Effect of Platelet Storage Temperature on Platelet Aggregation and Contractile Force Production
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Mary Gates Scholar
Mentor: Nathan Sniadecki, Mechanical Engineering
Mentor: Ava Obenaus, Mechanical Engineering

Platelet transfusions are used to treat patients with acute hemorrhage, low platelet count, and dysfunctioning platelets, but the ideal storage conditions for platelets prior to transfusion is poorly understood. Storage at room temperature is the current gold standard for platelets, but there is a limited shelf life of 5-7 days due to storage lesions and risk of bacterial growth. As a result, there is a need for alternative approaches that can increase the shelf life of platelets and increase their availability in remote locations or during major catastrophes. Here, we examined whether cold storage of platelets, e.g. 4°C, which can extend their shelf life to 14 days, has an impact on their hemostatic function. Specifically, the function of cold-stored and room temperature-stored platelets were investigated by utilizing a microfluidic shear assay that measures platelet aggregation and contractile force during the formation of a thrombus. Blood was perfused through the microfluidic channels, and platelet aggregation and production of contractile forces were calculated using custom MATLAB code. The previous code required user input at several steps to complete the analysis, so improved code was written and tested that quantified area and force with significantly less user input. Results from experiments and subsequent analysis found that cold-stored platelets formed larger aggregates and similar contractile forces as room temperature-stored platelets, indicating that their hemostatic function was not impaired. This investigation highlights the need for further investigations on cold-stored platelets as a means to increase shelf life and availability for transfusions.

Identifying Functional Non-Coding Genetic Variants Associated with Type 1 Diabetes
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UW Post-Baccalaureate Research Education Program
Mentor: David Hawkins, Medicine & Genome Sciences, University of Washington School of Medicine

Type 1 diabetes (T1D) is an autoimmune disease in which the pancreas is unable to produce enough insulin to effectively regulate sugar into the body’s cells. Recent literature suggests that T1D has a prevalence and incidence of 9.5% and 15 per 100,000 people respectively. T1D risk is multifactorial but is heavily dependent on genetics and the environment. Twin studies have shown that although disease risk for the general population is 0.4%, children of diabetic patients are 2 to 9% at risk and identical twins are up to 70% at risk of developing T1D. Genome-wide association studies have shown that many genetic variants associated with T1D risk are found in gene regulatory regions such as enhancer elements. As T1D is an autoimmune disease with a strong genetic component particularly in non-coding, regulatory regions, it follows that thorough genomic profiling of immune cells such as T cells can help identify functional non-coding genetic variants that alter gene regulation for target genes associated with disease risk. Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) allows for unbiased identification of cis-regulatory elements (CREs). By utilizing ATAC-seq on various T cell types of both healthy control donors and T1D patients, chromatin accessibility can be collected, and the underlying sequence data can be used to determine allelic differences in transcription factor binding due to T1D-associated genetic variants. I am currently applying ATAC-seq to three T cell subtypes isolated from both donor cohorts. Through these data functional links will be made between non-coding genetic variants and associated target genes to better understand how they impact disease risk in T1D.
Chronic Nonbacterial Osteomyelitis: Investigating the Effects of Serum Type On Osteoclastogenesis
Xinrui Bao, Senior, Biochemistry, Microbiology
Megan Cheung, Junior, Pre-Sciences
Mentor: Yongdong Zhao

Chronic nonbacterial osteomyelitis (CNO) is an autoimmune condition characterized by excessive activity of osteoclasts predominantly in children. It remains unclear what roles the precursor cells present in peripheral blood mononucleocyte cells (PBMCs) and their environment (serum) play in the disease pathogenesis. We hypothesize that the serum from children with active CNO have increased stimulating effects on osteoclastogenesis derived from PBMCs compared to the serum from patients with inactive CNO, inactive juvenile idiopathic arthritis (JIA), or healthy controls. With IRB approval, patient serum is utilized in varying concentrations of 0.1%, 1%, and 10% across different patient groups. PBMCs were ordered from ZenBio. In-vitro cultures of patient serum mixed with a donor PBMC sample were conducted for 7 days and supplemented with receptor activator of nuclear factor kappa-B ligand (RANKL) and macrophage colony stimulating factor (M-CSF) to simulate physiologic conditions. During the incubation period, media exchange was carried out twice. After 7 days, cells were stained with tartrate-resistant alkaline phosphatase (TRAP) and DAPI. Cells with 3 or more nuclei and stained positive for TRAP were counted as an osteoclast using ImageJ. Osteoclast counts from all groups were normalized to the counts of the serum-free well. The findings from this study are expected to inform on whether serum from patients with active CNO contribute to the process of osteoclastogenesis. Currently, the results suggest that as serum concentration increases there is a correlated increase in osteoclast counts, with the exception of patient samples from the inactive CNO group. As the study continues, we expect to gain a more comprehensive understanding of the association between serum concentration and cell types. Future studies can explore inflammatory markers in CNO samples that promote osteoclastogenesis as potential drug targets.

Enhancing Ascl1 Retinal Reprogramming Capabilities With Krüppel-Like Factor Gene Knockout
Faith Kierney, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Thomas Reh, Biological Structure

Blindness caused by retinal degeneration is untreatable, and is a condition currently suffered by millions. The field of retinal reprogramming aims to establish a way to treat this type of vision loss, by finding a way for the eye to essentially produce new retinal neurons after they have been lost to degeneration. Often, this is done through directing cell fate by expressing or repressing various transcription factors such as Ascl1, a potent pro-neural transcription factor involved in retinal reprogramming. Ascl1 expression in mouse Muller glia, a support cell in the retina, can stimulate the regeneration of certain subtypes of retinal neurons, but the variety of retinal cells produced through this strategy is still limited. The class of transcription factors known as ‘Krüppel-like factors,’ or KLFs, regulates important cell processes, such as cell proliferation and development, with several KLFs functioning in the development of neurons. Due to the inhibitory function of KLFs during neuron development, we propose that the loss of inhibitory KLF genes in Muller glia may allow for activation of neurogenesis. Therefore, coupling the knockout of KLF genes with Ascl1 expression in Muller glia may be a key to enhancing reprogramming capabilities of Ascl1. For this project, I knocked out KLF genes in young mouse Muller glia in culture using the CRISPR/Cas9 system, and induced Ascl1 expression to stimulate reprogramming of the Muller glia. We used scRNA sequencing to determine if the knockout reprogrammed cells were molecularly similar to various retinal cell types that are typically lost with degenerative blindness. Preliminary sequencing results revealed that knockout of one candidate, KLF15, appears to promote neurogenesis. Findings from this experiment will allow us to increase our understanding of the role of KLF genes in retinal cell development as we work towards a future treatment for degenerative vision loss.

The Ongoing Arms Race between Host Cell Antiviral Factors and Viral Antagonists
Magdalena Sotelo, Recent Graduate, Molecular Biology, University of Washington
Mentor: Adam Geballe, Allergy and Infectious Diseases

Protein kinase R (PKR) is a host antiviral factor that shuts off translation in response to double-stranded RNA that accumulates during viral infection. Many viruses, including cytomegaloviruses (CMVs), encode proteins that inhibit PKR and rescue viral protein synthesis in infected cells. In the case of CMV, the “terminal repeat short 1” (TRS1) protein antagonizes PKR. Analyses have shown that PKR has been evolving under positive selection among primates, likely in order to evade TRS1 and other viral antagonists. Consequently, the ability of TRS1 to block PKR is quite species-specific. For example, the TRS1 homolog in human and Old-World monkeys (OWMs) CMVs only inhibit PKR from great apes and OWMs, respectively. Thus, it is surprising that TRS1 encoded by a New World monkey (NWM) virus, squirrel monkey CMV, antagonizes hominoid and OWM PKRs. I hypothesize that squirrel monkey CMV TRS1 has broad activity against diverse PKRs because it interacts with a conserved domain of PKR that is different from the region bound by TRS1 encoded by human CMV or OWM CMVs. To test this hypothesis, I studied how TRS1 and PKR compete to control protein synthesis in infected cells by using assays of protein synthesis and of replication of wild-type and mutant viruses. Because there has been no study of squirrel monkey PKR
Evaluating the Secreted Protein Profile of Different Probiotic Bacteria Strains in both Healthy and Irritable Bowel Syndrome Gut Conditions

Mallica Cary, Sophomore, Biology, Bellevue Coll
Mentor: Grady Blacken, Chemistry, Bellevue College

Irritable bowel syndrome (IBS) is a disease that affects many people worldwide. Clinical trials using probiotic bacteria to treat IBS show promising outcomes, although the molecular mechanisms of probiotics and their effect on gut health is not completely understood. Multiple studies comparing different strains of probiotics show *Lactobacillus plantarum* and *Bifidobacterium longum* as the most effective strains in inhibiting the release of proinflammatory factors in the gut and promoting proliferation of healthy gut bacteria. The purpose of this study is to evaluate the protein profile of probiotic bacteria *Lactobacillus plantarum* and *Bifidobacterium longum* in both healthy gut and IBS gut conditions. To observe how *Lactobacillus plantarum* and *Bifidobacterium longum* respond to different simulated gut conditions, samples of the bacteria will be isolated from probiotic supplements. These samples will be grown in aliquots representing different simulated anaerobic gut conditions for 12-16 hours at 37°C. *Lactobacillus plantarum* and *Bifidobacterium longum* will be grown separately and grown combined within these conditions. To enrich secreted proteins, the supernatant from each aliquot sample will be clarified using centrifugation. Secreted proteins will be solubilized and denatured with sodium deoxycholate. The denatured proteins will be reduced with dithiothreitol and alkylated with iodoacetamide. The sample will be clarified using centrifugation. Secreted proteins will be solubilized and denatured with sodium deoxycholate. The denatured proteins will be reduced with dithiothreitol and alkylated with iodoacetamide. The sample will be clarified using centrifugation. Secreted proteins will be solubilized and denatured with sodium deoxycholate.

Tryptic peptides will be identified using the SEQUEST search engine with a reference database. I anticipate the probiotic strains will demonstrate differential proteomics when isolated and combined in the healthy gut and IBS gut conditions. The secreted protein analysis completed in this study will further the understanding of the probiotic molecular mechanism of promoting gut health.

Improving Disease Models in Organoids Heterozygous for Polycystic Kidney Disease

Chardai J Thomas, Senior, Biology (Bothell Campus)
Washington Research Foundation Fellow
Mentor: Benjamin Freedman, Medicine/Nephrology

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder that affects people who have mutations in the PKD1 or PKD2 genes. The prominent feature of this disease is the formation of cysts in the kidneys. Despite ADPKD being genetically heterozygous, cystic tissue from patients with ADPKD also contains cells with homozygous mutations. This causes speculation within the PKD community about whether heterozygous mutations with one mutated copy of the PKD1 or PKD2 gene are sufficient to induce cystogenesis or if two mutated copies are needed. This concept is referred to as the two-hit hypothesis, a somatic mutation (second hit) occurs during the lifetime of the kidney and gives rise to clonal populations of cells that give rise to cysts. The goal of my project is to investigate the two-hit hypothesis and determine if there is cyst formation in the heterozygous organoids. To do so, I use patient-derived hSPC lines modified using CRISPR to express PKD1 or PKD2 mutations. I follow a 21 day differentiation process to produce PKD organoids.

In heterozygous organoids, I then pick individual organoids to place in suspension for 14 days and track cyst growth by area and amount. If cysts form in heterozygous PKD organoids at the same rate as the control, it would suggest that heterozygous mutants are unlikely to cause cyst formation, indicating the two-hit hypothesis applies to PKD. Current data collected on mutations in PKD2 have shown heterozygous organoids do not form cysts, however more data is needed to test the PKD1 site. Future applications involving inducing cyst formation in heterozygous mutants using forskolin, an agonist of adenyl cyclase shown to induce cyst growth. Establishing if heterozygous mutant organoids form cysts has significant implications in ADPKD research by potentially creating a better representation of the disease process, which has been difficult to study in other models.

Investigating the Killing Effect of Designed Protein Nanocages.

Jordan Jackson, Recent Graduate,
UW Post-Baccalaureate Research Education Program
Mentor: Julie Mathieu, Comparative Medicine
Mentor: Gabriele Varani, Chemistry

Renal Cell Carcinoma (RCC) kills more than 14,800 people annually in the United States alone. While immune and targeted therapies have shown some promise, late stage RCC is an aggressive and lethal disease, and so, new therapies that can treat RCC are urgently needed. TRAIL (Tumor necrosis factor (TNF)-related apoptosis-inducing ligand) has been shown previously to induce apoptosis in cancer cells by binding to TRAIL receptors DR4 and DR5. Unfortunately, DR5
antibodies have failed to improve patient survival in phase II clinical trials due, in part, to the inability to cluster enough membrane receptors and kill RCC cells in the body. This project is investigating the effects that computationally designed protein nanocages bound to DR5 antibodies have on kidney cancer cells in culture. In collaboration with the Institute for Protein Design, we have recently shown that these nanoparticles induce significant activation of caspase 8 and caspase 3 in renal carcinoma TRAIL resistant cells, but not in healthy primary kidney tubular cells. To further investigate the nanocage-induced apoptosis specificity to DR5 and the involvement of other TRAIL receptors, we used CRISPR/Cas9 to generate knockouts of TRAIL receptors in RCC. We are also investigating the effects of these nanocages on other downstream pathways including the NFκB and cFLIP pathways. This project advances the knowledge of mechanisms involved in TRAIL receptor-mediated apoptosis, and our results suggest a potential new treatment for RCC.

**Metabolic Determinants of Ferroptosis Sensitivity in Renal Cell Carcinomas**

Christina Chloe Kuismi, Senior, Biology (Molecular, Cellular & Developmental)

CoMotion Mary Gates Innovation Scholar, UW Honors Program, Washington Research Foundation Fellow

Mentor: Lucas Sullivan, Biochemistry/Human Biology, UW/Fred Hutch

Mentor: Evan Quon, Human Biology, Fred Hutchinson Cancer Research Center

Altered cellular metabolism is intimately linked to cancer, both by supporting the increased metabolic demands of cell proliferation and through changes in metabolism that initiate tumorigenesis. For example, loss of function mutations to the metabolic enzymes Succinate Dehydrogenase (SDH) or Fumarase (FH) of the tricarboxylic acid cycle are sufficient to promote renal cell carcinomas (RCC). Normally, these enzymes fulfill metabolic roles, where SDH converts succinate to fumarate, which is then converted to malate by FH. Inactivating mutations to SDH and FH cause the accumulation of their substrates succinate and fumarate, respectively, which can drive cancer relevant signaling changes. Recently, studies have shown that perturbations to SDH cause resistance to ferroptosis, an iron-dependent form of nonapoptotic cell death. Inversely, another study has shown that FH-inactivation causes ferroptosis sensitivity. Considering SDH and FH are metabolically adjacent and have similar oncogenic consequences, it is unknown why they have opposing effects on ferroptosis induction. Cell death by ferroptosis occurs in response to the accumulation of oxidized polyunsaturated fatty acid-containing membrane lipids. I hypothesize that FH and SDH mutations have different effects on RCC cell lipid metabolism that cause divergent responses to ferroptosis induction. To test how SDH and FH alterations affect ferroptosis induction, I am performing a series of dose response assays with pro-ferroptosis treatments and measuring the effect on proliferation and lipid peroxidation in wild type, SDH-impaired, or FH-impaired cells. To inhibit SDH and FH, I will use two different patients derived RCC cell lines, UOK269 and UOK262, which have endogenous SDH and FH mutations, respectively. Each cell line will be compared to their respective SDH or FH addbacks. Ferroptosis induction is a potential opportunity for cancer treatment. Thus, it is essential to understand how different metabolic alterations affect ferroptosis sensitivity to identify conditions that may be most amenable to ferroptosis induction therapy.

**Prevalence Studies of MAGEA1 Expression in Head and Neck and Hepatocellular Cancers to Inform Efficacy of MAGEA1 Specific Cytotoxic T cells**

Michelle (Cing-Yu) Hsu, Senior, Medical Laboratory Science, Biology (General)

Mentor: Cecilia Yeung, DLMP, UW/FH

Mentor: Pooja Sandhuria

The purpose of this prevalence study is to determine if MAGEA1 specific cytotoxic T cells are good therapy for head and neck squamous cell carcinoma (HNSCC) and hepatocellular carcinoma (HCC). MAGEA1 gene is a member of the MAGEA gene family and MAGE-A antigens are expressed in a variety of cancers. Previous studies have shown a correlation between the MAGEA1 gene with HNSCC and HCC but further studies were not performed to validate it. We performed a prevalence study by immunohistochemistry staining (IHC) using tissue microarray (TMA) slides. We stained a total of 9 representative formalin-fixed paraffin embedded cores of HNSCC (HN804 and HN602) and 124 cores of HCC (LV809b and LV631) tumor tissue using mouse monoclonal antibody clone MA454 at a concentration of 8 ug/ml. We performed antigen retrieval using pH 9.0 Tris-EDTA buffer and achieved visualization via deposition of DAB chromogen by horseradish peroxidase reaction. Then, we use IHC to test and validate this protein marker and evaluate whether it is a good biological target in treatments for certain population groups. Two pathologists defined positive staining tumors as those demonstrating an intensity of 1+ or greater on nuclear or cytoplasmic compartments of the cell. Staining seen on stroma, artifactual folds, or necrotic tissue was not counted. By analyzing the percentage that is positive for this biomarker, we can determine if the MAGEA1 gene is a useful target for these cancer patients. After examining 129 cases of HNSCC, 10 positive cases indicated a prevalence rate of 7.75%; of the 124 HCC (liver cancer) cases examined, 14 positive cases showed a prevalence rate of 11.29%. In conclusion, about 8% of HNSCC and 11% of HCC cases can be targeted with the TCR treatment so it can be potentially beneficial as a targeted therapy worthwhile to pursue.
Merkel Cell Carcinoma has Significant Chance of Recurrence beyond Pathologically Clear Margins: An Analysis of 926 Cases

Alex Fu, Senior, Biology (Physiology)
Mentor: Song Park, Medicine/Dermatology
Mentor: Paul Nghiem, Dermatology
Mentor: Neha Singh

Early-stage melanoma, squamous and basal cell carcinomas have local control rates of >90% with wide excision after pathologically clear margins and >95% with Mohs micrographic surgery. Local control rates for these approaches are not well defined in Merkel cell carcinoma (MCC). Herein, we analyzed data from 80 patients (pts) in a Seattle-based IRB-approved registry who had local MCC and underwent surgical excision with pathologically clear margins. Patients who had local radiation therapy after surgery were excluded as radiation affects recurrence rate independent of surgery. We also performed meta-analysis of 13 published studies (846 pts) based on a random-effects model. For the 80 pt cohort, local recurrence rate (LRR; ≤2 cm from the primary tumor) was 10%. In-transit recurrence rate (ITR; >2cm from primary) was 1%. Regional nodal recurrence rate (RRR) was 5%. This cohort had low-risk characteristics with small primary tumors (74% were ≤1 cm, 23% were 1-2, and 4% were >2 cm). No residual tumor was found in 60% of re-excisions while 29% had closest pathologic margins <1 cm. Meta-analysis of 9 published studies (745 pts) who underwent excision with clear pathological margins yielded 16.4% LRR [95% CI 8.3-26.5], 9.5% ITR [95% CI 5.4-14.6], and 32.1% RRR [95% CI 19.1-46.7]. Data from 4 studies (101 pts) who underwent Mohs yielded 3.6% LRR [95% CI 0-16.3], 12.8% ITR [95% CI 6.4-21.1], and 20.7% RRR [95% CI 13.8-38.3]. In each cohort, LRR/IRR following surgical excision with pathologically clear margins was >10%. This suggests MCC is more likely to recur near the excision site than other skin cancers and may reflect biological difference in the MCC local extension pattern (discontinuous spread beyond pathologically clear margins). Even for pathologically negative excisions, higher risk tumors may benefit from adjuvant radiotherapy.