3. NOTCH ACTIVATION DURING MESODERM INDUCTION MODULATES EMERGENCE OF THE T/NK CELL LINEAGE FROM HUMAN IPSC

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Introduction: A robust method of producing mature T cells from iPSCs is needed to realize their therapeutic potential.

Hypothesis: Notch 1 is known to be required for the production of definitive hematopoietic stem cells *in vivo*, leading us to hypothesize that activation of the Notch pathway would drive access to the T/NK cell lineage.

Methods: Using an optimized hematopoietic progenitor differentiation protocol followed by co-culture with OP9:hDLL4:hMHCII feeder cells, we found Notch activation from day 0-2 of differentiation yielded a robust increase in T/NK lineage cells.

Results: We confirmed T cell identity with surface markers, the upregulation of T cell genes, and robust proliferation of stimulated cultures. Our hematopoietic progenitors were also capable of differentiating into a natural-killer-like cell. The T/NK cell lineage decision was influenced by the plating density of hematopoietic progenitors into the co-culture system with low density favoring T cells. Single cell RNA sequencing during differentiation showed a clear developmental trajectory toward the T cell lineage. Day 42 T cells shared a highly similar transcriptional profile with human primary thymocytes. Sequencing of day 12 cultures showed that early Notch activation yielded a 6-fold increase in the putative HSPC population.

Conclusions: We conclude that Notch activation during early mesoderm induction yields an increased population of hematopoietic progenitors with robust access to the T/NK cell lineage.

6. COVID-19 ASSOCIATED WITH INCREASED IMMUNE CELL INFILTRATION IN THE PLACENTA

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Introduction: Maternal COVID-19 infections are common in pregnancy, yet rates of infant SARS-CoV-2 transmission are low. As the interface between mother and fetus during pregnancy, the placenta may form a protective barrier against vertical transmission of COVID-19. Interestingly, the intrauterine immune response to maternal COVID-19 remains undefined. Major immune populations at the maternal-fetal interface include macrophages and natural killer (NK) cells which can mount cellular and inflammatory cytokine responses against viral infections. Understanding whether these cells respond to COVID-19 in the placenta may help understand intrauterine mechanisms preventing fetal transmission of SARS-CoV-2 in pregnancy.

Objective: Our objective was to examine the relative abundance of maternal CD14+ macrophages and CD56+ NK cells in human placental tissues in pregnancies affected by COVID-19 in comparison to contemporary controls.

Methods: Placental biopsies (including decidual tissue) were obtained from 8 SARS-CoV-2 positive pregnancies (COVID) and 5 contemporary SARS-CoV-2 negative pregnancies (Control) during a peak period of COVID-19 admissions at Boston Medical Center. Formalin-fixed cryosections were stained with primary antibodies (CD14 or CD56) and fluorescently labeled secondary antibodies. Immunofluorescence images of decidual tissue were obtained by automated acquisition and used to calculate a fluorescence ratio (FR) of corrected total cell fluorescence (CTCF) of target antigens over secondary-only negative background. FR of COVID and Control specimens were compared using independent t-tests.

Results: Using this methodology, we see an improvement in the stability and accuracy of network inference for each subtype individually – especially when the sample size is small – resulting in models that represent a broader and more accurate picture of the regulatory landscape.

Results: CD14+ macrophages and CD56+ NK cells were observed in all decidual tissues examined. Quantitative microscopy revealed significantly greater macrophage infiltrates in COVID samples compared to Control samples ($p < 0.01$, Figure 1). There were also significantly greater NK cell infiltrates in COVID samples compared to Controls ($p < 0.05$, Figure 2).

Conclusions: Our data demonstrate that NK cells and macrophages accumulate at the maternal-fetal interface in pregnancies complicated by COVID-19, suggesting these leukocyte populations may be important for preventing vertical transmission of SARS-CoV-2 during pregnancy.

14. INVESTIGATING COPD GWAS IN IPSC-DERIVATED ALVEOLAR EPITHELIAL CELLS USING CRISPI

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Introduction: The distal lung is lined by type 1 (AT1) and type 2 alveolar epithelial cells (AT2), with the latter important for producing surfactant and epithelial regeneration. Chronic obstructive pulmonary disease (COPD) is a debilitating disease affecting the lung epithelium. Poor lung function in adulthood precedes COPD diagnosis, and GWAS demonstrate overlapping risk variants with both COPD and low lung function. However, how these gene variants contribute mechanistically to lung function and/or epithelial dysfunction is poorly understood.

Hypothesis: In this study, we aim to assess how lung function genes affect epithelial cells and hypothesize that knock-down of genes of interest will modulate AT2 function.

Methods: We elected to use CRISPR interference (CRISPRi) to knock-down genes of interest. We engineered human induced pluripotent stem cell (iPSCs) to stably express an inducible, catalytically inactive Cas9 (dCas9) and used a lentiviral platform to deliver gRNAs targeted to the transcriptional start site of genes of interest.

Results: We used established lung directed differentiation protocols to produce iPSC-derived lung progenitors (expressing NKX2-1) then type 2 alveolar epithelial cells (iAT2s). Knock-down of many of the genes of interest commencing at the lung progenitor stage substantially affected differentiation of subsequent iAT2s. We also assessed the effect of knock-down in established iAT2s, including surfactant gene expression and proliferation. We found the absence of certain genes (e.g. HHIP, DSP or FAM13A) altered proliferation and/or increased surfactactant gene expression (SFTPC, SFTPA1 and SFTPA2) expression in iAT2 cells.

Conclusions: In summary, we have developed a CRISPRi platform to knock-down genes of interest identified from COPD and lung function GWAS. We found that the majority of genes assessed affected at least one aspect of AT2 function (differentiation, proliferation or surfactant expression). Future studies will determine the molecular mechanisms by which these genes of interest control key functions of AT2 cells.

30. CO-SEGMENTATION OF GLOMERULI ON HISTOLOGICAL IMAGES FROM MULTIPLE STAINS USING DEEP LEARNING

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Introduction: Pathologists rely on multiple histological stains as part of routine renal biopsy workup in patients with kidney disease.

Objective: We evaluated if a deep learning framework can facilitate assessment of glomeruli from digitized images derived from four different histological stains.

Methods: We developed a computational pipeline to identify and segment the glomeruli from whole slide images (WSIs) of Periodic Acid-Schiff (PAS), Hematoxylin and Eosin (H&E), Jones Methenamine Silver (Jones), and Trichrome stains obtained from kidney biopsies of 60 patients at Ohio State University Wexner Medical Center. Image registration was performed on all four WSIs obtained per patient biopsy and a sliding window operation was defined to crop each histological image to smaller patches. Each image patch was then assigned one of the following labels: no glomerulus, normal or partially sclerosed (NPS) glomerulus, or globally sclerosed (GS) glomerulus, based on whether the patch contained the pathologist-driven annotation of a glomerulus. Four independent patch-level convolutional neural network (CNN) models were trained with stain-specific image patches as inputs and corresponding labels as output. Using these models, an image processing algorithm was developed to process the test WSIs and map the identified glomeruli across different stains. A report identifying glomeruli across different stains and percentage of glomerulosclerosis was generated.

Results: Performance on test data for PAS images is as follows: Accuracy = 97.7 ± 0.98%, Kappa = 0.667± 0.13.

Conclusions: Through deep learning, our work has the potential to directly assist pathologists to examine human kidney biopsies.