Exploring early neuronal interactions in the developing human intestine using iPSC-derived organoids: implications in Hirschsprung's disease

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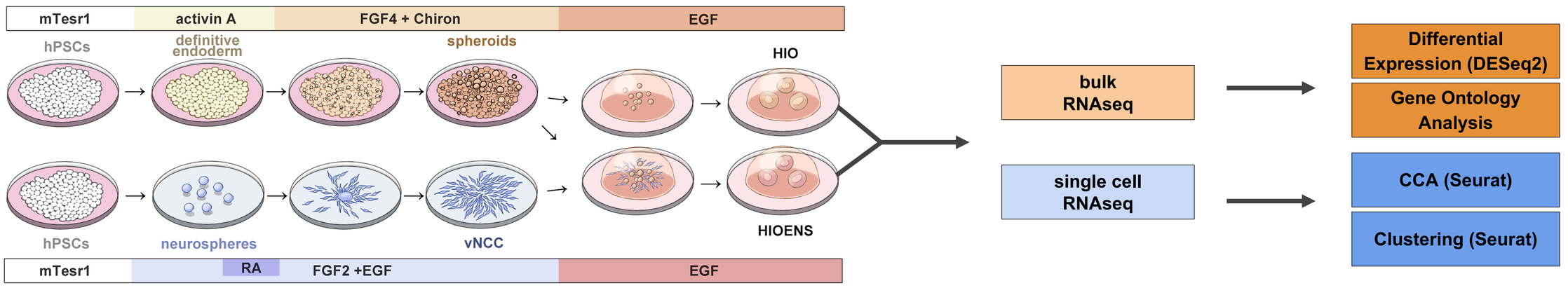
Introduction – The Enteric Nervous System (ENS) and intestinal development

The enteric nervous system (ENS) is one of the key regulators of the intestinal cellular microenvironment. However, its role in the maturation of the developing digestive tract remains poorly understood. ENS progenitor cells (vagal neural crest cells or vNCCs), intestinal endoderm and mesoderm co-develop during gut formation. **In this context, we hypothesized that the ENS contributes to gut development by influencing the patterning of mesodermal cells.** We further questionned whether ENS-mediated patterning would be affected during Hirschsprung's disease, a congenital gut disorder where the ENS is absent.

Methods – Using organoids to understand human intestinal development

Human intestinal organoids (HIOs) are human intestinal tissue produced *in vitro* from directed differentiation of human pluripotent stem cells (hPSCs). The HIO model can also include hPSC-derived enteric neuro-glial cells to obtain innervated intestinal organoids (HIOENS). To address our hypothesis, we used HIOs with and without an ENS. After generating HIO and HIOENS, we performed bulk RNA and single-cell RNA sequencing to identify differential expression and cell types that are conserved or different across our conditions.



Results 1 – ENS-progenitor cells patterned intestinal organoids

1.

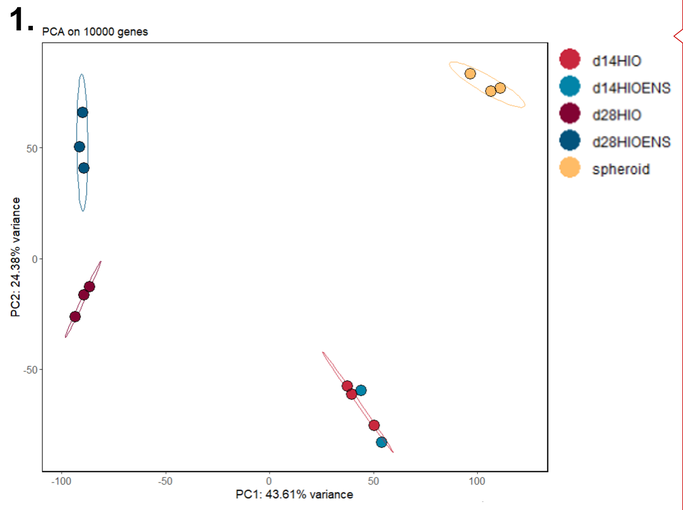
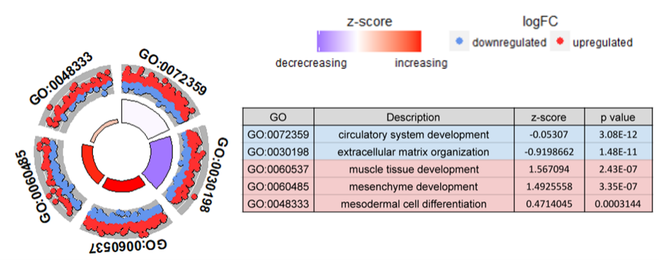


Fig 1. : Principal Component Analysis (PCA) on RNAseq data of spheroids and HIOs with and without an ENS at 14 and 28 days of *in vitro* culture.

Genes that were differentially expressed (log2FC > 1.5; p value < 0.05) between HIO and HIOENS at 28 days of culture were subsequently extracted and used for gene ontology analysis (using https://toppgene.cchmc.org/ and R package GOplot)

2.



GO	Description	z-score	p value
GO:0072359	circulatory system development	-0.05307	3.08E-12
GO:0030198	extracellular matrix organization	-0.9198662	1.48E-11
GO:0060537	muscle tissue development	1.567094	2.43E-07
GO:0060485	mesenchyme development	1.4925558	3.35E-07
GO:0048333	mesodermal cell differentiation	0.4714045	0.0003144

Fig 2. : Gene Ontology analysis on genes that are differentially expressed between HIO and HIOENS at 28 days. Z-score represents association with HIOENS. Ontologies with the highest z-score and lowest p-value were linked to neurogenesis or similar processes. Other relevant ontologies are represented on Fig.2 and include mesoderm and muscle development

3.

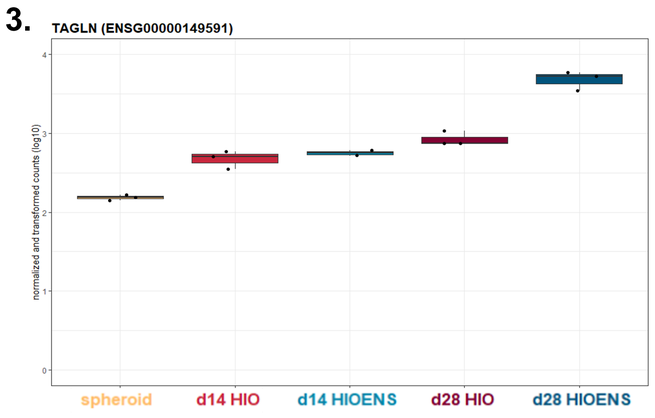


Fig 3. : Box plot of TAGLN expression (normalized and transformed read count, log10 scale)
TAGLN (sm22) is an early marker of visceral and vascular smooth muscle. TAGLN expression was upregulated in HIOENS suggesting that the ENS further muscle and vascular development.

Results 2 – ENS cells promoted mesoderm-derived cell fate

4.

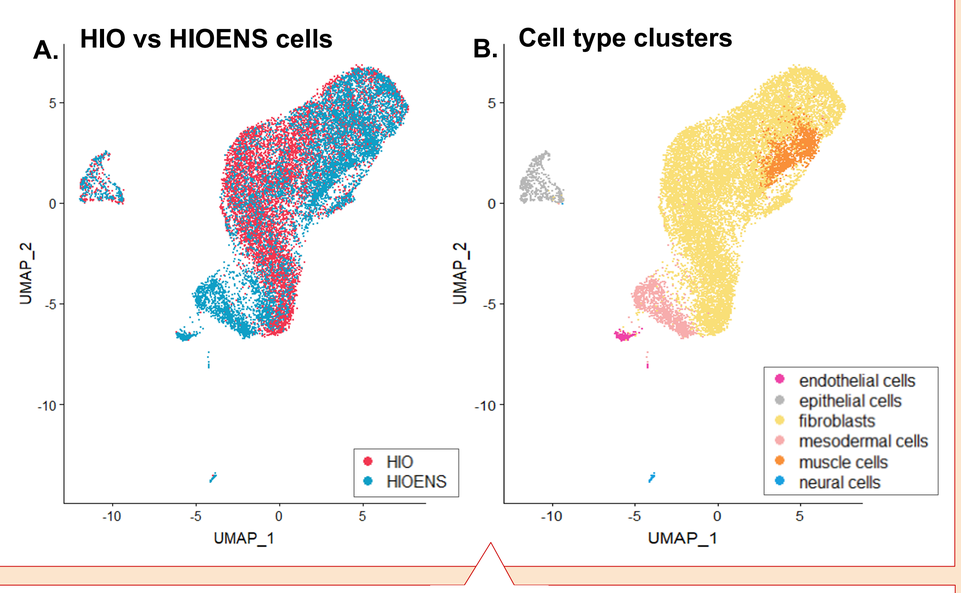


Fig 4.A-B. : UMAP of scRNAseq data of HIO and HIOENS at 28 days of culture. HIO and HIOENS data were integrated with R package Seurat (using a Canonical Correlation Analysis) and manual annotation of clusters identified by Seurat. Clusters of mesodermal, vascular and fibroblast-like cells were upregulated in HIOENS.

Results 3 – Organoid model to study Hirschsprung's disease (HSCR)

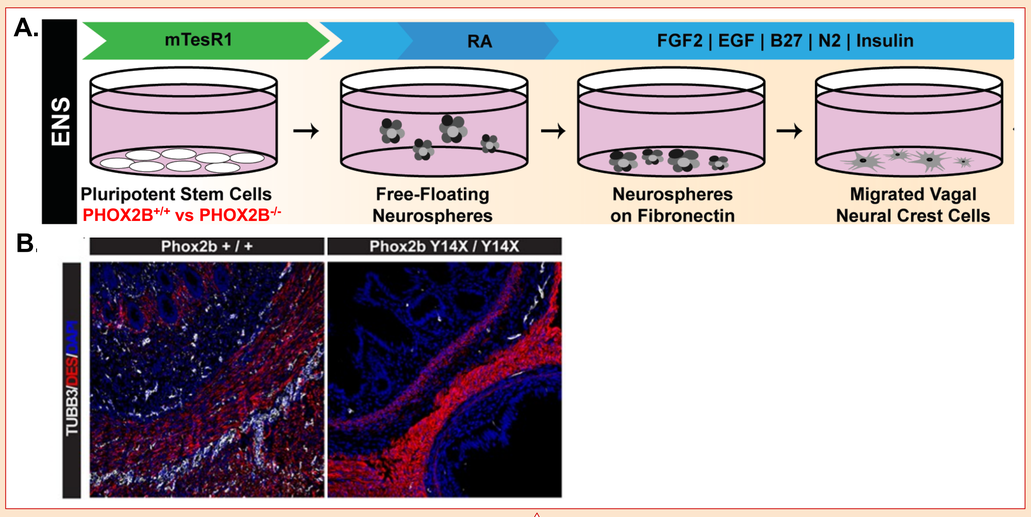


Fig 5.A-B : HIOENS were generated *in vitro* from human pluripotent stem cells bearing a PHOX2B mutation. This mutation is associated to severe HSCR phenotype. HIOENS presented altered ENS features compared to control, reminiscent of HSCR .

Conclusion

These results strongly suggest that ENS progenitor cells impact the development and patterning of intestinal mesoderm-derived cells. This needs to be confirmed by further experimentation to decipher the cell fate transitions and the mechanisms involved in the gut mesoderm patterning using advanced single cell modalities. In addition, we will pursue this line of work to address the role of the ENS on mesoderm-related tissue in Hirschsprung's disease (HSCR). This work may highlight additional pathways that are dysregulated during HSCR.

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