Single-cell RNA (scRNA) analysis to evaluate the anti-fibrotic effect of steroid in mouse model of biliary atresia

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Purpose: Biliary Atresia (BA) is a rare neonatal disorder characterized by jaundice and progressive liver fibrosis. The objective of this study is to explore the mechanism of steroid in reversing liver fibrosis in BA mouse model using organoid and scRNA technologies.

Methods: Mouse models of BA were created by inoculating post-natal day 1 mice with Rhesus Rotavirus (RRV). BA symptoms including cholestatic jaundice appeared around day 7 after RRV injection. Steroid or PBS solution was injected to these mice from day 21 to 28/34. Serums were collected to check liver function indexes. Liver samples were collected and CK19 expression was tested using IHC staining. Hepatic necrosis was evaluated through HE staining and fibrosis was analyzed by Sirius Red staining. Liver organoids and single cells were developed from selected mice and were subjected to Bulk RNA and scRNA sequencing.

Results: Mice developed BA features after RRV injection were sacrificed on day 21, 28 and 34. The mice that received steroid were found have a better growth rate, serum liver function and less liver fibrosis/necrosis compared with those receiving PBS. The results were consistent for all the mice that were sacrificed on day 28/34. We then performed analysis on the liver organoids to determine the expression differences between the steroid and PBS treated mice. Bulk RNA sequencing revealed that there were 6359 differentially expressed genes (DEGs) between the two groups that were sacrificed on day 34, and those genes targeted to metabolism pathway. Hub genes and the corresponding transcription factors were predicted in most significant lipid metabolism process. UMAP analysis revealed significant difference in the cell proportion (macrophage, B cell, endothelial and epithelial) between steroid and PBS treated mice that were sacrificed on day 28 and 34. Specifically, further analysis on the macrophage found that the expression of M1 macrophage decreased in steroid group compared to PBS group. Furthermore, it was found out that while the expression of M2 macrophage showed an opposite trend, i.e. steroid group has a higher expression than PBS group, only on 28 days' samples but not on 34 days' samples.

Conclusions: Organoid and scRNA analysis on BA mouse models indicated that steroid mitigated liver fibrosis through lipid metabolism pathway. It altered the expression of macrophages and other immune cells.