BIOGRAPHICAL SKETCH

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NAME: Julie A Siegenthaler

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

POSITION TITLE: Associate Professor

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
Mount Holyoke College, South Hadley MA	B.A.	05/2000	Neuroscience & Behavior
SUNY Upstate Medical Univ, Syracuse NY	Ph.D.	07/2005	Neuroscience
SUNY Upstate Medical Univ, Syracuse NY	Postdoctoral	05/2006	Developmental Neurobiology
University of California, San Francisco San Francisco CA	Postdoctoral	06/2012	Developmental Neurobiology

A. Personal Statement

The over-arching goal of my research is to elucidate important signaling between non-neural structures, the meninges and cells of the neuro-vasculature, and the brain required for normal development, adult function and how these are perturbed in CNS disease and brain injury. Projects in my lab use mouse genetic models, ex vivo and culture models, single cell transcriptional profiling and animal models of adult brain injury and disease (stroke, traumatic brain injury, viral encephalitis) to address these important questions in CNS biology.

I have a long-standing interest and expertise in the meninges, CNS vasculature and blood brain barrier (BBB) in both development and disease. I have made important discoveries in the field of meninges and CNS vascular biology. As a post-doctoral fellow, I was an integral part of characterizing mice with mutations in the meningeal-expressed gene *Foxc1* and developing these mice as a model to study how the meninges regulate brain development. I discovered that retinoic acid synthesized by the meninges regulates symmetric and asymmetric divisions in neocortical progenitors (Siegenthaler et al., 2009 *Cell*). Over the last 8 years, my independent group at the University of Colorado, Anschutz Medical Campus (CU AMC) has made important progress toward understanding how meningeal-derived retinoic acid functions in CNS vascular development (Bonney et al., 2016 *J of Neurosci*; Mishra et al., 2016 *Dev Bio;* Bonney et al., 2017 *eNeuro*; Bonney et al., 2018 *Frontiers in Cellular Neuroscience*) and adult neurogenesis (Mishra et al., 2018 *Stem Cell Reports*). My lab has generated a first-ever single cell RNAseq atlas of the developing mouse meninges (DeSisto et al., 2020 *Developmental Cell*). This data set has been an important resource for our work studying the arachnoid barrier, the topic of this proposal. Our work on CNS vasculature and BBB in injury (ischemic stroke, Kelly et al., 2016 *BMC Neuroscience*) disease (viral encephalitis, Bonney et al., 2019 *mBio*) has been the result of two fruitful collaborations with our colleagues at the University of Colorado (Paco Herson, PhD and Ken Tyler, MD). I am excited to continue this string of successful collaborations with the Doran lab to work on the arachnoid barrier in bacterial meningitis.

My extensive and distinctive combined knowledge of CNS barriers and the meninges makes me the ideal researcher to lead this project to study arachnoid barrier development, function and it's breakdown in bacterial meningitis, the latter in collaboration with Kelly Doran (Co-investigator).

B. Positions and Honors

Positions and Employment

08/00-07/05	Graduate Student, Department of Neuroscience and Physiology, SUNY Upstate Medical Syracuse, NY
08/05-4/05	Postdoctoral fellow, Department of Neuroscience and Physiology, SUNY Upstate Medical Syracuse, NY
05/06-06/12	Postdoctoral fellow, Department of Neurology, University of California, San Francisco, San Francisco, CA
07/12-07/2019	Assistant Professor, Career Track, Department of Pediatrics, Section of Developmental Biology, University of Colorado School of Medicine, Aurora, CO
07/19-present	Associate Professor, Career Track, Department of Pediatrics, Section of Developmental Biology, University of Colorado School of Medicine, Aurora, CO

Other Experience and Professional Memberships

2006-Present	Member, Society for Neuroscience
2014-Present	Member, North American Vascular Biology Organization

Honors and Awards

2006	Fetal Alcohol Syndrome Study Group Travel Award (Research Society on Alcoholism)
2007-2009	Lawrence M. Brass, M.D. Stroke Research Postdoctoral Fellowship Award (AHA/AAN)
2010-2015	K99/R00 Pathway to Independence Award (NS070920) National Institutes of Health/NINDS

C. Contribution to Science

1. During my PhD and a one year post-doc at SUNY Upstate Medical University, I investigated the molecular mechanisms underlying teratogenic and genetic causes of microcephaly. For my PhD work, I studied Fetal Alcohol Syndrome (FAS) associated defects in neocortical development, demonstrating that Transforming growth factor-β (TGFβ) signaling was involved in promoting both progenitor cell cycle exit and neocortical migration and that these functions of TGFβ were impaired by alcohol exposure. This work provided important new insight into how a pregnant mother's alcohol consumption perturbs normal brain development through modulation of TGFβ1. *FOXG1* is a disease gene for Rett syndrome of which microcephaly and developmental delay is a major feature. We found that heterozygosity of *Foxg1*, of which some Rett syndrome patients have, caused reduced production of intermediate progenitor cells in the neocortex and reduced neuron generation. We identified loss of Foxg1-mediated inhibition of CKI p21 as a mechanism leading to premature exit of ventricular zone progenitors from the cell cycle. Our work on Foxg1 in telencephalic development not only provided knowledge regarding the cause of microcephaly in human patients with *FOXG1* mutations but also outlined a novel mechanism by which TGFβ signaling regulates birth of Cajal-Retzius cells during early brain development.

a. **Siegenthaler, JA** and Miller, MW (2004) "Transforming growth factor β1 modulates cell migration in rat cortex: effects of ethanol." *Cerebral Cortex*, 14: 791-802.

b. **Siegenthaler, JA** and Miller, MW (2005) "TGFβ1 promotes cell cycle exit through the CKI p21 in the developing cerebral cortex." *Journal of Neuroscience*, 25(38):8627-8636.

c. **Siegenthaler, JA** and Miller, MW (2007) "Generation of Cajal-Retzius neurons in mouse forebrain is regulated by transforming growth factor-β-Fox signaling pathways." *Developmental Biology,* 313: 35-46.

d. **Siegenthaler*, JA**, Tremper-Wells*, B, and Miller, MW (2008) "Foxg1 haplo-insufficiency reduces the population of cortical intermediate progenitor cells: effect of increased p21 expression." *Cerebral Cortex,* 8:1865-1875. *Co-first authors

2. During my postdoctoral training at UCSF, I began an incredible research 'odyssey' to understand how signals made by the meninges are needed to regulate brain development. We found that mice with mutations in the forkhead transcription factor *Foxc1* fail to form meninges around the neocortex and, as a result, have enlarged

cerebral hemispheres. We showed that these defects in neocortical development result from failure of neocortical progenitors to switch from symmetric to asymmetric, neuron generating divisions due to lack of meningeal derived retinoic acid. This work represented a significant advancement in our understanding of corticogenesis, solidified the meninges as a major regulator of neocortical development and identified defects in meningeal development as a possible cause of focal cortical dysplasia in human patients. In my independent lab at CU AMC, we have shown that retinoic acid plays an important role in adult stem cell niches and, in particular for the adult hippocampus, the meninges are likely an important source of retinoic acid for this adult niche. To advance our studies on meninges development and function, we have generated a molecular atlas of the embryonic forebrain meningeal fibroblasts. Mining of this data has been important for this project, yielding our discovery of Wnt-β-catenin as an important player in arachnoid barrier development.

a. Zarbalis*, K, **Siegenthaler*, JA**, Choe, Y, May, SR, Peterson, AS, Pleasure, SJ (2007) "A novel hypomorphic *Foxc1* allele with disruption of meningeal differentiation causes cortical dysplasia and skull defects." *PNAS* 104:14002-14007. *Co-first authors

b. **Siegenthaler, JA**, Ashique, AM, ,Zarbalis, K, Patterson, KP, Hecht, JH, Kane, MA, Folias, AE, Choe, Y, May, SR, Kume, T, Napoli, JL, Peterson, AS, Pleasure, SJ (2009) "Retinoic acid from the meninges regulates cortical neuron generation." *Cell*, 139:597-609.

c. Mishra, S, Kelly, KK, Rumian, NL, and **Siegenthaler, JA** (2018) "Retinoic acid is required for neural stem and progenitor proliferation in the adult hippocampus" *Stem Cell Reports* 10 (6):1705-1720.

d. DeSisto, J, O'Rourke, R, Jones, HE, Pawlikowski, B, Malek, A, Bonney, S, Guimiot, F, Jones, KL, **Siegenthaler, JA** (2020) "Single cell transcriptomic analyses of the developing meninges reveals meningeal fibroblast diversity and function." *Developmental Cell* 54 (1):43-59.

3. Supported AHA/AAN grant and later by my K99-R00 Pathway to Independence Award and an AHA Beginning Grant-in-Aid, I focused the latter half of my post-doctoral work and now work in my lab at CU AMC brain pericyte function in vascular development and the response of pericyte subpopulations to brain injury. I have shown that Foxc1 has important roles in pericytes during brain vascular development. Using pericyte-conditional *Foxc1* KOs, we show that Foxc1 is needed to regulate pericyte proliferation. Also, *Foxc1*-deficient pericytes fail to inhibit endothelial cell proliferation leading to dysplastic, hemorrhage-prone vessels in the conditional mutants. This phenotype is particularly relevant in light of several publications describing cerebrovascular bleeds in patients with point mutations in or deletions that include *FOXC1*. Our lab has also taken the lead on characterizing the developmental origins and functions of a poorly understood but important pericyte subtype termed perivascular fibroblasts (PVFs). PVFs have recently been shown to have a differential response to brain injury. Unlike pericytes that die in the injury core, PVFs proliferate to increase numbers and are the source of most fibrotic scar material. We have shown these cells are potentially derived from the meninges during post-natal brain development and that PVFs may have a separate function in the injury site, specifically a source of bioactive signals like retinoic acid that have the potential to be neuroprotective and anti-inflammatory.

a. **Siegenthaler, JA**, Choe, Y., Patterson, K., Hsieh, I., Li, D., Jaminet, S., Daneman, R., Kume, T., Huang, E. and Pleasure, S. (2013). Foxc1 is required by pericytes during fetal brain angiogenesis. *Biology Open: The Company of Biologists* 0:1-13 DOI: 10.1242; bio.20135009.

b. **Siegenthaler, JA**, Sohet, F and Daneman, R (2013) "Sealing off the CNS: cellular and molecular regulation of blood brain barriergenesis." *Current Opinion in Neurobiology.* 23(6): 1057-64.

c. Kelly, KK, MacPherson ,A, Grewal, H, Strnad , Jones, J, Yu , J, Pierzchalski , K, Kane, M, Herson, P, **Siegenthaler, JA** (2016) "Col1a1+ perivascular cells in the brain are a source of retinoic acid following stroke." *BMC Neuroscience* 17(49) DOI: 10.1186/s12868-016-0284-5

4. Brain vascular development and vascular breakdown in disease is a major research focus for my lab at CU AMC. Using several animal models of RA deficiency and targeted disruption of RA signaling in brain endothelial cells, my lab has uncovered a previously unknown interaction between RA and the WNT pathway. Endothelial WNT signaling is absolutely required for development of the CNS vasculature however no pathways have been identified upstream of WNT in the CNS vasculature. We have found that RA is required to promote endothelial WNT signaling specifically in the neocortex via inhibiting expression soluble WNT inhibitors by cortical neural progenitors. Surprisingly, we have found that RA is required in brain endothelial cells to directly inhibit WNT signaling to prevent ectopic expression of WNT target Sox17. We have extended our interest in brain vascular development to understanding mechanisms of blood-brain barrier (BBB) breakdown in disease. Through a collaboration with neurovirologist Ken Tyler in the Dept of Neurology, we identified IFN-gamma as a key mediator

blood-brain barrier (BBB) breakdown in Reo virus encephalitis. Our discoveries are providing important insight into signaling mechanisms underlying brain vascular development and BBB breakdown in disease that we believe can be eventually applied to help 'normalize' vasculature integrity in brain pathologies.

a. Bonney*, S, Harrison-Uy*, S, Mishra, S, MacPherson, A, Choe, Y, Li, D, Jaminet, SC, Fruttiger, M, Pleasure, SJ and **Siegenthaler, JA** (2016) "Diverse functions of retinoic acid in brain vascular development." *Journal of Neuroscience* 36(29):7786-801. *Co-first authors

b. Mishra, S, Choe, Y, Pleasure, SJ, **Siegenthaler JA** (2016) "Cerebrovascular defects in *Foxc1* mutants correlate with aberrant WNT and VEGFA pathways downstream of retinoic acid from the meninges." *Developmental Biology* doi: 10.1016/j.ydbio.2016.09.019.

c. Bonney S, Dennison, BJC, Wendlandt, M, **Siegenthaler, JA** (2018) "Retinoic acid regulates endothelial β-catenin expression and pericyte numbers in the developing brain vasculature." *Frontiers in Cellular Neuroscience*

d. Bonney, S, Seitz, S, Ryan, CA, Jones, KL, Clarke, P, Tyler, KL, **Siegenthaler, JA** (2019) "IFNγ alters junctional integrity via Rho-kinase resulting in BBB leakage in experimental viral encephalitis." *mBio* Aug 6;10(4). pii: e01675-19

Complete List of Published Work in My Bibliography:

https://www.ncbi.nlm.nih.gov/myncbi/14IcotnlN2hkr/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing

R03 NS104566 – NIH/NINDS

Title: "Foxc1 control of meninges formation and function" 9/1/2018-8/31/2020

The project pilot grant is intended to create a molecular atlas of the developing forebrain meninges in wildtype and *Foxc1-KO* mice. This will generate a resource for investigations into meninges development and function and foster new lines of research into the function of Foxc1 in meninges formation. *There is no overlap with the R01 application, which will focus specifically on the meninges arachnoid barrier.*

Role: Principal Investigator (5% effort)

R01NS098273 – NIH/NINDS 6/1/2016-5/31/2021

Title: "Retinoic acid in the development of the CNS vasculature"

Projects in this grant will identify a role for RA signaling in controlling brain endothelial cell and pericyte proliferation required for vascular stability through regulation of WNT signaling and its target Sox17. Further, we will elucidate the function of RA in retinal vascular development and identify a role for RA deficiency in the developmental vascular pathology retinopathy-of-prematurity. *This proposal is a renewal of this award.*

Role: Principal Investigator (30% effort)

R01 MH123971 - NIH/NIMH (PIs: Gama, Bellan Vanderbilt University) 7/1/2020-6/30/2023

Title "Modeling developmental gradients and supportive tissue signaling networks using iPSC-derived forebrain organoids embedded in fluidic hydrogels"

Projects in the grant will 1) develop human polarized organoids using novel bio-instructive gelatin-based hydrogel to create forebrain organoids with enhanced levels of biomimetic topographic complexity and 2) elucidate and mimic the contributions of the meninges to cortical development. My role is to help develop the meninges-forebrain organoid model and help with efforts to generate human iPSC derived meningeal fibroblast subtypes utilizing meningeal fibroblast markers generated in our fetal meninges single cell project. *There is no overlap with this proposal.*

Role: Co-investigator (5% effort)