

SCHEDULE

1:00 pm

Opening Remarks

1:10–1:55 pm

Daphne Superville

Gupta Lab

Mary Clare Beytagh

Jacks Lab

Meenakshi Chakraborty

Sharp Lab

1:55 BREAK

2:10–2:55 pm

Chittampalli Yashaswini

Walker Lab

Rhogerry Deshycka

Lodish Lab/Ploegh Lab

Surya Tripathi

Yilmaz Lab

2:55 BREAK

3:10–3:55 pm

Jonathan Guzman

Ploegh Lab

Camilo Espinosa

Ploegh Lab

Alissandra Hillis

Vander Heiden Lab

3:55 CLOSING REMARKS

BIOLOGY UNDERGRADUATE RESEARCH SYMPOSIUM 2017

February 2nd | 1–4:00 pm | 68-181

ALL ARE WELCOME!
REFRESHMENTS WILL BE SERVED.

Biology Undergraduate Symposium 2017

- 4 Daphne Superville**, Regulation of Mammary Stem Cell Differentiation by BCL11B
- 5 Mary Clare Beytagh**, Dissecting Antigen-Specific T-cell Response in a Novel Mouse Model of Colon Cancer
- 6 Meenakshi Chakraborty**, The Impact of Intermediate-Expressed miRNA on Gene Expression Variability and Cell State Transitions
- 7 Chittampalli Yashaswini**, Obstruction of the Bacterial Secretory Pathway May Create Lethal Reactive Oxygen Species
- 8 Rhogerry Deshycka**, Engineered RBCs Equipped with PCSK9 Inhibitors as Cholesterol Blockbuster
- 9 Surya Tripathi**, Fasting-Activated Fatty Acid Oxidation Enhances Intestinal Stem Cell Function in Aging
- 10 Jonathan Guzman**, Chemokine-Nanobody Fusions for Enhanced Tumor Immunotherapy
- 11 Camilo Espinosa**, Creating an Immune Tolerance Model Using an antiCD47 Single Domain Antibody
- 12 Alissandra Hillis**, Identifying the Effect of PKM2 Deletion in Pancreatic Ductal Adenocarcinoma

Regulation of Mammary Stem Cell Differentiation by BCL11B

Daphne Superville, Daniel Miller, Piyush Gupta

Whitehead Institute for Biomedical Research, MIT, Cambridge, MA 02139, USA

The human mammary gland is a model organ for studying stem cell differentiation. The estrous cycle causes regular turnover of mammary epithelial cells, which maintain proper function and homeostasis of the gland. It is well known that a small population of self-renewing bipotent mammary stem cells (MaSCs) is responsible for producing the progenitors, which eventually differentiate into the luminal and basal cells that make up the bulk of the tissue. However, relatively little is known about what factors within the MaSC prompt this decision for it to either self-renew or differentiate. This knowledge gap is largely due to the lack of markers uniquely identifying MaSCs, making it very difficult to study them on a molecular level. Recently, the Gupta lab has identified BCL11B as a transcription factor that regulates MaSC self-renewal and lineage commitment. In this study we aimed to understand how BCL11B regulates MaSC renewal and differentiation. We found that depletion of BCL11B caused primary MaSCs to preferentially differentiate into basal cells. Furthermore, BCL11B depletion resulted in a decrease of stem cells. These results suggest that BCL11B acts by promoting MaSC self-renewal and inhibiting MaSC differentiation into the basal cell lineage. It is thought that different breast cancer subtypes arise from different cell types within the mammary gland, so by furthering our understanding of MaSC differentiation we may gain new insight into the development of different cancer subtypes.

Faculty Supervisor: Piyush Gupta

Graduate Student Mentor: Daniel Miller

Dissecting Antigen-Specific T-cell Response in a Novel Mouse Model of Colon Cancer

Mary Clare Beytagh, Peter Westcott, Olivia Smith,

Jatin Roper, Omer Yilmaz, Tyler Jacks

Dept. of Biology, MIT, Cambridge, MA 02139

Immune checkpoint blockade therapies, which unleash the immune system's ability to recognize and eliminate cancer cells, are currently among the most promising cancer treatments. Antibodies that block the immune inhibitory receptors CTLA4 and PD1 have shown clinical success, with objective response rates >20% and durable responses >10 years^{1,2}. However, some cancers, including colorectal cancer (CRC), are particularly refractory to immunotherapy. Although the colon is known to be an immunosuppressive environment, the mechanism of immune suppression remains unclear. Studies of human CRC show that T-cell infiltration is an important predictor of clinical outcome, yet current CRC mouse models are poorly suited to studying T-cell responses against tumor-specific antigens. Syngeneic cell line transplants, currently the most common models to study adaptive immune response in CRC, are characterized by a highly complex tumor antigen landscape, and have not employed orthotopic transplant into the colon. To track specific T-cell responses in CRC, we developed a novel colon organoid-based system, in which transformed organoids expressing model antigens are orthotopically transplanted into the colon wall using a method pioneered in the Yilmaz lab. Organoids were harvested from *Villin^{CreER}; Apc^{fl/fl}* mice and grown in the presence of Wnt ligand; addition of tamoxifen and removal of Wnt from the media facilitated expression of the colon-specific Cre, deletion of *Apc*, and selection of transformed organoids capable of growing in the absence of Wnt. To induce expression of model antigens, organoids were infected with a lentiviral vector that, in addition to a fluorescent reporter, expressed the T-cell antigen SIYRYGL and two ovalbumin antigens—SIINFEKL and OVA323–339—fused to the C-terminus of luciferase to monitor antigen expression *in vivo*. Organoids were then sorted and injected into the colon wall. This novel model system provides a powerful tool to dissect anti-tumor T-cell responses in the poorly understood context of CRC.

¹ Snyder, A et al. (2014) *N Engl J Med.* 371, 2189-2199

² Le, DT et al. (2015) *N Engl J Med.* 372, 2509-2520.

Faculty supervisor: Tyler Jacks

Post-doc mentor: Peter Westcott

The Impact of Intermediate-Expressed miRNA on Gene Expression Variability and Cell State Transitions

Meenakshi (Meena) Chakraborty, Salil Garg, Phillip A. Sharp

Koch Institute for Integrative Cancer Research, Dept. of Biology, MIT, Cambridge, MA 02139

The cells of the adult vertebrate organism derive from pluripotent embryonic stem cells (ESC). ESC can both differentiate and self-renew, and can be isolated and cultured *in vitro*. An emerging idea is that ESC fate pathways are not irreversible^{1,2}; rather, individual ESC interconvert between a variety of “cell states,” each characterized by particular gene expression programs that determine their capacity to self-renew or to differentiate into a particular cell type. Fluctuations of gene groups such as the network of pluripotency genes (Nanog, Esrrb, Sox2, etc.) are likely to play a key role in cell state transitions^{2,3}. Identifying the molecular controllers of variability, and therefore fluctuations, of pluripotency gene expression will be important for understanding these transitions. MicroRNAs (miRNAs), small non-coding RNAs that prevent messenger RNA from being translated to protein, hold promise as a molecular controller due to their ability to bind and control expression of many genes.

Here we report our initial studies of the impact of both intermediate and highly-expressed miRNA on gene expression variability. We find that a majority of expressed miRNA increase gene expression variability (noise) in the target gene, and find evidence that ESC newly transitioning from Nanog-high to Nanog-low cell states show differential activity for intermediate-expressed miRNA. We propose that variability derived from miRNA activity can cause fluctuation of pluripotency genes, leading to transitions in cell state. Future investigations will include defining the relative contributions of miRNA expression level, binding site type, and affinity to variability. The generation and control of variability by miRNA may have further implications for development and cancer biology.

¹ Chambers, I. et al. (2007) *Nature*. 450, 1230-34.

² Klein, A.M. et al. (2015) *Cell*. 161(5), 1187-1201.

³ Kumar, R.M. et al. (2014) *Nature*. 516, 56-61.

Faculty Supervisor: Phillip A. Sharp

Postdoc Mentor: Salil Garg

Obstruction of the Bacterial Secretory Pathway May Create Lethal Reactive Oxygen Species

Chittampalli Yashaswini, Charley Gruber, Graham Walker

Dept. of Biology, MIT, Cambridge, MA 02139, USA

Drug resistance is a far-reaching problem, with increasing numbers of dangerous bacteria gaining resistance to antibiotics. Specific antibiotics stall translation, which can jam the secretory pathway. This obstruction may result in lethality or delayed bacterial growth. However, recent findings have shown that antibiotics also generate reactive oxygen species (ROS), which can generate potentially lethal double-stranded DNA breaks (DSBs). Our goal is to show that ROS are produced from secretory pathway jamming and contribute to bacterial cell death. The model we utilized for antibiotic-treated bacteria was MM18, an E.coli strain with a MalE-LacZ fusion gene that exhibits significant jamming of the secretion pathway and a 100-fold decrease in cell count when treated with maltose. We aimed to establish a causative relationship between secretory jamming and ROS generation by conducting kill curves with the MM18 strain and MM18 mutants lacking candidate genes believed to be involved in ROS. We found that maltose-treated MM18 cells experience a three-fold increase in cell count when grown with ROS-combating agents. We also found that MM18 mutants lacking ROS scavenging abilities or DSB repair machinery experience a two- to three-fold decrease in cell count. We will conduct additional experiments to establish ROS as a contributing factor to MM18 cell death; by doing so, we can show that secretory jamming generates ROS, and thus lend support to the larger idea that ROS play a role in the mechanism of antibiotics. Establishing ROS as a contributing factor in antibiotics will help us elucidate the mechanism underlying antibiotic action, and may be instrumental in combating antibiotic resistance in the future.

Faculty Supervisor: Graham Walker

Postdoc Mentors: Charley Gruber

Engineered RBCs Equipped with PCSK9 Inhibitors as Cholesterol Blockbuster

Rhogerry Deshycka, Novalia Pishesha, Harvey Lodish and Hidde Ploegh

Department of Biology, MIT, Cambridge, MA 02139, USA

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A low plasma level of proprotein convertase subtilisin/kexin 9 (PCSK9) is associated with decreased low-density lipoprotein (LDL) cholesterol and a reduced risk of coronary heart diseases. PCSK9 accelerates hepatic low-density lipoprotein receptor (LDLR) degradation by binding to the epidermal growth factor-like repeat A (EGF-A) domain of LDLRs on the cell surface, increasing plasma LDL cholesterol levels. Monoclonal antibodies and inhibitor peptides against PCSK9 have been shown to reduce cholesterol-associated cardiac events. However, such treatments require inconvenient biweekly injections, alter the immune system, induce side effects due to high injection of bolus, and tend to be very costly. Utilizing genetic engineering and Sortase A-mediated protein modification strategies, we have engineered red blood cells covalently carrying EGF-A domain (RBC-EGFA) on the surface as a cholesterol blockbuster. This engineered strategy preserves the normal biological property of native RBCs as shown by the normal circulatory lifespan of the RBC-EGFA *in vivo*. We further found that transfusing a single dose of RBC-EGFA into a cohort of recipient mice lead to 50% decreases in plasma cholesterol levels for more than 14 days, 5 times longer than the reduction caused by administration of the free EGF-A inhibitory peptides at recommended dosage. Utilizing red blood cells as a PCSK9 inhibitor also opens the possibility of stem cells therapy as a one time cure for patients with familial hypercholesterolemia. We have shown mice that transplanted with modified red blood cells progenitors have significantly lower plasma LDL. These results highlight the potential of engineered red blood cells carrying PCSK9 inhibitors on the surface as a more practical and longer-term strategy for tackling hyperlipidemia.

Faculty Supervisor: Harvey Lodish and Hidde Ploegh

Graduate Student Supervisor: Novalia Pishesha

Fasting-Activated Fatty Acid Oxidation Enhances Intestinal Stem Cell Function in Aging

Surya Tripathi Maria Mihaylova, Chia-Wei Chang, Miyeko D. Mana, Amanda Q. Cao, Khristian E. Bauer-Rowe, Monther Abu-Remaileh, Laura Clavain, Aysegul Erdemir, Elizaveta Freinkman, Yanmei Huang, Peter Carmeliet, Pekka Katajisto, David M. Sabatini and Ömer H. Yilmaz

Koch Institute of Cancer Research, MIT, Cambridge, MA 02139, USA

Diet has a profound influence on tissue regeneration and aging in diverse organisms. Low caloric states such as intermittent fasting, for example, have beneficial effects on organismal health and slow the progressive loss of tissue function observed in old age. Whether mammalian adult stem and progenitor cells respond to short-term fasting regimens and, if so, how these responses improve tissue regeneration in old age is unclear. Here, we show that in old mice intestinal stem cell (ISC) numbers are reduced and that aged ISCs are less functional in an organoid assay, indicating that cell autonomous changes contribute to intestinal stem cell aging. We find that aged ISCs have dampened fatty acid oxidation (FAO), the main pathway for generating cellular energy from lipids. Interestingly, a short-term fasting regimen augments ISCs function in aged mice by inducing a peroxisome proliferator-activated receptor delta (PPAR-d) driven fatty acid oxidation (FAO) program, and pharmacological activation of this program mimics many of the effects of short-term fasting. Genetic disruption of Cpt1a, which encodes the rate-limiting step in FAO, abrogated the ISC-enhancing effects of short-term fasting. These observations provide a potential dietary and metabolic strategy for improving intestinal regeneration in old age.

Faculty supervisor: Ömer H. Yilmaz

Postdoc Mentor: Chia-Wei Cheng

Chemokine-Nanobody Fusions for Enhanced Tumor Immunotherapy

Jonathan Guzman, Tao Fang, Hidde Ploegh
Whitehead Institute for Biomedical Research, Cambridge, MA 02142

The discovery of negative checkpoint regulators in the immune system was significant, especially after the recognition that many cancer cells overexpress programmed death ligand-1 (PD-L1) to inactivate cytotoxic T cells which would normally eliminate them. Monoclonal antibodies to block the interaction of PD-L1 with its receptor PD-1 achieved impressive improvements in patient survival. However, the overall response rates remain low or even completely resistant in cases such as pancreatic cancer. New insight suggested that tumor infiltration by immune cells is a prerequisite for response to blockade therapy, and this in turn is dependent on the presence of chemokines within the local milieu. Chemokines are a subset of cytokine signaling proteins used to direct immune cells based on their functional gradient. In an effort to improve checkpoint blockade therapies, we have developed chemokine-nanobody fusions in order to block PD-L1 as well as deliver chemokines to the tumor microenvironment. An anti-PD-L1 nanobody has already been characterized by the Ploegh Lab, so the project has focused on chemokine expression, purification, successful chemical fusion reaction with nanobodies, and functional evaluation of *in vitro* and *in vivo* systems. A combination of sortase technology and click chemistry was used to create fusions that retain their bioactivity, especially the integrity of the native, signal-transducing N-terminus of chemokines.

Faculty Supervisor: Hidde Ploegh

Postdoc mentor: Tao Fang, PhD

Creating an Immune Tolerance Model Using an antiCD47 Single Domain Antibody

Camilo Espinosa, Michael Dougan, Hidde Ploegh
WIBR, Dept. of Biology, MIT, Cambridge, MA 02139, USA

Some diseases have treatments consisting of daily injections of recombinant protein, which can produce an immune response. The treatment protein then gets neutralized, preventing recovery. It has been shown that the response against a protein can be reduced by attaching the protein to a peptide binding Glycophorin-A, expressed on erythrocytes, before injection into mice. We aimed to develop a system where expressing any protein of interest linked to a single domain antibody (VHH) and injecting into mice would achieve a similar result. We chose to target transmembrane protein CD47, expressed in erythrocytes, using the antiCD47 VHH clone A4 which has low picomolar affinity for CD47. We injected antiCD47-A4 into mice (n=6) and measured antibody titer against VHHs, using a VHH control for comparison (n=5). We observed that antiCD47-A4 produced no response. We then injected the two together but unlinked (n=9) and observed that injection of antiCD47-A4 reduced the response against the control VHH. We then designed a protein construct consisting of antiCD47-A4 and a model protein (Sortase) connected via a serine-glycine linker. We cloned and optimized its expression to generate enough protein for in-vivo study, and tested it by FACS to ensure it bound to CD47 in isolated erythrocytes. We then tested the construct in mice (n=10) and measured antibody titer against Sortase. We compared it to mice that received only the control protein (n=5) and concluded that our construct produced an increased immune response. In order to test if injection route played a role in this finding, we repeated the experiment using intra-venous injection instead of intra-peritoneal, observing that our construct (n=5) still produced an increased immune response over the control (n=5). We concluded that conjugation to A4 does not prevent anti-Sortase responses, with a possible reason for the higher antibody titer being increased serum half-life of the construct.

Faculty Supervisor: Hidde Ploegh

Postdoc Mentor: Michael Dougan

Identifying the Effect of PKM2 Deletion in Pancreatic Ductal Adenocarcinoma

Alissandra Hillis, Allison Lau, Matthew Vander Heiden

Koch Institute for Integrative Cancer Research, Dept. of Biology, MIT, Cambridge, MA 02138, USA

Pancreatic cancer has the highest mortality rate of all cancers with a five-year survival rate of only 7%¹. Limited treatments for pancreatic cancer exist, in part because patients are diagnosed with advanced disease. Since tumor cells in most cancers express the PKM2 isoform of pyruvate kinase (PK) rather than the more active PKM1 form of the enzyme, we questioned whether PKM2 expression was essential for pancreatic tumorigenesis². PK catalyzes the final step in glycolysis, in which a phosphate group from phosphoenolpyruvate (PEP) is transferred to ADP, yielding pyruvate and ATP³. Both PKM1 and PKM2 carry out this same catalytic activity, and the differential requirement for PKM2 versus PKM1 is incompletely understood⁴. We studied the effects of PKM2 deletion in a pancreatic ductal adenocarcinoma (PDAC) mouse model of pancreatic cancer. Analysis of mouse survival showed no statistically significant difference in survival between the PKM2 deletion mice and PKM2 wild-type mice (0.2398, Log-rank (Mantel-Cox) test). However, the male PKM2 deletion mice trended towards shorter survival compared to PKM2 wild-type males. Furthermore, these male PKM2 deletion mice had a greater percent tumor weight than the PKM2 wild-type mice, suggesting that PKM2 is not necessary for pancreatic tumorigenesis, but rather could play a role in suppressing pancreatic tumor development. Immunohistochemical staining showed that PKM2 deletion mice were expressing higher levels of PKM1 compared to wild-type mice. This result suggests that tumor cells may require some form of PK, but PKM2 is not essential for pancreatic tumorigenesis.

¹ Hirshberg Foundation for Pancreatic Cancer Research

² Christofk, H. R. et al. (2008) *Nature*. 452(7184), 230–233

³ Dong, G. et al. (2016) *Oncology Letters*

⁴ Dayton, T. L. et al. (2016) *EMBO Reports*. 17(12), 1721–1730

Faculty Supervisor: Matthew Vander Heiden

Postdoc Mentor: Allison Lau

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