Perinatal Institute

Division Details

RESEARCH AND TRAINING DETAILS	
Faculty	**56**
Joint Appointment Faculty	**1**
Research Fellows and Post Docs	**13**
Research Graduate Students	**31**
Total Annual Grant Award Dollars	**$12,629,518**
Total Annual Industry Award Dollars	**$575,404**
Total Publications	**143**

CLINICAL ACTIVITIES AND TRAINING	
Staff Physicians	**34**
Clinical Fellows	**15**

Left to Right: L Muglia, J Whitsett, J Greenberg

Research Highlights

SINCERA: A Pipeline for Single-Cell RNA-Seq Profiling Analysis

A major challenge in developmental biology is to understand the genetic and cellular processes/programs driving organ formation and differentiation of the diverse cell types that comprise the embryo. While recent studies using single cell transcriptome analysis illustrate the power to measure and understand cellular heterogeneity in complex biological systems, processing large amounts of RNA-seq data from heterogeneous cell populations creates the need for readily accessible tools for the analysis of single-cell RNA-seq (scRNA-seq) profiles. The present study presents a generally applicable analytic pipeline (SINCERA: a computational pipeline for SINgle CEll RNA-seq profiling Analysis) for processing scRNA-seq data from a whole organ or sorted cells. The pipeline supports the analysis for: 1) the distinction and identification of major cell types; 2) the identification of cell type specific gene signatures; and 3) the determination of driving forces of given cell types. We applied this pipeline to the RNA-seq analysis of single cells isolated from embryonic mouse lung at E16.5. Through the pipeline analysis, we distinguished major cell types of fetal mouse lung, including epithelial, endothelial, smooth muscle, pericyte, and fibroblast-like cell types, and identified cell type specific gene signatures, bioprocesses, and key regulators. SINCERA is implemented in R, licensed under the GNU General Public License v3, and freely available from Cincinnati Children's PBGE website.

Metagenomic Sequencing with Strain-Level Resolution Implicates Uropathogenic E. coli in Necrotizing Enterocolitis and Mortality in Preterm Infants

In this study, researchers found necrotizing enterocolitis (NEC) afflicts approximately 10% of extremely preterm infants with high fatality. Inappropriate bacterial colonization with Enterobacteriaceae is implicated, but no specific pathogen was identified. We identified uropathogenic E. coli (UPEC) colonization as a significant risk factor for the development of NEC and subsequent mortality. We described a large-scale deep shotgun metagenomic sequence analysis of the early intestinal microbiome of 144 preterm, and 22 term

infants. Using a pan-genomic approach to functionally subtype the E. coli, we identify genes associated with NEC and mortality that indicate colonization by UPEC. Metagenomic multilocus sequence typing analysis further defined NEC-associated strains as sequence types often associated with urinary tract infections, including ST69, ST73, ST95, ST127, ST131, and ST144. Although other factors associated with prematurity may also contribute, this report suggests a link between UPEC and NEC, and indicates the need for further attention to these sequence types as potential causal agents.

Functional and Structural Connectivity of the Visual System in Infants with Perinatal Brain Injury

In this study, we evaluated term and preterm infants with perinatal brain injury and term controls in the first eight weeks of life using task-based functional MRI, functional connectivity during a visual task, and structural connectivity using diffusion tensor imaging. We found that infants with brain injury had reduced functional and structural connectivity compared to term control infants. Specifically, infants with brain injury had reduced activation in the expected area of the occipital cortex, weaker connectivity between visual areas and other areas of the brain during the visual task, and reduced fractional anisotropy (a measure of white matter integrity) in white matter tracts projecting to visual regions. Our next steps will be to correlate these early neuroimaging findings with later visual outcomes in this cohort.

Significant Publications

Cai Y, Bolte C, Le T, Goda C, **Xu Y, Kalin TV, Kalinichenko VV**. **FOXF1 maintains endothelial barrier function and prevents edema after lung injury**. *Sci Signal*. 2016 Apr 19;9(424):ra40.

The regulation of endothelial barrier function involve multiple signaling pathways, structural proteins, and transcription factors. The forkhead protein FOXF1 is a key transcriptional regulator of embryonic lung development, and we used a conditional knockout approach to examine the role of FOXF1 in adult lung homeostasis, injury, and repair. Tamoxifen-regulated deletion of both Foxf1 alleles in endothelial cells of adult mice (Pdgfb-iCreER/Foxf1(-/-)) caused lung inflammation and edema, leading to respiratory insufficiency and death. Deletion of a single Foxf1 allele made heterozygous Pdgfb-iCreER/Foxf1(+/-)mice more susceptible to acute lung injury. FOXF1 abundance decreased in pulmonary endothelial cells of human patients with acute lung injury. Gene expression analysis of pulmonary endothelial cells with homozygous FOXF1 deletion indicated reduced expression of genes critical for maintenance and regulation of adherens junctions. FOXF1 knockdown in vitro and in vivo disrupted adherens junctions, enhanced lung endothelial permeability, and increased the abundance of the mRNA and protein for sphingosine 1-phosphate receptor 1 (S1PR1), a key regulator of endothelial barrier function. Chromatin immunoprecipitation and luciferase reporter assays demonstrated that FOXF1 directly bound to and induced the transcriptional activity of the S1pr1 promoter. Pharmacological administration of S1P to injured Pdgfb-iCreER/Foxf1(+/-)mice restored endothelial barrier function, decreased lung edema, and improved survival. Thus, FOXF1 promotes normal lung homeostasis and repair, in part, by enhancing endothelial barrier function through activation of the S1P/S1PR1 signaling pathway.

Zhang G, Bacelis J, **Lengyel C**, **Teramo K**, Hallman M, Helgeland O, Johansson S, Myhre R, Sengpiel V, Njolstad PR, Jacobsson B, **Muglia L**. **Assessing the Causal Relationship of Maternal Height on Birth Size and Gestational Age at Birth: A Mendelian Randomization Analysis**. *PLoS Med*. 2015 Aug 18;12(8):e1001865.

Observational epidemiological studies indicate that maternal height is often associated with gestational age at birth and fetal growth measures (i.e., shorter mothers deliver infants at earlier gestational ages with lower birth weight and birth length). To explain these associations, postulating of different mechanisms has occurred. This study aimed to investigate the casual relationships behind the strong association of maternal height with fetal growth measures (i.e., birth length and birth weight) and gestational age by a Mendelian randomization approach. Our results demonstrate that the observed association between maternal height and fetal growth measures (i.e., birth length and birth weight) is mainly defined by fetal genetics. In contrast, the association between maternal height and gestational age is more likely to be causal. In addition, our approach that utilizes the genetic score derived from the nontransmitted maternal haplotype as a genetic instrument is a novel extension to the Mendelian randomization methodology in casual inference between parental phenotype (or exposure) and outcomes in offspring.

Division Publications

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3. Agricola ZN, Jagpal AK, Allbee AW, Prewitt AR, Shifley ET, Rankin SA, Zorn AM, Kenny AP. **Identification of Genes Expressed in the Migrating Primitive Myeloid Lineage of Xenopus Laevis.** *Dev Dyn*. 2016; 245:47-55.

4. Ahlfeld SK, Wang J, Gao Y, Snider P, Conway SJ. **Initial Suppression of Transforming Growth Factor-Beta Signaling and Loss of Tgfbi Causes Early Alveolar Structural Defects Resulting in Bronchopulmonary Dysplasia.** *Am J Pathol*. 2016; 186:777-93.

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13. Cai Y, Bolte C, Le T, Goda C, Xu Y, Kalin TV, Kalinichenko VV. **Foxf1 Maintains Endothelial Barrier Function and Prevents Edema after Lung Injury.** *Sci Signal*. 2016; 9:ra40.

14. Cortezzo DE, Sanders MR, Brownell EA, Moss K. **End-of-Life Care in the Neonatal Intensive Care Unit: Experiences of Staff and Parents.** *Am J Perinatol*. 2015; 32:713-24.

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