

Presenter	Info	Lab PI	Category	Title	Funding Source and Year	Abstract
Battle, Michele, PhD	Associate Professor	N/A-Seed Grant Recipient	Seed Grant/Basic	Novel roles for GATA4 in defining the squamocolumnar junction in the GI tract: Implications for Barrett's esophagus	MCWCC Pilot Program Philanthropy	<p>Introduction: Reactivation of pathways used during development to pattern tissue fate and function can play a role in disease. Our work explores the idea that abnormal re-activation of the developmentally important transcription factor GATA4 in the stratified epithelium of the esophagus contributes to the pathology of Barrett's esophagus (BE), a premalignant metaplasia preceding esophageal adenocarcinoma (EAC). Our previous studies identified GATA4 as an essential regionalizing factor within the small intestinal epithelium. GATA4 is also differentially expressed at the squamocolumnar junction, where it is present within the columnar epithelium of the glandular stomach but is absent from the stratified epithelium of the esophagus/forestomach. In BE, this boundary is disrupted, and the esophageal stratified epithelium is replaced by columnar epithelium. The lack of GATA4 in normal esophageal epithelium and its presence in BE metaplasia along with the observation that the GATA4 gene is frequently amplified and expressed in EAC suggest a role for GATA4 in BE/EAC pathogenesis.</p> <p>Objective: The objective of this study is to test the hypothesis that exclusion of GATA4 from esophageal/forestomach epithelium during development is essential to establish a normal squamocolumnar junction.</p> <p>Methods: We used Gata4 conditional knockout and knock-in mice with Sonic Hedgehog Cre to eliminate GATA4 in the columnar epithelium of the mouse hindstomach or to induce GATA4 in the stratified epithelium of mouse forestomach during development. We examined phenotypes in conditional mutants by histochemistry, immunohistochemistry, and RNA-Seq. We used ChIP-Seq to map the GATA4 binding profile in normal mouse hindstomach and combined binding data with gene expression data from mutants to identify high-confidence GATA4 direct targets implicated in human BE.</p> <p>Results: We found that GATA4-deficient hindstomach epithelium was stratified rather than columnar, resembling the epithelium of the forestomach/esophagus. Conversely, GATA4-expressing forestomach contained columnar epithelium whereas control forestomach consisted solely of stratified epithelium. RNA-Seq revealed alterations in the transcriptomes of GATA4 mutants. The transcriptome of hindstomach epithelium lacking GATA4 shifted toward that of forestomach epithelium, and the transcriptome of forestomach epithelium expressing GATA4 shifted toward that of hindstomach epithelium. We found a network of esophageal and gastric transcription factors to have altered expression in GATA4 conditional mutants. One key altered factor was p63, a master regulator of stratified squamous epithelial development. Importantly, many transcripts associated with human BE were similarly mis-regulated in GATA4 conditional mutants.</p> <p>Conclusions: Together, these data support the hypothesis that GATA4 has an essential role in squamocolumnar junction development. Our data suggest that GATA4 promotes columnar over stratified squamous epithelial development at the squamocolumnar junction by regulating expression of a transcription factor network, including GATA4-mediated repression of the stratified epithelial cell master regulator p63. Finally, altered expression of many BE-associated genes in GATA4 mutants further links GATA4 transcription factor function to human BE.</p> <p>Significance: We expect our studies to identify the GATA4-dependent molecular mechanisms that guide normal squamocolumnar development and contribute to the molecular pathogenesis of Barrett's esophagus. Identifying the role of GATA4 and its downstream targets in squamocolumnar development and disease has great potential to lead to new therapeutic targets and biomarkers for Barrett's esophagus. By reducing Barrett's esophagus, important progress can be made toward reducing esophageal cancer.</p>
Bluemn, Theresa	Graduate Student	Zhu, Nan, PhD/Pilot Grant	Seed Grant/Translational	ARID1B but not ARID2 is a novel tumor suppressor in MLL-AF9 leukemogenesis	MCWCC ACS-IRG-14-247-29 IRG	<p>Introduction: ARID1B and ARID2 are unique subunits of the Switch/Sugar Non-Fermenting (SWI/SNF) chromatin remodeling complex, BRG1 associated factor (BAF) and Polybromo-associated BRG1 associated factor (PBAF), respectively. These complexes utilize an ATPase, BRG1 or BRM, to reposition nucleosomes within existing chromatin, which alters the chromatin accessibility and ultimately, induces or represses gene expression. ARID1B and ARID2 are mutated in various malignancies, including head and neck, melanoma, hepatocellular, DLBCL, and CLL, and typically exhibit tumor suppressor activity. Recent investigations have additionally proposed a role for ARID1B and ARID2 in MLL (mixed lineage leukemia) -rearranged (MLLr) leukemias. MLL (mixed lineage leukemia) -rearranged (MLLr) leukemias have an intermediate to poor prognosis and occur in more than 70% of infant leukemia's and 5-10% of adult. MLLr leukemias involve the translocation of the MLL gene to over 50 partners fusion partners. In AML, the most frequently found fusion partner is AF9. The resulting MLL-AF9 is a driver oncogene in leukemia. Chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) identified ARID1B and ARID2 as novel targets for the oncogenic fusion protein MLL-AF9 and an in vivo pooled shRNA screen of MLL-AF9 target genes demonstrated that ARID1B and ARID2 may play a role in MLL-AF9 leukemia maintenance. Furthermore, gene alternations in ARID1B and ARID2 are found in AML patient samples.</p> <p>Objective: To determine the role of ARID1B and ARID2 in MLL-AF9 leukemogenesis.</p> <p>Methods: To evaluate the role of ARID1B and ARID2 in vitro, MLL-AF9 transformed HSPCs were analyzed by flow cytometry to assess cell cycle, cell proliferation, differentiation and apoptosis. To evaluate their role in vivo, two hematopoietic specific, deficient mouse models were assessed. VavCRE, a constitutively active CRE-recombinase, to evaluate leukemic initiation and MxCRE, an inducible CRE-recombinase, to evaluate leukemic maintenance. Recipient mice were assessed for disease burden and survival.</p> <p>Results: In vitro characterization of the loss of ARID1B or ARID2 in MLL-AF9 transformed HSPCs revealed no aberrant defects in differentiation, cell proliferation, cell cycle, or apoptosis. Studies using ARID2 deficient MLL-AF9 in mouse models show no role in leukemic initiation or maintenance. The requirement of ARID1B in MLL-AF9 is still being investigated. Our preliminary results showed that ARID1B deficiency leads to accelerated leukemia progression in recipient mice. Peripheral blood MLL-AF9 cells percentage is higher in mice transplanted with ARID1B deficient cells than that of the control four weeks post transplantation. Moreover, loss of ARID1B resulted in decreased latency of the recipient comparing to controls.</p> <p>Conclusions: ARID1B, but not ARID2, plays a more prominent role in MLL-AF9 leukemia by acting as a tumor suppressor. Further investigation is necessary to fully understand their role and mechanism of action.</p> <p>Significance: Elucidating the role of epigenetic regulators and their contributions to leukemogenesis is essential for the development of novel therapeutics to improve patient care and disease outcomes.</p>

Chaluvally-Raghavan, Pradeep, PhD	Assistant Professor	N/A-Seed Grant Recipient	Seed Grant/Basic	Unexpected Role of microRNAs in the Nucleus for Transcriptional activation	Ovarian Cancer Research Fund Alliance (OCRFA), DoD Breast Cancer Research Program (W81XWH-18-1-0024), the Women's Health Research Program (WHRP) at MCW, American Cancer Society's IRG, and research funds from MCW Cancer Center.	<p>Introduction: Ovarian cancer is unique amongst solid tumors in that Copy Number Variations (CNV) are an essential feature of tumor development, progression, and metastasis. Our recent study identified that 3q26.2 is a critical CNV, which is amplified in ~40% of ovarian cancer patients (Cancer Cell, 2014). Later, we discovered that a novel microRNA, miR551b is overexpressed as a direct result of 3q26.2 locus CNV in ovarian cancer (Cell Reports, 2016). We have identified that miR551b induce the transcription of STAT3 oncogene. We further demonstrate that STAT3 induced Oncostatin M Receptor and its ligand (OSM) activates a feedforward autocrine loop, on which tumor cells can rely for proliferation and metastasis (Manuscript is under revision in Cell Reports, 2019). However, the exact mechanism of how miR551b mediates the transcriptional activation of STAT3 is not well known.</p> <p>Objective: This study aims to evaluate the mechanism of nuclear functions of miR551b for the transcriptional activation of STAT3 oncogene for the growth and metastasis of cancer cells.</p> <p>Methods: miR551b expression in human breast and ovarian cancer cells and tissues determined by qPCR. We have either knocked down or overexpressed miR551b in breast and ovarian cancer cells and performed Reverse Phase Protein Array (RPPA) to identify the changes in the levels of total and phosphorylated forms of proteins in cancer cells. RNA immunoprecipitation (RIP) assays followed by immunoblot analysis was performed to identify the proteins bind to miR551b, that facilitate the translocation of miR551b from the cytoplasm to nucleus. Confocal microscopy was performed to determine the nuclear translocation of microRNAs and to identify the key proteins, which co-localizes with miR551b.</p> <p>Results: Our analysis identified that miR551b is highly amplified in breast and ovarian cancers and miR551b is the key regulator of STAT3 for oncogenic addiction. We further showed that miR551b-STAT3 axis leads to the upregulation of OSMR and OSM which activate an autocrine signaling loop in cancer cells for hyper-proliferation, migration, and metastasis of cancer cells. Our results demonstrate that the interaction of miR551b on STAT3 promoter leads to conformational changes in the promoters, that improves the occupancy of transcription factors (TFs) and RNA-Polymerase-II (RNA-Pol-II) on the promoter for transcriptional activation (RNAa). As a consequence of RNAa, miR551b upregulates the expression of important cytokines and growth factors such as Oncostatin M (OSM), Interleukin-31 (IL31) and their receptors Oncostatin M Receptor (OSMR) and Interleukin-31 receptor (IL-31R).</p> <p>We found that Importin-8 (IPO-8) is critical for the nuclear translocation of miR551b. In complement, we found that the use of nuclear transport inhibitors like KPT185 which inhibit the action of importin-8 abolished the miR551b-induced STAT3 transcription. Our immunoblot assays followed by RIP assays identified that IPO8 acts as an adaptor, which connects the binding of miR551b-AGO1 complex with RNA-Polymerase-II for the transcriptional activation of STAT3. In conjunction with our in vitro data, ectopic expression of miR551b promoted the tumor growth and metastasis of breast and ovarian cancers in vivo. Conversely, inhibiting miR551b using anti-microRNAs reduced the tumor growth and metastasis in vivo.</p> <p>Conclusions: Our study identified that miR-551b directly interacts with STAT3 promoter and activates its transcription. This novel mechanism is called RNA activation (RNAa), which is a significant departure from the status quo that microRNA bind on the 3'UTR of genes and downregulates its expression. We identified that tumor cells</p>
Neuner, Joan, PhD	Associate Professor	N/A-Seed Grant Recipient	Seed Grant/Pop Sci	The association of pharmacy fill synchronization with breast cancer endocrine therapy adherence	MCWCC Pilot Program Philanthropy, (Multi PI) 2018	<p>Introduction: One-third to one-half of patients prescribed adjuvant endocrine therapy with tamoxifen or an aromatase inhibitor either discontinue early or skip a substantial number of pills. Research to improve this has been disappointing.</p> <p>Objective: We investigated whether poor pharmacy synchronization of medications is an unrecognized barrier to adherence.</p> <p>Methods: A cohort of women aged 66-90 years old with Stage 0-3 hormone receptor-positive breast cancer were identified from the Surveillance, Epidemiology and End Result (SEER)-Medicare claims-linked cancer registry. Women with Medicare pharmacy claims documenting at least one endocrine therapy prescription fill and at least one other medication fill were identified, and the 3-month synchronization of their medication fills was calculated as the quotient of the number of pharmacy visits and the number of filled medications subtracted from 1. Logistic regression was used to assess the association between synchronization stratified by the number of medications and adherence to endocrine therapy (defined as medication possession ratio >80%) over the subsequent year.</p> <p>Results: The study cohort included 3,112 women treated with endocrine therapy for breast cancer (Table 1). Over 31% were age 70 or younger, and 38.0% had stage 2 or 3 disease. During the three months after the first endocrine therapy prescription, the mean number of unique medications was 8.0 (S.D. 6.0) and the mean number of pharmacy visits was 8.6 (S.D. 4.7). In an adjusted model, compared with the highest adjusted synchronization, those in the lowest adjusted synchronization quartile were less likely to be adherent (MPR >=80%) with an Odds Ratio 0.71 (95% CI 0.57, 0.88), as were those in the second-lowest (0.81 (0.65, 0.99). Neither age nor race/ethnicity were associated with adherence, and results were unchanged with inclusion of information about duration of fills (30 vs 90 days)</p> <p>Conclusions: Prescription fill synchronization is strongly associated with adherence to endocrine therapy. Research into interventions to improve adherence should include prescription synchronization and other health systems barriers.</p> <p>Significance: Our study provides an important target for future efforts to improve adherence to oral medications in cancer patients. With the growth in oral anti-oncologic agents, interventions that simplify patients' experiences are needed to foster optimal cancer outcomes.</p>

Spellecy, Ryan, PhD	Professor	N/A-Seed Grant Recipient	Seed Grant/Pop Sci	Mistrust, the Obstruction of Medicine: Repairing the Breach between Medical Research and the African American Community in Milwaukee.	MCW Community Engagement Core and MCWCC Philanthropy, 2017	<p>Introduction: It is well known that participation rates in cancer clinical trials among African Americans both nationally (Team, Gohagan, Prorok, Hayes, & Kramer, 2000) and in Milwaukee are unacceptably low. While the reasons for this have been documented in other regions (Blocker et al., 2006; Robinson, Ashley, & Haynes, 1996) in Milwaukee they are currently unknown. Until the reasons for low participation among African Americans in Milwaukee are known, attempts to increase participation will be uninformed and unsuccessful, as they will not address the root causes and, in turn, will fail to ameliorate cancer care inequalities that stem from a lack of research, as in prostate cancer among African American males.</p> <p>Objective: To understand and document the reasons for low participation in cancer clinical trials in the African American Community in Milwaukee. In addition, we documented the factors that participants believed would positively influence their participation in cancer clinical trials.</p> <p>Methods: We initially conducted a focus group with members of Pastor's United, a group of pastors from African American churches in Milwaukee, to inform our future focus groups. We then conducted 3 focus groups with members of the African American community identified by members of Pastor's United. Focus groups were facilitated by our community partners at the churches, recorded and transcribed. Transcripts were analyzed using the methods of grounded theory to identify themes, and discordant themes were discussed to achieve consensus.</p> <p>Results: We found that 1) Trust is key for positive interactions with the healthcare system, including participation in clinical research. 2) There is an enduring fear that abuse, mistreatment and dishonesty still exist in healthcare and research. 3) The community recognizes the value of being included in research, and how it may improve cancer screening, diagnosis, treatment and overall health for them. Lastly, 4) Many participants lacked knowledge to what clinical trials are, and the language used to educate on and discuss clinical trials is very important.</p> <p>Conclusions: While there is clearly distrust of medicine generally speaking, some focus group participants reported trusting relationships with their physicians, regardless of race. In addition to barriers of mistrust, there is also a lack of knowledge regarding clinical trials (which focus group participants did not like, preferring the term research) and a desire for more education in the community.</p> <p>Significance: Moving forward, the conversation about clinical trials must be part of a larger, holistic focus on building a healthy community and overall health equity in Milwaukee. Focusing the conversation only on research, to the exclusion of a focus on holistic and community health, will cause more mistrust.</p>
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