BIOGRAPHICAL SKETCH

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NAME: Robert C Rostomily

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

POSITION TITLE: Professor of Neurosurgery

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Yale University; New Haven CT	BS	05/1980	Biology
Case Western Reserve University; Cleveland OH	MD	06/1987	Medicine
Department of Neurosurgery, University of Washington School of Medicine; Seattle WA		07/1995	Neurosurgery Residency

A. Personal Statement

I have engaged in the study of human glioblastoma (GBM) for over 20 years and been NIH funded throughout. As a practicing surgical neuro-oncologist, I have personally experienced the devastation of this disease. Our lab's focus has been to discover mechanisms of TWIST1 mediated mesenchymal changes in GBM and glioma stem cells (GSCs). In collaboration with Andrei Mikheev I also developed novel syngeneic models for the study of aging which recapitulate key features of human GBM including invasive growth and aging dependent increases in malignancy and hypoxic response. We also profiled GSC ion channel expression and ion channel drug responses to support their repurposing for clinical application where disruption of GSC electrophysiologic properties will deregulate cancer stem cell phenotypes and promote treatment resistance and self-renewal. Using a degenerate screen for tissue specific bHLHs we identified TWIST1 (TW) in gliomas cells and human gliomas and GBMs for the first time. We also first suggested that an EMT like process may contribute to GBM malignancy which was borne out by subsequent functional studies showing TW promotes cell survival, invasion, stemness and mesenchymal phenotypes. The potential of TW and its regulated pathways as a therapeutic target in GBM was demonstrated in TW loss of function studies, which markedly prolonged animal survival *in vivo*. Through my clinical and research experiences it became evident that intra-tumoral heterogeneity was among if not the greatest challenge to realize progress in treating GBM. Our work with aging GBM models that demonstrated age-dependent genomic instability reinforced this as an underlying mechanism and our study of regional mutational profiling revealed the potential limitations of using biopsies to inform targeted therapy. This motivated our work using high-throughput drug screening which applied to multiple biopsies could potentially provide a practical readout of treatment responses accounting for regional molecular and cellular heterogeneity. My clinical and research experiences also drove an interest in pursuing new approaches to therapy based on modulation of glioma stem cell (GSC) electrophysiology. We have identified ion channel expression in GSCs that could be targeted to achieve electrical reprogramming through modulation of voltage membrane potential (Vmem). This led to NCI funding to develop high throughput screening platforms to identify ion channel drugs that drive GSC reprogramming through hyperpolarization and NINDS funding to apply optogenetic based Vmem modulation on GSC phenotypes. A fundamental unmet challenge is how to modulate Vmem in GBM in a clinically practical way. To this end, I have established collaborations to test novel implantable electrical recording and stimulation devices using rodent and porcine glioma models to better understand the evolution of glioma electrophysiology and the therapeutic potential of direct neuromodulation as a standalone or adjunctive treatment modality. My clinical insights, expertise with glioma surgery, development of rodent and glioma

models and current work with GSC electrophysiology position our lab to spearhead multidisciplinary efforts required to realize the potential of neuromodulation in treating GBM.

Ongoing, pending and recently completed projects that I would like to highlight include:
Funding:

R21CA287157 NCI 09/01/2024-08/31/2026
Development of a pre-clinical syngeneic pig glioma model for research and translational studies
This proposal examines transient versus chronic immune/inflammatory suppression to optimize porcine glioma formation and immune competence after orthotopic implants of allogeneic GBM cells or in situ viral oncogenesis in a transgenic Oncopig brains.
PD/PI: Robert **Rostomily** (lead), Phil Horner and Monika Vishnoi

R21NS127229 NINDS 1/9/2024 –12/31/2025
Patterned optogenetic neuromodulation to reprogram glioma stem cells
Use of optogenetic approaches to determine impacts of patterned modulation of Vmem on GBM stem cell phenotypes in vitro and in vivo.
PD/PI: **Rostomily**, Robert (lead) & St. Pierre, Francois

R61CA278458 NCI 4/1/2024 – 3/31/27**.**
A multiplexed high-throughput platform to report pharmacologic alteration of cancer stem cell membrane potential and cell cycle state
Integration of genetically encoded voltage and cell cycle sensors will provide a robust and novel high-throughput platform to identify modulators of membrane voltage and cell proliferation.
PD/PI: **Rostomily**, Robert (Lead) & St. Pierre, Francois

NIH/NINDS 1 R01 NS129720-01A1 7/1/23-6/30/28
Pharmacologic targeting of NR4A1 and NR4A2 to activate glioblastoma treatment response.
Rostomily (lead PI), Andrei Mikheev, Steven Safe (co-PIs)
This proposal studies the role of NR4A1/2 for concurrent regulation of the EMT GSC phenotype by inhibition of TWIST1 and immune suppressive TME through PD-L1 regulation using syngeneic glioma models.

***Manuscripts*:**
Pollak, J., K. G. Rai, C. C. Funk, S. Arora, E. Lee, J. Zhu, N. D. Price, P. J. Paddison, J. M. Ramirez, and R. C. **Rostomily**. “Ion Channel Expression Patterns in Glioblastoma Stem Cells with Functional and Therapeutic Implications for Malignancy.” PLoS One 12, no. 3 (2017): e0172884.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2016-Present	Professor, Director, Translational Research, Houston Methodist Hospital Department of Neurosurgery and Research Institute
	Professor, Weill Cornell School of Medicine, Department of Neurosurgery
	Adjunct Affiliate Professor, Neurological Surgery, University of Washington, Seattle, WA
2013-2016	Member, Alvord Brain Tumor Center, University of Washington School of Medicine
2012-2016	Professor, Neurological Surgery, University of Washington, Seattle, WA
2008-2016	Faculty Member, Institute of Stem Cell and Regenerative Medicine (UW)
2007-2016	Co-Director, Gamma Knife Radiosurgery Center, UW, Harborview Medical Center
2004-2016	Research Affiliate, Center on Human Development and Disability (UW)
2004-2016	Member, Fred Hutchinson/UW Cancer Consortium
2004-2013	Head, Neuro-Oncology Affinity Group, Fred Hutchinson//UW Cancer Consortium
2004-2012	Associate Professor, Neurological Surgery, University of Washington, Seattle, WA
2000-2004	Assistant Professor, Neurological Surgery, University of Washington, Seattle, WA

1999-2000	Acting Clinical Assistant Professor, Department of Neurological Surgery
1999	Cranial Base Surgery Fellowship; GWU, Washington DC: Dr. Laligam Sekhar
1996-1998	Staff Neurosurgeon, USAF, Wilford Hall Medical Center, San Antonio, TX
1995-1996	Acting Assistant Professor, Neurological Surgery, University of Washington, Seattle, WA
1993-1996	NIH NRSA postdoctoral research fellow; Tom Reh PhD, Mentor
1994-1995	Chief Resident/Acting Instructor, Department of Neurological Surgery

Honors

2011-2013	Tietze Stem Cell Scientist Award, University of Washington
1995-1996	Charles A. Elsberg Fellowship Award in Neurological Surgery, NY Academy of Medicine
1990-1991	ACS Clinical Fellowship in Neuro-Oncology (Drs. Alexander Spence and Mitchel S. Berger)

C. Contributions to Science

1. The role of TWIST1 and EMT in glioma phenotypes. Early in my clinical training and research career, I realized that diffuse invasion of glioma cells is arguably the major impediment to effective treatment and the underlying cause of their dismal prognosis. The similarities between glioma invasion and the migratory behaviors of normal neural stem and progenitor cells prompted me to investigate the potential relevance of regulatory mechanisms governing gliogenesis and neurogenesis for glioma invasion and other aspects of glioma malignancy. I hypothesized that developmentally regulated bHLH transcription factors which define specific developmental stages in neurogenesis/gliogenesis define malignant states and contribute to phenotypes of neural derived tumors. After showing that differential expression of developmentally regulated neural bHLHs partially recapitulated specific stages of neurogenesis in medulloblastoma and sPNET I screened a glioma cell line for tissue specific bHLHs and identified TWIST1, a developmentally regulated mesodermal bHLH not previously reported in glioma. Additional studies demonstrated the robust association between TWIST1 and malignant clinical grade as well as functional activity in regulating invasion, cell adhesion, cytoskeletal reorganization and cancer stem cell properties. These findings were the first demonstration that a process similar to the epithelial mesenchymal transition (EMT) that drives carcinoma metastasis is relevant for glioma malignancy as well. Further we showed that the EMT associated and TWIST1 regulated protein, periostin (POSTN), recapitulates EMT like phenotypes in glioma stem cells (GSCs) and is upregulated at tumor recurrence. Recently, we identified novel mechanisms regulating POSTN expression and invasion through site-specific TWIST1 phosphorylation and dimerization motifs which may provide targets for therapy. Collectively this work has been seminal to an emerging understanding of the importance of deregulated developmental mechanisms like EMT in promoting malignant properties of glioma such as invasion and stem cell phenotypes which have proven to be of great clinical and therapeutic relevance.

a. Mikheeva, S., Mikheev, A.M., Petit, A., Beyer, R.P., Oxford, R.G., Khorasani, L., Maxwell, J.P., Glackin, C.A., Wakimoto, H., Gonzalez-Herrero, I., Sanchez-Garcia, I., Silber, J.R., Horner, P.J. & **Rostomily, R.C.** (2010). TWIST1 promotes invasion through mesenchymal change in human glioblastoma. Molecular Cancer, 9, 194. PMID: 20646316 (*highly accessed*)
b. Mikheev, A.M., Mikheeva, S.A., Trister, A.D., Tokita, M.J., Emerson, S.N., Parada, C.A., Born, D.E., Carnemolla, B., Frankel, S., Kim, D.H., Oxford, R.G., Kosai, Y., Tozer-Fink, K.R., Manning, T.C., Silber, J.R. & **Rostomily, R.C.** (2014). Periostin is a novel therapeutic target that predicts and regulates glioma malignancy. Neuro Oncology, 17(3), 372-382. PMID: 25140038
c. Mikheev, A.M., Mikheeva, S.A., Severs, L.J., Funk, C.C., Huang, L., McFaline-Figueroa, J.L., Schwensen, J., Trapnell, C., Price, N.D., Wong, S. & **Rostomily, R.C.** (2018). Targeting TWIST1 through loss of function inhibits tumorigenicity of human glioblastoma. Mol Oncol., 12(7), 1188-1202. PMID: 29754406
d. Mikheeva, S.A., Camp, N.D., Huang, L., Jain, A., Jung, S.Y., Avci, N.G., Tokita, M., Wolf-Yadlin, A., Zhang, J., Tapscott, S.J., **Rostomily, R.C. &** Mikheev, A.M. (2019). TWIST1 Heterodimerization with E12 requires coordinated protein phosphorylation to regulate periostin expression. Cancers (Basel), 11(9). pii: E1392. PMID:31540485

2. The effects of aging in putative glioma cells of origin and glioma malignancy. Identifying potential cells of origin for glioma is of great significance for understanding how deregulation of development or aging

contributes to glioma malignancy and also informs the development of relevant models of gliomagenesis and malignancy. While the resident SVZ neural stem cell was considered a leading candidate, our finding of overlapping co-expression of Olig2 in a majority of proliferative glioma and non-neoplastic cells strongly suggested that gliomas may also arise from a reservoir of resident extra-ventricular neural progenitor cells. Despite the striking associations between patient age and adult glioma incidence, degree of malignancy and outcome, the impact of aging glioma cells of origin had not been comprehensively addressed in animal models until we developed a syngeneic model that could interrogate the relative impact of cell of origin and host aging. Transformed NSPCs from old donor mouse brains were markedly more malignant than transformed NSPCs from young donor brains, regardless of host age. This was the first demonstration that aging in the cell of origin could impact malignancy and correlated closely with additional findings of aging related proliferation and metabolic phenotypes in normal neural progenitors. Together these studies indicated that changes that occur during normal aging in putative glioma cells of origin contribute to the differential malignancy observed in their transformed counterparts. Of further importance correlation between aging dependent differential hypoxic responses, genomic stability and responses to chemotherapy/radiation and observations in glioma patients provided strong validation for the clinical relevance of this model.

a. Mikheev, A.M., Stoll, E.A., Mikheeva, S.A., Maxwell, J.-P., Jankowski, P.P., Ray, S., Uo, T., Morrison, R.S., Horner, P.J. & **Rostomily, R.C.** (2009). A syngeneic glioma model to assess the impact of neural progenitor target cell age on tumor malignancy. Aging Cell, 8(4), 499-501. PMID: 19489742
b. Mikheev, A.M., Stoll, E.A., Ramakrishna, R., Mikheeva, S.A., Horner, P.J. & Rostomily, R.C. (2012). Geropotency: increased malignant potential of aging neural progenitors. Aging, 4(12) 854-5. PMID: 23257545
c. Stoll, E.A., Habibi, B.A., Mikheev, A.M., Lasiene, J., Massey, S.C., Swanson, K.R., **Rostomily, R.C.** & Horner, P. (2011). Increased re-entry into cell cycle mitigates age-related neurogenic decline in the murine subventricular zone. Stem Cells, 29(12), 2005-2017. PMID: 21948688
d. Mikheev, A.M., Ramakrishna, R., Stoll, E.A., Mikheeva, S.A., Beyer, R.P., Plotnik, D.A., Schwartz, J.L., Rockhill, J.K., Silber, J.R., Born, D.E., Kosai, Y., Horner, P.J. & **Rostomily, R.C.** (2012). Increased age of transformed mouse neural progenitor/stem cells recapitulates age-dependent clinical features of human glioma malignancy. Aging Cell, 11(6), 1027-1035. PMID: 22958206

3. Intratumor Heterogeneity, Precision Medicine and Pre-clinical screening. Our studies of aging (see above) supported the importance of genomic instability as a mechanism contributing to glioma. A practical deduction from this observation with profound clinical ramifications was that genomic instability would confound the utility of single biopsy samples used to inform precision medicine by generating mutational heterogeneity. This was confirmed through deep sequencing of selected oncogenes and tumor suppressors from multiple samples obtained from individual patient tumors revealed a surprising degree of mutational heterogeneity. This work added significant confirmation for the importance of accounting for mutational heterogeneity when deploying paradigms of precision medicine and suggested that a pre-clinical screening platform to validate patient responses to genomically informed therapy could enhance precision medicine. However, in vitro screens based on cultured cells fail to account for the impact of the tumor microenvironment while patient derived xenografts are costly, not easily established, and are unable to provide readouts in a clinically relevant time frame. The development of a microfluidic delivery system using organotypic slice cultures from multiple regions of a single patient tumor addresses many of these challenges. This platform permits delivery of drugs to the tissues at multiple doses and in a sequenced fashion to provide readouts for combinatorial therapy. I also contributed to the generation of the IVY Gap Glioblastoma Atlas which provides an invaluable tool for the study of regional molecular heterogeneity in GBM.

a. Kumar, A., Boyle, E.A., Tokita, M., Mikheev, A., Sanger, M.C., Girard, E., Silber, J.R., Gonzalez-Cuyar, L.F., Hiatt, J.B., Adey, A., Lee, C., Kitzman, J.O., Born, D.E., Silbergeld, D.L., Olson, J.M., **Rostomily, R.C.*** & Shendure, J.* (2014). Deep sequencing of multiple regions of glial tumors reveals spatial heterogeneity for mutations in clinically relevant genes. Genome Biol., 15(12), 530. PMID: 25608559 (**co-senior authors*)
b. Bomsztyk K, Mar D, Denisenko O, Powell S, Vishnoi M, Delegard J, Patel A, Ellenbogen RG, Ramakrishna R, **Rostomily R**. (2024) Analysis of gliomas DNA methylation: Assessment of pre-analytical variables.

c. Rodriguez, A.D., Horowitz, L.F., Castro, K., Kenerson, H., Bhattacharjee, N., Gandhe, G., Raman, A., Monnat, R.J., Yeung, R., **Rostomily, R.C.** & Folch, A. (2020). A microfluidic platform for functional testing of cancer drugs on intact tumor slices. Lab Chip, 2020 Apr 9. PMID: 32270149
d. Horowitz, L.F., Rodriguez, A.D., Dereli-Korkut, Z., Lin, R., Castro, K., Mikheev, A.M., Monnat Jr., R.J., Folch, A. & **Rostomily, R.C.** Multiplexed drug testing of tumor slices using a microfluidic platform. NPJ Precis. Oncol. 2020, 4:12, PMC7237421

Complete List of Published Work in PubMed: http://www.ncbi.nlm.nih.gov/pubmed/?term=rostomily

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