiological links between PE, AD, and TBI and that the exosomal cargo plays a role in exposing trophoblasts or neurons to the pathology-causing protein aggregates and other detrimental factors.

Xiaoli Tang, PhD
Assistant Professor of Medicine, Division of Infectious Diseases, Rhode Island Hospital, Warren Alpert Medical School of Brown University

Eliminating HIV/AIDS with novel biologics

Xiaoli Tang, Huafei Lu, Mark Dooner, Stacey Chapman, Peter J. Quesenberry and Bharat Ramratnam

Replication competent HIV-1 persists in a subpopulation of CD4+ T lymphocytes despite prolonged antiretroviral treatment. This residual reservoir of infected cells harbors transcriptionally silent provirus capable of reigniting productive infection upon discontinuation of antiretroviral therapy. A commonly accepted strategy to eliminate HIV-1 from its reservoir is so called "Shock and Kill". Recently, we have engineered human cellular exosomes to express HIV-1 Tat, a protein that is a potent transactivator of viral transcription. Preparations of exosomal Tat activated HIV-1 in primary, resting CD4+ T lymphocytes isolated from antiretroviral treated individuals with prolonged periods of viral suppression and led to the production of replication competent HIV-1. To eliminate the CD4+ T cells hosting reactivated HIV-1 viruses, we engineered a dual affinity retargeting (DART) protein to mediate an HIV-1 specific immune clearance. The domain 1 and domain 2 of CD4 was fused to the single chain variable fragment of CD3ε antibody to generate this DART protein. An HA-tag was added to its C-terminus to facilitate the measurement of its expression. The DART protein (CD4-αCD3HA) could be detected in cell culture medium when an expression vector pAAV-CD4-αCD3HA was transfected into HEK293T or MOLT-4 cells. CD4-αCD3HA mediated killing of MOLT-4 cells transfected with a gp160 vector, U1 cells which constitutively express HIV-1. CD4-αCD3HA mediated killing of HIV-1 infected primary CD4+ T cells from HIV-1 infected and ART-treated patients. Furthermore, as a proof of concept, CD4-αCD3HA mediated the elimination of replication competent latent HIV-1 in an HIVE assay, warranting further investigation of its effectiveness in humanized mice.

Sicheng Wen MD, PhD
Assistant Professor of Medicine, Division of Hematology/Oncology, Rhode Island Hospital, Warren Alpert Medical School of Brown University

Mesenchymal stem cell derived vesicles therapy for mitigation of radiation damage

Sicheng Wen, Mark Dooner, Elaine Papa, Michael Del Tatto, Mandy Pereira, Yan Cheng, Theodor Borgovan, Laura Goldberg, Peter Quesenberry

Extracellular vesicles (EVs) including exosomes and microvesicles, have been studied for many years and were initially felt to be cell junk largely from platelets and erythrocytes. More recently, EVs have been found to deliver both mRNA and transcriptional modulators to target cells and affect their phenotype. Mesenchymal stem cells (MSC) have been shown to reverse radiation damage to bone marrow stem cells. In this study, we ask if MSC-EVs would reverse radiation damage to marrow stem cells and GI tract. Our data have shown that pretreated irradiated murine bone marrow stem cells with human or murine MSC-EV in vitro, could significantly improve the engraftment capacity of radiation-damaged stem cells up to 9 months post-transplantation. We have further assessed the therapeutic capacity of MSC-EVs by injecting them into C57BL/6J mice after 500 cGy whole body irradiation up to 378days after EVs injection. There was a significant WBC, RBC, HGB and PLT restoration in EV treated radiated mice compared to untreated mice in early period. The effect of MSC-EVs on reversal of radiation to bone marrow stem cells was further investigated by the competitive transplantation with whole bone marrow cells from 378 days post EV injection mice to lethally radiated mice. The engraftment rates in EV treated mice were significantly higher than untreated mice, indicating a long-term reversal effect on bone marrow stem cells. The in vivo biodistribution and localization of EVs detected by fluorescence molecular tomography indicated a radiation dose dependent accumulation of EVs at the site of injury bone marrow and spleen tissue, but undetectable in lung, heart and kidney. We also evaluated if MSC-EVs could rescue acute radiation damage in GI tract. After 24 hours post 1000 cGy TBI, mice were injected with MSC-EV daily for three days, the intestinal tissues were collected at day 4, 6 and 9 post radiation. Histopathological analysis of intestine from radiation exposed animals showed significant damage in the intestinal villi compared to normal mice from day 4 to 9 post-radiation. The lengths of the villi were markedly reduced and structure was largely destroyed. However, there was much less damage in EV treated mice. We also found that the expression of intestine stem cell (ISC) marker OLFM4 in crypts from MSC-EV treated, radiation exposed mice was higher than mice without MSC-EV treatment. We further isolated intestinal crypts from mice exposed to 1000 cGy radiation with/without EV treatment and cultured in Matrigel for 7 days to evaluate the enteroids formation. There was a significant improvement of enteroids formation in mice exposed to 1000 cGy radiation with EV treatment compared to EV untreatment control group. Thus, our preliminary data suggested that the effect of MSC-EVs on the mitigation of radiation damage in intestine by rescuing ISCs. In summary, our data indicate that MSC-EVs have the capacity to ameliorate radiation-induced damage to marrow and GI tract.

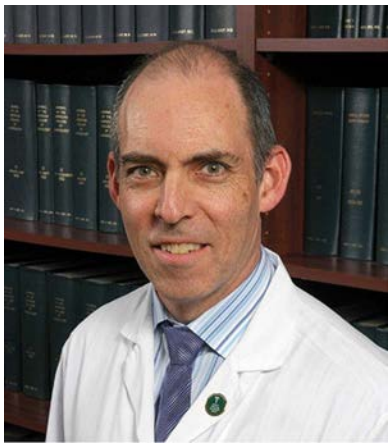
PRESENTER BIOGRAPHIES

Theodor Borgovan, MD PhD is a clinical oncology fellow spending some dedicated time with Dr. Peter Quesenberry and the Rhode Island Hospital Division of Hematology Oncology Research exploring the role of human cell line and patient-derived extracellular vesicles in Leukemia and other hematologic malignancies.

Yan Cheng, PhD is a Postdoc research fellow at Division of Hematology/Oncology in Rhode Island Hospital. Her research interests focus on investigation of human salivary extracellular vesicle biomarker studies and reversal radiation injury by mesenchymal stem cell-derived extracellular vesicles. She recently contributed to the publication "Potential biomarkers to detect traumatic brain injury by the profiling of salivary extracellular vesicles" published in the Journal of Cell Physiology.

Michael Del Tatto joined the Quesenberry laboratory at Rhode Island Hospital in 2005. His experience includes many projects including stem cell transplantation, pulmonary hypertension and cancer extracellular vesicles. Current he is working on techniques that will enhance the production and isolation of extracellular vesicles from primary mouse and human cells. His publications in the extracellular vesicle field include Marrow cell genetic phenotype change induced by human lung cancer cells Del Tatto, Michael et al. Experimental Hematology, Volume 39, Issue 11, 1072 – 1080

Laura Goldberg, MD PhD is currently an Assistant Professor at Brown University, Providence RI, in the Division of Hematology/Oncology at Rhode Island Hospital with interests in hematopoietic stem cell biology and extracellular vesicles. She obtained her MD and PhD in Molecular Genetics and Biochemistry from the Medical Scientist Training Program at the University of Pittsburgh, completed her residency in Internal Medicine at the University of Pittsburgh and her Hematology/Oncology Fellowship at the Warren Alpert Medical School of Brown University. Her current work is focused on the cycling nature of hematopoietic stem cells and the role of circadian rhythm in modulating stem cell function and influencing extracellular vesicle-marrow cell interactions.



James Klinger, MD is a member of the Division of Pulmonary, Sleep and Critical Care Medicine at Rhode Island Hospital and Professor of Medicine at the Warren Alpert Medical School of Brown University. His career has focused on both basic and clinical science aspects of pulmonary vascular disease including the role of the natriuretic peptides and nitric oxide and the importance of cGMP signaling in the lung. These areas of study contributed to the vast body of data in the 1990s that ultimately led to the development of phosphodiesterase inhibitors and soluble guanylyl cyclase stimulators for the treatment of patients with pulmonary arterial hypertension. More recently, Dr. Klinger's interests have examined the role of endothelial progenitor cells in the pathogenesis of pulmonary vascular disease and the use of mesenchymal stem cell extracellular vesicles for the treatment of pulmonary hypertension.

Dr. Klinger has served as the medical director of the Rhode Island Hospital Pulmonary Hypertension Center for the last 18 years and has served as the site PI on numerous investigator-initiated and industry-sponsored clinical trials in pulmonary hypertension. He has authored or co-authored over 100 publications in peer reviewed journals and edited 2 text books on pulmonary hypertension. Dr. Klinger has held numerous leadership positions in professional societies such as the American Thoracic Society, American College of

Physicians and the Pulmonary Vascular Institute. He has also served on the Scientific Liaison Committee of the Pulmonary Hypertension Association and the Pathology and Pathobiology Task Force for the World Symposium on Pulmonary Hypertension. He currently co-chairs the ACCP committee for treatment guidelines for pulmonary hypertension and serves as the pulmonary vascular disease editor for the medical journals "Lung" and "Current Hypertension Reports".

Olin D. Liang PhD is a graduate of Lund University in Sweden. He received rigorous training in molecular and cell biology in leading laboratories of renowned investigators at Boston Children's Hospital, Harvard Stem Cell Institute and Harvard Medical School. He joined the Rhode Island Hospital in 2013 and is currently an Assistant Professor of Medicine at the Warren Alpert Medical School of Brown University.

Dr. Liang is interested in several areas of scientific inquiry including stem and progenitor cell biology, cardiopulmonary vascular biology, and bone marrow hematopoiesis. His research encompasses a wide range of advanced in vitro and in vivo approaches as well as interrogation of clinical samples. A number of peer-reviewed publications including several seminal papers have resulted from his scientific endeavor.

Dr. Liang collaborates with other investigators across the Brown campus, RIH and Providence VA and participates in research training of undergraduate and graduate students as well as clinical fellows. Dr. Liang's lab is currently supported by grants from the National Institutes of Health and the American Heart Association.

James Padbury, MD PhD is the Oh–Zopfi Professor of Pediatrics and Perinatal Research at Brown University and Pediatrician-In-Chief at Women and Infants' Hospital. Dr. Padbury received his BS in biology from the UC Irvine and his MD degree from UCLA. His postgraduate training in pediatrics and neonatology was taken at UCSF and Boston Children's Hospital. He was recruited to Brown University from UCLA in 1995. He has long-standing research interests in cardiovascular and placental developmental biology and perinatal genetics. Among the awards since coming to Brown University are three

Center of Biomedical Research Excellence Awards (COBRE), grants from the March of Dimes to study the genetics of prematurity and NIH support to study the genetics of preeclampsia. He serves as a reviewer for NIH, NSF, the American Heart Association and the March of Dimes. He is past Chair of the NIH Pregnancy and Neonatology Study Section. He is Principal Investigator of the IDeA Clinical and Translational Research Award, *Advance-CTR*. This is a state-wide, $19.5M translational research award from NIGMS, based at Brown University, involving the University of Rhode Island, the Rhode Island Quality Institute and all three of the affiliated hospital systems.

Mandy Pereira has been a part of the Rhode Island Hospital Hematology Oncology Division of research since 2007. Her work includes pulmonary hypertension studies in both rodents and humans, and human salivary extracellular vesicle biomarker studies. She recently contributed to the publication "Potential biomarkers to detect traumatic brain injury by the profiling of salivary extracellular vesicles" published in the *Journal of Cell Physiology*.

Peter J Quesenberry is the Paul Calabresi, MD Professor of Oncology and Director of Hematology/Oncology Research at The Warren Alpert Medical School of Brown University in Providence, RI. He is a leading investigator in stem cell biology and extracellular vesicle research, both cutting edge areas. He lists over 500 publications, 345 on PubMed, and has previously been the Director of the Division of Hematology/Oncology at the University of Virginia, Director of the Cancer Center at UMass, Director of the Bone Marrow Transplant Program and Cancer Center at Roger Williams Medical Center, Director of the Division of Hematology/Oncology at The Warren Alpert Medical School of Brown University, Rhode Island Hospital and The Miriam Hospital, and most recently has been named the Director of Hematology/Oncology Research at The Warren Alpert Medical School of Brown University in Providence, RI. He is the American editor of the Journal of Extracellular Vesicles, and a previous editor of the Journal of Experimental Hematology. Dr. Quesenberry also served time in the United States Navy in Vietnam. Prior to this he was captain of the University of Virginia Lacrosse team and an honorable mention all American. He lives on the East Side of Providence with his wife Marilyn. He has two sons, Matthew Quesenberry who is in practice with him at Brown, and Preston Quesenberry who is a tax expert in the IRS and lives in Takoma Park, MD.

Bharat Ramratnam, MD is chief science officer at Lifespan, medical director of the Lifespan Clinical Research Center, and vice chair of research for the department of medicine at The Warren Alpert Medical School of Brown University, with appointments in the divisions of infectious diseases and hematology/oncology. He also leads the laboratory of Rhode Island Hospital's NIH-funded COBRE Center for Cancer Research Development.
In his role as chief science officer, Dr. Ramratnam provides scientific guidance to the vice president for research administration and to senior Lifespan management on matters of biomedical and translational science. In addition, he serves as co-chair of Lifespan's Research Advisory Committee, helps determine the goals and status of institutional core labs, and advises on the ongoing laboratory space management and new construction.

Dr. Ramratnam received his bachelor's and medical degrees from Brown University; and completed his internal medicine residency and chief residency at The Miriam Hospital. He was a clinical scholar at Rockefeller University in New York and completed a postdoctoral fellowship in virology at the Aaron Diamond AIDS Research Center at Rockefeller University in New York.

Dr. Ramratnam has received numerous awards including the NIH Career Development Award, the Doris Duke Clinical Scientist Award, the Daland Fellowship in Clinical Investigation from the American Philosophical Society, and the Culpepper Award from the Rockefeller Brothers Fund. Locally, he received the Lifespan Bruce Selya award for Research Excellence and the Dean's Teaching Excellence Award from the Warren Alpert Medical School of Brown University. He serves as a permanent member of the NIH AIDS Immunology and Pathogenesis Study Section.

Dr. Ramratnam's current research focuses on host factors that impact HIV-1 replication, including histone metabolism and noncoding RNA. His laboratory has made important contributions in multiple fields including virology, basic RNA biology, extra-cellular communication and translational.

Juan Sanchez-Esteban, MD is a neonatologist physician/scientist at Women & Infants Hospital of Rhode Island and Brown University. He has served on several NIH study sections. His long-standing research interest is to investigate the mechanisms regulating fetal lung development. He has made contributions to understanding how mechanical signals regulate fetal lung development. Current interest is to test the hypothesis that lung development is mediated via extracellular vesicles. His laboratory has extensive experience investigating cell signaling mechanisms in cultured fetal lung cells exposed to mechanical strain. As a result of these studies, they have identified signaling pathways by which mechanical forces promote differentiation of fetal distal lung epithelial cells. Interestingly, some of these molecules have been found inside the exosomes.

Jessica S. Schuster, PhD received her BA in Mathematics from Cornell University and her Masters and PhD in Applied Mathematics with a concentration in Genetics/Bioinformatics from Brown University in 2014. She completed her postdoctoral training at Women and Infants under the mentorship of Dr. James Padbury as part of the March of Dimes Grant for Prematurity**.**

Dr. Schuster currently serves as an Instructor of Pediatrics (Research) at Women & Infants Hospital of Rhode Island. Her research interest is in bioinformatics and genetics with a focus on reproductive disorders of pregnancy, including preterm birth and preeclampsia.

Surendra Sharma, MD, PhD is Professor of Pediatrics and Director of the COBRE for Reproductive Health at Women and Infants Hospital-Brown University, Providence, Rhode Island, USA. In the recent past, he served as President of the American Society of Reproductive Immunology. He has served as the organizer and/or the Scientific Chair of numerous National and International Conference on Reproductive

Biology and Immunology. He has been well -funded from the NIH and other funding sources and has served as a review panel member of the NIH and American Diabetes review panels. He has trained MD and PhD students as well as clinical fellows. His research focuses on mechanistic underpinnings, predictive assays, and therapeutic options for pregnancy complications. His recent work describing an etiological and epidemiological link between preeclampsia and Alzheimer's disease has been widely received. He has successfully established well-defined mouse models for fetal loss, preeclampsia, preterm birth and gestational diabetes. He currently serves as Editor-in-Chief of the American Journal of Reproductive Immunology.

Xiaoli Tang, PhD is an Assistant Professor of Medicine (research) at the Division of Infectious Diseases, Department of Medicine, Rhode Island Hospital, Warren Alpert Medical School of Brown University. His research is focused on creating novel biologics to reactivate latent HIV-1 and eliminate HIV-1 reservoirs. Recently, he engineered Exo-Tat exosomes to specifically target CD4+ T cells to reactivate latent HIV-1. Currently he is working on engineering a DART molecule to mediate the killing of cells hosting activated HIV-1.

Sicheng Wen, MD PhD is an assistant professor (research) at Division of Hematology/Oncology in Rhode Island Hospital. His major research interests focus on investigation of reversal radiation injury by mesenchymal stem cell-derived extracellular vesicles and understanding the mechanisms underlying these biological functions.

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