**Transcriptome profiling of** **hyperoxia-exposed preterm rabbit model of bronchopulmonary dysplasia (BPD)**

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**Background:**

The present PhD project is focused on the expression profiling characterization of the hyperoxia-exposed preterm rabbit model of BPD recently set-up and validated in Chiesi Farmaceutici laboratories for supporting a new line dedicated to bronchopulmonary dysplasia (BPD) drugs R&D.

BPD is the most common chronic lung disease in preterm neonates, with an incidence of 5–68% inversely related to the gestational age (1). BPD phenotype is characterized by an arrested of lung and microvascular development and inflammation (2). However, further studies are needed to fully understand the molecular mechanisms behind the lung development arrest. For this reason, BPD animal models are key tools to investigate how these mechanisms are impaired during the disease and to perform preclinical development of new pharmacological treatment. In this scenario, the hyperoxia-induced preterm rabbit BPD model recently validated in Chiesi laboratories could represent an ideal preclinical tool for mimicking at lab-scale level the clinical BPD phenotype (4).

The aim of the study is the identification of the main gene/protein-sets involved in the development of the BPD phenotype. based on a biomolecular and histopathological characterization of the preterm rabbit model at multiple time points.

**Objectives:**

The aim of the PhD project is the molecular characterization of the preterm rabbit BPD model, focusing on the identification, via transcriptome profiling, of genes and pathways dysregulated during hyperoxia exposure. The study includes a full characterization of multiple time points during the whole experiment. The transcriptomics data will be a unique resource for identifying a dedicated PCR panel to be used for monitoring the expression levels of key BPD genes.

The research activities of this first year of PhD were focused on the collection and processing of the different biological samples and on the preliminary analysis of mRNA transcriptomic results.

In vivo activities: Preterm rabbits were delivered at the 28th day of gestational age through a C-section and randomized at birth in two groups (normoxia (21% O2) or hyperoxia (95% O2)) and maintained for 7 days in ad-hoc customized incubators. For both group, lungs were harvested at different time-points: at birth (one hour after C-section), at day 3, 5 and 7. The whole lungs were removed, washed in saline solution, and weighed. After this step, right lungs were separated and stored for gene expression analysis, while left lungs were collected for histology and undergone a fixation process (4 ml 10% formalin buffer).

RNA analysis: Right lungs were collected in RNAlater™ for total mRNA and miRNA transcriptomic analysis. Besides, tissue samples are homogenized in QIAzol® Lysis Reagent. Subsequently, phenol/chloroform RNA extraction was performed using QIAGEN spin-column kits. After checking quality and quantity of the extracted RNA, a transcriptome analysis was conducted through RNA sequencing, using an Illumina NexSeq500 platform (analysis performed by an external laboratory).

Tissue analysis: Histological analysis will be performed on the left lungs, conserved in formalin solution, by an external laboratory.

**Results:**

Optimized rabbit RNA extraction protocol permitted to obtain high RNA concentration avoiding protein and phenol contamination. Denaturating agarose gel electrophoresis indicated high RNA integrity suitable for high-throughput RNA-seq.

Principal Component analysis showed different clusters, representing the 4 time-points considered. Gene analysis highlighted that more than 1000 genes are dysregulated at day 0 after 1 hour in hyperoxia compared to normoxia group, indicating that the first hour in hyperoxia significantly impacts on genes expression. Although at day 3 there are no significant differences between normoxia and hyperoxia group, at day 5 the amount of dysregulated genes significantly increased reaching at day 7 more than 2000 dysregulated genes. In addition, the results showed that the genes and pathways deregulated during the day 0 are different to those dysregulated at day 5 and day 7, underlining different involved processes. Comparing the preliminary results on hyperoxia model with the expression profiling of the normal lung development, using heatmap analysis, highlighted that hyperoxia exposure significantly impairs lung and vascular development.

A comparative analysis between our data and the literature-available transcriptome data on BPD is currently ongoing, permitting to evaluate the translational potential of the hyperoxia-exposed preterm rabbit model for the BPD disease. Moreover, it is important for PCR array setting-up and validation.

In parallel, an external group is analyzing the lung histological samples evaluating several parameters to measure the progressive BPD development.

**Conclusions and next steps:**

Molecular characterization of hyperoxia-exposed preterm rabbit BPD model is an important starting point to identify dysregulated genes and pathways during the pathology development. In this first year of PhD program, RNA seq analysis on preterm rabbits exposed either hyperoxia or normoxia at different time points was performed. Transcriptomic preliminary results demonstrated that hyperoxia leads to dysregulated genes compared to normoxia since day 5. These results are in line with historical histological analysis previously performed on this model, characterized by an arrest of lung development after five days in hyperoxia condition. Unexpectedly,more than 1000 genes are already dysregulated after just 1-hour exposure to high oxygen levels. In order to obtain a more comprehensive transcriptomic time course we foresee to add samples at days 1, 2 and 4.

These are of course still preliminary results and further investigations are required. In the next two years, a complete analysis on the BPD transcriptome results will be finalized. miRNA sequencing will be performed to identify hyperoxia-induced miRNAs that could influence gene expression. In addition, a PCR assay will be set-up and validated. Moreover, BPD transcriptomic results will be compared to lung development transcriptomic analysis in order to distinguish between physiological developmental transitions and hyperoxia-induced molecular changes.

Finally, additional samples will be collected for proteomic analysis that will be performed by the Chiesi Corporate R&D Preclinical Analytic & Formulation group. The transcriptome results will be then correlated with histological and proteomic analysis to complete the biomolecular and histopathological characterization.

In conclusion, this PhD study will ideally bring us to identify novel biomarkers and their modulation in the disease process. This in turn could be then critical to develop new and more targeted therapeutic approaches.

**References:**

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