BIOGRAPHICAL SKETCH

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NAME: Chang, Mario Carlo

eRA COMMONS USER NAME (credential, e.g., agency login): marioc14

POSITION TITLE: Graduate Student Research Assistant

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Florida, Gainesville, FL	BS	08/2014	08/2018	Chemistry
University of Florida, Gainesville, FL	MS	08/2018	12/2020	Biochemistry and Molecular Biology
University of Florida, Gainesville, FL	PhD	08/2020	–	Biomedical Sciences

A. Personal Statement

My current research is focused on establishing the production of singly deuterated water (HDO) from [$^2H_7$]glucose tracer as a biomarker of in vivo tumor metabolism using magnetic resonance imaging and deuterium metabolic imaging. To this end, we plan on imaging and measuring changes in HDO production in melanoma cancer cells implanted into the flanks of BL6 mice infused with [$^2H_7$]glucose. I am confident that I have the proficiency, technical skills and expertise, and determination to complete the proposed project successfully. My background is in biochemistry and molecular biology, with research training specifically needed to accomplish the work proposed. I became proficient in mouse handling including tail clipping for genotyping, xenograft imaging, and oral gavage during my undergraduate research, and gained early experience in mammalian cell culture. During my Master's degree I expanded my mammalian cell culture technique, learned the work flow for cell and media GC-MS analysis, and how to analyze LC-MS data. My early work in the PhD program helped establish HDO production as a quantitative biomarker of drug induced alterations in cancer cell metabolism in culture as detailed in my co-first author publication "Measuring NQO1 Bioactivation Using [$^2H_7$]Glucose". Overall these studies will be important to establish widely applicable methodology that would allow a noninvasive and serial means to monitoring tumor progression and treatment efficacy with imaging. These past experiences make me the best candidate for pursuing this project.

In addition to this work I plan on utilizing in vivo deuterium metabolic imaging of HDO production in tumors to work towards developing adaptive dosing strategies that would maximize therapeutic effects while minimizing drug toxicities based on continuous reassessment supported by imaging data. I am also working on establishing novel combinatorial cancer therapies by identifying compounds synergistic with the chemotherapeutic agent, β-lapachone, by employing ^2H and ^{13}C isotope tracers to assess drug induced metabolic alterations as an indication of treatment efficacy. Extensive research has shown significant anti-proliferative effects of β-lapachone against several cancers in vitro and in vivo models, and in human clinical trials. However, it has been reported that β-lapachone alone causes serious off target effects. Identifying synergistic compounds with β-lapachone could significantly improve cancer treatment efficacy and overall patient outcome, which is an area of urgent need in the field. These studies will be valuable to identifying novel cancer treatment regimens.

Citations:

1. Mahar* R, **Chang* MC**, Merritt, ME. Measuring NQO1 Bioactivation Using [$^2H_7$]Glucose. *Cancers* **2021**, *13*, 4165. **(*Equally Contributed)**
2. Staklinski* SJ, **Chang* MC**, Kilberg MS. Measuring the Activity of Human Asparagine Synthetase (ASNS) Disease Variants. *Analytical Biochemistry* (Under Review). **(*Equally Contributed)**
3. **Chang MC**, Mahmud I, Guingab-Cagmat JD, Pillai S, Gillies R, Garrett TJ. Deeper Lipidome Analysis of Acidosis Induced Triple Negative Breast Cancer Cells by Iterative Exclusion Omics. *Analytical Chemistry* (Under Review).

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021 – Present	Discussion Leader of Fundamentals of Biomedical Sciences, Graduate Program in Biomedical Sciences, College of Medicine, University of Florida, Gainesville, FL
2020 – Present	Ph.D. Graduate Student, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
2018 – 2020	M.S. Graduate Student, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
2018 – 2020	Supplemental Instruction Leader, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
2015 – 2017	Undergraduate Researcher, Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL

Honors

2020	Grinter Scholarship Award, University of Florida College of Medicine, Gainesville, FL
2019	Glenview Scholarship Award, Collier County Community Foundation, Naples, FL

C. Contributions to Science

My contribution to science during Master's degree and PhD program:

1. **Measuring the Activity of Human Asparagine Synthetase Disease Variants**: Asparagine synthetase (ASNS) is a metabolic enzyme that mediates the catalytic conversion of aspartate to asparagine. To our knowledge, ASNS is the only human enzyme responsible for producing asparagine in an ATP-dependent manner. In 2001, adaptive upregulation of ASNS was discovered in childhood acute lymphoblastic leukemia, and since then, the adaptive upregulation of ASNS has also been to be associated with a variety of different cancers. Interestingly, ASNS expression has also been linked to another disease. In 2013, asparagine synthetase deficiency (ASNSD) was found to be caused by biallelic mutations in the human ASNS gene. As of today, less than 50 cases of ASNSD have been reported globally, with many more potentially undiagnosed cases missed because of a lack of efficient testing. Currently, ASNSD diagnosis is exclusively determined by whole exome sequencing upon the onset of distinctly apparent clinical symptoms. Whole exome sequencing is limited by cost, time, and wide accessibility for patients. A rapid, reliable, and inexpensive enzymatic activity assay would be extremely valuable for diagnosis, monitoring activity, and expanding our understanding of the ramifications of ASNS in humans. To this mean, I established two different assays capable of measuring ASNS enzymatic activity. One is a commercially obtainable assay that measures AMP production and the other is an LC-MS based assay that directly quantitates asparagine production.

    a) Staklinski* SJ, **Chang* MC**, Kilberg MS. Measuring the Activity of Human Asparagine Synthetase Disease Variants. *Analytical Biochemistry* (Under Review). **(*Equally Contributed)**

2. **Measuring NQO1 Bioactivation Using [$^2H_7$]Glucose:** Treating cancers with the chemotherapeutic agent β-lapachone causes NAD(P)H: quinone oxidoreductase 1 to produce a highly unstable hydroquinone that reestablishes itself while generating reactive oxygen species in the form of hydrogen peroxide. Accelerated accumulation of ROS causes large amounts of DNA damage, triggers poly-ADP-ribose polymerase-I hyperactivity, induces global depletion of NAD^+ and ATP, and perturbs glycolytic metabolism. Cancer cells that overexpress NQO1 successively die through NAD^+-keresis. Measuring changes in glycolysis caused by the bioactivation of NQO1 would establish a platform of assessing the efficacy of treatment, potentially allowing the reduction of chemotherapeutic dosage and diminishing off-target toxicities. Changes in glycolysis caused by NQO1 bioactivation were readily detected in A549 lung carcinoma, MiaPaCa2 pancreatic carcinoma, and HCT-116 colorectal carcinoma cells by deuterium NMR after supplementation of [$^2H_7$]glucose. Deuterated lactate and HDO were feasibly quantitated, and correlative linear relationships of both deuterated products with [$^2H_7$]glucose were observed. The high concentration of HDO detected in comparison to ^{2}H-lactate grants for a more sensitive assesment of glycolytic metabolism in cancer cells. Gas chromatography-mass spectrometry analysis matched NMR results and validated downregulated energy metabolism in $NQO1^+$ cancer cells treated with β-lapachone. The reported method is highly beneficial for measuring rates of glycolytic metabolism, the efficacy of chemotherapeutic agents that specifically hamper glycolysis, and can be potentially translated to in vivo models.

    a) Mahar* R, **Chang* MC**, Merritt ME. Measuring NQO1 Bioactivation Using [$^2H_7$]Glucose. *Cancers* 2021, *13*, 4165. **(*Equally Contributed)**

3. **Utilizing Iterative Exclusion for Deep Lipidome Analysis of Triple Negative Breast Cancer**: Metabolic reprograming is a hallmark for enhanced cancer cell adaptation in adverse cellular environments. The altered redox balance, biosynthetic, bioenergetic demands of cancer cells lead to the significant elevation of glycolytic lactic acid fermentation that further drives acidosis in the tumor microenvironment. Recently, several reports have elucidated a clear correlation between microenvironment acidification and dysregulated lipogenesis in aggressively propagating cancers, however there lacks a clear understanding of the exact molecular insights at play. Here, we utilize iterative exclusion (IE) liquid chromatography-high resolution mass spectrometry LC-HRMS/MS to establish a deep lipidome analysis of acidosis induced triple negative breast cancer (TNBC) to elucidate insights into the underlying molecular mechanisms of this highly proliferative cancer. Our present results clearly demonstrate the applicability of IE to extend the coverage of lipidomic analysis by traditional data-dependent tandem mass spectrometry acquisition (ddMS^2-topN). We show that IE is capable of annotating 6 times more distinct lipid species and 14 more lipid classes than traditional ddMS^2-topN in MDA-MB-231 breast cancer cells. Statistical analysis shows that while both IE and ddMS^2-topN can identify the same trend between control and acidosis induced cells, IE identifies a higher fold change in the lipid composition of acidified cells. Comparative clustering of statistically significant differences in lipid expression in acidified TNBC show that IE and ddMS^2-topN discretely select for unique lipid molecules. Lipid pathway analysis of the top 24 lipid ontologies by FDR q-values show clear correlations to cancer related lipogenesis alterations strongly identified by IE-omics. This study opens a high-throughput screening to improve the lipidome coverage and annotation of cancer cells for the potential discovery of biomarkers and therapeutic targets.

    a) **Chang MC**, Mahmud I, Guingab-Cagmat JD, Pillai S, Gillies R, Garrett TJ. Deeper Lipidome Analysis of Acidosis Induced Triple Negative Breast Cancer Cells by Iterative Exclusion Omics. *Analytical Chemistry* (Under Review).

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	MASTER OF SCIENCE	
2018	Independent Studies	A
2018	Supervised Research	P
2018	Biochemistry Seminar	A
2018	Fundamentals of Biochemistry and Molecular Biology	A-
2019	Supervised Research	P
2019	Biochemistry Seminar	A
2019	Fundamentals of Cancer Biology	A
2019	Cell Biology	A-
2019	Advanced Metabolism: Role of Membranes in Signal Transduction	B+
2019	Advanced Metabolism: Regulation of Key Reactions in Amino Acid Metabolism	B
2019	Independent Studies	A
2019	Supervised Research	P
2019	Biochemistry Seminar	A
2020	Independent Studies	A
2020	Independent Studies	A
2020	Masters Research	P
	DOCTOR OF PHILOSOPHY	
2020	Masters Research	P
2020	Fundamentals of Biomedical Sciences	A
2020	Research/Professional Development	P
2020	Biomedical Sciences Rotation 1	P
2020	Biomedical Sciences Rotation 2	P
2021	Advanced Molecular & Cell Biology	A
2021	Advanced Physical Biochemistry	A
2021	Biochemistry Seminar	A
2021	Lab Rotation 3	P
2021	Responsible Conduct in Biomedical Sciences	A
2021	Advanced Research	P