BIOGRAPHICAL SKETCH

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NAME: Gracz, Adam David

eRA COMMONS USER NAME (credential, e.g., agency login): ADAM_GRACZ

POSITION TITLE: Assistant Professor of Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of North Carolina at Chapel Hill	B.A.	12/2008	Biology
University of North Carolina at Chapel Hill	M.S.	05/2011	Cell & Molecular Physiology
University of North Carolina at Chapel Hill	Ph.D.	11/2013	Cell Biology & Physiology
University of Pennsylvania	Postdoctoral	06/2014	Cardiac development
University of North Carolina at Chapel Hill	Postdoctoral	06/2016	Intestinal stem cells

A. Personal Statement

My primary research interest is understanding how chromatin regulatory mechanisms establish and maintain cellular identity, especially in the context of regenerative biology. The current research objective of my lab is to understand transcriptional and chromatin regulation of epithelial biology in the intestine and intrahepatic bile ducts.

My training focused on developing and applying novel tools for studying intestinal stem cell (ISC) biology. I have contributed to research at the forefront of ISC identification, isolation, and manipulation *in vitro*. This work resulted in transgenic and cell surface markers of mouse and human ISCs, culture conditions for active and reserve human ISCs, and a high-throughput platform for functional and genetic manipulation of single ISCs and organoids for downstream analysis. In the past 5 years, I have shifted my focus to transcriptional regulation and genomics. My K01 focused on acquiring skills in genomics and computational biology to dissect gene regulatory networks associated with chromatin modifying enzymes.

I have developed expertise in mouse models, flow/FACS analysis, three-dimensional and monolayer organoid cultures, transgenic organoids, genomics assays on rare primary cell populations (wet bench and computation), and high-throughput functional/genetic screening of primary stem cells and organoids. I have 15 years of combined experience in techniques related to the study of cell biology and genetics in animal models of glioma, ISCs, cardiopulmonary development, and biliary homeostasis/regeneration. I aim to apply my expertise in GI epithelial biology to basic questions at the interface of cell biology and genomics with fundamental relevance to human health and disease.

I have a strong commitment to scientific mentorship and am passionate about facilitating success in student populations from underrepresented backgrounds. I have mentored 13 undergraduates and post-bac technicians and served as a mentor in research programs for underrepresented minorities and transfer students. In my current tenure-track position, I plan to focus the professional service aspect of my career on improving research training opportunities and outcomes for undergraduates, graduate students, and postdoctoral trainees.

Recent and ongoing projects that I would like to highlight include:

R35 GM142503
Gracz (PI)

8/1/2021-7/31/2026
Chromatin regulation of epithelial stem cell function

R03 DK122111
Gracz (PI)
8/1/2019-7/31/2021
Determining the role of Tet1 in intestinal stem cell differentiation and self-renewal

K01 DK111709
Gracz (PI)
9/1/2016-7/31/2020
Mechanisms and function of Tet1 regulated gene networks in intestinal stem cell biology

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2020-current Assistant Professor (*tenure track*), Division of Digestive Diseases, Emory University
2016-2020 Assistant Professor (*non-tenure track*), Department of Genetics, UNC Chapel Hill
2008-2009 Research Technician, Center for Gastrointestinal Biology and Disease, UNC Chapel Hill
2006-2008 Undergraduate Researcher, Division of Neurosurgery. Duke University Medical Center

Other Experience and Professional Memberships

2020-current Member, American Society for Cell Biology
2016-2020 Member, Center for Gastrointestinal Biology and Disease, UNC Chapel Hill
2015-current Member, American Gastroenterological Association

Honors

2016 Research Scholar Award, American Gastroenterological Association
2015 Postdoctoral Award for Research Excellence, UNC
2015 Collaborative Research Travel Grant, Burroughs Wellcome Foundation
2014 Gastrointestinal Basic Science Training Grant (T32, PI: Sartor)
2013 Impact Award, UNC Graduate Education Advancement Board
2013 Dissertation Completion Fellowship (UNC Graduate School)
2011 Honorable Mention, NSF Graduate Research Fellowship
2009 Poster contest winner, CGIBD Annual Meeting
2008 Travel Fellowship, UNC Office of Undergraduate Research

C. Contributions to Science

1. Genetic regulation of ISCs. In my postdoc, I demonstrated that the transcription factor *Sox4* is highly expressed in a subset of early secretory progenitors and required for: (1) proper enteroendocrine and tuft cell specification, and (2) ISC function. *Sox4* is also required for tuft cell hyperplasia in the setting of parasitic infection. Further, *Sox4* is capable of driving enteroendocrine and tuft cell differentiation in the absence of the canonical master secretory transcription factor, *Atoh1*. These studies represent the first demonstration that secretory lineages can be specified by transcription factors in the absence of *Atoh1*.

My own lab has extended an interest in genetic regulation of ISCs toward genomic studies of chromatin dynamics in intestinal epithelial differentiation. Using bulk RNA-seq, ATAC-seq, and 5hmC-seq, we have demonstrated that intestinal chromatin can be “static” or “dynamic” at loci associated with differentially expressed genes. We found that static/dynamic chromatin is associated with transcription factor binding motifs in a predictable manner. Additionally, we found that intragenic levels of 5hmC correlate with gene expression in a manner that predicts sensitivity of gene expression to inhibition of TET enzyme activity. Collectively, this work has revealed paradigms for ISC regulation at the level of individual transcriptional networks as well as large-scale chromatin dynamics.

a) **Gracz AD***, Samsa LA, Fordham MF, Trotier DC, Altizer BA, Lo Y, Bao K, Starmer J, Shroyer NF, Reinhardt RL, Magness ST*. *Sox4* drives *Atoh1*-independent intestinal secretory differentiation toward tuft and enteroendocrine fates. *Gastroenterology,* 2018 (Nov);155(5):1508-1523. Epub 2018 Jul 25. *co-corresponding authorship.

b) Raab JR, Wager KE, Morowitz JM, Magness ST, **Gracz AD**. Quantitative classification of chromatin dynamics reveals regulators of intestinal stem cell differentiation. *Development, 147*(1). doi:10.1242/dev.181966.

2. Cellular heterogeneity in intrahepatic bile ducts. Intrahepatic bile ducts undergo significant remodeling following injury and are involved in a number of pathological conditions, but cellular heterogeneity in the biliary epithelium remains poorly characterized. No unique molecular markers or transcriptomic signatures exist for potential stem and/or progenitor populations in the bile ducts. Building on my work with the *Sox9*^{EGFP} reporter mouse in the intestinal epithelium, my lab has demonstrated that *Sox9* expression levels can be used to identify distinct biliary epithelial cell (BEC) populations. We have utilized bulk and single cell RNA-seq (scRNA-seq) to delineate transcriptomic heterogeneity in BECs. Clonal organoid-forming assays demonstrate that functional stemness is a shared property across *Sox9* populations. Semi-quantitative confocal microscopy and 3D tissue clearing/light sheet imaging reveal that small bile and large bile ducts are comprised of different ratios of *Sox9* subpopulations. We have also demonstrated that organoid-forming populations isolated following damage to the liver demonstrate enhanced potential for differentiation into hepatocyte-like cells when cultured in differentiation-promoting media. These data establish cellular diversity in the biliary epithelium and facilitate mechanistic studies examining the role of BEC subpopulations in liver biology and disease.

a) Tulasi DY, Martinez Castaneda D, Wager K, Alcedo KP, Raab JR, **Gracz AD**. Sox9^{EGFP} defines biliary epithelial heterogeneity downstream of Yap activity. *Cellular and Molecular Gastroenterology and Hepatology,* In Press. Published online: Jan 23, 2021. doi: https://doi.org/10.1016/j.jcmgh.2021.01.009.

b) Gomez I, Wager K, Fordham MJ, **Gracz AD**. *In vivo* damage primes biliary organoids for enhanced transdifferentiation *in vitro. Abstract, Digestive Disease Week. Gastroenterology,* 2018 May;154(6):S-7

3. Identification and isolation of ISCs. My graduate studies focused mainly on the identification and isolation of ISCs, beginning shortly after the discovery of *Lgr5* as the first validated ISC biomarker. I used a *Sox9*^{EGFP} BAC transgenic mouse model to isolate and characterize different crypt-based cell populations and demonstrated that different levels of *Sox9* expression are associated with distinct cell types. I applied used this model to identify CD24 as the first cell surface marker suitable for enrichment of ISCs from non-transgenic mice using commercially available antibodies. The identification of CD24 as an enrichment factor for ISCs was subsequently leveraged into translational studies in human tissue, where I demonstrated that CD24-/CD44+ cells represent active ISCs and CD24+/CD44+ cells represent reserve ISCs.

a) **Gracz AD**, Ramalingam S, Magness ST. Sox9-expression marks a subset of CD24-expressing small intestine epithelial stem cells that form organoids *in* vitro. *Am J Physiol Gastrointest Liver Physiol.* 2010 May; 298(5): G590-600.

b) von Furstenberg RJ, Gulati AS, Baxi A, Doherty JM, Stappenbeck TS, **Gracz AD**, Magness ST, Henning SJ. Sorting mouse jejunal epithelial cells with CD24 yields a population with characteristics of intestinal epithelial stem cells. *Am J Physiol Gastrointest Liver Physiol.* 2011 Mar; 300(3): G409-17.

c) **Gracz AD***, Fuller MK*, Wang F, Stelzner M, Dunn JC, Martin M, Li L, Magness ST. CD24- and CD44-expression profiles facilitate differential isolation of 'active' and 'reserve' intestinal epithelial stem cell populations from human small intestine. *Stem Cells.* 2013 Sep; 31(9): 2024-30. *authors contributed equally to this work.

d) Wang F, Scoville D, He XC, Box A, Perry J, Smith NR, Nanye NL, Davies PS, Fuller M, Haug JS, McClain M, **Gracz AD**, Ding S, Stelzner M, Dunn J, Magness ST, Wong M, Martin M, Helmrath M, Li L. Isolation and characterization of intestinal stem cells based on surface marker combinations and colony-formation assay. *Gastroenterology*. 2013 Aug; 145(2): 383-95.e1-21.

4. *In vitro* culture of mouse and human ISCs. My work has also focused on the development of culture systems for the *in vitro* studies of primary intestinal and colonic stem cells. The lack of *in vitro* conditions for primary intestinal tissue was a major hurdle in the field when I started my graduate studies. I utilized newly described organoid cultures to demonstrate functional stemness in *Sox9*^{lo} cell populations. Due to the broad expression of *Sox9* in the intestinal crypts, this approach allowed us to define a stem cell population with greater resolution than possible with traditional *in vivo* lineage-tracing assays. In my postdoctoral research, I developed a high-throughput platform for clonal ISC culture. This platform also facilitated retrieval of single ISCs and organoids across multiple developmental stages for downstream gene expression analysis. A major advance of this platform is the ability to expose single ISCs to different environments or conditions and retrieve these cells for analysis at very early time points, prior to cell division.

a) Ramalingam S, Daughtridge GW, Johnston MJ, **Gracz AD**, Magness ST. Distinct levels of Sox9 expression mark colon epithelial stem cells that form colonoids in culture. *Am J Physiol Gastrointest Liver Physiol.* 2012 Jan; 302(1): G10-20.

b) **Gracz AD**, Puthoff BJ, Magness ST, Identification, isolation, and culture of intestinal epithelial stem cells from murine intestine. *Methods Mol Biol.* 2012; 879:89-107.

c) **Gracz AD**, Williamson IA, Roche KC, Johnston MJ, Wang F, Wang Y, Attayek P, Balowski J, Liu X, Laurenza RJ, Gaynor L, Sims CE, Galanko J, Li L, Allbritton NL, Magness ST. A high throughput platform for stem cell-niche co-cultures and downstream gene expression analysis. *Nature Cell Biology,* 2015 Mar; 17(3):340-9.

5. Activation and maintenance of reserve ISCs. The mechanistic regulation of stem cell plasticity in the intestine remains poorly characterized. I contributed to studies demonstrating characteristics of stemness in the *Sox9*^{hi} population, which consists of secretory progenitors and enteroendocrine cells in homeostasis. These studies demonstrated that *Sox9*^{hi} cells proliferate following radiation damage. I contributed to mechanistic studies that demonstrated a requirement for *Sox9* in the maintenance of reserve, “label retaining” ISCs. These studies also demonstrated a requirement for *Sox9* in intestinal regeneration following radiation injury. Together, these studies have been important in advancing the concept of facultative stemness, wherein non-canonical stem cells reacquire properties of self-renewal and multipotency to contribute to damage responses.

a) Van Landeghem LM, Santoro A, Krebs A, Mah AT, Dehmer JJ, **Gracz AD**, Scull BP, McNaughton K, Magness ST, and Lund PK. Activation of two distinct Sox9-EGFP expressing intestinal stem cell populations during crypt regeneration after irradiation. *Am J Physiol Gastrointest Liver Physiol.* 2012 May 15; 302(10):G1111-32.

b) Roche KC, **Gracz AD**, Lui X, Newton V, Magness ST. SOX9 maintains reserve stem cells and preserves radio-resistance in mouse small intestine. *Gastroenterology*, 2015 Nov;149(6):1553-1563.e10.

Complete list of published work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/adam.gracz.1/bibliography/49582339/public/?sort=date&direction=ascending