BIOGRAPHICAL SKETCH

NAME: Ihrie, Rebecca Ann

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

POSITION TITLE: Assistant Professor of Cell & Developmental Biology and Neurological Surgery, Vanderbilt University

EDUCATION/TRAINING*:*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Michigan, Ann Arbor, MI	B.S. Honors.	04/00	Biochemistry
Stanford University, Stanford. CA	Ph.D.	06/06	Cancer Biology
University of California, San Francisco, CA	Postdoctoral	12/11	Neural Stem Cells

A. Personal Statement

I am interested in the signaling pathways regulating proliferation and differentiation in neural stem/progenitor cells and brain tumor cells. My long-term goals are to reveal how the normal proliferation and differentiation of stem cells is controlled in the brain, dissect how perturbation of these pathways results in neurological diseases, and leverage these findings to treat human disease. My laboratory uses single-cell measurements of protein activation to 1) identify proliferative pathways that are differentially active within cell subsets in the ventricular-subventricular zone), 2) determine how this pattern of activation is altered in disease states, particularly brain tumors in Tuberous Sclerosis Complex, and 3) directly identify clinically relevant subpopulations of brain tumor cells, including immune cells, using multiparameter single-cell protein analyses (**Contribution 1**). I have worked collaboratively with Vanderbilt neurosurgeons, neurologists, and basic researchers since 2012 to map cell-to-cell differences in proliferative signaling in neural stem cells and immune infiltrate in all grades of brain tumors.

My postdoctoral research focused on neural precursors in the adult brain and their involvement in brain tumors, and my independent laboratory has continued on this track. The germinal region which is our focus, the ventricular-subventricular zone (V-SVZ), extends over a large area contacting both the cerebrospinal fluid and an extensive vascular network, and is therefore exposed to many secreted factors which modulate cell function. I identified a pathway – Sonic hedgehog (Shh) signaling – that is activated in specific subregions of the stem cell niche and, by ectopically activating this pathway, accomplished the first *in vivo* fate switch in mouse neural stem cells (**Contribution 3**). In my independent group, we have identified radiographic contact of glioblastoma with the lateral ventricles as an independent negative prognostic factor. Further, we have used single-cell measurements of protein phosphorylation to identify differences in signaling activity between distinct populations of V-SVZ progenitor cells, and have shown that these differences affect tumor development in a mouse TSC tumor model developed by the Ess lab (**Contribution 2**). I have over 15 years of experience in cytometric studies of mouse and human brain tissue, and have worked on multiple successful collaborative projects to map the features of the V-SVZ across species, including first-in-human studies (Ihrie et al Cell 2006, Sanai et al Nature 2011, Ihrie et al Neuron 2011, Yang et al Cell Reports 2017, and others). These projects formed the foundation for a robust professional network available to my trainees, including ongoing interactions with research centers such as the Barrow Neurological Institute. I and my group members have relevant expertise in tumor modeling in the mouse, stereotaxic injection of patient-derived xenografts, monolayer, slice, and sphere culture of stem cells from mouse and human brain, and the analysis of all of these models using flow and imaging cytometry.

B. Positions and Honors

Employment

1997-2000 Honors Undergraduate Researcher, Laboratory of E. Neil G. Marsh, Ph.D. – Professor, Chemistry Department, University of Michigan

2000-2006 Graduate Student, Laboratory of Laura D. Attardi, Ph.D. – Professor, Cancer Biology Program and Departments of Radiation Oncology and Genetics, Stanford University

2006-2011 Postdoctoral Fellow, Laboratory of Arturo Alvarez-Buylla, Ph.D. – Professor, Department of Neurosurgery and Eli and Edythe Broad Institute of Regeneration Medicine and Stem Cell Research, University of California – San Francisco

2012-present Assistant Professor, Departments of Cell & Developmental Biology (primary) and Neurological Surgery (secondary) Vanderbilt University School of Medicine

Member, Vanderbilt Center for Stem Cell Biology, Vanderbilt Brain Institute,
Vanderbilt-Ingram Cancer Center, and Programs in Developmental Biology and Cancer Biology

Activities and Honors

Fellowships

2005-2006 Gerald Lieberman Dissertation Fellowship,
Stanford University School of Medicine -1 awarded per year
2007-2009 Damon Runyon Cancer Research Foundation Postdoctoral Fellowship
2009-2010 American Association for Cancer Research / National Brain Tumor Society Fellowship,
in memory of Bonnie Brooks - 1 of 2 awarded nationally

Select Other Awards and Academic Honors

1998, 1999 Abbott Labs Summer Research Fellowship, Chemistry Department, University of Michigan
2000 Seyhan N. Ege Women in Science and Engineering Award, University of Michigan
2003 Outstanding Talk Award, Cancer Biology Program, Stanford University
2003, 2009 Graduate Scholar-in-Training Awards, American Association for Cancer Research
2010 Outstanding Poster Award, International Society for Stem Cell Research Annual Meeting
2011 Postdoctoral Scholar-in-Training Award, American Association for Cancer Research
2017 Ann Faulkenberry Memorial Award, Southeastern Brain Tumor Foundation

Select Invited and Abstract Selected Presentations

2009 "Hedgehog Signaling Specifies Fate in Adult Neural Stem Cells."
Cold Spring Harbor Meeting on Stem Cell Biology
2011 "Hedgehog Signaling Specifies Positional Identity and Fate in Adult Neural Stem Cells."
AACR Special Conference on Stem Cells, Development, and Cancer
2013 "Persistent Hedgehog Signaling and Neuronal Fate in the Adult Brain."
Southeast Regional Society for Developmental Biology Annual Meeting
2015 "Quantifying the Effects of Heterogeneity in Brain Tumors at the Single Cell Level."
Telethon Kids Institute, Perth, Australia
2016 "Dissecting the Multicellular Ecosystem of Glioblastoma Using Single-Cell Mass Cytometry."
Barrow Neurological Institute, Phoenix, Arizona
2017 "Dissecting Glioblastoma (and its Immune Component) Using Mass Cytometry."
McGill University Workshop on Cancer and the Immune System, Bellairs Research Institute
2017 "Quantification of Neural Stem Cell Heterogeneity in Health and Disease."
Cincinnati Childrens' Hospital, Cincinnati, OH
2017 "Differential mTORc1 Activity in Subdomains of the Mouse Ventricular-Subventricular Zone Leads to Location Specific Tumor Development."
International Conference on Tuberous Sclerosis Complex and LAM, Washington, DC
2018 "Dissecting Neural Lineages and Brain Tumors Using Single-Cell Measurements of Signaling."
Cancer Sciences, King's College London
2018 "Dissecting Neural Lineages and Brain Tumors Using Single-Cell Snapshot Proteomics."
Brain & Spine Institute (ICM), Hôpital de la Pitié-Salpêtrière, Paris
2018 "Identifying Clinically Relevant Features of Brain Tumors Using Single-Cell Measurements of Signaling."
University of Texas MD Anderson Cancer Center Brain Tumor Center
2018 "Neural Stem Cell Signaling in Health and Disease."
Stanford University Cancer Biology Seminar Series
2019 "Single-Cell Snapshot Proteomics Reveal an Intrinsic, Differential Susceptibility to TSC Tumor Development Within a Persistent Stem Cell Niche."
International Conference on Tuberous Sclerosis Complex and LAM
2019 "Location-Dependent Maintenance of an Intrinsic, Differential Susceptibility to mTORC1-Driven Tumor Growth in a Persistent Stem Cell Niche."
International Society for Stem Cell Research Annual Meeting

Academic Service

1998 Curriculum Committee, Chemistry Department, University of Michigan
1998-2000 Undergraduate Student Instructor, "Structure and Reactivity" course, University of Michigan
2001 Graduate Instructor, "Study and Treatment of Cancer" graduate course, Stanford University

2004 Chair, “Evolution of Cancer Therapy: from Pathways to Patients” symposium, Stanford
2005 Cancer Biology Program Graduate Admissions Committee, Stanford University
2012 Faculty Preceptor, Hematology – Oncology Fellows Journal Club, Vanderbilt University
2012-present “Brain Tumors” lecturer, Advanced Concepts in Cancer Biology, Vanderbilt University
2012-2013 Junior Faculty Leadership Development Program, Vanderbilt University
2013-2016 Dean’s Honor Fellowship Committee, Vanderbilt University
2013-present IMPACT (Intensive Mentoring Program for Advancement and Career Training) leader
2014-present Program in Developmental Biology steering committee, Vanderbilt University
2015-present “Neural Development” lecturer, Fundamentals of Neuroscience I, Vanderbilt University
2015-2016 “Brain Tumors” lecturer, Neurobiology of Disease, Vanderbilt University
2017 Co-director, Cancer and Development graduate course, Vanderbilt University
2017-present Director, Introduction to Developmental Biology graduate course, Vanderbilt University
2018-present Chair, Outreach Committee, Vanderbilt Brain Institute

Professional Associations and Service

2008-present American Association for Cancer Research
2009-present *Ad hoc* reviewer, Oncogene, Cancer Cell, Neuron, Frontiers in Cellular Neuroscience, Cold Spring Harbor Perspectives, Developmental Neuroscience, PNAS, Development, Laboratory Investigation, Neuropharmacology, Eur J Neuroscience, Cell Reports, PLoS Genetics
2009-2011 Associate Faculty Member, Faculty of 1000 (Neurobiology of Disease and Regeneration)
2010-present International Society for Stem Cell Research
2012-present Society for Neuro-Oncology
2014-present *Ad hoc* grant reviewer, Medical Research Council UK, Agence Nationale Recherche (France), Tuberous Sclerosis Alliance, Oak Ridge Associated Universities, Department of Defense Congressionally Directed Medical Research Program
2015 Expert reviewer, Developmental Biology, 11th edition (eds. Gilbert and Barresi)
2016-present Editorial Board member, Scientific Reports
2018 Minisymposium co-chair, Society for Neuroscience annual meeting

C. Contributions to Science

1. Analysis of Brain Tumors Using Mass Cytometry.

The stem-like population within malignant brain tumors is thought to be a major driver of resistance to therapy, but examination of these populations has, until recently, been complicated by substantial intratumoral heterogeneity and a paucity of validated protocols for tumor dissociation. In collaboration with the Irish Lab, we have developed and published novel protocols for the dissociation and cryopreservation of intraoperative solid tumor samples (including glioblastoma) for analysis using mass cytometry, enabling the measurement of 35+ proteins of interest in millions of cells per sample.

a) Leelatian N., D.B. Doxie, A.R. Greenplate, B.C. Mobley, J.M. Lehman, J. Sinnaeve, R.M. Kauffman, J. A. Werkhaven, A.M. Mistry, K.D. Weaver, R.C. Thompson, P.P. Massion, M.A. Hooks, M.C. Kelley, L.B. Chambless, R.A. Ihrie, and J.M. Irish. Single cell analysis of human tissues and solid tumors with mass cytometry. *Cytometry B*. (2017) 92(1): 68-78. PMID: 27598832
b) N. Leelatian, J. Sinnaeve, B.C. Mobley, K.D. Weaver, R.C. Thompson, L.B. Chambless, R.A. Ihrie*, and J.M. Irish*. (* - co-corresponding) Dissecting the Multicellular Ecosystem of Human Glioblastoma Tumors Using Single Cell Mass Cytometry. Presented at 2016 Annual Society for Neuro-Oncology Meeting, abstract published in Neuro Oncol (2016) 18 (suppl 6):vi39. PMID:
c) Leelatian, N., K.E. Diggins, A.A. Brockman, L.B. Chambless, R.A. Ihrie*, and J.M. Irish* (* - co-corresponding) A Protocol for Preparing Viable Single Cells from Human Tissue and Tumors for Cytomic Analysis. *Current Protocols in Molecular Biology* (2017) 118:25C.1.1-25C.1.23. PMID: 28369679
d) Mistry A.M., Greenplate A.R., Ihrie R.A., Irish J.M. Beyond the message: advantages of snapshot proteomics with single-cell mass cytometry in solid tumors. *FEBS J.* (2018) Dec 13. doi: 10.1111/febs.14730. [Epub ahead of print] PMID: 30549207

2. Quantifying the Effects of Stem Cell Niche Contact and Location in Brain Tumors.

My laboratory is focused on the ventricular-subventricular zone (V-SVZ), the largest stem cell niche in the adult brain. The substantial proliferative capacity of stem and progenitor cells in a largely quiescent tissue suggests that they are particularly susceptible to tumor-initiating events, and that this niche may be especially supportive

of brain tumor growth. We are now investigating heterogeneity within the normal neural stem cell niche, how this heterogeneity contributes to localized tumor development, and how V-SVZ-derived factors may alter the progression or therapy resistance of malignant brain tumors. Most recently, we have identified V-SVZ contact as an independent negative prognostic factor in glioblastoma outcome, and have linked differing levels of normal signaling activity in stem cells to differences in tumorigenic capacity in Tuberous Sclerosis Complex.

a) Sinnaeve J., B.C. Mobley, and R.A. Ihrie. Space Invaders: Brain Tumor Exploitation of the Stem Cell Niche. *Am J Pathol.* (2018) 188(1):29-38. PMID: 29024634
b) Mistry A.M., A.T. Hale, L.B. Chambless, K.D. Weaver, R.C. Thompson, and R.A. Ihrie. Influence of Glioblastoma Contact with the Subventricular Zone on Survival: A Meta-Analysis. *J Neurooncol*. (2017) 131(1):125-133. PMID: 27644688
c) Mistry A.M., D. Wooten, T. Davis, B.C. Mobler, V. Quaranta, R.A. Ihrie. Ventricular-Subventricular Zone Contact by Glioblastoma is Not Associated with Molecular Signatures in Bulk Tumor Data. *Scientific Reports* (2019) 9(1):1842. PMID: 30755636
d) Rushing G.V., A.A. Brockman, M.K. Bollig, N. Leelatian, B.C. Mobley, J.M. Irish, K. C. Ess, C. Fu, R.A. Ihrie. Location-dependent maintenance of an intrinsic susceptibility to mTORC1-driven tumorigenesis. *Life Sciences Alliance* (2019) 2:2. PMID: 30910807

3. Identifying Regulators of Neural Stem Cell Fate and Human-Specific Features of the Stem Cell Niche.

During my postdoctoral research, I focused on three major aspects of V-SVZ biology: 1) heterogeneity within the stem cell population; 2) comparison of the mouse stem cell niche to the equivalent structure in pediatric human brain; and 3) investigating how V-SVZ progenitors contribute to brain tumor development. In the mouse, I identified a pathway (Sonic hedgehog signaling) that is highly active in specific stem cells and not others, and demonstrated that this pathway activity is necessary and sufficient to alter the fate of neuronal progeny derived from these cells. This work was the first example of *in vivo* fate reprogramming within the V-SVZ. In collaboration with a neurosurgeon (N. Sanai – first author), I co-supervised a research assistant (T. Nguyen – second author) and completed a series of analyses of pediatric human brain sections to map the composition of the V-SVZ in young human brain. These studies identified several novel features specific to pediatric human (vs. older human or young mouse), and were the first detailed studies of their kind at this age. I also contributed to collaborative work using genetically relevant manipulations of V-SVZ progenitors and orthotopic injection of cells to model pediatric glioblastoma. Subsequently, I used my expertise in targeting the germinal zone via microinjection or localized cannula implantation to drive multiple collaborative projects targeting specific subregions within the adult brain. Each of these has resulted in publications, including four since the start of my independent lab.

a) Ihrie R.A., J.K. Shah, C.C. Harwell, J.H. Levine, C.D. Guinto, A.R. Kriegstein, and A. Alvarez-Buylla (2011). Sonic hedgehog Regulates the Positional Identity of Neural Stem Cells in the Adult Brain. *Neuron* 71 (2), 250-262. PMID: 21791285
b) Sanai N., T. Nguyen, R.A. Ihrie, Z. Mirzadeh, H-H. Tsai, M. Wong, N. Gupta, M.S. Berger, E. Huang, J-M.Garcia-Verdugo, D. H. Rowitch, and A. Alvarez-Buylla (2011). Corridors of Migrating Neurons in the Human Brain and Their Decline During Infancy. *Nature* 478(7369):382-6. PMID: 21964341 Highlighted in *F1000*, *Nature*, *Nat Rev Neurosci*, and *Cell Stem Cell*
c) Huillard E., R. Hashizume, J.J. Phillips, A. Griveau, R.A. Ihrie, Y. Aoki, T. Nicolaides, A. Perry, T. Waldman, M. McMahon, W.A. Weiss, C. Petritsch, C.D. James, D. H. Rowitch (2012). Cooperative interactions of BRAFV600E kinase and CDKN2A locus deficiency in pediatric malignant astrocytoma as a basis for rational therapy. *PNAS* 109(22):8710-5. PMID: 22586120
d) Yang Y.P., H. Ma, A. Starchenko, W.J. Huh, W. Li, F.E. Hickman, Q. Zhang, J.L. Franklin, D.P. Mortlock, S. Fuhrmann, B.D. Carter, R.A. Ihrie, R.J. Coffey (2017). A Chimeric Egfr Protein Reporter Mouse Reveals Egfr Localization and Trafficking In Vivo. *Cell Rep* 19(6):1257-1267. PMID: 28494873

4. Revealing a Novel Function of a Tumor Suppressor Target Gene

My graduate research focused on the downstream transcriptional targets of the P53 tumor suppressor. *TRP53* is the most frequently mutated gene in human cancer, and the P53 pathway is thought to be abrogated in nearly all tumors. Activation of P53 can result in cell death (apoptosis), cell cycle arrest, or senescence. At the time of this work, very few P53 target genes had been shown to be specifically associated with only one of these outcomes. *PERP* was identified in a screen for apoptosis-specific p53 target genes. During my thesis research, I used multiple primary cell types to demonstrate a requirement for PERP in the P53-dependent cell death response. In addition, my analyses of the *PERP*-deficient mouse line revealed a novel role for PERP downstream of the related factor P63. Revealing the mechanistic basis for neonatal lethality in PERP-deficient animals

ultimately sparked a new line of investigation in my graduate lab – leading to 8 total publications, with the most recent in 2011 – and identified PERP as a novel component of the desmosome, a critical cell-cell adhesion complex that is affected in human blistering diseases. I also contributed to a collaborative project on age-specific tumorigenic effects of the MYC oncogene, demonstrating differential activation of P53 target genes in tumors.

a) Ihrie R.A., E. Reczek, J.S. Horner, L. Khachatrian, J. Sage, T. Jacks, and L.D. Attardi (2003). *Perp* is a Mediator of p53-Dependent Apoptosis in Diverse Cell Types. *Current Biology* 13(22), 1985-90. PMID: 14614825
b) Ihrie R.A., M.R. Marques, B.T. Nguyen, J.S. Horner, C. Papazoglu, R. T. Bronson, A.A. Mills, and L.D. Attardi (2005). *Perp* is a p63-Regulated Gene Essential for Epithelial Integrity. *Cell*, 120(6), 843-56. PMID: 15797384 – Highlighted in *F1000* and *Science*
c) Ihrie R.A., R.T. Bronson, and L.D. Attardi (2006). Adult Mice Lacking the p53/p63 Target Gene *Perp* are not Predisposed to Spontaneous Tumorigenesis but Display Features of Ectodermal Dysplasia Syndromes. *Cell Death and Differentiation*, 13:1614-8. PMID: 16485031
d) Beer S., A. Zetterberg, R.A. Ihrie, R.A. McTaggart, Q. Yang, N. Bradon, C. Arvanitis, L.D. Attardi, S. Feng, B. Ruebner, R.D. Cardiff, D.W. Felsher (2004). Developmental Context Determines Latency of MYC-Induced Tumorigenesis. *PLoS Biology* 2(11):e332. PMID: 15455033

Complete List of Published Works in My Bibliography (myNCBI):
http://www.ncbi.nlm.nih.gov/sites/myncbi/rebecca.ihrie.1/bibliography/42842697/public/?sort=date&direction=ascending

D. Research Support

Active

UG3 TR002097 NIH/NCATS (PI: Wikswo) 07/21/2017-06/30/2019
Project Title: Drug development for tuberous sclerosis complex and other pediatric epileptogenic diseases using neurovascular and cardiac microphysiological models
Project Goal: Use organ-on-a-chip models to test novel candidate drugs identified in Ihrie lab for use in TSC.

SBTA-0001199 Southeastern Brain Tumor Foundation (PIs: Ihrie & Irish) 07/01/2017-06/30/2019
Project Title: Dissecting the Contribution of the Niche and Immune Cells to Patient Outcome Using Single-Cell Protein Measurements
Project Goal: Develop a panel of heavy metal-conjugated antibodies to study GBM and immune cells.

W81XWH-16-1-0171 DOD/CDMRP TSCRP Idea Development (PI: Ihrie) 05/01/2016 – 08/31/2019
Project Title: Identifying Novel Candidate Therapies for SEGAs Using Quantitative Single Cell Assays
Project Goal: Perform a high-throughput screen for modifiers of mTOR activity in groups of neural stem cells.

R01 NS096238 NIH/NINDS (PI: Ihrie) 04/15/2016 – 03/31/2021
Project Title: Quantifying Differences in mTOR Activity and Tumor Development Between Neural Stem Cell Microdomains
Project Goal: Determine the causes and effects of differential mTOR activity within the subventricular zone.

R01 GM117916 NIH/NIGMS (PI: Weaver) 02/05/2016 – 11/30/2019
Project Title: Exosome-filopodia interactions
Project Goal: Test the effects of manipulating key exosome-modulating proteins in the subgranular zone.

NIH/NCI R21CA227483 (PI: Gama) 04/01/2019 – 03/31/2021
Project Title: Assessing the contribution of mitochondrial heterogeneity to gliomagenesis using single cell approaches
Project Goal: Study mitochondrial heterogeneity in the tumor-propagating fraction of glioblastoma patient tissue.