Presenter: Anzel Hennop

The potential role of RIPK3 in PARP-1-mediated memory T cell death

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Introduction: Alloreactive memory T (Tₘ) cells have emerged as a major barrier to transplant tolerance. Not only are these cells unsusceptible to traditional immunosuppressive therapies, they are also inherently resistant to apoptosis. Necroptosis, necrosis that is dependent on receptor-interacting protein kinase 1 and 3 (RIPK1, RIPK3), has been newly identified as a form of regulated cell death. Parthanatos, genotoxic stress-induced poly-(ADP-ribose)-polymerase-1 (PARP-1) mediated cell death, represents another such regulated cell death process. Recently, RIPK1 and RIPK3 have been suggested to play a role in PARP-1-mediated cell death. Necroptosis and parthanatos have yet to be studied in Tₘ cells. This present study aims to identify the role of RIPK3 in PARP-1-mediated Tₘ cell death.

Methods: Wild-type and RIPK3-null Tₘ cells were treated with MNNG (1-methyl-3-nitro-1-nitroso-guanidine), a potent activator of PARP-1. The effect of PARP-1 hyperactivity on the survival of Tₘ cells was determined by flow cytometry with Annexin V and Propidium Iodide labeling.

Results: Following MNNG treatment, both CD4+ and CD8+ Tₘ cells showed an increase in Annexin V-PI positivity (85% vs. 47.9% and 76.6% vs. 12.6%, respectively). Annexin V-PI positivity increased for both wild-type and RIPK3-null Tₘ cells (85% vs. 47.9% and 87.3% vs. 17.2%, respectively).

Conclusion: Tₘ cells were found to be susceptible to PARP-1-mediated cell death, independent of RIPK3 and caspases. Further investigation of regulated cell death in Tₘ cells is warranted as it holds the potential to identify novel therapeutic targets for eliminating Tₘ cells that mediate transplant rejection.

Keywords: memory T cells, cell death, regulated necrosis, PARP-1, RIPK3
The Effects of Mechanical Stress on Human Trabecular Meshwork Cells

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Introduction: High intraocular pressure (IOP) is a major risk factor for glaucoma. Resistance to outflow of aqueous humor through the trabecular meshwork cells (HTMCs) is believed to cause high IOP. However, the exact mechanism is unknown. The aim of the study is to develop a clear dose-and time-response relationship between degree of stretch and HTMCs viability, study specific downstream effects of mechanical stretch on changes in gap junction Connexin 43 expression.

Methods: Primary HTMCs various donors were obtained and cultured. Upon reaching near-confluency, HTMCs were stretched at 5%, 10%, and 15%, each for 24hr, 48hr, 72hr. Cell health was then measured using vital Trypan blue stain, lactate dehydrogenase (LDH) assay, and ELISA apoptosis assay. Expression of connexin 43 was measured using real-time qPCR and western blotting.

Results: No significant changes in viability upon stretching of HTMCs as measured by Trypan blue stain. LDH levels increased in a dose-and time-response manner with increasing % stretch. Interestingly, there is a significant decrease in apoptosis after 10% stretch. We also detected an up-regulation of connexin 43 protein concentration but slight decrease in RNA concentration under high % stretch conditions.

Conclusion: To date, the cause of glaucoma remains elusive and there is scarce information on ocular cell response to mechanical stress. Our studies have the potential to provide insight into the effect mechanical stretch has on the trabecular meshwork in glaucoma. In addition, if a stretch-stressor response is identified, this knowledge may uncover potential new targets for drug therapy targeting the trabecular outflow.

Keywords: Trabecular meshwork, glaucoma, mechanical stress, intraocular pressure, gap junction, cyclical stretch
**Poster Presentation**

**Presenter:** Lacey Brennan

**Determining Global Cytogenomic Changes in Hodgkin Lymphoma**

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**Introduction:** Classical Hodgkin lymphoma (cHL) is the most prevalent lymphoma in the Western world, yet its pathogenesis is largely unknown. The neoplastic cells, called Hodgkin Reed-Sternberg (HRS) cells, are greatly outnumbered by surrounding inflammatory cells, making it a difficult malignancy to study. We employ laser-capture micro-dissection (LCM) technology on formalin-fixed paraffin-embedded (FFPE) tissue samples to obtain an enriched population of HRS cells for genomic studies.

**Methods:** Five cHL cases were selected. Following LCM and DNA isolation, we used whole genome amplification (WGA) to increase our double stranded DNA (dsDNA) yield. We used the Affymetrix Cytoscan HD array, which provides both copy number and genotype information, to compare cytogenomic changes in HRS cells with normal lymphocytes from the same specimens. Array results were analyzed using Affymetrix’s Chromosome Analysis Suite (ChAS).

**Results:** We proceeded to the Cytoscan HD array with 2 cases. However, the majority of the calls made by ChAS were determined to be false calls.

**Discussion:** FFPE samples are an underutilized and important resource for genomic studies, but are challenging to work with due to poor quality of nucleic acids. Uncovering cytogenomic changes in HRS cells will allow us to further our understanding of cHL and may have future implications in developing targeted therapies.

**Keywords:** Hodgkin lymphoma, Reed-Sternberg cells, micro-dissection, microarray, DNA, cytogenomics
Presenters: Matthew Chan

Tissue Engineering a Corneal Stroma for Transplantation through Keratocyte-mediated Mechanotransduction

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Introduction: The cornea has been a target for early applications of tissue engineering due to its avascularity and relatively simple organization. However, mechanical instability in the stroma is a major problem tissue engineers face when attempting to generate a viable human cornea. Previous studies have demonstrated the importance of keratocyte/integrin-mediated mechanotransduction in the normal development of the cornea; when mechanotransduction is inhibited, resultant corneas are thinned and weakened. Therefore, this study examined the effect of eliciting keratocyte/integrin-mediated mechanotransduction on the mechanical stability of a tissue engineered model cornea.

Methods: To explore this problem, our study used classical tissue engineering techniques to generate a model corneal stroma by seeding keratocytes in a collagen gel. Mechanotransduction was elicited by adding various concentrations of TGF-β2 during the development of the model stromata, and the mechanical stability of the tissues was determined through stretch tests.

Results: Our preliminary data show that TGF-β2 is effective in eliciting mechanotransduction in our model stromata, and that mechanotransduction may increase the mechanical stability and resilience of the tissues.

Conclusion: These findings provide evidence that the elicitation of mechanotransduction should be considered in novel tissue engineering projects.

Keywords: Corneal stroma, tissue engineering, mechanotransduction, TGF-β2, keratocyte, tensile strength
Poster Presentation

Presenter: Yuxin Chang

Effect of Dietary Modifications during Pregnancy With and Without Diabetes on Offspring Pancreas Development

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Introduction: Maternal type 1 diabetes mellitus (T1DM) impacts fetal development and results in glucose intolerance later in life. Previous research has shown that dietary supplementation with 6% olive oil given to diabetic mothers increased the numbers of term births and decreased neonatal malformations by reducing the inflammatory intrauterine environment. Additionally, it has been shown that prolonged high-fat diets in non-diabetic mothers increased the likelihood of fetal death and health complications by altering vascular development in the placenta. Although these were seen at birth, it is still unclear how they may affect overall predisposition to disease later in life. Our aim was to elucidate the effect of these supplementations on fetal and neonatal pancreatic development and its impacts in early adulthood.

Methods: We established a maternal T1DM model by injecting rats with either streptozotocin (90mg/kg) diluted with fresh citrate buffer or citrate buffer alone. The rats were fed with control chow diet, diet supplemented with 6% olive oil, or diet supplemented with 25% saturated fat. Body and pancreatic weight were recorded as well as fasting glycemia value. Dual immunohistochemistry was performed to detect α and β cells in islets at day 2 and 4 months postnatal. Morphometric analysis was carried out to study islet area, islet distribution by size, and α and β cell area.

Results: Our results showed that olive oil supplementation had a restorative effect on islet and beta cell area, as well as beta cell mass at 4 months. There was also a significant increase in the number of large islets at 4 months in the offspring of diabetic mothers whose diet was supplemented with olive oil.

Conclusion: These findings addressed the importance of diet quality in mothers with T1DM to prevent disease on their offspring.

Keywords: Type 1 diabetes mellitus, pancreas, olive oil, saturated fat, beta cell, alpha cell
Presenter: Samik Doshi

The Role of microRNA-346 in Breast Cancer

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Introduction: In the recently developing field of micro RNA research, many micro RNAs are being implicated in cancer pathogenesis. miR-346 is an intronic micro RNA that has been associated with thyroid and prostate cancer, and has been shown to target genes that are related to breast cancer. The present study is aimed at exploring the role of miR-346 in breast cancer tumorigenesis using an in vitro model.

Methods: Mouse mammary carcinoma cells (4T1) were transfected with miR-346 mimic and inhibitor. The effect of miR-346 on tumor cell functions was characterized through migration, viability, and cell-cycle assays.

Results: Our results show that miR-346 mimic transfection increases proliferation and migration of 4T1 cells, while miR-346 inhibitor has the opposite function. These findings contrast with what was hypothesized based on the function of predicted target genes (such as LIF and RIP140). Cell cycle analysis yielded unclear results. We aim to explore the mechanisms for these effects through analysis of target gene expression and downstream apoptosis and proliferation pathway-associated gene expression.

Conclusion: Our results indicate for the first time that miR-346 expression level may be related to breast cancer tumorigenesis. Though continued experimentation is needed to yield more conclusive results, these findings suggest a novel mechanism that maybe relevant in the development of novel diagnostic/prognostic disease biomarkers or therapeutic approaches in breast cancer.

Keywords: micro RNA, miR-346, breast cancer, leukemia inhibitory factor (LIF), 4T1 cells, proliferation, tumorigenesis
Presenter: Doy B. Kagan, Greg Douglas, Aamir Azeem

Levels of the Growth Hormone Secretagogue Receptor in Human Cardiomyopathies

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Introduction: Growth hormone secretagogue receptor 1a (GHS-R1a), which binds the peptide hormone ghrelin, is a novel putative biomarker for diagnosing human cardiomyopathies. To study GHS-R1a, we have previously synthesized a Cy5-Ghrelin(1-19) analog and demonstrated that it binds specifically to the ghrelin receptor in murine myocardial tissue. In this present study, we used Cy5-ghrelin to measure GHS-R1a levels in human transplant patients, who have end-stage heart disease, as well as cardiac surgery patients with a broader spectrum of cardiomyopathies.

Methods: Samples from two cardiac transplant patients and eight cardiac surgery patients were incubated with 10 µM Cy5-ghrelin and imaged using fluorescence microscopy. Mean fluorescence intensity, computed using ImageJ, was used to measure GHS-R1a levels.

Results: Levels of GHS-R1a were significantly increased (P<0.05) in the diseased explanted hearts of transplant patients relative to their healthy implanted hearts. Levels of the ghrelin receptor were also significantly higher (P<0.05) in the left ventricle and right atrium than in other sampled regions, such as the right ventricles of transplant patients and the aortae, right auricles and pectoral major muscles of cardiac surgery patients.

Conclusion: Our study represents the first mapping of GHS-R1a in the diseased human heart and also provides the first evidence that the receptor may be a biomarker of human cardiomyopathies.

Keywords: Ghrelin, GHS-R1a, Cardiomyopathy, Biomarker, Myocardium
Presenter: Aaron Leung

The Effects of Human DnaJ Proteins (DnaJA1andDnaJB1) on Huntington Aggregation and Toxicity

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Introduction: Huntington’s Disease (HD) is a neurodegenerative disorder characterized by an abnormally long polyglutamine (polyQ) region in the Huntingtin protein (HTT), resulting in protein misfolding, aggregation and the subsequent death of neurons. Eukaryotic cells possess protein quality control mechanisms to guide proper protein folding, prevent protein misfolding and its cytotoxic consequences. Key players in protein quality control are molecular chaperones, including DnaJ proteins, which we have expressed in yeast models to study their effects on polyQ expanded HTT’s aggregation and toxicity. Two yeast DnaJ proteins, Ydj1andSis1, modulate polyQ toxicity and aggregation; it remains unknown, however, if these DnaJ-mediated effects are unique to yeast. To address this knowledge gap, our study focuses on the human homologs’ (DnaJA1forYdj1andDnaJB1forSis1) effects on polyQ toxicity and we hypothesized that the human homologs will have a similar effect as their yeast counterparts.

Methods: Yeast strains expressing polyQ expanded HTT (tagged with CFP, cyan fluorescent protein) will be transformed with either DnaJA1, DnaJA2 (a negative control) or DnaJB1 with a carboxy-terminal YFP (yellow fluorescent protein) tag. We will monitor the effect of these human DnaJ proteins on polyQ toxicity using growth assays and fluorescent microscopy to assess the aggregation and toxicity of polyQ expanded HTT as well as the localizations of both HTT and DnaJ proteins.

Results: Preliminary results show that the human DnaJ proteins are not toxic to yeast, and they appear to have similar effects on polyQ toxicity and aggregation as their yeast homologs.

Conclusion: Our studies will thus establish yeast as a model to explore cellular and molecular mechanisms underlying the interactions between human molecular chaperones, particularly DnaJ proteins, and misfolded proteins in neurodegenerative diseases.

Keywords: Huntington’s Disease, polyglutamine protein, neurodegeneration, DnaJ proteins, HSP40, protein aggregation
Presenter: Sandra Mekhaiel

Understanding the mechanism of carboplatin-induced vascular dysregulation in ovarian serous adenocarcinoma
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Introduction: Ovarian cancer is one of the leading causes of cancer-related mortality among women. Although advances have been made in surgical and chemotherapy strategies, long-term survival remains poor. Carboplatin, a platinum-based drug, has become the chemotherapy agent of choice in ovarian cancer due to its favourable therapeutic profile. However, there remains a high rate of resistance to chemotherapy treatment in ovarian cancer. Since, angiogenesis has been linked to tumour progression and chemotherapy resistance in several malignancies including ovarian cancer, we aim to examine whether carboplatin treatment can alter the expression of angiogenic factors and subsequently promote angiogenesis in ovarian tumours.

Methods: We extracted RNA from samples of ovarian serous adenocarcinomas obtained from patients treated with carboplatin and those without treatment. Quantitative RT-PCR was used to determine the gene expression profiles of angiogenic factors in the tissue samples and immunohistochemistry was performed on selected candidates to confirm PCR findings.

Results: Our results showed that carboplatin-treated tumours had higher expression levels of several genes involved in regulating angiogenesis and vascular permeability. These genes included matrix proteins such as fibronectin, and secreted growth factors such as vascular endothelial growth factor-A and angiopoietin1. In addition, we noted high mRNA levels of several chemokines including CCL2, CCL11, and CXCL5 in samples obtained from patients treated with carboplatin.

Conclusion: These findings suggest that carboplatin treatment may modulate the tumour microenvironment in ovarian carcinomas by increasing the expression of various angiogenic factors and that inflammatory cells may play a role in potentiating angiogenesis in ovarian carcinomas.

Keywords: Ovarian cancer, angiogenesis, carboplatin, chemokines, VEGF, CXCL5
**Presenter: Kelsey Watson**

**Interaction of Primary Human Trabecular Meshwork Cells with Metal Alloy Candidates for Microinvasive Glaucoma Surgery**

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**Introduction:** As a novel therapy for glaucoma, small metallic stents are inserted into the trabecular meshwork to increase the drainage of aqueous humour. It has been predicted that this microsurgical stent approach may become the first line therapy in glaucoma management due to the favourable side effect profile. Remarkably, there have been no published studies on the effect of the metal alloys used in the stents on human trabecular meshwork cells (HTMCs). This study aims to determine the morphological and functional response of HTMCs to metal alloys used in these stents.

**Methods:** HTMCs were cultured on the surface of titanium, titanium-nickel (nitinol) alloy and a Hydrus Microstent, with glass as control substrata. Titanium samples with different surface textures (sandblasted vs. machine polished) were also compared. Fluorescent imaging studies were conducted to measure cell adhesion and spreading. Finally, a BrdU proliferation assay, LDH assay and cell death detection ELISA were conducted.

**Results:** Our results showed that the cells cultured on the sand blasted titanium surface had significantly greater cell spreading ($p=0.012$) than the cells cultured on other substrata. HTMCs on the nitinol and Hydrus Microstent surface were poorly spread. Also, HTMCs cultured on the machine polished titanium and nitinol followed a parallel growth pattern along the surface grooves. Finally, there was significantly less cell proliferation on the nitinol ($p=0.01$) compared to glass after 24 hours of incubation.

**Conclusion:** These findings suggest that the elemental composition and texture of a metal surface impact the functional and morphological properties of HTMCs and identify cellular effects that may influence short- and long-term function of microinvasive glaucoma shunts.

**Keywords:** trabecular meshwork, glaucoma, biomaterials, stent, titanium alloy, nitinol
Poster Presentation

Presenter: Mark Woo

**Immunohistochemical Characterization of mTOR Pathway Activation in Gastroenteropancreatic Neuroendocrine Tumours**

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**Introduction:** Gastroenteropancreatic neuroendocrine tumours (GEP-NETs) are increasingly prevalent and highly heterogeneous neoplasms with suboptimal outcomes. This stems from the lack of useful prognostic and predictive markers, reflecting our lack of understanding of their molecular pathogenesis. The mTOR pathway has ample evidence suggesting a role in tumourigenesis, and appears to correlate with outcome.

**Methods:** In this immunohistochemistry study, we elucidate mTOR pathway activation in GEP-NETs to better characterize their pathogenesis. We investigate the phosphorylation status of 4EBP1 and mTOR, and expression of the repressor PTEN, using tissue microarrays of primary and metastatic GEP-NETs.

**Results:** p-mTOR, p-4EBP1, 4EBP1 and PTEN were detected in both primary and metastatic tissue, with highly variable expression. The pathway was well characterized, with expression patterns matching what has been described in previous literature. In some cases, phosphorylation of mTOR was observed in patterns suggesting an association with progression, such as within mitotically active cells figures and at the periphery of tumour nests. There were however some unexpected protein expression patterns, possibly indicating a lack of constitutive activation.

**Conclusion:** We propose a mechanism by which p-mTOR is activated and deactivated based on tumour behaviour. Correlation of results with patient outcomes is warranted, and could have implications on the use of mTOR inhibitors to treat these tumours.

**Keywords:** GEP-NETs, biomarkers, mTOR pathway, 4EBP1, PTEN, immunohistochemistry
Presenter: Jin Hui Yan

Impact of Different Indoleamine-2, 3-Dioxygenase (IDO) Isoforms in Melanoma Cells

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Introduction: Many cancer types express the tryptophan-metabolizing enzyme indoleamine-2, 3-dioxygenase (IDO), thus conveying them T cell suppression and cancer immune escape. Though IDO1 and its effects have been known for many years, the second isoform of IDO (IDO2) is not as well studied. This study is aimed at studying the effects of the two IDO isoforms on cancer cell growth in vitro and determining the differences in their effects. We expected that IDO-mediated tryptophan depletion through both IDO isoforms would inhibit BL6 proliferation in vitro.

Methods: Both IDO1 and IDO2 isoforms are expressed in the BL6 strain of mice melanoma cells at basal levels. BL6 cells were induced to express high levels of IDO (either IDO1 and/or IDO2) through cDNA transfection or knocked down to suppress IDO expression using siRNA transfection. BL6 cells were then studied on the basis of their proliferation, cell cycles status, and migration to determine the effects of high and low IDO1 vs. IDO2 expression on cancer cell phenotypes.

Results: Our results have shown IDO knockdown has increased the proliferation, migration of BL6 cells. IDO1 knockdown exhibited a stronger increase in proliferation than IDO2.

Conclusion: These results suggest that IDO1 has greater level of activity than IDO2. Whether this difference is solely due to differences in affinity for tryptophan or due to secondary actions needs to be further explored in future studies.

Keywords: melanoma, tryptophan, cDNA, siRNA, immune tolerance, T cells, indoleamine2, 3-dioxygenase, IDO1, IDO2
Pr**esenter:** James R. Roos

**Trauma in Adult Pedestrians due to Frontal Motor Vehicle Collisions**

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**Introduction:** Various injury patterns have been described for upright adult pedestrians struck by the front end of a motor vehicle. “Bumper” fractures of the lower extremity are commonly observed in upright adult pedestrian collisions. In this study, we aimed to determine the type and distribution of lower extremity injuries, and other injuries unique to motor vehicle frontal impacts. We hypothesize that lower extremity injuries are invariable when an upright pedestrian is struck by the front of a vehicle and that certain trauma due to secondary impacts with the vehicle is unique to the speed-dependent pedestrian trajectories.

**Methods:** To test this hypothesis, we collected and analyzed human, environmental, and postmortem data related to adult pedestrian fatality cases contained within the coroner’s reports of postmortem examinations from the Office of the Chief Coroner for Ontario in order to identify injury patterns and possible hallmark indicators of upright pedestrian collisions.

**Results:** Lower extremity injuries were not invariable and were sometimes absent in upright adult pedestrians. Pelvic fractures and femur fractures were identified as positive indicators of upright pedestrian status and were found significantly likely to accompany “bumper” fractures. Human, environmental, and vehicular variables related to the collision were not correlated to “bumper” fractures, pelvic fractures, or femur fractures.

**Conclusion:** These findings show that that pelvic fractures and femur fractures can serve as an indicator of upright pedestrian orientation in frontal motor vehicle collisions when bumper fractures are not observed.

**Keywords:** “Bumper” fracture, pedestrian collision, lower extremity injury, frontal impact, postmortem examination, primary impact, secondary impact