BIOGRAPHICAL SKETCH

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NNAME: Borok, Zea

POSITION TITLE: Professor of Medicine and Biochemistry & Molecular Biology

eRA COMMONS USER NAME

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
University of Cape Town, Cape Town, South Africa	M.B., Ch.B.	12/1979	Medicine
University of Pittsburgh, Pittsburgh, PA	Internship/ Residency	06/1985	Internal Medicine
University of Pittsburgh, Pittsburgh, PA	Fellowship	06/1987	Critical Care Medicine
National Institutes of Health, Bethesda, MD	Post-doc	06/1990	Pulmonary Medicine

A. Personal Statement

I am a physician-scientist who directs the basic science research activities of the Division of Pulmonary, Critical Care and Sleep Medicine at the University of Southern California (USC) Keck School of Medicine and the Hastings Center for Pulmonary Research. I have extensive expertise and experience in cell and molecular biology of lung alveolar epithelium, with particular focus on transcriptional and epigenetic mechanisms that regulate alveolar epithelial cell (AEC) differentiation and plasticity. I have been continuously funded by the NIH since 1993 for studies of transcriptional regulation of AEC differentiation by the forkhead transcription factor, FOXO1 (MERIT award), the role of Wnt and transforming growth factor-β signaling in AEC plasticity in the context of pulmonary fibrosis, and characterization of epigenetic and transcriptomic profiles of normal and diseased AEC and recently received an R35 Outstanding Investigator Award to fund my research program on alveolar epithelial cell biology. My laboratory has developed methods for isolation and culture of primary rat, mouse and human alveolar epithelial type II cells. We have established *in vitro* models for culture of alveolar epithelial type II (AT2) cells that enable detailed investigation of pathways that regulate phenotypic transitions at baseline and in response to exogenous stimuli, as well as *in vivo* animal models of lung injury (bleomycin, hyperoxia, lipopolysaccharide) including measurement of lung function. AT2 cells serve as progenitors for adult alveolar epithelium, thus understanding mechanisms that regulate their self-renewal and differentiation to a type I (AT1 cell) phenotype are key to understanding mechanisms of repair following injury, as well as aberrant differentiation in the context of carcinogenesis. My laboratory was among the first to demonstrate epithelial-mesenchymal transition (EMT) of AEC in the context of lung fibrosis and to suggest that 'reprogrammed' AEC contribute to fibrosis via abnormal epithelial-mesenchymal crosstalk and, in collaboration with Dr. Michael Kahn, that selective inhibition of β-catenin/CBP interactions inhibits fibrosis and preserves epithelial phenotype and integrity. Through a long-standing collaboration with Dr. Ite Laird-Offringa, we undertook integrated analysis of transcriptomic and epigenomic changes during human AEC differentiation from an AT2 to an AT1 cell phenotype. We identified RXR signaling as an important pathway in normal AEC differentiation and a number of transcription factors that regulate the process. This collaboration has led to further studies to immortalize AT2 cells, to investigate effects of environmental factors (e.g., cigarette smoking) on AEC transcriptomes and epigenomes and to explore epigenetic changes in AEC from patients with idiopathic pulmonary fibrosis (IPF) to understand how these contribute to the abnormal AEC phenotype in fibrosis. We recently generated mice with knockout of claudin-18, (CLDN18) a tight junction protein that is highly enriched in lung alveolar epithelium. Cldn18 knockout mice show increased proliferation of a number of lung progenitor cells, including AT2 cells. We are currently investigating mechanisms whereby CLDN18 regulates cell proliferation/progenitor function and the role of epigenetic changes in regulating CLDN expression.

B. Positions and honors

Professional Experience

2005-present: Professor, University of Southern California
2000-2005: Associate Professor, University of Southern California
1992-2000: Assistant Professor, University of Southern California
1990-1992: Instructor, University of Southern California
1985-1986: Instructor of Medicine, University of Pittsburgh
1984-1985: Chief Medical Resident, Montefiore Hospital, University of Pittsburgh
1981-1982: Fellow in Thrombosis Research, College of Physicians and Surgeons, Columbia University

Awards and Activities

2017: Recipient of R35 Outstanding Investigator Award from NHLBI, NIH
2017: Elizabeth Rich Award from the American Thoracic Society for significant contributions by an outstanding woman to pulmonary, critical care and sleep medicine,
2017: Mayo Soley Award from the Western Society for Clinical Investigation
2017: Deputy Editor, American Journal of Physiology, Lung Cellular and Molecular Physiology
2014: Recognition Award for Scientific Accomplishment, American Thoracic Society, 2014
2014: Elected to Association for American Physicians
2010: Recipient of NHLBI Method to Extend Research in Time (MERIT) Award
2008-present: Chief, Division of Pulmonary and Critical Care Medicine, USC
2008-2016: Associate Editor, American Journal of Respiratory Cell and Molecular Biology
2007: Western Society for Clinical Investigation Outstanding Investigator Award
2000-2013: Director, Pulmonary and Critical Care Medicine Fellowship Training Program, USC
2000-2013: Editorial Board, American Journal of Physiology: Lung Cellular and Molecular Physiology
1993: NIH Clinical Investigator Development Award
1979: Graduated in first position with distinction in the first, second, third and final professional examinations and the M.B., Ch.B. degree with first class honors

Member, Western Society for Clinical Investigation

C. Contributions to Science

1. Transcriptional and epigenetic mechanisms regulating alveolar epithelial cell differentiation. Normal distal lung alveolar epithelium is comprised of type I (AT1) and type II (AT2) cells. In the adult, AT2 cells both self-renew and serve as progenitors for AT1 cells during normal maintenance and following injury. Understanding mechanisms that regulate normal AT2 to AT1 cell differentiation is key to elucidating mechanisms of repair following injury. We developed methods for isolation of rat, mouse and human AT2 cells (and more recently rat AT1 cells) and *in vitro* models in which AT2 to AT1 cell differentiation can be modulated. We were one of the first to demonstrate that AT2 cells in primary culture undergo transition to an AT1 cell phenotype and that both exogenous soluble factors and changes in cell shape induce AT1-like cells to revert to an AT2 cell phenotype, indicating both active regulation of AEC phenotype and phenotypic plasticity. We initially investigated the role of individual transcription factors (e.g., GATA-6 and FoxP2) in regulating AEC phenotype. In a productive collaboration with Dr. Ite Laird-Offringa, we extended these studies to perform genome-wide analyses and integrated epigenetic and transcriptomic data during AEC differentiation. Cross-specifies transcriptomic profiling led to the recent identification of GRAMD2 as a novel AT1 cell-specific surface marker that should be useful for AT1 cell isolation and generation of an AT1 cell-specific Cre driver line.

1. Marconett CN, B. Zhou B, M. Sunohara , T.M. Pouldar, H. Wang, Y. Liu, M.E. Rieger, E.Tran, P. Flodby, K.D. Siegmund, E.D. Crandall, I.A. Laird-Offringa, **Z. Borok**. Cross-species transcriptome profiling identifies new alveolar epithelial type I cell-specific genes. *Am J Respir Cell Mol Biol* 56:310-321, 2017. [PMID 27749084]
2. Marconett, C., B. Zhou, M.E. Rieger, S.A. Selamat, M. Dubourd, X. Fang, S.K. Lynch, T.R. Stueve, K.D. Siegmund, B.P. Berman, **Z. Borok***, and I.A. Laird-Offringa. Integrated transcriptomic and epigenomic analysis of primary human lung epithelial cell differentiation. *PLoS Genet* 9:e1003513, 2013. [PMC3688557] *Zea Borok and Ite Laird-Offringa contributed equally to this work.
3. Marconett, C.N., B. Zhou, K.D. Siegmund, **Z. Borok**, and I.A. Laird-Offringa. Transcriptomic profiling of primary alveolar epithelial cell differentiation in human and rat. *Genom Data* 1:105-109, 2014. [PMC4203668]

4. Zhou, B., T.A. Francis, H. Yang, W. Tseng, Q. Zhong, B. Frenkel, E.E. Morrisey, D.K. Ann, P. Minoo, E.D. Crandall, and **Z. Borok**. GATA-6 mediates transcriptional activation of aquaporin-5 through interactions with Sp1. *Am J Physiol* 295:C1141-C1150, 2008. [PMC2584994]
5. **Borok, Z**., R.L. Lubman, S.I. Danto, X.L. Zhang, S.M Zabski, L.S. King, P. Agre, and E.D. Crandall. Keratinocyte growth factor modulates alveolar epithelial cell phenotype *in vitro*: expression of aquaporin 5 (AQP5). *Am J Respir Cell Mol Biol* 18:554-561, 1998. [PMID9533944]

2. Epithelial-mesenchymal transition and alveolar epithelial cell plasticity in pulmonary fibrosis: roles of transforming growth factor-β and Wnt signaling. Idiopathic pulmonary fibrosis (IPF) is a fatal disease of unknown etiology characterized by progressive fibrosis leading to lung destruction. AT2 cells in lungs of IPF patients are morphologically abnormal, demonstrating a hyperplastic phenotype with increased proliferation and impaired differentiation to an AT1 cell phenotype. We showed that AEC in IPF express mesenchymal markers, consistent with EMT, suggesting that the epithelium in IPF is 'reprogrammed' perhaps as a result of chronic injury (e.g., endoplasmic reticulum (ER) stress). Effects of ER stress are dose-dependent, with lower amounts of ER stress inducing EMT, and higher amounts apoptosis, suggesting that upregulation of mesenchymal markers in AEC may be an attempt at survival. We further showed that epithelial abnormalities in IPF are abrogated by treatment with the selective inhibitor of β-catenin/CBP interactions, ICG-001, which normalizes epithelial differentiation, and that interactions between β-catenin and TGF-β mediate EMT. Together these findings suggest an important role for epithelial abnormalities in lung fibrosis that may be amenable to combinatorial treatment with inhibitors of β-catenin and TGF-β signaling.

1. Rieger M.E., Zhou B., Solomon N., Sunohara M., Li C., Nguyen C., Liu Y., Pan J., Minoo P., Crandall E.D., Brody S.L., Kahn M. and **Borok Z**. p300/β-catenin interactions regulate adult progenitor cell differentiation downstream of Wnt5a/protein kinase C (PKC). *J Biol Chem* 291: 6569-82, 2016. [PMC4813575]
2. Zhou, B., Y. Liu, M. Kahn, D.K. Ann, A Han, H. Wang, C. Nguyen, P. Flodby, Q. Zhong, M.S. Krishnaveni, J.M. Liebler, P. Minoo, E.D. Crandall, and **Z. Borok**. β-catenin/CBP-dependent regulation of TGF-β-mediated epithelial-mesenchymal transition (EMT) by Smad3. *J Biol Chem* 287:7026-7038, 2012. [PMC3293544]
3. Henderson, W.R. Jr, E.Y. Chi, X. Ye, C. Nguyen, Y.T. Tien, B. Zhou, **Z. Borok**, D.A. Knight, and M. Kahn. Inhibition of Wnt/beta-catenin/CREB binding protein (CBP) signaling reverses pulmonary fibrosis. *Proc Natl Acad Sci USA* 107:14309-14314, 2010. [PMC2922550]
4. Willis, B.C., J.M. Liebler, A.G. Nicholson, E.D. Crandall, R.M. duBois, and **Z. Borok**. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-β1: potential role in idiopathic pulmonary fibrosis. *Am J Pathol* 166:1321-1332, 2005. [PMC1606388]

3. Elucidation of characteristics of alveolar epithelial tight junctions. Claudins are a family of integral tight junction proteins that regulate paracellular permeability to ions and solutes. Claudin 18 (C18) is highly expressed in lung alveolar epithelium. We recently generated C18 knockout (KO) mice that demonstrate increased solute permeability and alveolar fluid clearance (AFC) with normal survival compared to wild type (WT) controls. Increased AFC in C18 KO mice was associated with increased β-adrenergic signaling with activation of cystic fibrosis transmembrane conductance regulator (CFTR), higher epithelial sodium channel (ENaC) and Na-K-ATPase (Na pump) activity, and increased Na-K-ATPase β1 subunit expression. C18 KO AEC monolayers exhibited lower transepithelial resistance (Rt) and increased solute and ion permeability with unchanged ion selectivity, demonstrating a crucial role for C18 in alveolar epithelial tight junction (TJ) composition and permeability properties. Claudin 4 (C4) has been reported to function as an epithelial paracellular Na transport barrier. Unexpectedly, C4 KO mice exhibited normal phenotype, despite increased permeability to 5-carboxyfluorescein and decreased AFC C4 KO alveolar epithelial cell (AEC) monolayers exhibited unchanged ion permeability, higher solute permeability and lower short-circuit current. Interestingly, C4 expression was markedly upregulated in WT mice in response to ventilator-induced lung injury or hyperoxia, although C4 KO mice showed greater degrees of lung injury in these conditions. RNA sequencing suggested Egr1, Tnf and Il1b as potential mediators of increased lung injury in C4 KO mice. These results demonstrate that C4 has little effect on normal lung physiology but may protect against acute lung injury.

1. Li, G., P. Flodby, J. Luo, H. Kage, A. Sipos, D. Gao, Y. Ji, L.L. Beard, C.N. Marconett, L. Demaio, Y.H. Kim, K.J. Kim, I.A. Laird-Offringa, P. Minoo, J.M. Liebler, B. Zhou, E.D. Crandall, and **Z. Borok**. Knockout mice reveal key roles for claudin 18 in alveolar barrier properties and fluid homeostasis. *Am J Respir Cell Mol Biol* 51:210-222, 2014. [PMC4148039]
2. Kage, H., P. Flodby, D. Gao, Y.H. Kim, C.N. Marconett, L. DeMaio, K.J. Kim, E.D. Crandall, and **Z.**

Borok. Claudin 4 knockout mice: normal physiological phenotype with increased susceptibility to lung injury. *Am J Physiol Lung Cell Mol Physiol* 307:L524-L536, 2014. [PMC4187039]

4. Contributions of alveolar epithelial cell transport and barrier properties to alveolar homeostasis: role of type I cells. We developed methods for isolation and culture of primary rat, mouse and more recently human AT2 cells. AT2 cells cultivated on tissue culture-treated polycarbonate filters form confluent monolayers with high transepithelial resistance, serving as useful models for investigating transport and barrier function of AEC. Using these monolayer cultures, we showed that ion transport is regulated by epidermal growth factor (EGF) and keratinocyte growth factor (KGF). We also developed methods for isolation of rat AT1 cells and reported expression of α1 and β1 Na pump subunits and α-rENaC in isolated AT1 cells, indicating an important role for AT1 cells in alveolar homeostasis. We also developed a lung slice preparation that enables us to positively identify AT1 cells within an intact and viable alveolar epithelium using living cell immunohistochemistry in order to assess functional ion channels in AT1 cells. This technique has allowed, for the first time, single ion channels of identified AT1 cells to be recorded using the cell-attached configuration of the patch-clamp technique, facilitating the assignment of function to AT1 cells.

1. Bourke, S., H.S. Mason, **Z. Borok**, K.J. Kim, E.D. Crandall, and P.J. Kemp. Development of a lung slice preparation for recording ion channel activity in alveolar epithelial type I cells. *Resp Res* 6:40, 2005. [PMC1131928]
2. **Borok, Z.**, J.M. Liebler, R.L. Lubman, M.J. Foster, B. Zhou, X. Li, S.M. Zabski, K.J. Kim, and E.D. Crandall. Sodium transport proteins are expressed by rat alveolar epithelial type I (AT1) cells. *Am J Physiol* 282:L599-L608, 2002. [PMID11880284]
3. **Borok, Z.**, S.I. Danto, L.L. Dimen, X.L. Zhang, and R.L. Lubman. Na^+, K^+- ATPase expression in alveolar epithelial cells: upregulation of active ion transport by KGF. *Am J Physiol* 274:L149-L158, 1998. [PMID9458813]
4. **Borok, Z.**, A. Hami, S.I Danto, R.L. Lubman, K.J. Kim, and E.D. Crandall. Effects of EGF on alveolar epithelial junctional permeability and active sodium transport. *Am J Physiol* 270:L559-L566, 1996. [PMID8928815]

5. Development of novel transgenic mouse models with AT1 cell- and AT2 cell-specific knockout/knockin of genes of interest. We characterized the rat aquaporin-5 (Aqp5) promoter and demonstrate that the 4.3-kb 5'-flanking region differentially regulates expression in salivary gland and lung. We identified a highly conserved region in the 3' portion of intron 1 (ci1) by alignment of rat, mouse and human AQP5 genomic sequences that functions as an Aqp5 promoter-specific enhancer. We generated mice with knockin of Cre-IRES-DsRed into the AT1 cell-specific gene Aqp5, resulting in Aqp5-Cre-IRES-DsRed (ACID) mice. ACID mice were crossed to two Cre reporter strains (R26LacZ and mT/mG). Offspring demonstrated reporter gene expression in a very high fraction of AT1 cells, whereas AT2 cells were negative suggesting utility of these mice for investigating AT1 cell biology. Utilizing ACID mice (and Sftpc mice that enable specific gene alteration in whole alveolar epithelium due to transdifferentiation of AT2 into AT1 cells), we recently generated mouse lines that exhibit deletion of the Na pump β1 subunit gene specifically in AT1 cells alone or in the entire alveolar epithelium. allowing us to estimate the relative contribution of AT1 cells to lung fluid clearance. Analysis of these mice revealed for the first time *in vivo* a major role for AT1 cells in alveolar fluid homeostasis.

1. Knockout mice reveal a major role for alveolar epithelial type I cells in alveolar fluid clearance. Flodby P, Kim YH, Beard LL, Gao D, Ji Y, Kage H, Liebler JM, Minoo P, Kim KJ, **Borok Z***, and Crandall ED*. *Am J Respir Cell Mol Biol* 55: 395-406, 2016. * contributed equally to the manuscript.
2. Flodby, P*., **Z. Borok**, A. Banafalvi, B. Zhou, D. Gao, P. Minoo, E.E. Morrisey, and E.D. Crandall. Directed expression of Cre in alveolar epithelial type 1 cells. *Am J Respir Cell Mol Biol* 43:173-178, 2010. [PMC2937230] *contributed equally to this work.
3. Zhou B, Ann DK, Flodby P, Minoo P, Liebler JM, Crandall ED, and **Borok Z**. Rat aquaporin-5 4.3-kb 5'-flanking region differentially regulates expression in salivary gland and lung *in vivo*. *Am J Physiol Cell Physiol*, 295:C111-C120, 2008. [PMC2493550]
4. Flodby, P., B. Zhou, D.K. Ann, K.J. Kim, P. Minoo, E.D. Crandall, and **Z. Borok**. Conserved elements within first intron of aquaporin-5 (aqp5) function as transcriptional enhancers. *Biochem Biophys Res Comm* 356:26-31, 2007. [PMC2366028]
5. **Borok, Z.**, X. Li, V.F.J. Fernandes, B. Zhou, D.K Ann, and E.D. Crandall. Differential regulation of rat aquaporin-5 promoter/enhancer activities in lung and salivary epithelial cells. *J Biol Chem* 275:26507-26514, 2000. [PMID10849430]

URL for Complete List of my Published Work:
https://www.ncbi.nlm.nih.gov/pubmed/?term=Borok+Z

D. Research support

Ongoing Research Support

"Beyond the barrier: alveolar epithelial cell biology in health and disease"
PI: Zea Borok, M.D.
Agency: National Institutes of Health/NHLBI
Type: R35 HL135747 (PI: Borok) 07/17 – 06/24

This proposal is thematically focused on alveolar epithelial cell (AEC) plasticity/'reprogramming', with the overall goal of characterizing the molecular basis of AEC function and phenotype in order to elucidate mechanisms leading to aberrant repair and develop strategies for improving outcome following injury. This grant is to fund my overall research program and is not directed to Specific Aims. No overlap in scientific goals or financial obligations.

"Epithelial abnormalities in IPF: role of ER stress and GRP78/BiP"
PI: Beiyun Zhou, Ph.D. Role: Co-investigator
Agency: National Institutes of Health/NHLBI
Type: R01 HL114959 08/13 - 06/18

The major goals of this proposal are to investigate mechanisms underlying endoplasmic reticulum (ER) stress-induced epithelial abnormalities implicated in the pathogenesis of fibrosis by modulating the key unfolded protein response (UPR) signaling component, GRP78, utilizing mice with lung epithelial cell-specific deletion of this ER chaperone. Specific aims are to 1) evaluate effects of ER stress mediated by loss of GRP78 on pulmonary fibrosis in inducible AEC-specific Grp78 knockout mice; 2) investigate the role of ER stress in AEC abnormalities (apoptosis vs EMT vs aberrant epithelial-fibroblast crosstalk) implicated in fibrosis in inducible AEC-specific Grp78 knockout mice; and 3) determine mechanisms underlying ER stress-induced AED injury/fibrosis. No overlap in scientific goals or financial obligations.

"Mechanisms of lung development & injury"
PI: Parviz Minoo, Ph.D. Role: Co-investigator
Agency: National Institutes of Health
Type: R01 HL095349 05/09 - 04/19

This grant is focused on the exclusive role of transforming growth factor-β receptor II signaling in lung development and pathogenesis of pulmonary fibrosis in a murine model. No overlap.

"Novel targeted therapies for pulmonary fibrosis"
PI: Jan Schnitzer, M.D. Role: PI of Project 3 (subcontract)
Agency: National Institutes of Health
Type: P01HL119165-01 03/15 - 02/20

The goals of this project are to investigate the ability of lung-specific delivery of inhibitors of transforming growth factor-β signaling to ameliorate lung fibrosis. Conjugation of the inhibitors to a caveolae-targeted antibody is expected to significantly increase concentrations in the lung, thereby enhancing therapeutic efficacy while avoiding off-target effects in other organs. No overlap in scientific goals or financial obligations.

"Regulation of CLDN18.1 in pulmonary epithelial differentiation, barrier function and repair"
PI: Amy Firth, Ph.D. Role: Co-investigator (no costs)
Agency: American Lung Association
Type: Biomedical Research Grant 07/17 – 06/18

The goal of this proposial is to investigate the role of tight junction protein CLDN18.1 in airway epithelial progenitor cell maintenance and differentiation and repair of the proximal epithelial barrier using complementary studies in induced pluripotent stem cells and Cldn18$^{-/-}$ mice. No overlap.