Hyperoxia induced loss of Electron Transfer Chain annotated proteins in a lung model of neonatal dysplasia revealed via global proteomic analysis using Bruker TIMS-TOF Ultra MS system

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Mark R. Chace[2], Diego Assis[1], Danie Schlatzer[2], Filipa Lopes[2], Anantha Harijith[3], Matthew Willetts[1], Tara Sudhadevi[3]

1-Bruker Scientific LLC, Billerica-MA
2-Department of Nutrition, Center for Proteomics and Bioinformatics, School of Medicine, Case Western Reserve University, Cleveland, Ohio
Department of Pediatrics, Case Western Reserve University
3-Department of Pediatrics, School of Medicine, Case Western Reserve University, Cleveland, Ohio

Introduction

Bronchopulmonary dysplasia (BPD) is an intractable lung disease affecting up to 40% of extreme low birth weight preterm newborns who require exposure to supplemental oxygen i.e. hyperoxia (HO) for survival. HO overwhelms the immature antioxidant systems of the preterm infant, promoting excess reactive oxygen species (ROS) generation, causing lung damage leading to long term morbidity in survivors. NADPH oxidase 4 (NOX4) plays a significant role in generating ROS under both physiological and pathological circumstances and we have previously reported its role in promoting HO-induced acute lung injury. In this study, we have confirmed and reported additional proteins after testing the direct effect of hyperoxia on lung protein abundances in neonatal mice lung using the latest timsTOF Ultra MS instrument.

Methods

Mouse lung samples from 2 different groups were prepared using Easy Pep kit (Thermo Scientific). Peptides were separated within 52-minute ACN gradients on a 25cm x 75µm column (Ion Opticks) using a nanoElute2 LC. The LC system was connected via a CaptiveSpray Ultra source to a trapped ion mobility – quadrupole time-of-flight MS (timsTOF Ultra, Bruker Daltonik). The MS was operated in dia-PASEF mode with an optimized acquisition scheme the py_diAID software with dia-windows isolation starting from 7 to 95 Da according to the multiple charge ion cloud density map (Figure 1). Raw files were processed in Spectronaut 18 (Biognosys) in library-free mode (directDIA+).

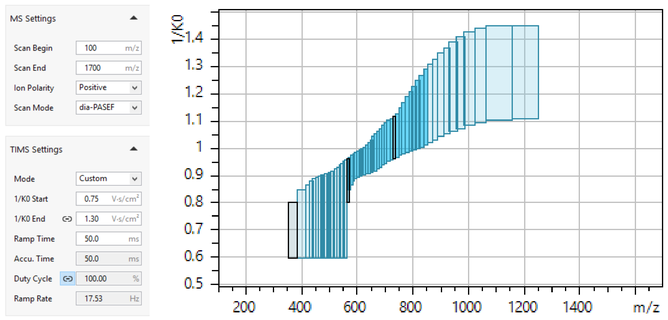

Figure 1: MS method showing dia-PASEF window placement scheme. The cycle time was 1.2 s

Results

Using a neonatal mouse model of BPD, we conducted deep lung proteome analysis using the timsTOF Ultra combined with the nanoElute 2 system. In dia-PASEF mode, we quantified over 11,800 protein groups (1% peptide and protein false discovery rate) and 225,000 peptides from just 175 ng on-column injections, with excellent reproducibility (over 93% of proteins had CVs <20%). Figure 2 shows the number of identifications from 12 patients distributed in 2 groups.

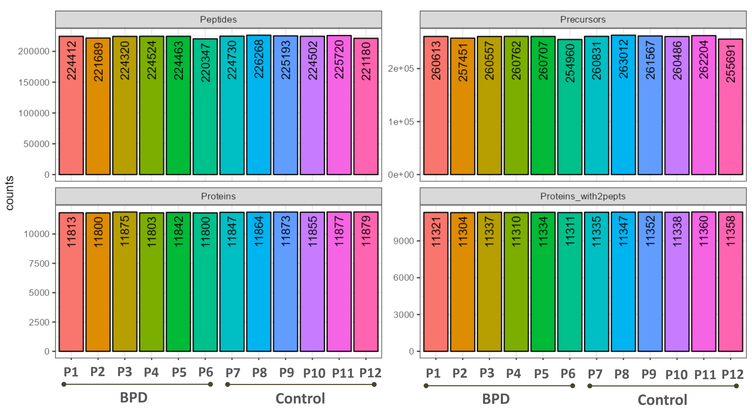

Figure 2: Number of precursors, peptides, protein groups and protein groups with at least 2 peptides from 12 patients (6 control and 6 with Bronchopulmonary dysplasia – BPD).

Using a neonatal mouse model of BPD, we conducted deep lung proteome analysis using the timsTOF Ultra combined with the nanoElute 2 system. In dia-PASEF mode, we quantified over 11,800 protein groups (1% peptide and protein false discovery rate) and 225,000 peptides from just 175 ng on-column injections, with excellent reproducibility (over 93% of proteins had CVs <20%). A comparison of hyperoxia (HO) to room air (RA) controls revealed 708 significantly changed proteins indicating substantial proteome perturbation in BPD. Proteins were significantly downregulated for multiple functional pathways including mitochondrial electron transport chain (ETC) activity and oxidative phosphorylation (OxPhos) (Figure 3 and 4). For example, levels of proteins in Complex I (NADH dehydrogenase), Complex II (Succinate dehydrogenase) were broadly decreased. The coherent downregulation of ETC and OxPhos machinery provides strong evidence that hyperoxia causes substantial mitochondrial functional impairment and potential energy deficits that likely contribute to arrested alveologenesis.

Beyond confirming mitochondrial injury, the in-depth proteome analysis enabled by the timsTOF Ultra revealed additional novel pathways affected by hyperoxia that provide clues into disease mechanisms. For example, For example, marked reductions were observed in central regulators of angiogenesis in HO vs RA. Reduced blood vessel development likely worsens tissue oxygenation. We also found significant changes in complement and coagulation cascades and attendant inflammatory responses reduced in HA vs RA, together promoting tissue inflammation and damage. By significantly expanding lung proteome coverage, the ultrasensitive timsTOF Ultra platform provided unique biological insights beyond prior efforts. The depth of analysis details specific protein-level changes that may causatively link to functional deficits underlying arrested alveolar formation in this neonatal hyperoxia BPD model. Our team is further investigating key pathways as potential therapeutic targets or biomarkers.

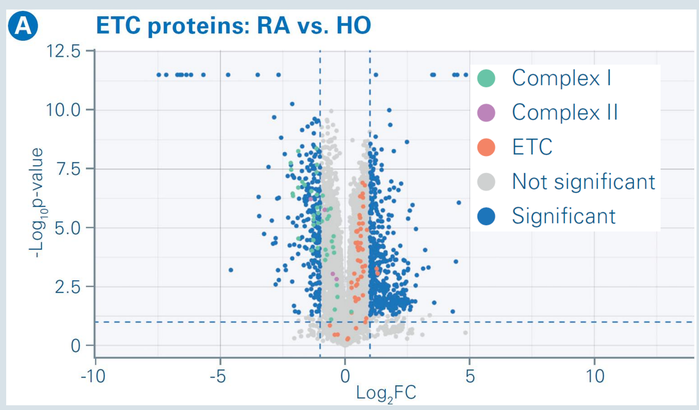

Figure 3: Volcano plot showing broad downregulation of mitochondrial ETC and OxPhos proteins

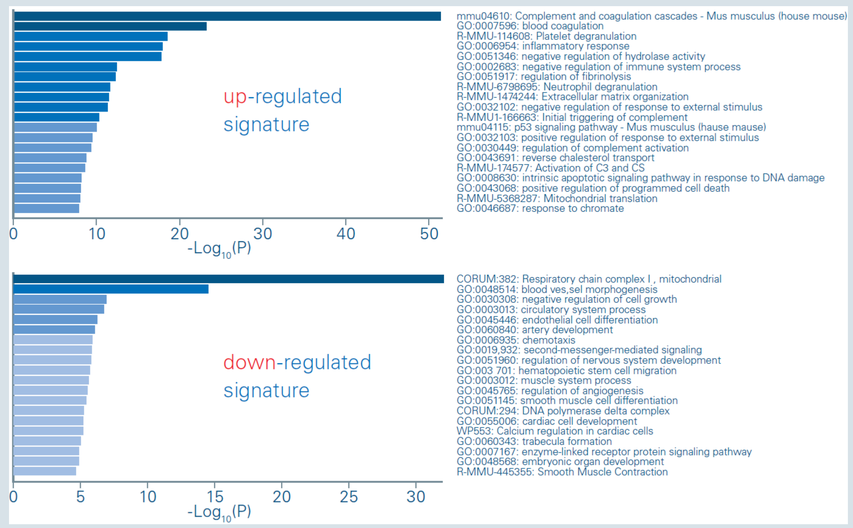

Figure 4: Enrichment pathways. Proteins were significantly downregulated for multiple functional pathways including mitochondrial electron transport chain (ETC) activity and oxidative phosphorylation (OxPhos).

Conclusions

The timsTOF Ultra proteomics system enabled full-proteome characterization of a complex disease model, capturing the breadth of pathway dysregulation. The complete hyperoxia-induced proteome signature provides an important knowledge base of disease mechanisms that will accelerate development of targeted interventions for this chronic, debilitating condition affecting vulnerable premature infants.

timsTOF Ultra