Single Cell Gene Expression Analysis in Mouse Embryonic Brains through Microfluidics and RNA Sequencing

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INTRODUCTION

Prenatal exposure to toxic environmental stressors can be detrimental to proper neural development resulting in profound neurodevelopmental deficits. Several substances have been found to play significant roles in proper neurodevelopment. Heat Shock Proteins (HSPs) are major molecular chaperons involved in primary defense against cell damage. Prenatal overexposure of alcohol results in neurodevelopmental disorders. In addition, man-made chemicals used for industrial and commercial purposes have the potential to increase the chance of exposure to unknown and harmful environmental factors (EFs) such as heavy metals.

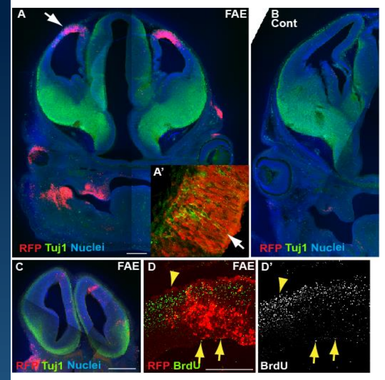

Figure 1: HS response to ETOH

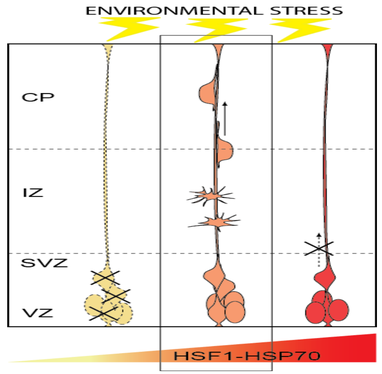

Figure 2: Levels of HS signaling

RESEARCH QUESTION

The proposed study aims to identify specific molecular pathways that cause prenatal neurodevelopmental disorders by testing cell-to-cell variability in response to environmental factors (EFs), and to see if various EFs have common target molecular pathways.

METHOD

To test the hypothesis, we use mouse neural progenitor cells (NPCs) dissociated from embryonic brains exposed to EFs such as alcohol and methylmercury. Through collaboration between Dr. Zhenyu Li and Dr. Kazue Hashimoto-Torii laboratories, a combination of microfluidics-based single-cell optics/separation as well as single cell RNA sequencing will be used to draw conclusive gene expression data. Downstream bioinformatics analysis will be performed to reveal functional links among coordinately fluctuating genes among single cells, and to identify key genes/pathways that show variability in response to EFs. Enrichment of specific biological functions and signaling in the list of the genes with increased cell-to-cell variability will also be tested using gene ontology (GO) analysis.

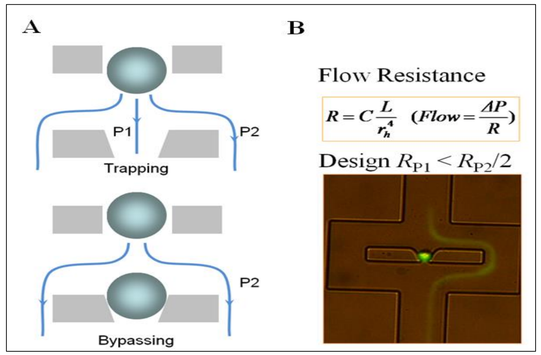

Figure 3: Microfluidics trapping mechanism

APRROACH

In order to begin these studies, the controller for the microfluidics chip must be built and integrated with a computer through a graphical user interface. The microfluidics chip must also be designed with the requirements and specifications in mind for this particular study. Not only must the cells be trapped, but they must be moved into separate chambers where different stressors and buffers can be applied to the cell and then analyzed.

DESIGN

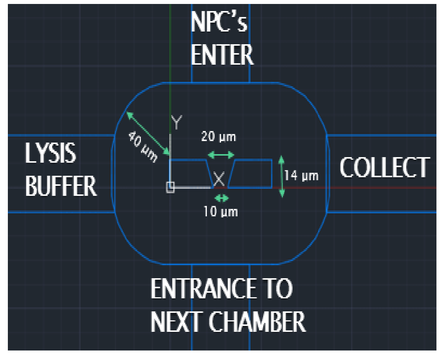

Figure 4: AutoCAD Trap Design

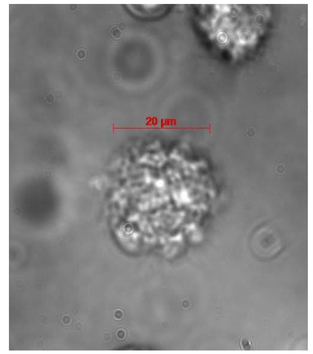

Figure 5: NPC cell dimension

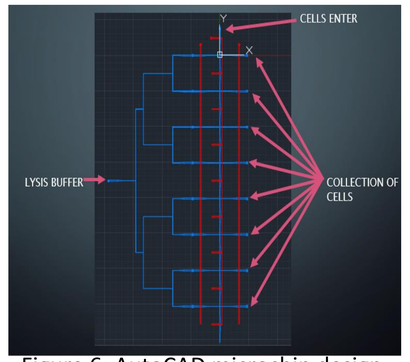

Figure 6: AutoCAD microchip design

ANTICIPATED RESULTS

The results of this study may provide crucial information leading to the discovery of target molecular pathways that cause these environmentally-induced neurodevelopmental disorders. The results can therefore help create new strategies for controlling cellular response against EFs. Each mechanism that can be decoded is a step forward in solving the puzzle behind neurodevelopmental disorders caused by EFs; identifying and characterizing target molecular pathways can lead to eventually preventing these disorders.