Conclusions: Mucosal humoral immunity is critical in preventing re-administration of Adenoviral vectors. Impairment of B-cell responses by αCD20 treatment prior to vector delivery allows re-administration and may help overcome low efficiencies of gene therapy approaches to CF treatment. AAV vectors may be less susceptible to neutralization by pre-existing immunity.

617

Pulmonary rehabilitation in patients with prior COVID-19 infections

A Mallik, A Motes, K Nugent. *Texas Tech University Health Science Center School of Medicine, Lubbock, TX*

Purpose of Study: Patients with prior COVID-19 infections can develop persistent dyspnea and physical limitation. This may reflect chronic lung disease, chronic heart disease, or neuromuscular disease. Treatment approaches include pulmonary rehabilitation. This study analyzed the benefits of conventional pulmonary rehabilitation in patients with chronic symptoms following COVID-19 infections.
Methods Used: 28 patients completed pulmonary rehabilitation at University Medical Center in Lubbock, Texas. The primary outcome was the time spent on four different types of exercise equipment during aerobic exercise sessions.
Summary of Results: This study included 15 women and 13 men. The mean age of 54.4 years. The racial distribution included 12 white patients, 10 Hispanic patients, and 6 black patients. Ten patients had a smoking history,19 patients used supplemental oxygen, 14 patients had hypertension, and 11 patients had diabetes. The median CAT score was 21.5 (Q1, Q3: 17.5,29), the median PHQ9 score was 8 (Q1, Q3: 4.5,15.5), and the median MRC score was 2. Twenty-six patients had the abnormal chest x-rays within 3 months of starting the rehab program; these included 5 patients with focal interstitial infiltrates, 9 patients with diffuse interstitial infiltrates, 6 patients with focal opacities, and 6 patients with bilateral opacities. These patients completed 22.9 (Q1, Q3: 6.8,36) exercise sessions. The table reports the change in machine times from baseline to the last session of rehab. There were statistically significant increases in all machine times on all 4 machines.

Aerobic Activity during Rehabilitation Sessions.

Machine Type	Baseline (minutes)	Post Rehabilitation (minutes)	Paired t-test
Treadmill	9.6*	14.4*	<0.01
NuStep	10.2*	14.8*	<0.01
Arm Ergometer	7.7*	11.4*	<0.01
Bike	8.0*	12.2*	<0.01

statistical significance*

Conclusions: This study indicates a pulmonary rehabilitation can significantly increase aerobic activity levels in patients with prior COVID-19 infections. This improvement occurred in patients with important comorbidity, abnormal chest x-rays, and chronic oxygen supplementation requirements. Patient with prior COVID-19 infection and persistent respiratory symptoms should be referred to pulmonary rehabilitation.

618

Comparative transcriptomics of hyperglycemic-induced BPD in olive baboons

M Petershack, A Moreira. *UT Health Science Center San Antiono, San Antonio, TX*

Purpose of Study: Bronchopulmonary Dysplasia (BPD) is a prevalent lung disease in extremely premature infants. Despite its prevalence, the clinical definition of BPD lacks consensus, which has hindered our understanding of its genetic underpinnings. To delve into the genetic aspects of BPD, we have harnessed high throughput sequencing techniques to pinpoint genes and biochemical pathways contributing to this disease.
Methods Used: Our study involved analyzing RNA sequencing data extracted from the lung tissue of 13 baboons, equivalent to extremely premature neonates at 25 weeks of gestation. These baboons were divided into two groups: 6 healthy controls and 7 baboons with hyperglycemia-induced BPD. The RNA-sequences were initially aligned using STAR, followed by differential gene expression analysis performed with edgeR. Subsequently, we applied a filter to select genes expressed in every sample. These data were then normalized to counts per million and further filtered for counts less than one. Genes were considered differentially expressed if they exhibited an absolute log fold change of 0.5 or greater, a p-value less than 0.05, and a false discovery rate of less than 0.05.
Summary of Results: The average weight of the baboons was 376 +/-8 grams for the control compared to 390 +/-31 grams for the BPD cohort ($p = 0.4$). The gestational age was 127 versus 125 days for the the control and BPD groups, respectively ($p = 0.07$). Our DGE analysis revealed 26 differentially expressed genes (DEGs), all of which were upregulated in BPD. Notably, the top upregulated genes included COX1, SFTPC, MACF1, and EPAS1. COX1 is associated with cellular respiration, while SFTPC plays a crucial role in lung function by encoding a surfactant. MACF1 codes for a cytoskeletal crosslinking protein, and EPAS1 has been linked to vascular structure and oxygen transport. The DEGs were enriched for gene ontology processes, including regulation of cellular adhesion, developmental maturation, and cell-cell adhesion. Additionally, our gene set enrichment analysis indicated an upregulation of tight junctions and phagosome activity.
Conclusions: Conclusion: Our study, utilizing high-throughput sequencing techniques in extremely premature baboons, revealed genetic underpinnings associated with BPD. Specifically, these genes are involved in cellular respiration, lung function, and cytoskeletal structure, contributing to our understanding of the complex molecular changes occurring in BPD-affected lung tissue and demonstrating biological and clinical plausibility.

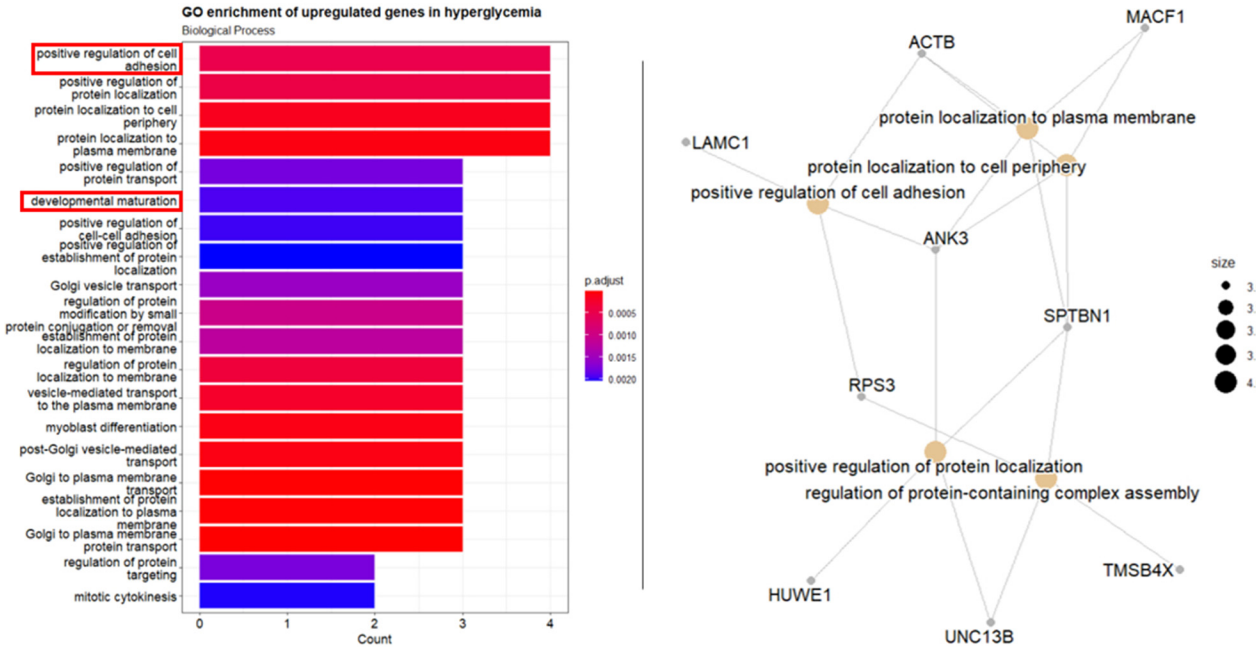

Figure (abstract 618).

619

Long-read sequencing reveals an expansive differentially espressed transcriptome in influenza-infected human airway organotypic cell cultures

E Hartsell[1], A Haastrup[1], J Roberts[1], K Harrod[2], RJ Langley[1]. [1]*University of South Alabama, Mobile, AL;* [2]*University of Alabama at Birmingham, Birmingham, AL*

Purpose of Study: Traditional sequencing techniques, such as Illumina, are limited by their reliance on short reads and therefore unsuited to the detection of splice isoform variants and require significant coverage to identify differentially expressed genes. Oxford Nanopore Technology (ONT) sequences full length transcripts by passing them through membrane bound nanopores causing an electrochemical disruption in the membrane potential which is measured and reported in real time. Here we use the ONT MinION to sequence RNA transcripts from Influenza A infected human lung organoids to examine bulk gene expression when compared to uninfected controls.

Methods Used: Lung organoids were grown from human bronchial epithelial cells taken from the lungs of transplant donors. They were cultured on a chip apparatus until they were confirmed by light microscopy to contain both ciliated and mucous producing cells. Half the organoids were subsequently exposed to Influenza A pH1N1 for 72h; the other half were uninfected controls. RNA was isolated from the organoids and sequenced using the ONT MinION.

Summary of Results: ONT sequencing generated 3.24 million reads per sample, with 21,891 total genes identified. A total of 13,128 genes were relevantly expressed (defined as ≥1 read per million in ≥50% of the samples). ANOVA with a standard Benjamini and Hochberg false discovery rate correction revealed 5,417 genes that were significantly differentially expressed in infected compared to control samples. We further applied a highly stringent Bonferroni correction which narrowed the significantly different genes to 551. Within this subset of genes, pathway analysis revealed downregulation in mucociliary clearance genes and upregulation in inflammatory markers. These changes illuminate the underlying pathophysiology of Influenza A infection. We also noted deficits in mitochondrial and ß-oxidation related genes alongside an upregulation in lactate dehydrogenase pointing to a Warburg-like phenotype not previously reported in Influenza A infection. Glutathione replenishment enzymes were also downregulated, while several S100 proteins were upregulated. We further determined the performance of the PromethION Solo sequencer. A single run provided more than 200 million reads at approximately 1.2 kilobases in length for more than 240 trillion total bases sequenced. This would provide adequate coverage (6 trillion bases/sample) for up to 40 samples at a cost of $59 per sample; this includes the cost of the flow cell, sample preparation and additional consumables.

Conclusions: The long-read data set generated by ONT provides insights into gene expression and the host splice isoform signature of Influenza A infection and sheds light on the mechanistic pathways of disease. Future comparisons will determine differential isoform usage following influenza infections. The cost per sample makes the ONT device an attractive proposition for many basic science labs interested in performing in-house transcriptome sequencing projects.