Abstract Booklet

EViND - *Extracellular Vesicles in Neurodegenerative Diseases*

Monday 13th May 2024

Queensland Brain Institute (QBI), The University of Queensland,

St Lucia, Building 79, Main Auditorium, Level 7

Organising Institutions:

Queensland Brain Institute

CJCADR Clem Jones Centre for Ageing Dementia Research

THE UNIVERSITY OF QUEENSLAND AUSTRALIA | Centre for Extracellular Vesicle Nanomedicine

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Characterising Nanoparticles, EVs and exosomes is now quicker, easier and more accurate

Monday 13th May 2024
Queensland Brain Institute (QBI), The University of Queensland, St Lucia

Attend our talk by Dr Yu-Su Chen

Talk Title: Characterisation of EVs with the NanoSight Pro

Abstract: Nanoparticle Tracking Analysis (NTA) has proven to be a valuable and an effective tool for extracellular vesicle (EVs) characterisation. NTA provides visual confirmation and high-resolution particle size and concentration data within minutes allowing the instant assessment of sample stability but also complexity. With the introduction of the new NanoSight Pro, characterisation of EVs is easier and quicker than before.

Powered by machine learning algorithms, measurement subjectivity is reduced and automated processing enabled to assure superior Nanoparticle Tracking Analysis. The NanoSight Pro is packed with smart features, providing greater sensitivity in biologicals detection, high reproducibility, and enhanced fluorescence measurements for detecting sample subpopulations.

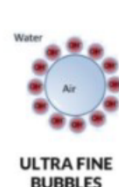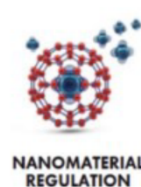### *You are invited to attend our lunchtime demo: 1-2pm*

The Malvern Panalytical NanoSight Pro is accessible to all levels of user and supports automated processing to remove subjectivity allowing for faster walk-away analysis and more accurate size and concentration data when operating in both Standard (light scatter) mode and in Fluorescent mode. Join us and try it yourself! Samples welcome.

GUEST SPEAKER

Dr Yu-Su Chen, is a Field Application Specialist, at Malvern Panalytical based in the UK. For close to 10 years Dr Chen has provided support and application advice to global biopharmaceutical research as well as manufacturers using a range of orthogonal analytical techniques like NTA, DLS, microcalorimetry and more. Her specialty is in the Pharma and Food sector supporting customers working with exosomes, extracellular vesicles and other drug delivery systems.

EXTRACELLULAR VESICLES

DRUG DELIVERY

VIRAL VACCINES

NANOMATERIAL REGULATION

PROTEIN AGGREGATION

ULTRA FINE BUBBLES

We also thank our Silver Sponsors for their contribution to making the EViND Symposium possible!

Talks from Invited Speakers

Monday, 13th May 2024

Neuron-microglia interaction via extracellular vesicles in Alzheimer's disease

Abstract: Extracellular vesicles (EVs) carry pathogenic molecules and play a role in the disease spread, including aggregated tau proteins. The Endosomal Sorting Complexes Required for Transport (ESCRT) machinery is responsible for the biogenesis of small EVs (exosomes); thus, targeting critical ESCRT molecules can disrupt EV synthesis. We hypothesize that microglia-specific targeting of ESCRT-I molecule Tsg101 suppresses EV-mediated propagation of tau pathology, leading to amelioration of the disease phenotype of the tauopathy mouse model. To test this hypothesis, we have studied microglia-specific deletion of Tsg101 in PS19 tau transgenic mice for the study. PS19 mice develop cognitive impairment as determined by Y-maze, forced alternation, novel object recognition and fear conditioning, which are reversed in PS19:Tsg101cKO mice. This is correlated with reduced Alz50+ tau accumulation, neurodegenerative microglial activation, neuroinflammation and complement pathway activation as determined by bulk RNA sequencing, ELISA, and neuropathology. Tsg101 cKO microglia show reduced expression of C3aR1 and CD68, and secretion of total and Tau+ EVs in vivo. Microglia-specific targeting of Tsg101 shows a beneficial effect for ameliorating the disease progression of the tauopathy mouse model via suppression of EV secretion, microglial activation, tau accumulation and complement-dependent synaptic pruning. Microglial Tsg101 is a potential therapeutic target of Alzheimer's disease and related tauopathy.

Biography: Dr. Tsuneya Ikezu is a Professor in the Department of Neuroscience and Director of Molecular NeuroTherapeutics Laboratory at the Mayo Clinic in Florida. After receiving his M.D. and Ph.D. at the University of Tokyo School of Medicine. Dr. Ikezu is best known for his groundbreaking work deciphering the role of extracellular vesicles (EVs) secreted from microglia on the progression of Alzheimer's disease (AD). Dr. Ikezu characterized the protein composition of EVs isolated from the brain tissues, cerebrospinal fluid, or plasma of AD patients and related disorders, and discovered pathological function of EVs and their potential as cell type-specific biomarkers for neurodegenerative diseases.

Prior to this, Dr. Ikezu was Professor of Pharmacology and Neurology at Boston University School of Medicine until 2020. He has authored more than 139 journal articles, which were cited over 23,000 times and served on several editorial boards including Journal of Extracellular Vesicles. Dr. Ikezu has received numerous awards including Inge Grundke-Iqbal Award from Alzheimer's Association (2016), Jack Spivack Excellence in Neuroscience Award (2018), and Investigator of the Year Award from Mayo Clinic (2023). Dr. Ikezu also serves as co-chair of Extracellular Vesicles in Nervous System Group sponsored by the International Society of Extracellular Vesicles.

Understanding the role of EVs in Tau pathology – A quest for therapeutic targets

Abstract: Tauopathies represent a spectrum of neurodegenerative conditions characterised by the accumulation of tau protein within neurons, resulting in the formation of neurofibrillary tangles—a hallmark feature of dementias such as Alzheimer's disease. Despite decades of research, our comprehension of the origins of dementia remains incomplete, and effective treatments for tauopathies are lacking. However, emerging evidence suggests that tau aggregates serve as proteopathic "seeds," catalysing the misfolding and aggregation of normal tau proteins, thereby perpetuating a self-propagating cycle that alters the conformation of soluble tau. Remarkably, this pathological amplification extends beyond the affected neurons, as tau seeds propagate trans-synaptically and traverse extracellular spaces. Our pioneering investigations have revealed that small extracellular vesicles (EVs), known as exosomes, have the capacity to harbour tau seeds capable of initiating tau aggregation. In my presentation, I will provide an overview of several groundbreaking studies that have significantly advanced our understanding of the critical roles played by EVs and vesicle-free tau seeds in both the initiation and propagation of tau pathology. These studies represent a critical step in our ongoing quest to identify novel therapeutic targets by exploring the deleterious effects, underlying mechanisms, and regulators of tau aggregation within various subcellular compartments.

Biography: Dr Juan Carlos Polanco leads the Exosomes and Tau Pathology research team within Prof Jürgen Götz's larger laboratory based in the Clem Jones Centre for Ageing Dementia Research (CJCADR) at the Queensland Brain Institute (QBI) of the University of Queensland. Supported by funding from the NHMRC, Dr Polanco's research focuses on three primary areas: investigating the involvement of small extracellular vesicles (EVs) called exosomes in the propagation of tau pathology in Alzheimer's disease, elucidating the mechanisms underlying exosomal cargo delivery to the cytosol, and exploring the genes and cellular processes associated with tau aggregation. His overarching objective is to uncover novel therapeutic approaches to combat tau pathology by identifying the cellular mechanisms driving tau aggregation. Dr Polanco has been at the forefront of groundbreaking research in exosomes and tau pathology. Notably, he pioneered the demonstration that exosome-like EVs containing tau seeds can induce tau aggregation. Furthermore, his team was instrumental in revealing that exosomes can trigger endolysosomal permeabilisation, providing an escape route for exosomal tau seeds into the cytosol. A core member of the International Society of Extracellular Vesicles Special Interest Group on Extracellular Vesicles in Nervous Systems, Dr Polanco also serves as Chair of the QBI's FACS Steering Committee. His impactful research has garnered widespread recognition, with his work being featured on the covers of esteemed journals such as Cell Reports and Acta Neuropathologica. Additionally, it has received coverage in prominent news outlets, including AlzForum, BioWorld, and The Australian, eliciting favourable attention and commentary.

It's in the blood: EV miRNA biomarkers associated with neurodegenerative diseases

Abstract: Several blood-based tests have been explored to detect Alzheimer's disease (AD) and other neurogenerative diseases however, evidence is required to determine whether blood sampling is an appropriate specimen to diagnose brain diseases. Exosomes are extracellular vesicles secreted by all cell types of different tissues and can be found in the bloodstream. They are enriched with miRNA and are involved in cell-cell communication. We have successfully isolated exosomes from brain tissue and hypothesize that exosomal miRNA in the brain are critical players in the pathogenesis of neurodegenerative diseases. Previously we isolated serum exosomes from AD patients which displayed an abnormal composition of 16 specific microRNA (miRNA) biomarkers compared to controls. To provide evidence that our serum exosomal miRNA biomarkers are suitable for the detection of a brain condition, we also profiled exosomes isolated from post-mortem human AD (n = 8), PD (n = 8), ALS (n = 7) and control (n = 5- 8 per group) brain tissues which were compared to profiles in the periphery. Brain derived exosomes (BDEs) from AD subjects were found to contain a unique profile of small RNA, including miRNA, compared controls which displayed some overlap between peripheral biomarkers. We further validated the serum exosomal biomarkers in a second study using PET (C-PiB-SUVR) classification (PET+, n = 171 and PET-, n = 139) to determine whether exosomal miRNAs could assist with managing costly PET referrals. Training towards PET classification provided an accuracy of >90% for predicting a PET+ result. This work has identified a highly specific panels of miRNA that is both present in the brain and blood of patients with neurodegenerative diseases. The miRNA candidates can be used to develop a blood-based diagnostic test highly relevant to a brain disease, equivalent to non-invasive brain biopsy.

Biography: Dr. Lesley Cheng is presently the Group Leader of the Neurodegeneration EV Biomarkers and Biology Lab at the La Trobe Institute for Molecular Science (LIMS), La Trobe University. Currently, her major focus is to develop a minimally invasive blood test for the early detection and monitoring of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. She has developed a stream-lined biomarker platform that uses next-generation sequencing to profile small RNA molecules extracted from extracellular vesicles (EVs) derived from biological fluids or tissues. Her lab uses this platform to identify various small RNA biomarkers for translational and commercial potential including applying this knowledge to understanding the role of EVs in disease pathology.

She was awarded a Bachelor of Medical Science with Honours and a PhD from Monash University. She is Deputy Director of the Research Centre of Extracellular Vesicles, La Trobe University and Principal Scientist at Carrier Biomed, China. She presented at TEDx Melbourne about her breakthrough work and her take on the '21st Century Innovator'.

Sources of variability in tissue EV composition: brain region and disease

Abstract: Not provided at the time of finalising the booklet.

Biography: Kenneth W. Witwer is an associate professor of molecular and comparative pathobiology and neurology at the Johns Hopkins University School of Medicine in Baltimore, United States. He is the current President of the International Society for Extracellular Vesicles (ISEV) and previously served as Secretary General and Executive Chair of Science and Meetings. His laboratory studies extracellular vesicles (EVs), noncoding and extracellular RNA (exRNA), and enveloped viruses, including HIV and SARS-CoV-2. Witwer is an Allen Distinguished Investigator, managing editor of the journal Cytotherapy, and a member of the Richman Family Precision Medicine Center of Excellence in Alzheimer's Disease. He has advised the US Environmental Protection Agency, the US National Institutes of Health, and various commercial entities.

Extracellular vesicles as a new paradigm of therapeutics

Abstract: Extracellular vesicles represent a paradigm shift in cell-free therapy with improved safety and easier handling/storage compared to cells. The extracellular vesicle field has a projected global market size of over $2.2B USD in 2030 (Emergen Research). The versatile bioactive cargo of extracellular vesicles makes them ideal candidates for treating multifaceted pathologies. My team is realising the potential of extracellular vesicles as effective, scalable, and safe therapeutics to alter the trajectory of life-threatening diseases. We are leveraging our key discoveries in extracellular vesicle manufacturing and the use of extracellular vesicles as drug carriers to enable clinically feasible and multitargeted approaches for treating disease, improving patient outcomes, and prolonging healthy lifespan.

Biography: Associate Professor Joy Wolfram leads a nanomedicine and extracellular vesicle research program with the goal of developing innovative approaches that bring the next generation of therapeutics directly to the clinic. She has joint appointments in the School of Chemical Engineering and the Australian Institute for Bioengineering and Nanotechnology at The University of Queensland. Her research program has three main focus areas: 1) developing improved methods for extracellular vesicle isolation from human biofluids, 2) designing hybrid drug delivery systems with extracellular vesicle and synthetic components for a 'best-of-both-worlds' approach to treat disease, and 3) understanding the role of extracellular vesicles in cancer immunoevasion and metastasis. Her research program has resulted in more than 90 publications in journals, such as, Nature Nanotechnology, Materials Today, and Nature Reviews Materials. These publications have been cited over 14,000 times (Google Scholar).

12.30pm – 12.45pm
Carlos Salomon – UQCCR, The University of Queensland, Australia

Shaping the Future of Extracellular Vesicle (EV) Research: Establishing the UQ Centre for EV Nanomedicine

Abstract: The field of nanomedicine is rapidly advancing, driven in part by the recent discovery of extracellular vesicles (EVs). EVs, small nanoparticles released by cells, play a crucial role in local and systemic cell communication by transferring bioactive cargo. With their potential for disease diagnosis and treatment, engineered EVs offer a promising avenue for delivering therapeutic agents with

enhanced precision and reduced side effects. The use of EVs in nanomedicine holds immense promise, with over 100 products currently in clinical trials. At The University of Queensland (UQ), numerous research groups are actively investigating EVs for disease diagnosis, treatment, and fundamental biological research. Recognizing the need to foster collaboration and advance research in EV nanomedicine, we established of The UQ Centre for EV Nanomedicine.

The primary objective of this centre is to facilitate interdisciplinary interactions among research groups focusing on EV nanomedicine. Through consultation and collaboration, the centre will provide training and education on experimental procedures and study design, ensuring that research conducted at UQ meets the highest international standards. By promoting excellence in EV nanomedicine research, the centre aims to maximize the potential for developing applications and products that ultimately benefit society.

Biography: Professor Salomon is the Director of the UQ Centre for Extracellular Vesicle Nanomedicine, Head of the Translational Extracellular Vesicles (EV) in Obstetrics and Gynae-Oncology Group, NHMRC Investigator Fellow (EL2) and a worldwide authority on EV biomarkers for complications of pregnancies. In the last 10 years, Professor Salomon's primary research and commercialisation activities have focused on the identification and validation of biomarkers, and development of In Vitro Multivariate Index Assays for clinically relevant complications (including ovarian cancers, and obstetrical syndromes) and their translation into clinical applications. He is a pioneer on investigation the release of extracellular vesicles (EVs) by the placenta and tumour cells and their utility as a biomarker for a wide range of pregnancy complications and ovarian cancer (OC). Prior to his research program, the field had little understanding of the changes in circulating EVs and their content across gestation and in OC progression. In pioneering this research, his program recruited, and collected biological samples, from over 20,000 participants in multiples studies in the USA, India, Chile, UK, and Australia in the last 10 years. He has optimised methods to isolate total and placenta and tumour-derived EVs present in circulation, and profiled their content by quantitative proteomic analysis, and miRNA sequencing; identifying for the first time, molecules within EVs associated with different complications of pregnancies, and at early stages of oncogenic transformation in OC. One of the most significant contributions of his research program has been the development of a test for early detection of ovarian cancer, OCRF-7, that displayed a classification efficiency of 98%. These outcomes of the research program provide a novel conceptual basis, and evidence for translation, resulting in changes in clinical practice and management.

12.45pm – 12.55pm

Yu-Su Chen – Malvern Panalytical, United Kingdom

Characterization of EVs with the NanoSight Pro

Abstract: Nanoparticle Tracking Analysis (NTA) has proven to be a valuable and an effective tool for extracellular vesicle (EVs) characterisation. NTA provides visual confirmation and high-resolution particle size and concentration data within minutes allowing the instant assessment of sample stability but also complexity. With the introduction of the new NanoSight Pro, characterisation of EVs is easier and quicker than before. Powered by machine learning algorithms, measurement subjectivity is reduced and automated processing enabled to assure superior Nanoparticle Tracking Analysis. The NanoSight Pro is packed with smart features, providing greater sensitivity in biologicals detection, high reproducibility, and enhanced fluorescence measurements for detecting sample subpopulations.

1.55pm - 2.30pm

Andrew Hill – Institute for Health & Sport, Victoria University, Australia

Extracellular vesicles and their role in prion-like mechanisms of protein misfolding and propagation

Abstract: Neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD) and prion diseases are associated with proteins that misfold and deposit in the brain. Many cell types, including neurons, release extracellular vesicles (EVs) which have been shown to be involved in processing of proteins such as APP, α-synuclein, and PrP which are those involved in AD, PD and prion diseases respectively. Roles for these vesicles include cell-cell signalling, removal of unwanted proteins, and transfer of pathogens (including prion-like misfolded proteins) between cells. Our group has shown that EV's contain distinct processed forms of these proteins and that, in the case of prion disease, they contain the transmissible form of the misfolded protein. Biophysical studies have also demonstrated that EVs can accelerate the misfolding of α-synuclein, suggesting they may be a platform for aggregation of these proteins. As EVs can be isolated from circulating fluids such as serum, urine, and cerebrospinal fluid (CSF), they provide a potential source of biomarkers for neurological conditions. This talk will review the roles these vesicles play in neurodegenerative disease and highlight their potential roles in disease pathogenesis.

The role of EVs in the initiation and progression of Parkinson's disease

Abstract: Over the past decade, our understanding of when Parkinson's disease starts, and how it can spread in the brain has changed significantly. We now know that the disease starts up to 20 years before motor symptoms are diagnosed, and that the transmission of α-synuclein within the brain is involved in the pathogenesis of the disease. However, little is known about the initial cellular event(s) that result in the propagation of pathology associated with Parkinson's disease. Defining this molecular event is of fundamental importance, as it would allow for the targeting of α-synuclein transmission before the irreversible spread of misfolded protein pathology. Here we have identified a mechanism for the loading of α-synuclein into exosomes, resulting in prion-like transmission that is mediated by the ubiquitin ligase activator, NDFIP1. Several common risk factors for Parkinson's disease can promote the interaction between α-synuclein and NDFIP1, resulting in ubiquitin-mediated trafficking of α-synuclein. We show that activating this pathway results in the loading of endogenous α-synuclein into exosomes, which when intranasally delivered to either wild-type or M83 transgenic mice, results in Parkinson's-like pathology including motor impairments and brain aggregates similar to Lewy bodies. Overall, our results demonstrate a novel mechanism to initiate the prion-like spread of α-synuclein in exosomes, following exposure to risk factors for Parkinson's disease. As such, we have identified a pathway for the initiation of Parkinson's disease, allowing for the development of new therapies.

Biography: Professor Jason Howitt is a neuroscientist who heads the Cell Signalling laboratory at Swinburne University. His interests span from single protein interactions, through to animal models of disease, and the analysis of human tissue. Dr Howitt's laboratory studies molecular signalling pathways in neurodevelopmental disorders, such as autism, and neurodegenerative diseases, with the majority of his lab working on Parkinson's disease. A focus of his laboratory is extracellular vesicle signalling, in both healthy and disease states. He is currently the Chair for the Department of Health Sciences, and was formally the Chair of the Florey Faculty at the Florey Institute of Neuroscience and Mental Health. Dr Howitt has held five CIA NHMRC grants over the past ten years and his laboratory is also funded by the Michael J Fox Foundation for Parkinson's Research.

A perfect circle: extracellular vesicles in the progression and treatment of retinal degeneration

Abstract: Aims. Age-related macular degeneration (AMD) is a significant cause of irreversible vision loss globally. Small endogenous cellular communication vehicles called extracellular vesicles (EVs) play essential roles in regulating retinal immune responses and maintaining homeostasis, with their depletion linked to hallmark pathological features of AMD. This study thereby examines whether restoring EV communication can alleviate retinal inflammation and cell death to slow the progression of AMD. **Method.** Retinal EV profiles (miRNA and proteins) were profiled in both healthy and degenerating mouse retinas using an established model of retinal degeneration; photo-oxidative damage (PD). Healthy mouse retinal EVs were intravitreally injected into mice undergoing retinal degenerations at a concentration of ~2.0x10^9 EVs/eye. Retinal function and structure were assessed using electroretinography (ERG) and optical coherence tomography (OCT) following 5 days PD post-EV administration. TUNEL and IBA-1+ immunohistochemistry were performed on retinal cryosections to gauge cell death and inflammation levels. **Results.** Results demonstrated a shift in the retinal EV concentration, and molecular profile (proteins and RNA) in response to retinal degeneration. Supplementation of healthy retinal-EVs in wild-type control animals were well-tolerated, with no signs of retinal inflammation or cell death. Mice subjected to retinal degeneration and receiving EV supplementation displayed a significant decrease in retinal degeneration. Compared to untreated controls, those mice injected with retinal EVs showed higher retinal function, reduced inflammation, and decreased photoreceptor cell death. **Conclusions.** Taken together, our data supports a central hypothesis in which a loss of retinal EV bioavailability is correlated to progressive retinal degeneration while supplementation of EVs reduces the pathological features of retinal degeneration. Results from this work, therefore, support the use of EV-based therapies to restore homeostatic communication pathways and slow the progression of retinal degeneration. Further, this work also demonstrates the use of the eye as an excellent model for studying EV and their potential neuroprotective properties.

Biography: I am currently the Associate Director of Research Development at the School of Medicine and Psychology (SMP) and Head of Clear Vision Research at The John Curtin School of Medical Research (JCSMR) at the Australian National University (ANU) in Canberra, Australia. My primary focus is on leading an internationally recognized research program aimed at reducing the severity and progression of Age-Related Macular Degeneration (AMD) using RNA, including microRNA (miRNA), and Extracellular vesicles (EVs). This groundbreaking work aims to understand and treat retinal degenerations, particularly focusing on the neuroinflammatory aspects of AMD. Additionally, I have a keen interest in commercializing research, holding patent positions on various technologies for treating retinal and neurodegenerative conditions, with plans to launch a startup company in 2024 based on our EV/miRNA research. My passion for community engagement, education, and leadership has led me to take a unique approach to nurturing the next generation of vision researchers and science communicators. This philosophy is reflected in my establishment of Clear Vision Research in 2017, providing a framework for supporting student and staff involvement in driving research goals, engaging with AMD-affected communities, and promoting science outreach. My support for Early Career Researchers (ECR) has been acknowledged through awards such as The Australian Institute of Policy and Science Tall Poppy Award (2019), the ANU College of Health and Medicine Dean's Commendation for Excellence in Supervision (2023), and the VC's Award for Excellence in Supervision (2023).