MOSES SCHANFIELD YOUNG INVESTIGATOR AWARD (MSYIA) PRESENTATIONS

Presentation number: YIA 01 Abstract number: ABS-63-ISABS-2024

A RAPID BLOOD TEST FOR BREAST CANCER DETECTION

Domljanovic Ivana[1], Loretan Morgane[2], Kempter Susanne[3], P. Acuna Guillermo[2], Kocabey Samet[1], Ruegg Curzio[1]

[1]Department of Oncology, Microbiology and Immunology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland; [2]Department of Physics, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland; [3]Department of Physics, Ludwig-Maximilians-University, Munich, Germany

ivana.domljanovic@unifr.ch

Breast cancer is the most frequent cancer in women. Mammography screening reduces breast cancer mortality through early diagnosis, but it has significant shortcomings. Therefore, an alternative rapid, accurate, safe, easy-to-use, and cheap test for breast cancer detection and monitoring is needed. Micro-RNAs (miRNAs) are non-coding RNA with cellular regulatory functions that are considered promising biomarkers since their expression is altered in cancers. However, routine measurement in blood is time-consuming, costly, and requires specialized labs. In this study, we designed and characterized an optical dynamic DNA origami book biosensor. It is precisely decorated with arrays of fluorophores acting as donors and acceptors and also with fluorescence quenchers that produce a strong optical readout upon exposure to external stimuli for the single or dual detection of target oligonucleotides and miRNAs. This biosensor allowed the detection of target molecules (miRNAs) either through the decrease of Förster resonance energy transfer (FRET) or an increase in the fluorescence intensity profile owing to a rotation of the constituent top layer of the structure. Single-DNA origami experiments showed that the detection of two targets can be achieved simultaneously within 10 min with a limit of detection in the range of 1–10 pM. Our DNA origami book biosensor showed sensitive and specific detection of synthetic target oligonucleotides and natural miRNAs extracted from breast cancer cells and blood from Women with breast cancer. Based on these results, we foresee that our DNA origami biosensor could be in future translated into an alternative rapid, accurate, safe, easy-to-use, and cheap test for breast cancer detection and monitoring, which will make personalized medicine more accessible to all women, advanced molecular biology, and eventually save lives.

Keywords: DNA origami biosensor, cancer biomarker, miRNA, breast cancer, diagnostic test

Presentation number: YIA 02 Abstract number: ABS-24-ISABS-2024

A COMPREHENSIVE ANALYSIS OF WHOLE MITOGENOME HETEROPLASMY PATTERNS IN DIFFERENT FORENSIC TISSUES

Claessens Floor[1], Farzad Maryam[1], Parson Walther[2,3], Børsting Claus[1], Pereira Vania[1]

[1]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [2]Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria; [3]Forensic Science Program, The Pennsylvania State University, University Park, PA, United States of America

floor.claessens@sund.ku.dk

Mitochondrial DNA (mtDNA) is a good candidate marker when nuclear DNA (nuDNA) analysis is not possible. This is particularly important in forensic samples containing small amounts of nuDNA such as hair shafts. However, mtDNA interpretation presents challenges, especially in the reporting of heteroplasmy, which is the presence of slightly different mtDNA genomes (mitogenomes) in a cell. Depending on the bottleneck size during transmission the proportion of detectable mtDNA sequences can vary between and even within tissues. This effect is pronounced in single hair shafts that are known for a tight bottleneck size. In this study, we aim to identify heteroplasmy patterns across three different human tissues using whole mitogenomes, analyzed with massively parallel sequencing. Blood and buccal reference samples from 36 unrelated individuals and 317 hair segments (each measuring 2 cm) were analyzed as part of this study. DNA extractions were performed using in-house pre-treatment protocols followed by DNA extraction with the EZ-1 extraction robot (Qiagen). Quantification of nuDNA in blood and buccal samples was performed using the Quantifiler Trio DNA quantification Kit (ThermoFisher Scientific, TFS). In the hair samples, mtDNA (and nuDNA if present) was quantified using the qPCR SD quants assay, with all quantification experiments performed on the 7500 Real-Time PCR system (Applied Biosystems). The Precision ID mtDNA Whole Genome Panel and the Ion Torrent Ion Gene Studio S5 sequencing platform with the Ion 530 Chip (both TFS), were used for library preparation and sequencing. This study provides insights into the heteroplasmic patterns observed across the different tissues, revealing high inter and intra-individual variations. The outcomes of this study hold the potential for the determination of mitochondrial somatic mutation rates and their incorporation in statistical evaluations, thus enhancing the interpretation and reporting of mtDNA data in forensic contexts.

Keywords: Mitochondrial DNA, Heteroplasmy, Human tissues, Massively Parallel Sequencing, Precision ID mtDNA Whole Genome Sequencing

Presentation number: YIA 03 Abstract number: ABS-50-ISABS-2024

CNS-ASSOCIATED MACROPHAGES CONTRIBUTE TO INTRACEREBRAL ANEURYSM PATHOPHYSIOLOGY

Glavan Martina[1,2], Jelic Ana[2], Levard Damien[2], Frösen Juhana[3], Keränen Sara[3], Franx Bart[4], Vivien Denis[2], Rubio Marina[2]

[1]Yale University, School of Medicine, New Haven, CT, United States of America; [2]Normandie University, UNICAEN, INSERM U1237, Etablissement Français du Sang, Physiopathology and Imaging of Neurological Disorders (PhIND), Cyceron, Institut Blood and Brain @ Caen-Normandie (BB@C), Caen, France; [3]Hemorrhagic Brain Pathology Research Group, Kuopio University Hospital, Kuopio and University of Tampere, Tampere, Finland; [4]Translational Neuroimaging Group, Center for Image Sciences, University Medical Center Utrecht and Utrecht University, Utrecht, Netherlands

martina.glavan@yale.edu

Intracerebral aneurysms (IAs) are pathological dilatations of cerebral arteries whose rupture leads to subarachnoid hemorrhage, a significant cause of disability and death. Inflammation is recognized as a critical contributor to the formation, growth, and rupture of IAs; however, its precise actors have not yet been fully elucidated. Here, we report CNS-associated macrophages (CAMs) as one of the key players in IA pathogenesis, acting as critical mediators of inflammatory processes related to IA ruptures. Using a new mouse model of middle cerebral artery (MCA) aneurysms we show that CAMs accumulate in the IA walls. This finding was confirmed in a human MCA aneurysm obtained after surgical clipping, together with other pathological characteristics found in the experimental model including morphological changes and inflammatory cell infiltration. In addition, in vivo longitudinal molecular MRI studies revealed vascular inflammation strongly associated with the aneurysm area, i.e., high expression of VCAM-1 and P-selectin adhesion molecules, which precedes and predicts the bleeding extent in the case of IA rupture. Specific CAM depletion by intracerebroventricular injection of clodronate liposomes prior to IA induction reduced IA formation and rupture rate. Moreover, the absence of CAMs ameliorated the outcome severity of IA ruptures resulting in smaller hemorrhages, accompanied by reduced neutrophil infiltration. In conclusion, our data suggest a previously unexplored role of CAMs as central actors orchestrating inflammation in the IA walls and promoting IA ruptures, and molecular MRI as a promising diagnostic clinical tool in future patient follow-up studies. Based on our data, we propose vascular inflammation as a potential diagnostic marker for the clinical management of unruptured IAs, and CAMs as important targets for novel therapeutic strategies in the treatment of aneurysms prone to rupture.

Keywords: CNS, macrophages, Intracerebral aneurysms, stroke, MRI

Presentation number: YIA 04 Abstract number: ABS-153-ISABS-2024

EFFECTS OF TREATING KNEE OSTEOARTHRITIS WITH AUTOLOGOUS MICROFRAGMENTED ADIPOSE TISSUE CONTAINING MESENCHYMAL STEM CELLS COMPARED TO HYALURONIC ACID – CLINICAL RESULTS FROM IRI2 PROJECT

Molnar Vilim[1], Jeleč Željko[1,2], Rod Eduard[1,2], Hudetz Damir[1,3], Vidović Dinko[1,4,5], Starešinić Mario[6,7], Glavaš Weinberger David[5], Čemerin Martin[1], Čukelj Fabijan[1,4,5], Blažević Dejan[5], Štivičić Josip[6], Dujmović Tomislav[6], Bobek Dubravka[8], Brlek Petar[1], Pavelić Eduard[1], Primorac Dragan[1,9,10,11,12,13,14,15,16,17]

[1]St. Catherine Specialty Hospital, Zagreb, Croatia; [2]Department of Nursing, University North, Varaždin, Croatia; [3]Department for Orthopaedic and Trauma Surgery, University Hospital Dubrava, Zagreb, Croatia; [4]School of Dental Medicine, University of Zagreb, Zagreb, Croatia; [5]Clinic for Traumatology, University Hospital Sestre Milosrdnice, Zagreb, Croatia; [6]Division of general and sport traumatology and orthopaedics, Department of surgery, Merkur University Hospital, Zagreb, Croatia; [7]School of Medicine, University of Zagreb, Zagreb, Croatia; [8]Department of Physical medicine and rehabilitation with rheumatology, University Hospital Dubrava, Zagreb, Croatia; [9]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia; [10]Medical School, University of Split, Split, Croatia; [11]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State Colllege, PA, United States of America; [12]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, United States of America; [13]Medical School REGIOMED, Coburg, Germany; [14]Medical School, University of Rijeka, Rijeka, Croatia; [15]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia; [16]Medical School, University of Mostar, Mostar, Bosnia and Herzegovina; [17]National Forensic Sciences University, Gujarat, India

vilim.molnar@svkatarina.hr

Regenerative medicine offers a novel approach to combating osteoarthritis (OA), the most common musculoskeletal progressive disease and a leading cause of disability worldwide. The study aimed to evaluate the effects of microfragmented adipose tissue (MFAT) and hyaluronic acid (HA) on knee symptoms, pain, and function using questionnaires such as the Visual Analog Scale (VAS), Western Ontario and McMaster Universities OA Index (WOMAC), and Knee Injury and OA Outcome Score (KOOS) before therapy, and at 1- and 6-months post-application in patients with OA. Conducted as part of the European research project IRI2 (KK.01.2.1.02.0173), as a continuation of previous work, the study included 53 patients aged 30-75 with mild to moderate knee OA, randomized into two groups. Detailed inclusion and exclusion criteria (available at isrctn.com/ISRCTN88966184) were implemented to form a cohort from the same phenotypic group corresponding to the inflammatory phenotype of knee OA. To facilitate blinding to the received therapy, lipoaspiration was performed on all patients. Patients treated with MFAT (7 mL, Lipogems®) and those with HA (Hyalubrix 60®) were followed for 6 months. Statistical analysis showed significant clinical improvement in both groups with changes in questionnaire scores (increase in KOOS, decrease in WOMAC, VAS, $p<0.05$). Particularly, the

MFAT group showed a statistically significant improvement in the KOOS Symptoms score after 6 months compared to the HA group, indicating a superior effect of MFAT in terms of mobility, effusion, and stiffness due to its high anti- inflammatory potential ($p=0.008$). Furthermore, patients treated with MFAT exhibited continued symptom improvement at 6 months compared to the 1-month post-treatment point, a trend not observed with HA, which showed its peak effect at 1 month. This was observed in all questionnaires ($p<0.05$). This suggests that while HA provides a quicker response, MFAT demonstrates a progressive improvement over time.

Keywords: microfragmented adipose tissue, mesenchymal stem cells, hyaluronic acid, knee

Presentation number: YIA 05 Abstract number: ABS-113-ISABS-2024

TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS OF MENSTRUAL BLOOD-DERIVED MESENCHYMAL STROMAL CELL EXTRACELLULAR VESICLES: NOVEL APPROACH FOR FUTURE DIAGNOSTICS OF UNEXPLAINED FEMALE INFERTILITY

Uzieliene Ilona[1], Vaiciuleviciute Raminta[1], Pachaleva Jolita[1], Brennan Kieran[2], McGee Margaret[2], Piesiniene Lina[3], Kasilovskiene Zaneta[4], Bironaite Daiva[1], Bernotiene Eiva[1]

[1]Innovative medicine centre, Vilnius, Lithuania; [2]University college Dublin, Conway institute, Dublin, Ireland; [3]Nanodiagnostika ltd., Vilnius, Lithuania; [4]Maxmeda ltd., Vilnius, Lithuania

ilona.uzieliene@imcentras.lt

Unexplained infertility (uIF) affects 30% of couples worldwide and the diagnosis is confirmed if female is not able to get pregnant after at least 12 cycles of unprotected intercourse. However, the pathogenesis of uIF is still poorly understood and currently there are no effective diagnostic and prognostic tools for this issue. The goal of the study was to evaluate the differences of transcriptomic and proteomic profiles of menstrual blood mesenchymal stromal cells (MenSCs), their extracellular vesicles (EVs), menstrual blood serum EVs from fertile (F) and uIF woman and detect possible molecular biomarkers of uIF. MenSCs were isolated from 8 F female volunteers and 8 uIF patients. MenSCs EVs were isolated using ultracentrifugation with density gradient. Transmembrane, cytosolic markers of EVs and lipoprotein markers were analysed by flow cytometry/Western blot. Transcriptome of all samples was performed using next generation Illumina NextSeq 500 Platform and analysis performed using GO (gene onthology). Proteomic analysis was performed using mass spectrometry. Transcriptomic and proteomic analysis of all samples revealed the highest amount of differently expressed proteins and miRNAs between F and uIF groups were detected in serum EVs. Bioinformatic gene ontology analysis revealed that cell adhesion is the mostly affected process by uIF, as demonstrated by altered proteins in MSC EVs. The changes in the serum EVs of uIF women seem mainly related to innate immune response and neutrophil degranulation. Gene expression profiles of uIF EV group revealed downregulation of miRNAs associated with endothelial cell proliferation, involved in sprouting angiogenesis and embryo implantation, as compared to F EVs. We demonstrated that both, F and uIF MenSCs EVs and menstrual blood serum EVs is a promising source for uIF biomarkers, while our detected miRNAs and proteins related to fertility are potential for further studies and use for uIF diagnostics.

Keywords: unexplained infertility, menstrual blood mesenchymal stromal cells, extracellular vesicles, transcriptomic analysis