BIOGRAPHICAL SKETCH

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NAME: **Major, Michael B.**

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

POSITION TITLE: **Professor of Cell Biology and Physiology**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Michigan State University, MI	**B.S.**	**05/1997**	**Microbiology**
University of Utah/Huntsman Cancer Institute, UT	**Ph.D**	**05/2004**	**Oncological Sciences**
University of Washington, WA	**Postdoctoral**	**07/2009**	**Pharmacology**

A. Personal Statement

My lab studies how perturbation of specific signal transduction pathways contributes to the initiation, progression and dissemination of cancer. We employ a “systems level” integrative discovery platform to characterize pathway dynamics in normal and cancer cell models. More specifically, we use mass spectrometry-based proteomics to define the protein-protein interaction and protein proximity networks, along with phosphorylation and ubiquitylation post-translational modifications. Much of our effort focuses on the WNT and NRF2/oxidative stress signaling pathway. We then annotate the nodes within the networks for function, as determined by established and novel functional genomic screening technologies. Integration of these data with disease-associated mutation and gene expression data yields a powerful tool for discovery—a disease annotated physical/functional map. Critical to our success is the development and implementation of computational scoring algorithms, relational database construction and data visualization. Ultimately, the models and hypotheses produced are challenged through mechanistic studies employing cultured cancer cells, new and established mouse models, clinical tissue and various biochemical and cell biological systems.

Using these technologies, a major focus of my lab is to interrogate the NRF2 signaling pathway in lung and upper aerodigestive cancers. To discover new disease-relevant regulators of the pathway and their mechanics, we have defined the KEAP1 and NRF2 protein-interaction networks and associated post translational modifications. Using a chemical proteomics, we are identifying NRF2-responsive kinases. We functionally annotated the NRF2 signaling through: 1) siRNA and Crispr screens, 2) kinome-centric drug screens and 3) gain-of-function kinome screens. More recently, we have also created a new conditionally active NRF2 mouse GEMM to support the physiological significance of our findings. The translational impact of our research is strengthened by a new RNA-based transcriptional classifier of NRF2 activity; this predicts outcome and response to radiation therapy. Collectively, these data and my previous publications demonstrate that I have the molecular tools and experience to successfully interrogate NRF2 signaling and its function in cancer.

Selected review articles and book chapters from my laboratory:

a. Erica W. Cloer, Dennis Goldfarb, Travis P. Schrank, Bernard E. Weissman, **Michael B. Major**. NRF2 Activation in Cancer: From DNA to Protein. Cancer Research, Feb. 13th, 2019
b. Tigist Y. Tamir, Kathleen M. Mulvaney and **M. Ben Major**. Dissecting the Keap1/Nrf2 pathway through proteomics, in Current Opinion in Toxicology. 2016, Elsevier. Pgs. 118-125.
c. Matthew P. Walker, Dennis Goldfarb, **Michael B. Major**. New Insights from proteomic analysis of WNT signaling, in Wnt Signaling in Development and Disease: Molecular Mechanisms and Biological Functions, Stefan P. Hoppler, Randall T. Moon. Wiley-Blackwell, 2014, Chapter 9.

d. Ma S, Paiboonrungruan C, Yan T, Williams KP, **Major MB**, Chen XL. Targeted therapy of esophageal squamous cell carcinoma: the NRF2 signaling pathway as target. Ann N Y Acad Sci. 2018 May 11

B. Positions and Honors

Positions and Employment

1997-2004	Dissertation, Huntsman Cancer Institute, Salt Lake City, UT (Prof. David. A. Jones)
2004-2009	HHMI Postdoctoral Fellow, U of Washington, Seattle, WA (Prof. Randall T. Moon)
2009	Visiting Professor, University of North Caolina at Chapel Hill
2010-2016	Assistant Professor of Cell Biology and Physiology, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill
2012-2019	Adjunct appointment, assistant professor of Computer Sciences, UNC at Chapel Hill
2015-2016	Joint appointment, assistant professor of Pharmacology, UNC at Chapel Hill
2016-2019	Associate Professor of Cell Biology and Physiology with Tenure, Associate Professor of Pharmacology with Tenure (Joint appointment), Associate Professor of Computer Science (Adjunct appointment), Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill
2019-	Director, Washington University Systems Medicine Center for Mass Spectrometry
2019-	Alan A. and Edith L. Wolff Professor of Cell Biology and Physiology, Departments of Cell Biology and Physiology, and Otolaryngology, Washington University, St. Louis, MO

Honors and Awards

2010	Sidney Kimmel Scholar Award
2010	NIH Director's New Innovator Award (DP2)
2014	American Cancer Society Research Scholar
2014	V Foundation Translational Scientist Award
2015	Gabrielle Angel Foundation Medical Scientist Award
2016	Keynote Speaker, American Cancer Society Regional Conference
2016	HHMI Gilliam Mentor to Tigist Tamir
2017	UNC ACCLAIM Academic and Executive Leadership Fellow
2018	HHMI Gilliam Mentor to Megan Agajanian
2018	UNC Excellence in Basic Science Mentoring

Other Experience and Profession Memberships

2012-2013	American Heart Association Peer Review, Committee. IRG Basic & Bioengineering Sciences, Basic Cell PC 5.
2013-2015	Howard Hughes Medical Institute review panel member, International Student Dissertation Research Fellowship program
2014-2019	Chair (2018/19), American Cancer Society review panel, Tumor Biochemistry and Endocrinology
2016-2019	Associate Director, Cancer Cell Biology Training Program at UNC (T32)
2018	NIH Study Section, ZRG1 OBT-M (ad hoc)
2010-current	Member, American Society of Mass Spectrometry (ASMS)
2012-current	Member, American Association for Cancer Research (AACR)
2014-current	Member, American Society for Microbiology (ASM)
2016-current	NIH F09B Oncological Sciences Fellowship Study Section (F30, F31, F32)
2018-current	NIH Study Section, CAMP (ad hoc);
2018-current	HHMI Gilliam Fellowship Study Section

C. Contribution to Science

1. *Proteomics, Functional Genomics and Computational Technologies.* A central tenet of my research program is discovery-based science, founded largely upon mass spectrometry-driven proteomics, functional genomics and disease annotation. The integration of these disparate data types eliminates false positives, rescues false negatives, and highlights neighborhoods within the global network of phenotypic, mechanistic and disease importance. As a postdoc in Randy Moon's laboratory, I was the first to integrate genome-wide siRNA screens

with protein-protein interaction networks. Our ability to integrate and evaluate large datasets has improved dramatically in recent years. My team developed a machine learning approach to probabilistically score and functionally annotate protein-protein interactions—this is the most accurate scoring platform currently available (Spotlite). We also developed an improved MS data acquisition simulator, which facilitates the development of new algorithms for improved protein sequencing. Most recently, we developed novel algorithms to approximate isotopic distributions of MS2 fragment ions; this allows decomplexing of chimeric spectra and improved peptide identification. Within the proteomics arena, we have devoted great effort to kinase enrichment mass spectrometry to capture, identify and quantify the kinome in a single mass spectrometry run. In a recent example, we used this approach in a competitive fashion to define the kinase target landscape for FDA-approved kinase inhibitors. We are also developing new functional genomic screening technologies, but rather than continue with the more traditional loss-of-function approaches (siRNA, haploid mutagenesis), our efforts have focused on gain-of-function genome annotation. To this end, we developed an arrayed lentiviral clone library and a mass spectrometry-coupled genome-wide hypermorphic functional annotation technology called CDt/MS (listed under the WNT section).

a. Goldfarb, Dennis; Lafferty, Michael; Herring, Laura; Wang, Wei; **Major, Michael**. Approximating isotope distributions of biomolecule fragments. *American Chemical Society Omega.* 2018. PMCID: PMC6166224.
b. Emily M Cousins, Dennis Goldfarb, Feng Yan, Jose Roques, David B. Darr, Gary L. Johnson, and **Michael B Major**. Competitive Kinase Enrichment Proteomics Reveals that Abemaciclib Inhibits GSK3β and Activates WNT Signaling. *Molecular Cancer Research*. 2017 Nov 13th. PMCID: PMC5805620
c. Dennis Goldfarb, Bridgid Hast, Wei Wang and **Michael B. Major**. Spotlite: An Improved Algorithm and Web Application for Predicting Co-Complexed Proteins from Affinity Purification – Mass Spectrometry Data. *Journal of Proteome Research*, 2014 Oct 10th, PMCID:PMC4360886. http://warlock.med.unc.edu/spotlite/
d. Dennis Goldfarb, Wei Wang and **Michael B. Major.** MSAcquisitionSimulator: data-dependent acquisition simulator for LC-MS shotgun proteomics. *Bioinformatics*. 2015 Dec. 17th. PMCID: PMC4894284

2. *KEAP1 and NRF2 Signal Transduction in Cancer*. KEAP1 is an E3 ubiquitin ligase important for cellular defense against oxidative stress, and in that context contributes fundamentally to aging, neurodegeneration, and a myriad of human cancers, most notably lung cancer. KEAP1 functions by ubiquitylating the NRF2 transcription factor, resulting in NRF2 degradation. In cancer, mutations within NRF2 or KEAP1 result in constitutive NRF2-driven expression of cytoprotective genes—this occurs in ~30% of lung cancer. In an effort to better understand KEAP1/NRF2, we were the first to define and validate a KEAP1 protein-protein interaction network. This revealed a group of KEAP1-associated proteins that competitively displaces NRF2, driving pathway activation. Our work has also begun to connect KEAP1 genotype with phenotype, wherein we functionally and biochemically characterized 19 lung cancer KEAP1 mutations. This revealed that ~half of the KEAP1 cancer mutations are hypomorphic, and surprisingly retain the ability to ubiquitylate NRF2. Ongoing work is focused on the mechanism by which these 'ANCHOR' mutants impair KEAP1-dependent degradation (but not ubiquitylation) of NRF2 function. Based off of proteomic and functional genomic screens, we are also pursuing novel NRF2-independent functions for KEAP1, including a cell cycle phenotype relevant for lung cancer cell proliferation (MCM3). Most recently, we have created a new NRF2-active mouse model, allowing us to test the importance of our discoveries and the various biological impacts of NRF2 in a physiologically relevant system.

a. Tamir TY, Bowman BM, Agajanian MJ, Goldfarb D, Schrank TP, Stohrer T, Hale AE, Siesser PF, Weir SJ, Murphy RM, LaPak KM, Weissman BE, Moorman NJ, **Major MB**. Gain-of-function genetic screen of the kinome reveals BRSK2 as an inhibitor of the NRF2 transcription factor. *Journal of Cell Science*. 2020 Jun 16:jcs.241356. doi: 10.1242/jcs.241356. Online ahead of print. PMID: 32546533 PMCID: in process
b. Cloer EW, Siesser PF, Cousins EM, Goldfarb D, Mowrey DD, Harrison JS, Weir SJ, Dokholyan NV, **Major MB**. p62-Dependent Phase Separation of Patient-Derived KEAP1 Mutations and NRF2. *Molecular and Cellular Biology*, 2018. Aug 20. pii: MCB.00644-17. PMCID: PMC6206457
c. Kathleen M. Mulvaney, Jacob P. Matson, Priscila Siesser, Tigist Y. Tamir, Dennis Goldfarb, Timothy Jacobs, Erica W. Cloer, Joseph S. Harrison, Cyrus Vaziri, Jeanette G. Cook & **Michael B. Major**. Identification and Characterization of MCM3 as a KEAP1 Substrate. *The Journal of Biological Chemistry*, 2016 Nov 4;291(45):23719-23733. PMCID: PMC5095425
d. Hast BE, Cloer EW, Goldfarb D, Li H, Siesser PF, Yan F, Walter V, Zheng N, Hayes DN, **Major MB**. Cancer-derived mutations in KEAP1 impair NRF2 degradation but not ubiquitination. *Cancer Research,* 2013, Dec. 9, 2013. PMCID: PMC3932503.

3. *Mechanistic Studies of WNT/β-catenin Signaling.* Of the relatively small number of signaling pathways that function as master regulators of development, adult tissue homeostasis and cancer, the β-catenin dependent

Wnt pathway (Wnt/β-catenin) figures prominently; it regulates the growth and fate of neoplastic cells in tissues of diverse origin, notably the colon, kidney, breast and skin. My group has performed an array of proteomic and functional genomic studies of WNT signaling, including protein-protein interaction screens, kinase enrichment profiling, phospho-proteomics, siRNA and haploid mutagenesis loss-of-function screens, and more recently, novel gain-of-function screens. Integration of these data has and continues to reveal mechanistic insight and new disease-relevant regulators of pathway activity. As an example, our gain-of-function genomic screens demonstrated that the FOXP1 transcription factor activates β-catenin dependent transcription. Proteomic analyses demonstrated that FOXP1 binds the β-catenin transcriptional complex on chromatin. Disease-focused studies in mice and human clinical samples demonstrated that FOXP1 overexpression in B-cell lymphoma activates WNT signaling to promote tumor growth. In more recent work, we discovered that the ubiquitin-specific protease (USP6) deubiquitylates the WNT receptor to govern its endocytosis. We also discovered a WNT-driven negative feedback loop that activates the AAK1 kinase to promote endocytosis of LRP6.

a. Megan J. Agajanian, Matthew P. Walker, Alison D. Axtman, Roberta R. Ruela-de-Sousa, Alex D. Rabinowitz, David M. Graham, Meagan Ryan, D. Stephen Serafin, James M. Bennett, Rafael M. Couñago, David H. Drewry, Jonathan M. Elkins, Carina Gileadi, Opher Gileadi, Paulo H. Godoi, Nirav Kapadia, Susanne Müller, André S. Santiago, Fiona J. Sorrell, Carrow I. Wells, Oleg Fedorov, Timothy M. Willson, William J. Zuercher, **Michael B. Major**. AAK1 inhibits WNT signaling by promoting clathrin-mediated endocytosis of LRP6. *Cell Reports*, 2019 Jan 2;26(1):79-93. PMCID: PMC6315376
b. Babita Madan, Matthew P. Walker, Robert Young, Laura Quick, Kelly A. Orgel, Meagan Ryan, Priti Gupta, Ian C. Henrich, Marc Ferrer, Shane Marine, Brian S. Roberts, William T. Arthur, Jason D. Berndt, Victor Kwan Min Lee, Andre M. Oliveira, Randall T. Moon, David M. Virshup, Margaret M. Chou and **Michael B. Major**. The USP6 Oncogene Promotes Wnt Signaling by Deubiquitylating Frizzleds. *Proceedings of the National Academy of Sciences*, 2016. May 9th. PMCID: PMC4889410.
c. Matthew P. Walker, Charles M. Stopford, Maria Cederlund, Fang Fang, Christopher Jahn, Alex D. Rabinowitz, Dennis Goldfarb, David M. Graham, Feng Yan, Allison M. Deal, Yuri Fedoriw, Kristy L. Richards, Ian J. Davis, Gilbert Weidinger, Blossom Damania, and **Michael B. Major**. FOXP1 Potentiates Wnt/β-catenin Signaling in Diffuse Large B-cell Lymphoma. *Science Signaling*. 2015 Feb. 3rd, PMCID:PMC4356208.
d. **Major MB**, Camp ND, Berndt JD, Yi X, Goldenberg SJ, Hubbert C, Biechele TL, Gingras AC, Zheng N, Maccoss MJ, Angers S, Moon RT. Wilms Tumor Suppressor WTX Negatively Regulates Wnt/β-catenin Signaling. *Science*. 2007. May 18;316(5827):1043-1046. PMID: 17510365

4. *Protein-protein interaction networks and E3 Ubiquitin Ligase Substrate Discovery.* E3 ubiquitin ligase complexes provide specificity and catalysis for the transfer of ubiquitin to target proteins, a post-translational modification that results in proteasome-mediated degradation, altered subcellular localization or changes in protein interaction. Traditional pull-down/MS approaches fail to identify many E3 substrates, in part because the E3 complex is catalytic in action and because substrates are often short-lived. We use pharmacological and genetic approaches to stabilize the E3-substrate interaction. To date, we have identified known and novel substrates for the βTrCP, KEAP1 and RAD18 E3 ubiquitin ligases. Work in my laboratory is exploiting these system to identify substrates for uncharacterized E3 ubiquitin ligases, specifically those with established connections to oxidative stress signaling, Wnt signaling and human disease.

a. Tai Young Kim, Priscila Siesser, Kent L Rossman, Dennis Goldfarb, Kathryn Mackinnon, Feng Yan, XianHua Yi, Michael MacCoss, Randall T. Moon , Channing .J Der and **Michael B. Major**. Substrate Trapping Proteomics Reveals Targets of the βTrCP2/FBXW11 Ubiquitin Ligase. *Molecular and Cellular Biology*, 2014 Oct 22nd, PMCID: PMC4295375.
b. Yanzhe Gao, Elizabeth Mutter-Rottmayer, Alicia M. Greenwalt, Dennis Goldfarb, Feng Yan, Yang Yang, Raquel C. Martinez-Chacin, Kenneth H. Pearce, Satoshi Tateishi, **Michael B. Major**, and Cyrus Vaziri. A Neomorphic Cancer Cell-Specific Role of MAGE-A4 in Trans-Lesion Synthesis (TLS). *Nature Communications*, 2016. July 5th, 7:12105. PMCID: PMC4935975.
c. Hast BE, Goldfarb D, Mulvaney KM, Hast MA, Siesser PF, Yan F, Hayes DN, **Major MB**. Proteomic analysis of ubiquitin ligase KEAP1 reveals associated proteins that inhibit NRF2 ubiquitination. *Cancer Research*, Apr 1st, 2013;73(7):2199-210. PMCID: PMC3618590.
d. Siesser PF, Motolese M, Walker MP, Goldfarb D, Gewain K, Yan F, Kulikauskas RM, Chien AJ, Wordeman L, Major MB. FAM123A Binds Microtubules and Inhibits the Guanine Nucleotide Exchange Factor ARHGEF2 to Decrease Actomyosin Contractility. *Science Signaling*. 2012 Sep 4; 240(5):ra64. PMCID: PMC3618590

Complete List of Published Work:
http://www.ncbi.nlm.nih.gov/sites/myncbi/michael.major.1/bibliography/40003073/public/?sort=date&direction=ascending

D. Additional Information: Research Support

R01CA187799 Major (PI) 8/4/15 6/30/21
National Inst. of Health
Role of FOXP1 and WNT signaling in B-cell Lymphoma
This grant will evaluate how FOXP1 and its alternative splice variants control WNT signaling. The identification of FOXP1 and WNT target genes and their coordinate regulation in DLBCL will be defined. Pharmacological inhibitors of WNT signaling will be evaluated in FOXP1-driven DLBCL.
Role: PI

R01CA216051 Major/Weissman (MPI) 12/1/18 11/30/22
National Inst. of Health
The Role of Protein Kinases in NRF2-driven Lung Squamous Cell Carcinoma
This proposal seeks to understand how protein kinases impact NRF2 activity and biology in lung squamous cell carcinoma. We will identify and characterize kinases that control NRF2 and kinases that respond to NRF2, leveraging 2D and 3D cell culture models and a new NRF2 mouse model.
Role: PI

U24DK116204 Johnson (PI) 9/15/17 8/31/23
National Inst. of Health
Illuminating Function of the Understudied Druggable Kinome
A subset of kinases, the understudied dark kinases (DKs), have received little or no attention because foundational data on their biochemical and biological functions is not available. This proposal will collect such data by perturbing DKs genetically and with small molecules and then measuring the cellular consequences using multiplex proteomic, gene expression, metabolomic and imaging assays.
Role: co-I

R01NS111588 Chi (PI) 7/1/19 3/21/23
National Inst. of Health
Metabolic Regulation of KLHL Proteins through Glycosylation
Working within the larger team, we are using proximity biotinylation mass spectrometry to determine how glycosylation impacts KEAP1 and its associated proteins.
Role: co-I

TLC Pilot grant (PI: Major, Holz) 1/1/20 – 12/31/20
American Cancer Society
Tamoxifen signaling in ER-negative breast cancer
We propose to uncover the mechanism of action of tamoxifen in TNBC, to allow for some patients who have no other treatment options besides conventional chemotherapy, to have a targeted treatment available. Both tamoxifen and mTOR inhibitor are FDA-approved for other forms of breast cancer. Therefore, successful results of our proposed research plan could be rapidly translated into the clinic.
Role: PI