NIH/NIDDK P30 Center for Molecular Studies in Digestive & Liver Diseases

NEWSLETTER

SPRING 2021

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Please remember to cite the Center (NIH-P30-DK050306) and its core facilities (MPIC, H-MARC, G-MMC, and BDSC) in your publications.

BRAD JOHNSON, PHD LAB UPDATE

The Johnson lab focuses on the biology of telomeres, structures that "cap" and protect the ends of chromosomes and thus serve critical roles in the normal function of the genome. Telomeres naturally lose function with age, and thus contribute to the onset of several age-related diseases. Telomere defects occur prematurely in the rare disease dyskeratosis congenita (DC), which is caused by a genetic deficiency in telomerase, an enzyme that naturally repairs telomeres. We have been investigating how telomere dysfunction interferes with the maintenance of stem cells, and the differentiation of stem and progenitor cells into mature tissues, so that we can intervene to alleviate disease in people with DC and with natural aging.

Our studies of intestinal defects in telomerase deficient mice revealed the existence of a positive feedback loop that reflects a mutual support between normal telomere capping and the Wnt signaling pathway, which is a form of short-range intercellular communication that is critical for stem cell function. Remarkably, this feedback loop enables the pharmacologic stimulation of Wnt signaling to restore telomere capping, and thus intestinal integrity, in telomerase deficient mice (*Yang TB et al., Nature Communications, 2017*). In collaboration with Chris Lengner's lab, we showed that similar biology holds true for human cultured intestinal organoids derived from DC mutant iPS cells (*Woo DH et al., Cell Stem Cell, 2016*), suggesting that Wnt pathway agonism may be a therapeutic approach to DC. Review of the features of other tissue pathologies in DC, including pulmonary fibrosis and liver cirrhosis, suggests that Wnt agonism may also help treat these problems (*Fernandez RJ and Johnson FB, Ann NY Acad Sci, 2018*), and together with the Lengner lab we are actively testing this idea using human iPS cell derived lung alveolar and liver organoids.

The Johnson lab is also working together with Chris Lengner's and Klaus Kaestner's labs to learn more about the cells that provide key support signals to the intestinal stem cell niche. We found previously that transplantation of bone marrow from normal donor mice can rescue the epithelial stem cell defects in telomerase-deficient mice. We're now testing the hypothesis that the rescuing cells are FoxL1-positive telocytes, recently discovered by the Kaestner lab, which reside in the stroma underlying the epithelium and which provide Wnt signals to the stem cells. These studies may help lead to cell-based therapeutic approaches to DC and other intestinal pathologies.

CENTER SYMPOSIUM

Save the date

21ST Annual NIH Center for Molecular Studies in Digestive and Liver Diseases Symposium

"Liver Disease Across the Age Spectrum: Studies of Genetics and the Environment"

National Constitution Center in Philadelphia.

September 22, 2021

NEWSLETTER
SPRING 2021 continued

GENETICALLY-MODIFIED MOUSE CORE UPDATE

Several years ago the University of Pennsylvania School of Medicine funded the creation of a dedicated mouse embryo cryopreservation storage facility. This facility is located in a secured room in the Anatomy-Chemistry Bldg and is overseen by the Core. The facility currently contains 9 liquid N_2 storage tanks (plus a working tank in the microinjection room) with alarms and a source tank for liquid N_2 refills. The Core bears responsibility for maintaining the integrity of the N_2 dewars, and maintaining up-to-date, computerized storage records that can be accessed in real time by P.I.s. There is a modest *per* line yearly charge to Core users for this cryostorage service ($24/line/yr for center members). The fee will be collected quarterly through our database according to the account number that the user provides. Users can monitor their inventories and arrange with the Core to have samples sent to collaborators. The user needs only to provide the contact info for the recipient and the name of the line to be sent and the Core will take care of the shipping process.

The Transgenic and Chimeric Mouse Facility began providing direct genome editing services using the CRISPR-cas9 technology two years ago. CRISPRs (clustered regularly interspersed short palindromic repeats) encode RNAs (guide RNA) target a CRISPR-associated protein (Cas9) to cleave a DNA sequence in a site-specific manner. Following the double-stranded break, non-homologous end joining generates targeted deletions of random size. The DNA cleavage can also be used to enhance high-fidelity homologous recombination using a co-injected single stranded or double stranded DNA template. These CRISPR/Cas9-based methodologies significantly reduce the time and resources involved in generating genetically modified mouse lines.

The direct genome modification service based on CRISPR-Cas9 was integrated into the Core services in 2014 and has rapidly increased in its utilization. The overwhelming majority of the projects use an injection mix of Cas9 RNA and sgRNA either with or without template DNAs. The mix is injected into the cytoplasm of fertilized mouse oocytes of the strain of choice requested by the user. Similar to the DNA injection service, injected eggs are cultured O/N and the embryos are surgically transferred into pseudopregnant females and allowed to go to term. For 'knock-in' and targeted sequence modification projects, the eggs are cultured O/N in the presence of 50 uM SCR7, an inhibitor of Ligase IV to enhance homologous recombination (vs. non-homologous end-joining) events. The success rate for the KO projects ranges from 5%-50% of the live-born with frequent occurrence of bi-allelic mutations. KI projects based on homologous recombination remain less successful (3-10%) and the success frequency varies tremendously based on multiple variable in the project (base substitution, LoxP or tag insertion, large segment insertion). The Core continues to monitor the outcome of all projects and collect data that would help improve the efficiency of this technology. http://www.med.upenn.edu/genetics/tcmf/

KATHRYN HAMILTON, PHD LAB UPDATE

Following her time as a postdoctoral fellow and Research Associate, Kate Hamilton joined the Department of Pediatrics as an Assistant Professor, tenure-track, and started her laboratory the Children's Hospital of Philadelphia in the fall of 2017. Located in the Abramson Research Center, 9th floor, the Hamilton laboratory aims to understand mechanisms of post-transcriptional regulation in intestinal homeostasis and disease, with a specific focus on pediatric and adult Inflammatory Bowel Disease (IBD) and pre-malignant transformation. Kate's lab uses mouse models and enteroid and colonoid cultures from mice and IBD patients to determine the functional role of previously undescribed genes or pathways in intestinal epithelial response to inflammation. In a separate but interrelated arm of the Hamilton lab, Kate is working to characterize novel epithelial defects in patients with Very Early Onset (VEO)-IBD in collaboration with Judith Kelsen, MD, Program Director of the CHOP VEO-IBD Clinic. These collaborative studies are supported via the Penn ITMAT as well as the Joint Penn/CHOP GI Center. The goal of this work is to identify previously unknown pathways amenable to therapeutic intervention in VEO-IBD patients, who often present with severe and refractory disease.

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