Introduction

Focused ultrasound (FUS) blood-brain barrier (BBB) opening transiently and non-invasively opens the BBB. The safety of this method has been established in mice, non-human primates and is currently undergoing clinical trials.

Efficacy and safety studies of FUS-BBB opening demonstrate immunotherapy effects, including pathology reduction and behavior improvement in transgenic Alzheimer's mice. These effects are hypothesized to be due to the stimulation of microglia, the brain's resident phagocyte. Preliminary studies support this hypothesis by demonstrating microglia morphological changes and alterations in the expression of associated chemokine and cytokines after treatment. This study utilizes single-cell RNA sequencing to understand microglia phenotypic changes after FUS-BBB opening.

Methods

Wild-type mice were split into three treatment groups - FUS-BBB opening (FUS/MB), sham and naive, with two sacrifice times - 24 and 72h post-treatment. At time of sacrifice, mice were perfused and the targeted area was extracted, homogenized and stained with both genetic and fluorescent antibodies prior to being combined for flow cytometry selection of the viable microglia. Cells underwent 10x microfluidic cell processing, library preparation, and Illumina Nextseq sequencing. Three 10x runs were performed to account for both technical and biological variation. Alignment was performed in CellRanger and integration and processing were performed on R with the Seurat and Bioconductor packages.

Results

Principal component analysis clustering assigns cells into three clusters - one homeostatic and two perturbed. The homeostatic cluster is dominated by cells from sham and naive mice. The perturbed clusters represent cells from 24 and 72h FUS/MB groups, respectively.

Cell cycle analysis reveals that the 24h perturbed cluster is primarily cells in the synthesis phase whereas the 72h perturbed cluster is primarily cells in the mitosis phase. Trajectory analysis connects the two - finding a distinct trajectory from the homeostatic through the cells undergoing synthesis and ending with the cells undergoing mitosis.

Discussion & Conclusion

This data suggests that microglia proliferation is a potential mechanism behind the previously demonstrated immunotherapy effects of FUS-BBB opening. Future work will include spatial localization of phenotypic changes and validation via immunohistochemistry.