Characterization of human brain organoids with single-cell spatial resolution and molecular specificity

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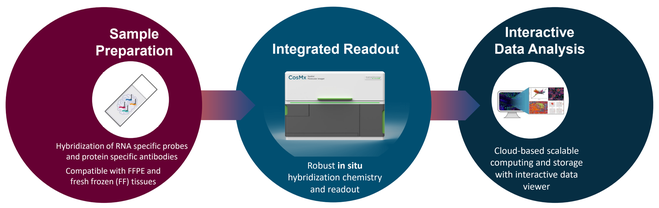
Abstract

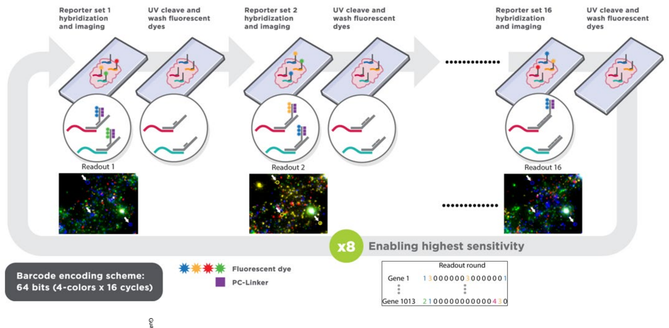
Microglia, the major tissue-resident macrophage population in the parenchyma of the central nervous system, play key roles in brain development, homeostasis, and neurological disorders. Under homeostatic conditions, microglia constantly survey their surrounding environment and remove damaged cells by phagocytosis. Hence, dysregulation of microglia functions causes neurodevelopmental and neurodegenerative diseases. A better understanding of microglial function and molecular crosstalk with brain cells is key to find new targets for disease treatment. Recent advanced stem cell research has made possible to generate from human induced pluripotent stem cells (iPSC) three-dimensional (3D) brain organoids with diverse neural cells and functions, recapitulating embryonic human brain development. To gain a deeper understanding of microglia biology in normal and disease contests, we aim to investigate the spatial location of microglia and their interaction with neuron precursor cell (NPC) and neurons at the single-cell level in iPSC derived brain organoids. Here, we first generated microglia-like cells (hiMicro) from iPSC-derived human primitive macrophages (hiMac) by co-culture them with brain organoids. Next, we implemented the CosMx™ spatial molecular imager (SMI, NanoString Technologies) to map the identity and status of IPSC-differentiated cell types in brain organoids. The CosMx SMI system features high-plex, high-throughput and high resolution, therefore conducive to assess the distribution, interaction and function of hiMicro. In particular, the CosMx SMI system enabled us to categorize different subgroups of hiMicro based on their unique spatial locations and transcriptional status. For instance, we profiled differential gene expression of hiMicro at anatomically distinct layers within brain organoids. Moreover, we observed a significant shift in the transcriptome of hiMicro when in the proximity of different cell types (e.g., NPC). Taken together, these pioneering efforts and preliminary findings revealed by single-cell spatial transcriptomic profiling laid the foundation for systematic investigation of microglia biology under various circumstances, as well as paved the way for studying neurobiology with single-molecule and nanometer resolutions.

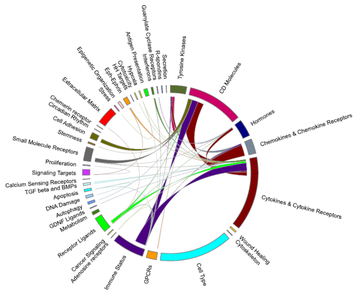
Introduction

CosMx™ SMI assay overview

CosMx™ SMI is a single instrument solution for subcellular spatial analysis







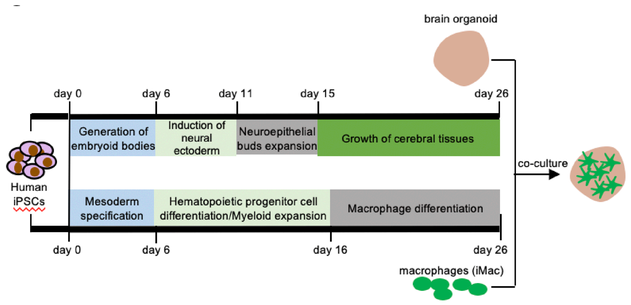
1000-Plex RNA Panel for Cell Typing and Cell-Cell Interaction Studies

Applications	# of genes
Cell typing and mapping	243 genes
Cell state and function	269 genes
Cell-cell interaction (ligand-receptor)	435 genes
Hormone activities	46 genes

Custom panel spike-in

2 custom genes for marking NPC

Generation of hiMicro and Brain Organoids



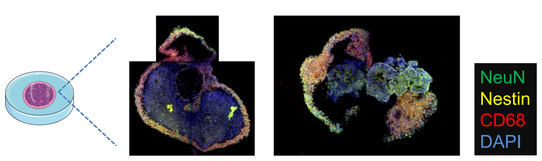
Study design

Goals

- Spatial atlas of hiMicro in iPSC-derived brain organoids
  - Map subpopulations of hiMicro in spatial context
    - Determine abundance and distribution of subpopulations in brain organoids
- Spatial atlas of NPC and neurons in brain organoids
  - Characterize gene expression of hiMicro in the proximity of different cell types

Samples

- FFPE tissue blocks of brain organoids

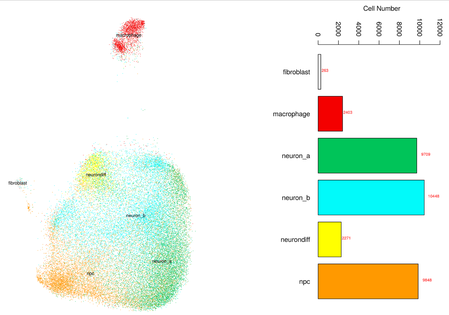


CosMx SMI reagents

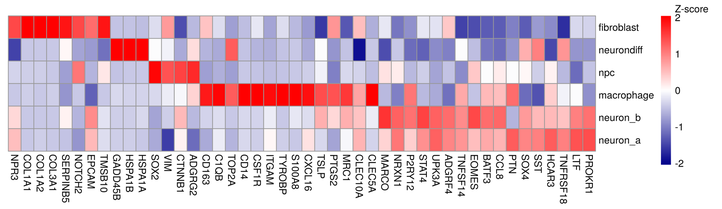
- RNA 1K-plex panel + custom panel of 2 genes [PTN, TMSB10]
- Morphology markers: GFAP, MAP2, NeuN, CD68, DAPI

CosMx™ SMI profiling of brain organoids

Unsupervised cell clustering



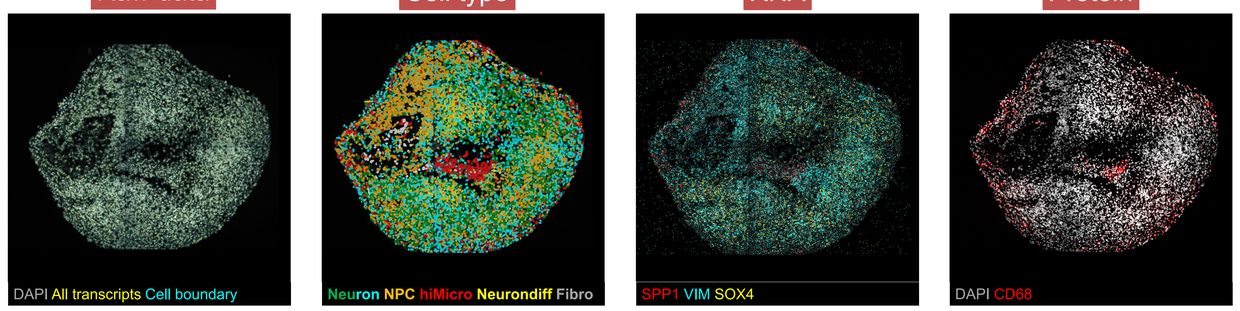
Marker gene heatmap



Summary

- In total about 35,000 cells from 4 brain organoid slices were profiled with the CosMx 1000-plex RNA panel
- Unsupervised cell clustering successfully detects all expected cell types: neuron, NPC, macrophage (hiMicro)
- Small group of neuron expressing GADD45B, HSPA1A and HSPA1B
- A few hundred fibroblast-like cells were annotated, suggesting the further development from mesoderm

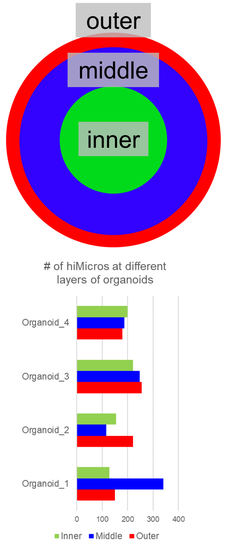
Visualization of CosMx™ data in space

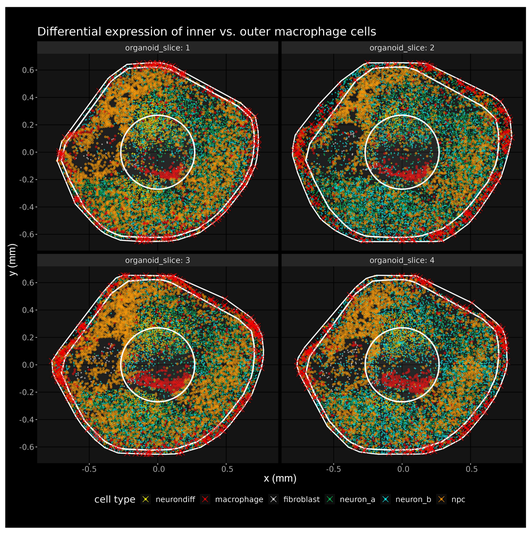


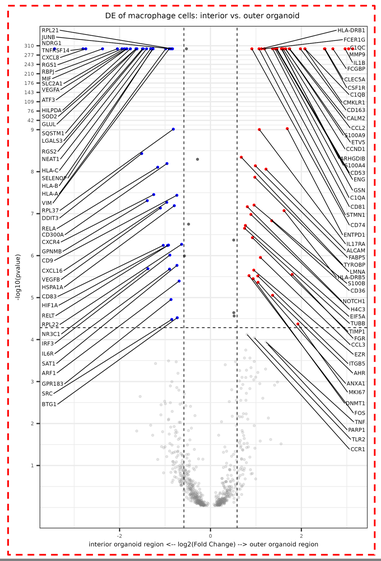
Summary

- The CosMx dataset is multidimensional, spanning over RNA, protein, cell type and all relevant spatial information
- The multilevel data structure obtained by CosMx is closely interconnected and enables in-depth studies of fundamental biology
- The unique distribution pattern for hiMicro in brain organoid was successfully revealed by CosMx profiling
- The standard spatial data format from CosMx supports systematic and customizable downstream analysis

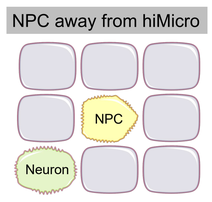
Spatial differential expression analysis of hiMicro

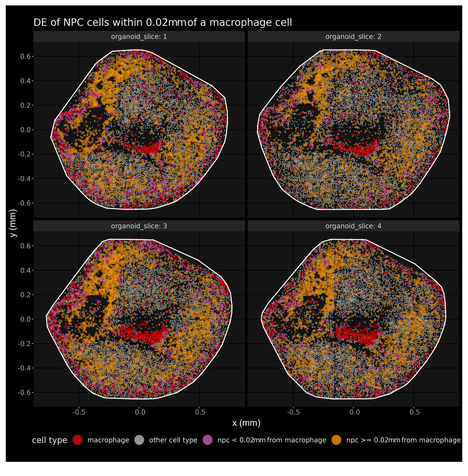


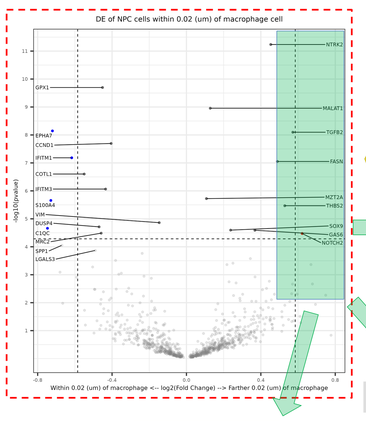


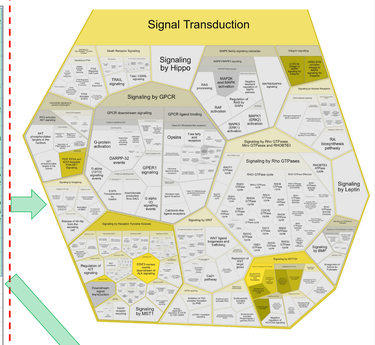


Differential expression analysis for NPC in proximity of hiMicro

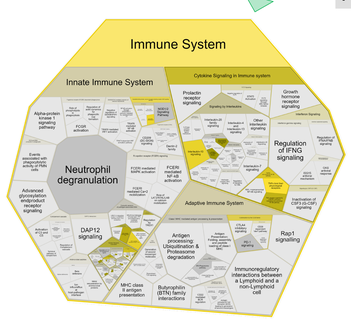


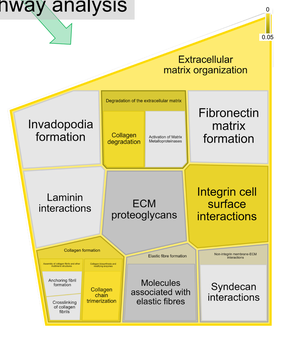






Pathway analysis

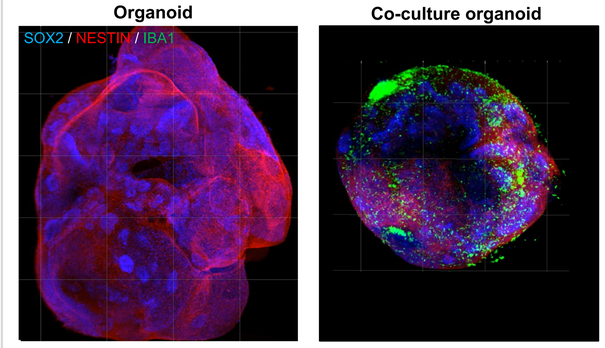




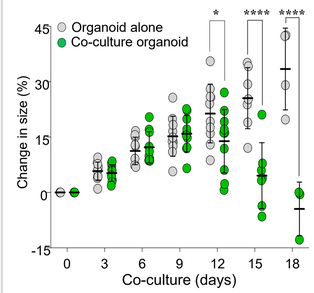
Summary

- Both hiMicro and NPC show highly location-specific gene expression profiles in brain organoids
- Spatial distancing from hiMicro up-regulates the expression of several key proliferation-related genes, including NTRK2, TGFB2 and NOTCH2, in NPC
- Pathway analysis suggests that hiMicro primarily impacts “Signal Transduction”, “Immune System” and “Extracellular Matrix Organization” of the NPC and brain organoids

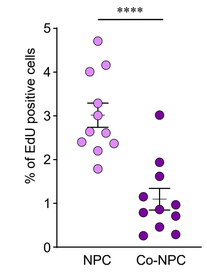
Comparison of brain organoid growth w/ or w/o hiMicro



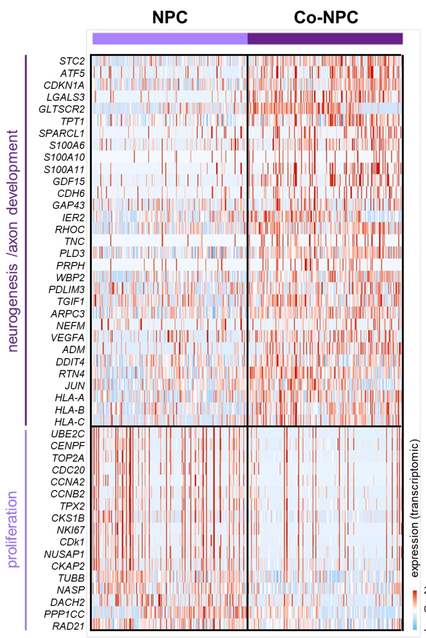
Representative 3D images of brain organoids cultured in the absence and presence of iMac for 18 days



Changes in organoid size was measured over time in the absence and presence of hiMicro



Proliferative activity for NPC cultured in organoids with or without hiMicro was evaluated with EdU incorporation assays



Heatmap showing the genes involved in neurogenesis and axon development whose expression is up-regulated in co-NPC; and those involved in proliferation, whose expression is down-regulated in co-NPC compared to NPC

Summary

- Co-culture with hiMicro reduces the size of brain organoids in growth
- The modulation of NPC differentiation by hiMicro is translated by limiting proliferation while promoting axonogenesis

Conclusions

- The CosMx spatial molecular imaging platform enables subcellular localization and quantification of 1000-plex genes in FFPE human brain organoids
- The multimodal profiling approach by CosMx helps identify and visualize key transcripts and cell types involved in the development of iPSC-induced brain organoids
- By spatial mapping, the location-dependent gene expression patterns in hiMicro were revealed while a hiMicro-NPC interaction may cause cell fate change
- DEG analysis showed that proliferation-related genes in NPC near from hiMicro are down-regulated
- The presence of hiMicro cause the reduction in the size of brain organoids
- Less expression of proliferation-related genes in co-NPC was validated by single-cell RNA-seq and less proliferation of co-NPC was confirmed by EdU incorporation assays
- The CosMx spatial RNA-seq technology is an unique tool for understanding microglial biology in co-cultured brain organoid systems

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