Doctoral Dissertation Defense Announcement

“Transcriptional Reprogramming by GATA4 Activation in Human Squamous Esophageal Epithelial Cells: Implications for Barrett’s Esophagus and Esophageal Adenocarcinoma”

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Basic & Translational Science Concentration
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Date: Tuesday, June 18, 2024
Time: 1:00PM (CST)

Defense Location: Kerrigan Auditorium

https://mcw-edu.zoom.us/j/96875071410?pwd=Wk92cGo4RjkwNUc4UGoxS09nNTZDQT09

Zoom Meeting ID: 968 7507 1410 Passcode: 9BggMFBa
Graduate Studies:
Foundations in Biomedical Sciences I
Foundations in Biomedical Sciences II
Foundations in Biomedical Sciences III
Foundations in Biomedical Sciences IV
Intro to Biomedical Research
Techniques in Molecular & Cell Biology
Professional Development I
Professional Development II
Statistics for Basic Sciences
Writing an Individual Fellowship
Developmental and Stem Cell Biology
Ethics & Integrity in Science
Advanced Cell Biology
Functional Genomics
Research Ethics Discussion Series
Basic & Translational Science Seminar
Boundaries of Science and Medical Practice
Reading and Research
Doctoral Dissertation
Dissertation

“Transcriptional Reprogramming by GATA4 Activation in Human Squamous Esophageal Epithelial Cells: Implications for Barrett’s Esophagus and Esophageal Adenocarcinoma”

Esophageal Adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC) are the two primary histological subtypes of esophageal cancer. In recent years, EAC diagnoses have begun to surpass diagnoses of ESCC while the prognosis for patients remains poor. Increased incidences of EAC are likely due to the increasing cases of obesity and gastroesophageal reflux disease (GERD) causing Barrett’s esophagus, the only known precursors for EAC. Barrett’s esophagus (BE) is a form of metaplasia that occurs in the distal esophagus proximal to the squamocolumnar junction (SCJ) in response to reflux-induced injury and inflammation. Barrett’s metaplasia is characterized by the emergence of columnar epithelial cells with gastric and intestinal features in the normally stratified squamous epithelium. There is much debate in the field regarding the cell of origin of Barrett’s metaplasia. Theories contemplate squamous cells in the esophagus, including esophageal progenitors and submucosal glands, versus migrating columnar epithelial progenitor cells to the site of reflux-induced injury. These theories are not mutually exclusive but demonstrate a need to define further the molecular changes that promote BE.

GATA4 is a zinc finger DNA-binding transcription factor that plays a critical role in the development and homeostasis of various organs and tissue types. During gastrointestinal development, GATA4 is expressed throughout the nascent foregut endoderm; the anterior foregut endoderm gives rise to the squamous esophageal epithelium; the posterior foregut endoderm gives rise to the columnar epithelium comprising gastric glands and intestinal crypts in the proximal duodenum. GATA4 expression is lost in the mature esophageal epithelium upon the initiation of squamous differentiation, and it is not found in the mature squamous epithelium. However, GATA4 expression remains in the gastric and intestinal columnar epithelium and plays a crucial role in columnar epithelial morphogenesis and the establishment of the SCJ. We have previously shown that aberrant reactivation of GATA4 in the developing mouse forestomach is sufficient to suppress the formation of the squamous epithelium and promote the formation of a glandular columnar-like epithelium. This study also found that GATA4 expression is required for columnar epithelial morphogenesis. Loss of GATA4 activity in the developing columnar epithelium of the mouse hindstomach resulted in the formation of a stratified squamous-like epithelium in its place.

We found abnormal reactivation of GATA4 in the metaplastic regions in BE and EAC. Given our observations of the effects of GATA4 on epithelial cell fate during development, we sought to determine if GATA4 could elicit similar effects on squamous epithelial cell fate to promote Barrett’s metaplasia. We demonstrated that GATA4 reactivation in squamous epithelial cells for 48h suppresses TP63, master-regulator of squamous differentiation, along with several squamous-associated cytokeratins, and promotes the expression of columnar-associated KRT8. These observations led us to hypothesize that GATA4 activation in human squamous epithelial cells would promote a columnar-like epithelial expression patterning resembling BE. We predicted that the changes in expression patterning in we observe in human squamous epithelial cells with GATA4 activation would be due to GATA4-direct transcriptional regulation. Several of the signaling pathways and transcription factors regulating anterior and posterior foregut morphogenesis are abnormally reactivated during Barrett’s esophagus. We further sought to determine the effects of GATA4 activation on these signaling pathways in squamous epithelial cells.

We address our hypothesis in the studies outlined in this thesis. (1) We first assessed the transcriptional effects of the abnormal reactivation of GATA4 for five days on biopsy-derived human squamous epithelial cells using bulk RNA sequencing. We determined the proportion of changes in gene expression due to GATA4 activity as a transcription factor through CUT&Tag to identify the genes bound by GATA4 in human squamous epithelial cells. (2) Next, we assessed esophageal cancer cell lines that express endogenous GATA4 and created GATA4 knock-out cell lines using CRISPR/Cas9. Using bulk RNA-sequencing, we determined the transcriptional changes that occur when GATA4 is lost in esophageal cancer cell lines. Again, we employed CUT&Tag to determine the genomic regions bound by endogenous
GATA4 in esophageal cancer cells. (3) Lastly, an in vivo mouse model of BE and EAC was employed by administering water containing the unconjugated bile acid deoxycholic acid (DCA) coupled with a high-fat diet for up to 12 months. Based on our in vitro studies, we predicted that GATA4 conditional knock-in (cKI) in the squamous epithelium would accelerate disease progression and promote esophageal cancer. Overall, we found that GATA4 activation in human squamous esophageal epithelial cells results in broad transcriptional reprogramming, suggesting loss of squamous cell identity and adopting transcriptional programming resembling gastric and intestinal columnar epithelial cells. Moreover, we found the abnormal reactivation of pathways and transcription factors implicated in pluripotency and posterior foregut morphogenesis in human squamous epithelial cells with GATA4 activity. We found a substantial overlap in the transcriptional changes that occur with GATA4 activation and the genes bound by GATA4. This demonstrates that the differential expression gene is due to GATA4 activity as a transcriptional regulator. We further determined that GATA4 may contribute to the innate immune response during reflux-induced injury and the activation of cellular repair mechanisms similar to those observed in metaplasia in different regions of the GI system. We observed overlapping expression patterns in human squamous epithelial cells with GATA4 activation of genes differentially expressed in BE and EAC. We found that the genes differentially expressed in GATA4KO esophageal cancer cell lines resulted in the enrichment of GO terms regarding epithelial morphogenesis and gland development similar to those found in human squamous epithelial cells with GATA4 activation, further demonstrating that GATA4 may act as a transcriptional regulator of columnar epithelial cell fate in esophageal cancer. Our in vivo study revealed substantial weight loss in mice in GATA4cKI mice compared to littermate controls and GATA4cKO mice subjected to the same treatment. We found that GATA4cKI mice develop esophageal cancer and intestinal metaplasia in the gastric epithelium as early as six months. Overall, male GATA4cKI mice had poorer prognosis and a more rapid and severe onset of disease. These findings can be attributed to the role of GATA4 in sex determination during development and or the role of GATA4 in hormonal signaling and homeostasis.

The findings of this study are significant in that they establish a potential relationship between GATA4 activation and epithelial cell plasticity during wound healing by promoting stem-like pluripotency and altering cell fate. This response has been observed in the stomach and pancreas metaplasia but has not been demonstrated in squamous epithelial cells or Barrett’s esophagus. This study further indicates that while cell plasticity during wound healing may be beneficial in chronic conditions, it may also promote metaplasia by promoting epithelial cell fate. There is a clinical need to determine the mechanisms outlining the onset of metaplasia; in this study, we propose a novel mechanism of squamous epithelial cell transformation directed by the morphogenic transcription factor GATA4. Moreover, we found several developmental signaling pathways activated downstream of GATA4 that can provide insights into novel therapeutic targets.
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Education:

Medical College of Wisconsin, Milwaukee, WI
Aug 2020- present
PhD Cellular and Developmental Biology
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Idaho State University Graduate School, Pocatello, ID
M.S., Anthropology
Thesis: Molecular Bioarcheological Approaches for Identifying Diet and Diagenetic Alteration in a Latte Period Assemblage from Saipan, Northern Mariana Islands (Accepted August 2018)

Winston-Salem State University, Winston-Salem, NC
May 2011- May 2015
Bachelor of Science, Biology

Experience:

Aug 2020-present
Graduate Research Assistant
Medical College of Wisconsin
(Dr Michele Battle, PI)

Sept 2018-Aug 2020
Research Technician I
Medical College of Wisconsin
(Dr Michele Battle, PI)

August 2015-August 2018
Career Path Internship
Idaho State University - Pocatello, ID
Graduate Assistant (Dr. John Dudgeon, PI)

August 2016- May 2017
Graduate Assistantship Anthropology Department
Idaho State University - Pocatello, ID
Teaching Assistant

MARC U* STAR Program
Winston-Salem State University- Winston-Salem, NC
Student Researcher/Scholar (Dr. Victor Pulgar, PI)

August 2012- August 2014
WSSU Department of Life Sciences
Winston-Salem State University- Winston Salem, NC
Student Researcher (Dr. Daniel Williams, PI)

Awards & Honors:

2022 Graduate Student Fellowship MCW Cancer Center
2022 Best Poster Award MCW Cancer Center Scientific Retreat
2022 Graduate Student Travel Award MCW CBNA
2022 Graduate Student Travel Award MCW GSA
2021 1st Place Poster Award in MCW Graduate School Research Symposium
2019 Best Poster Award MCW Cancer Center Scientific Retreat
2016 Graduate Assistantship Anthropology Dept. ISU
2016 1st Place Award in poster category, ISU Graduate Research Symposium
2014 MARC U STAR Program Research Fellowship
2014 Provost Undergraduate Summer Research Fellowship
Abstracts & Oral Presentations:

**Olivia D. Franklin** Defining the Role of GATA4 in Esophageal Diseases
Short Talk; MCW Cancer Center Trainee Symposium (2023)

**Olivia D. Franklin, Kirthi Pulakanti, Sridhar Rao, Thai Pham, David Wang, Michele A. Battle.** Defining the Role of GATA4 in Esophageal Diseases
Short Talk; FASEB GI (2022)

**Franklin, O. D., Pulakanti, K., Rao, S., Pham, T., Wang, D., & Battle, M. A.**

**Olivia D. Franklin, Kirthi Pulakanti, Sridhar Rao, Thai Pham, David Wang, Michele A. Battle.** Defining the Role of GATA4 in Esophageal Diseases
Poster Presentation; MCW Cancer Center Retreat (2022)


**Olivia D. Franklin, Kirthi Pulakanti, Sridhar Rao, Thai Pham, David Wang, Michele A. Battle.** Defining the Role of GATA4 in Esophageal Disease. Virtual Poster Presentation: MCW Graduate Student Research Symposium (2021)

**Olivia D. Franklin, Roman Stavniichuk, Thai Pham, Ann DeLaForest, Rhonda Souza, David Wang, Michele A. Battle.** Repression of Stratified Squamous Cell gene expression and induction of squamous lesions in GATA4 expressing esophageal cells. Virtual Poster Presentation: FASEB GI (2020)

**Olivia D. Franklin, Roman Stavniichuk, Thai Pham, Ann DeLaForest, Rhonda Souza, David Wang, Michele A. Battle.** Repression of Stratified Squamous Cell gene expression and induction of squamous lesions in GATA4 expressing esophageal cells Flash Talk & Poster Presentation; FASEB GI (2019)

**Olivia Franklin, John Dudgeon, Amy Commendador, Micheal Dega (SCS)**
Molecular bioarcheological approaches for identifying diet in a Latte period assemblage from Saipan, Northern Mariana Islands Short Talk: Society for American Archaeology Conference (2017)

Olivia Franklin, Daniel Williams. Bioinformatic Analysis of Presenilin 2 Poster Presentation; Emerging Researchers National Conference (2014)


Leadership, Service & Professional Memberships:

2020-2023: Co-Chair, Driving Equity and Inclusion for Student in Science Committee
2021-present: Student Member, American Gastroenterological Association
2022-2023: SPUR Ambassador, Summer Program for Undergraduate Research MCW