Erik Duane Hedrick, PhD
12000 Lake Cypress Circle, Apt A-306, Orlando, FL 32828
Phone: (440)-637-5578
Email: ehedrick@burdockgroup.com, hedman52@yahoo.com

EDUCATION:

INSTITUTION AND LOCATION	DEGREE	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Lakeland Community College, Kirtland, OH	A.S.	08/2003	05/2005	Science (PSEO program)
The Ohio State University, Columbus, OH	B.S.	09/2005	03/2010	Biochemistry/ Molecular Genetics
University of Nebraska Medical Center, Omaha NE	Ph.D	06/2010	03/2013	Colorectal Cancer
Texas A&M University, College Station, TX	Ph.D.	08/2013	12/2016	Toxicology, Breast Cancer, RMS, Pancreatic Cancer, Colorectal Cancer
Texas A&M University, College Station, TX	Postdoctoral	01/2017	07/2017	Lung Cancer, Breast Cancer, RMS, Toxicology
Lerner Research Institute, Cleveland, Clinic Foundation, Cleveland OH	Postdoctoral	08/2017	11/2021	Prostate Cancer

FIELDS OF SPECIALIZATION:
Molecular Toxicology
Molecular Oncology
Cell and Molecular biology
Biochemistry

A. Personal Statement

I have an unbridled level of ardor and professionalism for scientific research and understanding the regulatory space as it relates to occupational health and safety and ensuring the safety/compliance of new products. I have a Ph.D. in toxicology, with 10 years of experience in toxicology and over 13 years in molecular biology and biochemistry. I specialize in toxicological safety assessments, development and monitoring of safety/clinical studies, and regulatory consulting. This involves determining what information and studies are needed to ensure a client's product is safe and compliant with current regulations before it comes out to the market. My work includes composition of GRAS, GRAS notifications, FAP, CAP, NDIN, safety assessments, Novel Food Applications, study monitoring and clinical trial development with CROs, manuscript preparation, pesticide registrations, and other regulatory assessments/documentation. I have experience with the FDA, EPA, EFSA, FTC, and other regulatory bodies. I am also very familiar with OECD, GLP, GMP, GCP, and ICH guidelines and their application to study development and monitoring.

During my research career, my interests included the development of new chemotherapeutic studies to target metabolic pathways in cancer. I have a strong background in biochemistry, molecular biology, and toxicology which significantly augments my ability to accomplish the endeavors I have set forth for myself. My graduate work consisted of working with orphan nuclear receptors [specifically nuclear receptor family 4 A1 (NR4A1)] and studying the role of Sp transcription factors as nononcogene-addiction genes. During my doctoral tenure, I elucidated previously unknown signaling pathways in triple negative breast cancer, developed and demonstrated the chemotherapeutic potential of methylene-substituted diindolyl methane analogs (c-DIMS) in a multitude of solid tumor malignancies, and mastered many scientific techniques, including cell culture, animal husbandry, CRISPR-Cas9 and lentiviral gene editing, siRNA transient transfection, RT-qPCR, molecular cloning techniques, western blotting, ELISA, Immunoprecipitation, immunofluorescence, Chromatin Immunoprecipitation (ChIP), confocal microscopy, protein purification techniques, DNA site-directed mutagenesis, chromatography (ion exchange ,Gel, Hydrophobic, Reverse Phase, IMAC, and Affinity label), flow cytometry. I worked

as a postdoctoral research fellow at the Cleveland Clinic and am investigating mechanisms of metabolic resistance in prostate cancer. Prostate cancer is a highly metabolically driven cancer that relies heavily on androgens and the androgen receptor (AR). However advanced prostate cancer involves metabolic pathways that preclude the AR and these pathways need to be elucidated and investigated. I have elucidated a role of mTORC1 in AR-negative CRPC and how mTORC1 senses androgens and other steroids as a survival mechanism in treatment of refractory prostate cancer. This research has allowed me to apply my current skills and develop new skills in treatment refractory prostate cancer, including HPLC and mass spectrometry. In pursuing this project, I have contributed to the field of prostate cancer by exploring novel treatment therapies and demonstrated the therapeutic potential of the mTORC1/PGRMC1 interface as an effective treatment target in AR-negative CRPC.

B. Positions and Honors

Position	Start Date	End Date	Institution/Company	Supervisor
Undergraduate Researcher	10/2008	03/2010	The Ohio State University	Andrea Wolfe, Ph.D.
Graduate Researcher	06/2010	03/2013	University of Nebraska Medical Center	Michael Brattain, Ph.D.
Doctoral Researcher	08/2013	12/2016	Texas A&M University	Stephen H. Safe, Ph.D.
Postdoctoral Research Fellow	01/2017	07/2017	Texas A&M University	Stephen H. Safe, Ph.D.
Postdoctoral Research Fellow	08/2017	11/2021	Lerner Research Institute Cleveland Clinic Foundation	Nima Sharifi, M.D.
Toxicologist	12/2021	Present	Burdock Group Consultants	George Burdock

Academic and Professional Honors

2013-2016	CVM Merit Scholars Fellowship
2014-2015	Outstanding Graduate Student award
2015	CVM Research Training Grant
2015	George T. Edds award
2016	FASEB Travel award

Society memberships

2004-2005	Phi Theta Kappa
2015-Present	Phi Kappa Phi

C. Contributions to Science

Bibliography: https://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/57496362/

1. **Differential PKA activation and AKAP association determines cell fate in cancer cells.** The work in which I participated focused on, in Dr. Brattain's laboratory at Univeristy of Nebraska Medical Center, primary cancer progression and genetic mechanisms that lead to metastatic disease in colorectal cancer (CRC). One of these genes is TGFβRII, which in early CRC serves as a tumor suppressor and eventually acts as a tumor promoter and promotes EMT in later stage CRC. We focused on how this mechanism occurs and my project focused on a crosstalk between TGFβRII and insulin-like growth factor receptor type 1 (IGF-1R). IGF-1R is associated with the progression of many cancers including CRC. We elucidated that this crosstalk converged on protein kinase A (PKA) and its dichotomous role in cell survival. This dichotomy of PKA is orchestrated by A kinase anchoring proteins (AKAPs) and the presence or absence of cyclic adenosine monophosphate (cAMP). We observed that when IGF-1R was inhibited by a small molecule inhibitor (OSI-906), that TGFβRII would become activated and this would lead to a cAMP-independent activation of PKA by AKAP149. On the other hand when cells were treated with transferrin or insulin IGF-1R would become activated and this would lead to a rise in cAMP and a cAMP-dependent activation of PKA by an AKAP known as praja2. This dichotomy determined the cancer cell fate, with cAMP-dependent activation of PKA leading to cell survival and cAMP-independent activation of PKA leading to cell death.

a.) **Hedrick ED**, Agarwal E, Leiphrakpam PD, Haferbier KL, Brattain MG, Chowdhury S. Differential PKA activation and AKAP association determines cell fate in cancer cells. J Mol Signal. 2013 Oct 1;8(1):10.

2. Diindolylmethane analogs as novel NR4A1 antagonists and as a class of anticancer agents. NR4A1 is overexpressed in the majority of solid malignancies and has been demonstrated to be a prognostic factor for these cancers. During my graduate tenure at Dr. Stephen Safe's laboratory, we developed a novel class of 1,1-bis(3'-indolyl)-1-(p-substituted phenyl)methanes (C-DIMs) as NR4A1 antagonists. We specifically used 1,1-bis (3'-indolyl)-1-(p-hydroxyphenyl)methane (DIM-C-pPhOH) (C-DIM 8) and the p-carbomethoxy derivate (DIM-C-pPhCO2Me) (C-DIM 14) in our studies because they exhibited the strongest binding to NR4A1 while exhibiting the strongest efficacy towards inhibiting cancer cell growth. I established that NR4A1 is a master regulator of multiple oncogenic pathways including cell growth, inhibition of apoptosis, suppression of reactive oxygen species (ROS), and a promoter of cancer cell migration and invasion. NR4A1 controls activation of mTORC1 by suppressing activation of p53 and sestrin2. It also controls expression of genes such as bcl2 and surviving by forming a transactivational complex with specificity protein 1 (Sp1) and histone acetylase p300 by binding to GC rich regions within the promoters of these genes. NR4A1 also controls expression of thioredoxin domain containing 5 (TXNDC5) and isocitrate dehydrogenase 1 (IDH1) through a similar mechanism as bcl2 and surviving. TXNDC5 and IDH1 suppress production of ROS and there subsequent downregulation leads to ROS induced cancer cell death. These C-DIMs inhibited these pathways and promoted cancer cell death and inhibited cancer cell growth. Moreover, we demonstrated that NR4A1 controls cancer cell invasion and migration by targeting expression of multiple integrins (such as β1-integrin) and playing a critical role in TGF-β induced invasion. I demonstrated that NR4A1 binds to GC rich regions of multiple integrins (α2,5,6-) and (β1, 3, 4-) integrins. This also targets a phenomenon known as integrin switching, which one integrin will compensate for loss of the other (β3-integrin is upregulated in response to β1-integrin) in triple negative breast cancer (TNBC). I also demonstrate that NR4A1 in response to TGF-β NR4A1 translocates from the nucleus and forms a complex with arcadia, axin2, and RNF12 and promotes ubiquitination and subsequent proteasome-dependent degradation of smad7, which is the negative regulator of the TGF-β pathway in TNBC. Moreover, we demonstrated that TGF-β induced epithelial to mesenchymal transition (EMT) is NR4A1 and β-catenin dependent. I also established that this pathway is MAPK14 (p38α) dependent. Inhibition of NR4A1 nuclear export by leptomycin B (nuclear export inhibitor) or by C-DIMs inhibits this pathway. All these studies established NR4A1 as a drug-targetable prognostic factor in these cancers and is targetable through a novel class of compounds known as C-DIMs

a.) **Hedrick E**, Lee SO, Doddapaneni R, Singh M, Safe S. Nuclear receptor 4A1 as a drug target for breast cancer chemotherapy. *Endocr Relat Cancer.* 2015;22:831-40. PMID26229035
b.) **Hedrick E**, Lee SO, Doddapaneni R, Singh M, Safe S. NR4A1 Antagonists Inhibit β1-Integrin-Dependent Breast Cancer Cell Migration. *Mol Cell Biol.* 2016;36:1383-94. PMC4836213
c.) **Hedrick E**, Lee SO, Safe S. The nuclear orphan receptor NR4A1 regulates β1-integrin expression in pancreatic and colon cancer cells and can be targeted by NR4A1 antagonists. *Mol Carcinog.* 2017;56:2066-75. PMC5546981
d.) **Hedrick E**, Safe S. Transforming Growth Factor β/NR4A1-Inducible Breast Cancer Cell Migration and Epithelial-to-Mesenchymal Transition Is p38α (Mitogen-Activated Protein Kinase 14) Dependent. *Mol Cell Biol.* 2017 Aug 28;37(18). pii: e00306-17 PMC5574050

3.) Sp transcription factors as nononcogene addiction genes that are targets of ROS inducing agents. During my graduate tenure in the Safe laboratory, I also demonstrated that specificity proteins (Sp) are nononcogene addiction genes and they are drug targetable, specifically by ROS-inducing agents. Sp transcription factor (TF) Sp1 is overexpressed in multiple tumors and is a negative prognostic factor for patient survival. Sp1, Sp3 and Sp4 are highly expressed in cancer cells and in this study, using RNA interference (RNAi) to demonstrate that these TFs individually play a critical role in cancer cell growth, survival and migration/invasion in SKBR3, MDA-MB-231 breast, 786-0 kidney, L3.6pL, PANC1, Miapaca2 pancreatic, A549 lung and SW480 colon cancer cell lines.. Moreover, in athymic nude mice bearing L3.6pL pancreatic cancer cells, xenograft growth was significantly attenuated in cells depleted of Sp1, Sp3, and Sp4 in combination or Sp1 alone. Ingenuity Pathway Analysis (IPA) of changes in gene expression in Panc1 pancreatic cancer cells after individual knockdown of Sp1, Sp3 and Sp4 demonstrated that these TFs regulate gene sets/pathways that either positively correlate or inversely correlate with the functional responses observed after knockdown. However, causal IPA analysis, which integrates pathway-dependent changes in all genes strongly, predicted that Sp1-, Sp3- and Sp4-regulated genes were associated with the pro-oncogenic activity. These functional and genomic results coupled with overexpression of Sp transcription factors in tumor vs. non-tumor tissues and decreased Sp1 expression with age indicate that Sp1, Sp3 and Sp4 are non-oncogene addiction (NOA) genes and are attractive drug targets for individual and combined cancer chemotherapies.

I also demonstrated that Sp transcription factors are the target of ROS-inducing agents (eg, NSAIDS, phytochemicals, triterpenoids, HDACs, the antipsychotic penfluridol) and the mechanism behind this phenomenon. Many highly metabolically active cancers are very sensitive to ROS-inducing agents and the common mechanism is the downregulation of Sp transcription factors by the epigenetic downregulation of c-Myc. This leads to a downregulation in the miR17, 20a, 27a axis, which contains E-boxes that are controlled by c-Myc. This leads to an upregulation in the ZBTB proteins. These ZBTBs bind to Sp promoters and inhibit their expression. I demonstrated that penfluridol induced ROS and inhibited breast cancer cell growth and the HDAC inhibitor panobinostat inhibited both alveolar RMS (ARMS) and embryonic RMS (ERMS) growth by targeting these Sp proteins through this mechanism.

a.) **Hedrick E**, Crose L, Linardic CM, Safe S. Histone deacetylase inhibitors inhibit rhabdomyosarcoma by reactive oxygen species-dependent targeting of specificity protein transcription factors. *Mol Cancer Ther.* 2015;14:2143-53. PMC4618474

b.) **Hedrick E**, Cheng Y, Jin UH, Kim K, Safe S. Specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 are non-oncogene addiction genes in cancer cells. *Oncotarget.* 2016 Apr 19;7(16):22245-56. PMC5008359

c.) **Hedrick E**, Li X, Safe S. Penfluridol represses integrin expression in breast cancer through induction of reactive oxygen species and downregulation of Sp Transcription Factors. *Mol Cancer Ther.* 2017;16:205-16. PMC5222719

4.) NR4A1 in TGF-β dependent lung cancer migration and second-generation C-DIM derivatives as potent NR4A1 antagonists. During my postdoctoral tenure at the Safe laboratory I worked on a class of second-generation C-DIM derivatives. The original first-class compounds used in my graduate research involved *para*-substituted C-DIM compounds: 1,1-bis (3'-indolyl)-1-(p-hydroxyphenyl)methane (DIM-C-pPhOH) (C-DIM 8) and the p-carbomethoxy derivate (DIM-C-pPhCO2Me) (C-DIM 14). The second class derivatives involved substitution of the *para*-bound substituent with other functional groups and moieties. This second class of C-DIMs also involved *ortho* and *meta*-hydroxy C-DIMS. The rationale behind their putative enhanced potency is the different functional groups strengthen the affinity for the NR4A1 ligand binding domain and also extend the half life of the compound and prevent it from being metabolized by drug-metabolizing enzymes such as cytochrome P450s and glutathione–S-transferases. For the first group we identified three different compounds that had the strongest NR4A1 antagonistic activity: 1,1-bis(3'-indolyl)-1-(3-chloro-4-hydroxy-5-methoxyphenyl)methane (DIM-C-pPhOh-3-Cl-5-OCH_3), 1,1-bis(3'-indolyl)-1-(3,5-dibromo-4hydroxyphenyl)methane (DIM-C-pPhOh-3,5-Br_2), 1,1-bis(3'-indolyl)-1-(3-chloro-4-hydroxyphenyl)methane (DIM-C-pPhOh-3-Cl). We tested 9 different compounds and investigated their ability to inhibit NR4A1 induced luciferase activity using a GAL4-NR4A1/UAS-NBRE (Nur77 binding response element) system and by looking at transcription/translation of known NR4A1 dependent genes. The buttressed analogs were more potent than DIM-C-pPhOH in both in vitro assays and as inhibitors of mammary tumor growth. 1,1-bis(3'-indolyl)-1-(3-chloro-4-hydroxy-5-methoxyphenyl)methane (DIM-C-pPhOh-3-Cl-5-OCH3) was at least an order of magnitude more potent and significantly inhibited tumor growth at doses as low as 2 mg/kg/d. Thus we established that these buttressed analogs represent a more potent set of second-generation NR4A1 antagonists.

During my postdoctoral tenure at Safe laboratory, we also demonstrated that TGFβ induces migration of lung cancer cells (A549, H460, and H1299), dependent on activation of c-Jun N-terminal kinase (JNK1), and is inhibited by the JNK1 inhibitor SP600125. Moreover, TGFβ-induced migration of the cells is also blocked by the nuclear export inhibitor leptomycin B (LMB) and the orphan nuclear receptor 4A1 (NR4A1) ligand 1,1-bis(3'-indolyl)-1-(p-hydroxyphenyl)methane (CDIM8), which retains NR4A1 in the nucleus. We also showed that the TGFβ/TGFβ receptor/PKA/MKK4 and -7/JNK pathway cascade phosphorylates and induces nuclear export of NR4A1, which in turn forms an active complex with Axin2, Arkadia (RNF111), and RNF12 (RLIM) to induce proteasome-dependent degradation of SMAD7 and enhance lung cancer cell migration. This complex is similar to the one formed in breast cancer through TGF-β/p38α induced NR4A1 migration. Thus, we demonstrated that NR4A1 plays an integral role in mediating TGFβ-induced lung cancer invasion, and the NR4A1 ligand CDIM8, which binds nuclear NR4A1, represents a novel therapeutic approach for TGFβ-induced blocking of lung cancer migration/invasion. The implications of this include effective treatment of TGFβ-induced lung cancer progression using a number of agents including the CDIM/NR4A1 antagonists that block not only TGFβ-induced migration, but several other NR4A1-regulated pro-oncogenic genes/pathways in lung cancer cell lines.

a.) **Hedrick E**, Mohankumar K, Safe S. TGFβ-induced lung cancer cell migration is NR4A1-dependent. Mol Cancer Res. 2018;16:1991-2002. PMID30072581

b.) **Hedrick E**, Li X, Cheng Y, Lacey A, Mohankumar K, Zarei M, Safe S. Potent inhibition of breast cancer by bis-indole-derived nuclear receptor 4A1 (NR4A1) antagonists. Breast Cancer Res Treat. 2019 May 22. doi: 10.1007/s10549-019-05279-9.

5.) Sp transcription factors as regulators of transcription of ROS suppressors TXNDC5 and IDH1 and ROS as a mechanism of IL-24 induction and suppression of PAX3-FOXO1A axis in rhabdomyosarcoma. During my postdoctoral tenure at Safe laboratory I also worked on showing that Sp transcription factors are regulators of transcription of ROS suppressors, TXNDC5 and IDH1, and ROS as a mechanism of IL-24 induction and suppression of PAX3-FOXO1A axis in RMS. PAX3-FOXO1A is a genetic translocation that is found in alveolar RMS (ARMS). This translocation is a genetic driver of ARMS and leads to very poor clinical outcomes. Using RNA-sequencing studies we showed that PAX3-FOXO1 represses expression of interleukin-24 (IL24), and these two genes are inversely expressed in patient tumors. PAX3-FOXO1 also regulates histone deacetylase 5 (HDAC5) in ARMS cells, and results of RNA interference studies confirmed that PAX3-FOXO1-mediated repression of IL24 is HDAC5-dependent. Knockdown of PAX3-FOXO1 decreases ARMS cell proliferation, survival, and migration, and we also observed similar responses in cells after overexpression of IL24, consistent with results reported for this tumor suppressor-like cytokine in other solid tumors. We also observed in double knockdown studies that the inhibition of ARMS cell proliferation, survival, and migration after knockdown of PAX3-FOXO1 was significantly (>75%) reversed by knockdown of IL24. Adenoviral-expressed IL24 was directly injected into ARMS tumors in athymic nude mice, and this resulted in decreased tumor growth and weight. Because adenoviral IL24 has already successfully undergone phase I in clinical trials, this represents an alternative approach (alone and/or combination) for treating ARMS patients who currently undergo cytotoxic drug therapies. We demonstrated that the induction of ROS led to the upregulation of IL-24 and that this upregulation was abrogated in the presence of the antioxidant glutathione (GSH). The opposite was observed with the expression of PAX3-FOXO1A. We also demonstrated that PAX3-FOXO1A itself can directly regulate the expression of IL-24, demonstrating a cyclical feedback inhibition loop. I also investigated the effects of Sp on ROS induction itself. Using ChIP analysis, I determined that the genes TXNDC5 and IDH1 are directly transcribed by Sp proteins. This also led to upregulation of ROS and downregulation of Sp proteins. Treatment with GSH reversed all these effects.

a.) **Hedrick E**, Mohankumar K, Lacey A, Safe S. Inhibition of NR4A1 Promotes Ros Accumulation and IL24-Dependent Growth Arrest in Rhabdomyosarcoma. Mol Cancer Res. 2019 Aug 28. doi: 10.1158/1541-7786.MCR-19-0408.

b.) Lacey A, **Hedrick E**, Cheng Y, Mohankumar K, Warren M, Safe S. Interleukin-24 (IL24) is suppressed by PAX3-FOXO1 and is a novel therapy for rhabdomyosarcoma. Mol Cancer Ther. 2018;17:2756-66. PMID30190424

c.) Safe S, Abbruzzese J, Abdelrahim M, **Hedrick E**. Specificity protein transcription factors and cancer: opportunities for drug development. Cancer Prev Res (Phila). 2018 Jul;11(7):371-82. PMID29545399

6.) **Androgen-Dependent mTORC1 Activation in Advanced Androgen Receptor (AR)-Independent Prostate Cancer**. During my current postdoctoral tenure in Dr. Nima Sharifi's laboratory at the Cleveland Clinic I have focused on investigating noncanonical androgen signaling and metabolic mechanisms of advanced CRPC progression. Specifically I have investigated the mechanism of androgen-dependent mTORC1 activation in AR-negative cell lines PC-3 and DU-145. I demonstrated that androgens can stimulate cell growth in these cell lines through mTORC1, induce subcellular local mTORC1 to the lysosome, and promote the association of mTORC1 with the Ragulator complex. Through mass spectrometric analysis I have also demonstrated that a protein known as PGRMC1 binds to mTORC1 when cells are treated with DHT and that abrogation of PGRMC1 inhibits androgen-dependent mTORC1 activation. Moreover the cytochrome b binding domain of PGRMC1 and a critical residue (Y113) is essential for PGRMC1 function as demonstrated by mutational analysis. Other preliminary work I have performed has shown that other androgens, progestagens, and glucocorticoids can stimulate mTORC1 but not to the degree that DHT does. This reflects the promiscuity of the steroid binding site of PGRMC1 and gives some insight to the multiplicity of steroids that can stimulate mTORC1. This work was submitted for publication to *Molecular Cell* and was used for applications for the Early Investigator Research Award (FOA: W81XWH-19-PCRP-EIRA) by the Prostate Cancer Research Program through the Department of Defense (DoD) and the TL1 postdoctoral fellowship awarded by Case Western Reserve University. I was recommended for funding for both of these funding resources but formally accepted the Early Investigator Research Award due to the inability to have overlapping funding sources.

7.) Asparagine Utilization as a Metabolic Adaptation in Glutamine-Deprived Prostate Cancer through upregulation of Asparagine Synthetase (ASNS). Cancer cells as they progress in malignancy face glutamine deprivation as a metabolic hurdle. This involves a loss of dependency of glutamine and a number of metabolic adaptations to compensate for glutamine loss. I demonstrated that prostate cancer cells will utilize asparagine as a metabolic surrogate in glutamine deprived conditions. Asparagine will rescue cell proliferation and inhibit prostate cancer cell death in a cell environment depleted of

glutamine. This involves upregulation of asparagine synthetase (ASNS) and amino acid transporters (LAT1, LAT3, ASCT2), with a concomitant downregulation of glutamine metabolizing enzyme glutaminase (GLS). Androgen receptor cooperates with ATF4 to upregulate ASNS and use of enzalutamide blocks this upregulation. However, when cells are supplemented with media containing asparagine this rescues the inhibitory effect observed with enzalutamide. To further illustrate the importance of asparagine for prostate cell growth, use of asparaginase, the enzyme which hydrolyzes asparagine into aspartate, leads to decreased cell proliferation, increased apoptosis, and decreased cell migration. Asparagine will rescue cell proliferation as a result of glutamine depletion, serve as an anaplerotic surrogate for glutamine and rescue NEAA synthesis, TCA cycle anaplerosis, increase nucleotide synthesis, and increase glycolysis/gluconeogenesis. Upregulation of asparagine will also serve as an exchange factor for other amino acids (i.e. leucine, NEAAs, glutamate, and glutamine) through upregulation of these amino acid transporters.

8.) **mTORC1 controls prostate cancer progression by regulating stability of HSD3B1**. HSD3B1 is a major enzyme involved in prostate cancer, which catalyzes the initial rate-limiting step in conversion of the adrenal-derived steroid dehydroepiandrosterone (DHEA) to dihydrotestosterone (DHT). This leads to increased activation of AR, which drives prostate cancer progression. Sharifi Laboratory has demonstrated that 3β-Hydroxysteroid Dehydrogenase 1 (HSD3B1) acquires a gain of function mutation through a SNP (A1245C) which corresponds to N367T at the protein level. This advances CRPC growth by stabilizing HSD3B1, making it resistant to ubiquitination by E3 ubiquitin ligase autocrine mobility factor receptor (AMFR). I have discovered another mechanism of stabilization of HSD3B1 by amino acid stimulation of mTORC1. Amino acid stimulation of mTORC1 leads to enhanced protein expression of HSD3B1 and its respective enzymatic activity. This upregulation is inhibited by mTORC1 inhibitor everolimus (RAD001), and knockout of RAPTOR, an mTORC1 specific component. Moreover, knockdown of RICTOR, an mTORC2 specific component did not abrogate upregulation of HSD3B1 and had no effect on its ubiquitination. This suggests this pathway is independent of mTORC2. It has been demonstrated that mTORC1 phosphorylates HSD3B1, which can enhance binding ubiquitin-specific protease 20 (USP20). USP20 removes the ubiquitin chain from HSD3B1 and prevents proteasome dependent degradation of HSD3B1 by AMFR.

D. Additional Information: Research Support

COMPLETED SUPPORT

Grant - PC190380 "Androgen-Dependent mTORC1 Activation in Advanced Androgen Receptor (AR)-Independent Prostate Cancer" (PI: Hedrick, Erik PhD) (10/01/2020-9/30/2022)
Prostate Cancer Research Program, Early Investigator Research Award
W81XWH-19-PCRP-EIRA
Sponsoring Agency: The Assistant Secretary of Defense for Health Affairs endorsed by the Department of Defense
FOB: Destination
PURCHASE REQUEST NUMBER: 0011420173-0001 PSC CD: AN13
ESTIMATED COST $305,158.00
ACRN AA CIN: GFEBS001142017300001
Contact: Lymor Barnhard Science Officer CDMRP Email: lymor.r.barnhard.ctr@mail.mil
Phone: 301-619-7360

5T32CA059366-22(23) (Sharifi, Project Leader; Jackson, Program Director) 08/01/2017-08/01/2019
National Institute of Health $ 47,484 (direct) 08/01/2017-07/31/2018
National Institute of Health $ 48,900 (direct) 08/01/2018-07/31/2019
Project: noncanonical androgen signaling mechanisms by activating mTORC1 in AR negative castration resistant prostate cancer (CRPC) through the steroid receptor PGRMC1.
Contact: David Banks (NIH) banksdh@mail.nih.gov

5T32CA009476-22 (Brattain, Project Leader; Rizzino, Program Director) 05/25/2012-02/28-2013
National Institute of Health $ 16,380.00
Project: Noncanonical PKA activation determines cancer cell fate in colon cancer cells.

Major goals of project were to demonstrate the how PKA activation (whether through IGF1R or TGFβRII) can activate PKA (either through a cAMP dependent or independent mechanism, respectively) and how each activation mechanism utilized different AKAP proteins.
Contact: David Banks (NIH) banksdh@mail.nih.gov

PUBLICATIONS:

https://pubmed.ncbi.nlm.nih.gov/?term=erik%20hedrick&sort=date

1. **Hedrick ED**, Agarwal E, Leiphrakpam PD, Haferbier KL, Brattain MG, Chowdhury S. Differential PKA activation and AKAP association determines cell fate in cancer cells. J Mol Signal. 2013 Oct 1;8(1):10.
https://jmolecularsignaling.com/articles/10.1186/1750-2187-8-10

2. Safe S, Jin UH, **Hedrick E**, Reeder A, Lee SO. Minireview: role of orphan nuclear receptors in cancer and potential as drug targets. Mol Endocrinol. 2014 Feb;28(2):157-72.
https://academic.oup.com/mend/article/28/2/157/2556166?login=false

2. Lee SO, Li X, **Hedrick E**, Jin UH, Jalkens R, Backos D, Zijin L, Zhang Y, Wu Q, Safe S. Diindolylmethane Analogs Bind NR4A1 and Are NR4A1 Antagonists in Colon Cancer Cells. Mol Endocrinol. 2014 Oct;28(10):1729-39.
https://academic.oup.com/mend/article/28/10/1729/2623280?login=false

3. **Hedrick E**, Lee SO, Kim G, Abdelrahim M, Jin UH, Safe S, Abudayyeh A. Nuclear receptor 4A1(NR4A1) as a Drug Target for Renal Cell Adenocarcinoma. PLOS One. 2015 Jun 2;10(6):e0128308.
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0128308

4. **Hedrick E**, Crose L, Linardic CM, Safe S. Histone Deacetylase Inhibitors Inhibit Rhabdomyosarcoma by Reactive Oxygen Species-Dependent Targeting of Specificity Protein Transcription Factors. Mol Cancer Ther. 2015 Sep;14(9):2143-53.
https://aacrjournals.org/mct/article/14/9/2143/122654/Histone-Deacetylase-Inhibitors-Inhibit

5. **Hedrick E**, Lee SO, Doddapaneni R, Singh M, Safe S. Nuclear Receptor 4A1 (NR4A1) as a Drug Target for Breast Cancer Chemotherapy. Endocr Relat Cancer 2015 Oct;22(5):831-40.
https://erc.bioscientifica.com/view/journals/erc/22/5/831.xml

6. Gandhy SU, Imanirad P, Jin UH, NAIR V, **Hedrick E**, Cheng Y, Corton JC, Kim K, Safe S. Specificity protein (Sp) transcription factors and metformin regulate expression of the long non-coding RNA HULC. Oncotarget 2015 Sep 22;6(28):26359-72.
https://www.oncotarget.com/article/4560/text/

7. **Hedrick E**, Lee SO, Doddapaneni R, Singh M, Safe S. NR4A1 Antagonists Inhibit β-1 Integrin-Dependent Breast Cancer Cell Migration. Mol Cell Biol. 2016 Apr 15;36(9):1383-94.
https://www.tandfonline.com/doi/full/10.1128/MCB.00912-15

8. **Hedrick E**, Cheng Y, Jin UH, Kim K, Safe S. Specificity Protein (Sp) Transcription Factors as Non-Oncogene Addiction Genes in Cancer Cells Oncotarget. 2016 Apr 19;7(16):22245-56.
https://www.oncotarget.com/article/7925/text/

9. Lacey A, **Hedrick E**, Li X, Patel K, Doddapaneni R, Singh M, Safe S. Nuclear receptor 4A1 (NR4A1) as a drug target for treating rhabdomyosarcoma (RMS). Oncotarget. 2016 May 24;7(21):31257-69.
https://www.oncotarget.com/article/9112/text/

10. **Hedrick E**, Li X, Safe S. Penfluridol Represses Integrin Expression in Breast Cancer through Induction of Reactive Oxygen Species and Downregulation of Sp Transcription Factors Mol Cancer Ther 2017 Jan;16(1):205-216.
https://aacrjournals.org/mct/article/16/1/205/145994/Penfluridol-Represses-Integrin-Expression-in

11. Kasiappan R, Jutooru I, Karki K, **Hedrick E**, Safe S. Benzylisothiocyanate (BITC) Induces ROS-dependent Repression of STAT3 by Downregulation of Specificity Proteins in Pancreatic Cancer J Biol Chem 2016 Dec 30;291(53):27122-27133.
https://www.jbc.org/article/S0021-9258(20)34385-4/fulltext

12. **Hedrick E**, Lee SO, Safe S. The Nuclear Receptor NR4A1 Regulates β1-Integrin Expression in Pancreatic and Colon Cancer Cells and Can Be Targeted by NR4A1 Antagonists" Mol Carcinog 2017 Sep;56(9):2066-2075.
https://onlinelibrary.wiley.com/doi/10.1002/mc.22662

13. Karki K, **Hedrick E**, Kasiappan R, Jin UH, Safe S. Piperlongumine Induces Reactive Oxygen Species (ROS)-Dependent Downregulation of Specificity Protein Transcription Factors. Cancer Prev Res (Phila). 2017 Aug;10(8):467-477. doi: 10.1158/1940-6207.CAPR-17-0053. Epub 2017 Jul 3.
https://aacrjournals.org/cancerpreventionresearch/article/10/8/467/46876/Piperlongumine-Induces-Reactive-Oxygen-Species-ROS

14. **Hedrick E**, Safe S. Transforming Growth Factor β/NR4A1 Inducible Breast Cancer Cell Migration and Epithelial to Mesenchymal Transition is p38α (MAPK14) Dependent. Mol Cell Biol. 2017 Mol Cell Biol. 2017 Aug 28;37(18). pii: e00306-17.
https://www.tandfonline.com/doi/full/10.1128/MCB.00306-17

15. Cheng Y, Imanirad P, Jutooru I, **Hedrick E**, Jin UH, Rodrigues Hoffman A, Leal de Araujo J, Morpurgo B, Golovko A, Safe S. Role of metastasis-associated lung adenocarcinoma transcript-1 (MALAT-1) in pancreatic cancer. PLoS One. 2018 Feb 1;13(2):e0192264.
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0192264

16. Safe S, Abbruzzese JL, Abdelrahim M, **Hedrick E**. Specificity Protein Transcription Factors and Cancer: Opportunities for Drug Development. Cancer Prev Res (Phila). 2018 Mar 15. pii: canprevres.0407.2017.
https://aacrjournals.org/cancerpreventionresearch/article/11/7/371/47160/Specificity-Protein-Transcription-Factors-and

17. **Hedrick E**, Mohankumar K, Safe S. TGFβ-induced Lung Cancer Cell Migration is NR4A1-dependent. Mol Cancer Res. 2018 Aug 2. pii: molcanres.0366.2018. doi: 10.1158/1541-7786.MCR-18-0366.
https://aacrjournals.org/mcr/article/16/12/1991/89817/TGF-Induced-Lung-Cancer-Cell-Migration-Is-NR4A1

18. Lacey A, **Hedrick E**, Cheng Y, Mohankumar K, Warren M, Safe S. Interleukin-24 (IL-24) Is Suppressed By PAX3-FOXO1 and Is A Novel Therapy For Rhabdomyosarcoma. Mol Cancer Ther. 2018 Sep 6. doi: 10.1158/1535-7163.MCT-18-0118.
https://aacrjournals.org/mct/article/17/12/2756/273075/Interleukin-24-IL24-Is-Suppressed-by-PAX3-FOXO1

19. **Hedrick E**, Li X, Cheng Y, Lacey A, Mohankumar K, Zarei M, Safe S. Potent inhibition of breast cancer by bis-indole-derived nuclear receptor 4A1 (NR4A1) antagonists. Breast Cancer Res Treat. 2019 May 22. doi: 10.1007/s10549-019-05279-9.
https://link.springer.com/article/10.1007/s10549-019-05279-9

20. **Hedrick E**, Mohankumar K, Lacey A, Safe S. Inhibition of NR4A1 Promotes Ros Accumulation and IL24-Dependent Growth Arrest in Rhabdomyosarcoma. Mol Cancer Res. 2019 Aug 28. doi: 10.1158/1541-7786.MCR-19-0408.
https://aacrjournals.org/mcr/article/17/11/2221/89942/Inhibition-of-NR4A1-Promotes-ROS-Accumulation-and

21. Safe S, Shrestha R, Mohankumar K, Howard M, **Hedrick E**, Abdelrahim M. Transcription factors specificity protein and nuclear receptor 4A1 in pancreatic cancer.World J Gastroenterol. 2021 Oct 14. doi: 10.3748/wjg.v27.i38.6387.
https://www.wjgnet.com/1007-9327/full/v27/i38/6387.htm

22. **Hedrick E**. From Lab Bench to Consumer: The Regulatory and Safety Challenges of Cultivated Meat. Food Safety Magazine.
https://www.food-safety.com/articles/8660-from-lab-bench-to-consumer-the-regulatory-and-safety-challenges-of-cultivated-meat.

PRESENTATIONS/CONFERENCES:

- Erik Hedrick, Agarwal E, Leiphrakpam PD, Haferbier KL, Brattain MG, Chowdhury S. "Differential PKA activation and AKAP association determines cell fate in cancer cells." (UNMC Omaha NE)
- Erik Hedrick, Un-Ho Jin, Stephen Safe and Syng-Ook Lee "NR4A1 Antagonists inhibit Cancer Cell Growth, Survival and Invasion" (CVM Graduate/Postdoctoral Research Symposium College Station TX January 2014)
- Erik Hedrick, Un-Ho Jin, Stephen Safe and Syng-Ook Lee "NR4A1 Antagonists Inhibit Cancer Cell Growth, Survival and Invasion" SOT conference March 2014 (Phoenix AZ)
- Erik Hedrick, Lisa Crose, Corinne Linardic, Stephen Safe "Histone Deacetylase Inhibitors Inhibit Rhabdomyosarcoma by Reactive Oxygen Species-Dependent Targeting of Specificity Protein Transcription Factors"(CVM Graduate/Postdoctoral Research Symposium, College Station TX January 2015)
- Erik Hedrick, Lisa Crose, Corinne Linardic, Stephen Safe "Histone Deacetylase Inhibitors Inhibit Rhabdomyosarcoma by Reactive Oxygen Species-Dependent Targeting of Specificity Protein Transcription Factors"(IBT, Houston TX February 2015)
- Erik Hedrick, Syng-Ook Lee2, Jagun M. Somagoni3, Mandip Sachdeva Singh3 and Stephen Safe "Nuclear Receptor 4A1 (NR4A1) as a Drug Target for Breast Cancer Chemotherapy" (SOT Conference, San Diego CA March 2015)
- Erik Hedrick, Syng-Ook Lee, Gyungeun Kim, Un-Ho Jin, Stephen Safe, and Ala Abudayyeh "Nuclear receptor 4A1(NR4A1) as a Drug Target for Renal Cell Adenocarcinoma" (SOT Conference, San Diego CA March 2015)
- Erik Hedrick, Lisa Crose, Corinne Linardic, Stephen Safe "Histone Deacetylase Inhibitors Inhibit Rhabdomyosarcoma by Reactive Oxygen Species-Dependent Targeting of Specificity Protein Transcription Factors"(LSSOT conference, Houston TX October 2015)
- Erik Hedrick, Yating Cheng, Un-Ho Jin, Kyounghyun Kim, Stephen Safe "Specificity Protein (Sp) Transcription Factors as Non-oncogene Addiction Genes in Cancer Cells" (CVM Graduate/Postdoctoral Research Symposium, College Station TX, January 2016)
- Erik Hedrick, Lisa Crose, Corinne Linardic, Stephen Safe "Histone Deacetylase Inhibitors Inhibit Rhabdomyosarcoma by Reactive Oxygen Species-Dependent Targeting of Specificity Protein Transcription Factors"(SOT conference, New Orleans LA March 2016)
- Erik Hedrick, Yating Cheng, Un-Ho Jin, Kyounghyun Kim, Stephen Safe "Specificity Protein (Sp) Transcription Factors as Non-oncogene Addiction Genes in Cancer Cells" (SOT conference, New Orleans LA March 2016)
- Erik Hedrick, Lisa Crose, Corinne Linardic, Stephen Safe "Histone Deacetylase Inhibitors Inhibit Rhabdomyosarcoma by Reactive Oxygen Species-Dependent Targeting of Specificity Protein Transcription Factors" (FASEB conference; KLF/Sp transcription factors in Disease and Regenerative Medicine, Snowmass CO, August 2016)
- Erik Hedrick, Stephen Safe "TGFβ/NR4A1 Inducible Breast Cancer Cell Migration and Epithelial to Mesenchymal Transition is p38α (MAPK14) Dependent" (Imaging Sciences Spotlight Series, College Station TX October 2016)
- Erik Hedrick, Stephen Safe "TGF□/NR4A1 Inducible Breast Cancer Cell Migration and Epithelial to Mesenchymal Transition is p38α (MAPK14) Dependent" (Toxicology Forum, College Station TX December 2016)
- Erik Hedrick, Stephen Safe "TGF□/NR4A1 Inducible Breast Cancer Cell Migration and Epithelial to Mesenchymal Transition is p38α (MAPK14) Dependent" (Imaging Sciences Spotlight Series, CVM Graduate/Postdoctoral Research Symposium, College Station TX January 2017)
- TGFβ/NR4A1 Inducible Breast Cancer Cell Migration and Epithelial to Mesenchymal Transition is p38α (MAPK14) Dependent (SOT conference, Baltimore MD March 2017)
- Erik Hedrick, "DIINDOLYLMETHANE ANALOGS AS NOVEL NR4A1 ANTAGONISTS AND AS A NOVEL CLASS OF ANTICANCER AGENTS AND SP TRANSCRIPTION FACTORS AS NONONCOGENE ADDICTION GENES THAT ARE TARGETS OF ROS INDUCING AGENTS" (Cleveland Clinic Lerner Research Institute seminar, May 2017)
- Erik Hedrick "mTORC1 activation by androgens as a metabolic phenotype of advanced androgen receptor (AR)-independent prostate cancer" (Cleveland Clinic Lerner Research Institute seminar, July 24th, 2018)
- Erik Hedrick "Androgen-Dependent mTORC1 Activation in Advanced Androgen Receptor (AR)-Independent Prostate Cancer" (Cleveland Clinic Lerner Research Institute seminar, February 26th, 2020)
- Erik Hedrick "THE 50 SHADES OF CANNABIS: the gray pathway to regulatory compliance for non-THC/CBD cannabinoids" (Burdock Group Webinar, July 27th, 2022)
- Erik Hedrick "ASK THE EXPERT, Alternative Protein Sources- EU & US Regulatory Perspectives" (Burdock Group Webinar, Nov 16th, 2022)
- FDLI's Food and Dietary Supplement Safety and Regulation Conference. March 23-24th, 2023.
- IFT FIRST Annual Event and Expo. Burdock Group Booth. July 16th-19th, 2023.

- Erik Hedrick "Regulatory and Safety Considerations for Algal-based Food Ingredients and Dietary Supplements" (Algae Production, Products and Equipment Webinar, September 20th, 2023)
- Eurofins BioPharma Product Testing. Information requirements for biocidal product authorization. October 25th, 2023.
- RIFM's 2nd Annual Science Symposium. November 29th, 2023.