2021 Postdoc Research Symposium
12-Minute Research Talks
Life Science & Health Sciences: Abstract Book

1. **Dr. Aimee Potter, Microbiology & Immunology. "A genome scale metabolic model reveals changes in *Neisseria gonorrhoeae* central metabolism during neutrophil inflammation**

   Aimee Potter, Jason Papin, Alison Criss

Neisseria gonorrhoeae (the gonococcus, Gc) is the causitive agent of the sexualy transmitted infection gonorrhoeae. Gc is uniquely adapted to colonize human mucosal surfaces, where it survives despite initiating a robust inflammatory response and influx of innate immune defenses, specifically neutrophils, that typically clear bacteria. We hypothesize that Gc utilizes distinct metabolic pathways to circumvent human mucosal defenses. To test this hypothesis, we have developed a curated genome-scale metabolic network reconstruction of *Neisseria gonorrhoeae* strain FA1090. Data from published, genome scale mutant screens has been integrated into this model to validate *in silico* gene essentiality predictions in rich media. Successful validation of selected gene perturbations from in vivo screens demonstrates the utility of model-driven screening of therapeutic targets. Furthermore, using transcriptional data generated from RNAseq in which Gc was exposed to neutrophils, we have contextualized this model using transcriptomic abundances and parsimony of overall flux (RIPTiDe - Reaction Inclusion by Parsimony and Transcript Distribution). This contextualization identifies the most cost-effective usage of metabolism while also reflecting Gc's transcriptional investment. Our contextualized model reveals that Gc relies on distinct, metabolic pathways during interactions with neutrophils including glycolysis, acetogenesis, and purine biosynthesis, whereas the TCA cycle is predicted to be largely dispensible. These predictions will be tested in vitro and will reveal insights into potential metabolic targets for antimicrobial development.

2. **Dr. Alexandra Donlan, Immunology, Infectious Diseases & International Health. "IL-13 is a driver of COVID-19 severity"**

Keywords: COVID-19, IL-13, Dupilumab, Infection, Type 2 immunity

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Background: Immune dysregulation is characteristic of the more severe stages of SARS-CoV-2 infection. Understanding the mechanisms by which the immune system contributes to COVID-19 severity may open new avenues to treatment.

Methods: We measured Interleukin-13 (IL-13) levels in plasma from COVID-19 patients to assess the association of IL-13 with severe disease. We also performed a retrospective analysis on patients who received the IL-4Ra mAb, Dupilumab, prior to developing COVID-19. To test the role of IL-13 during disease, we used K18-hACE2 transgenic mice which are susceptible to SARS-CoV-2 infection, administered neutralizing antibodies against IL-13, and then assessed disease severity and survival. Additionally, we performed whole-lung RNAseq to identify key downstream mediators of IL-13 which we could subsequently target during disease.

Results: We found that elevated IL-13 was associated with the need for mechanical ventilation in two independent patient cohorts. In addition, patients who acquired COVID-19 while prescribed Dupilumab had less severe disease. In SARS-CoV-2 infected mice, IL-13 neutralization reduced death and disease severity without affecting viral load. Following anti-IL-13 treatment during infection, hyaluronan synthase 1 (*Has1*) was the most downregulated gene and accumulation of hyaluronan was decreased in the lung. In patients with COVID-19, hyaluronan was increased in the lungs and plasma. Blockade of the hyaluronan receptor, CD44, reduced mortality in infected mice, supporting the importance of hyaluronan as a pathogenic mediator. Finally, hyaluronan was directly induced in the lungs of mice by administration of IL-13, indicating a new role for IL-13 in lung disease.

3. **Dr. Atum Buo, Center for Public Health Genomics, Bone Genetics. "MPDZ, a potential causal gene for the bone mineral density association at chromosome locus 9p23, regulates *in vitro* osteoblast function and *in vivo* bone mass accrual."**

Osteoporosis is a highly debilitating disease of low bone mass and increased fracture risk. Genome-wide association studies (GWASs) have collectively identified many genetic loci that are associated with changes in bone mineral density (BMD). However, the mechanism by which these loci contribute to BMD and osteoporosis is poorly understood. One of these loci is located at chromosome 9p23, which contains a lead variant (rs12340775; $P = 3.8 \times 10^{-10}$) that resides in intron 3 of the Multiple PDZ Domain Crumbs Cell Polarity Complex Component (*MPDZ*) gene. Utilizing data from the Genotype-Tissue Expression (GTEx) consortium, we observe that the risk allele of the lead BMD variant is associated with reduced *MPDZ* expression, thereby establishing a possible causal link between differences in *MPDZ* expression and the BMD association at this locus. Here, we present evidence for a novel role for *MPDZ* in regulating bone mass accrual in mice, thus supporting its involvement in driving the genetic BMD association at 9p23. Given that *MPDZ* is expressed in osteoblasts, we hypothesized that loss of *Mpdz* impairs osteoblast function and bone mass accrual. To test this, we generated *Mpdz-deficient* primary osteoblast cultures and an *in vivo* Mpdz-heterozygous ($Mpdz^{\Delta/+}$) mouse. We demonstrated that knockdown of *Mpdz* in primary osteoblasts impairs the formation of mineralized nodules and reduces the expression of key osteoblast markers. Furthermore, we performed micro-computed tomography analysis of the skeletal architecture in wildtype and $Mpdz^{\Delta/+}$ mice and show that there is a 23% reduction in trabecular bone volume in the distal femurs of female Mpdz $^{\Delta/+}$ mice in comparison to sex-matched wildtype controls. Together, these results demonstrate that *MPDZ* impacts *in vitro* and *in vivo* bone function and further underscores *MPDZ* as the likely causal gene influencing the BMD association at 9p23.

4. **Dr. Bing Xu, Genome Editing, Animal Models. "zMADM (zebrafish Mosaic Analysis with Double Markers) for single-cell gene knockout and dual lineage tracing.**

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Gene knockout with high spatial resolution, achieved by conditional knockout (CKO) in mice, is critical for studying gene functions in vivo. As a model organism, zebrafish provides unique advantage with its transparent body, enabling real-time studies of development and disease progression. However, it's challenging to establish CKO in zebrafish due to technical difficulties of making floxed alleles. Even if successful, tissue- level CKO is still not optimal for spatial resolution, especially to distinguish cell autonomous from non-cell autonomous gene functions.

Here, we present a novel genetic mosaic system, termed zMADM (zebrafish Mosaic Analysis with Double Markers). Via Cre/loxP mediated *inter*-chromosomal mitotic recombination of two reciprocally chimeric fluorescent genes, zMADM generates sporadic, GFP^+ mutant cells along with their RFP^+sibling WT cells. To create zMADM, we knocked the zMADM cassettes into the pre- selected genomic region with CRISPR/Cas9. The successful establishment of zMADM was then confirmed by injecting Cre mRNA or plasmids into the eggs of zMADM zebrafish,achieving a labeling efficiency of ~0.5%, sparse enough for single-cell analysis. With liveimaging, we showed the birth of two sibling cells and their subsequent development in zMADM, demonstrating its application for dual lineage tracing. When combined with *nf1* mutation, we observed an over-expansion phenotype of *nf1* mutant cells in comparison to wildtype sibling cells in the same zebrafish. Once broadly distributed, we anticipate that zMADM should help unleash the full power of zebrafish genetics.

5. Dr. Karina Dias Teixeira, Molecular & Cell Biology. "Lacritin Rescue of Homeostasis Requires GPR87 Coupled to Syndecan-1

Karina Dias-Teixeira[1], Mazhar Adli[2], John Doench[3], Gordon W. Laurie[1]

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Introduction: Lacritin is a mitogenic tear and plasma glycoprotein with a critical role regulating ocular surface homeostasis. It does so by transiently accelerating epithelial autophagy thereby restoring oxidative phosphorylation, as validated in a recent 204 patient phase 2 clinical trial of human Sjögren's Syndrome Dry Eye. We identified the G protein-coupled receptor 87 (GPR87) as the lacritin signaling receptor out of a genome-wide CRISPR/Cas9 death screen using the C- terminal lacritin synthetic peptide N-94 as probe. Years ago, GPR87 was thought to be an LPA receptor, later disproven by Arfelt et al, '17. Here we further validate GPR87 as the lacritin receptor and work out how GPR87 couples with lacritin co-receptor syndecan-1.

Methods: Lacritin affinity binding to GPR87 was first studied out of recombinant lacritin- or lacritin C-25-intein pulldowns. Truncation mutant 'C-25', lacks lacritin's 'N-94' bioactive domain. Lacritin pulldowns were challenged using each of eleven synthetic peptides corresponding to or togetherspanning all eight extracellular or intracellular strands or loops of GPR87. To explore whether GPR87 can couple with SDC1, we blotted for GPR87 out of SDC-1 pulldowns from HEK2936E cells co-transfected with wild type or mutated GPR87 and SDC1 cDNA's.

Results: Lacritin, but not C-25, binds GPR87 via GPR87's outer three loops with outer loop 3 peptide most inhibitory. Not inhibitory were outer and inner tail, and inner loop peptides. GPR87 complexes with SDC1 in the absence of N-94, but not with SDC1 lacking the PDZ binding domainnor lacking N-terminal domain heparan sulfate at S15 nor membrane proximal chondroitin sulfateat S184 and S185. SDC1 failed to bind GPR87 lacking the G-protein binding site "DRY" motif.

Impact: Lacritin has therapeutic potential for treatment of dry eye and other inflammatory diseases. Characterization of the receptor will improve drug cross-reactivity and contribute to theadvance of new therapies.