A cerebral organoid model of progranulin-associated frontotemporal dementia

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Background

Heterozygous mutations in the progranulin gene (*GRN*) are the cause of 5-10% of frontotemporal dementia (FTD) cases, with patients consistently showing TDP-43 pathology at post-mortem. There is evidence to suggest that progranulin (PGRN) plays an important role in lysosomal function. PGRN is localised to the lysosome and has been shown to regulate lysosomal enzyme activity. Additionally, homozygous *GRN* mutations lead to neuronal ceroid lipofuscinosis (NCL), a group of lysosomal storage disorders, further highlighting PGRN's role in lysosomal function. PGRN is expressed throughout the brain with highest expression seen in microglia. Recent evidence suggests lysosomal function is particularly impaired in PGRN deficient microglia despite TDP-43 pathology being predominantly localised to neurons, therefore further studies looking at the interactions between microglia and neurons are essential to assess the potential non-cell autonomous mechanisms at play. The main aims of this study were to 1). establish cerebral organoid model of PGRN associated FTD and 2). establish a cerebral organoid – microglia co-culture model of PGRN associated FTD.

Methods

For this study, iPSC lines from the Kolf 2.1J background were used and included the wildtype (WT) line, R493X heterozygous and homozygous mutation. These were obtained from the human iPSC Neurodegenerative Disease (iNDI) Initiative (1).

Cerebral organoids (COs) were differentiated using the STEMdiff™ Cerebral Organoid Kit (StemCell Technologies, #08570) and following the well-established protocol from Lancaster et al. (2014), (2).

IPSC derived microglial (iMG) were differentiated using a protocol as previously described in Garcia-Reitboeck et al. (2018) and Xiang et al. (2018), (3,4). In brief, embryoid bodies (EBs) were differentiated in a 96-well ultra-low attachment plate using StemFlex media with 50ng/ml BMP4, 50ng/ml VEGF and 20ng/ml SCF and seeded at 10,000 cells per well. After 4 days, EBs were transferred to T75 flasks and kept in X-Vivo 15 media with 100ng/ml MCSF and 25ng/ml IL3. IMGs were harvested and plated into 6-well plates and fed on DMEM F-12 media with 100ng/ml IL34, 25ng/ml MCSF and 5ng/ml TGFb1 for 2 weeks of maturation.

For the **CO-iMG co-cultures,** WT iMGs were seeded onto COs at three densities of $1x10^5$, $2.5x10^5$ and $5x10^5$ (total cells) per organoid. This was achieved by adding the iMGs cells directly to the 6-well plate that the organoids were cultured in and then left off the shaker for 6 hours. Co-cultures were then harvested at Day 7, Day 14 and Day 21 after initial seeding for characterisation.

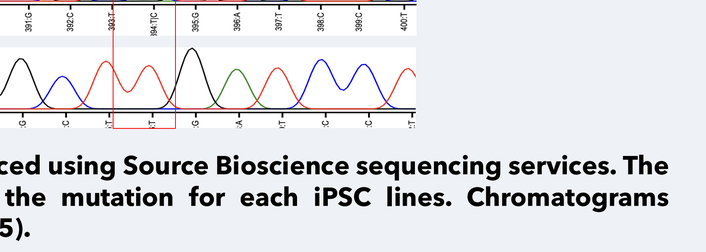


Figure 1. Images of neuroepithelial loop formation in cerebral organoids 12 days in vitro (DIV) (left) and iPSC derived microglia (right).

Results

All iPSC lines were sequenced to confirm the absence, in the case of the Kolf 2.1J WT line, or presence of the *GRN* mutation in the *R493X* heterozygous and homozygous lines. See *Figure 2* below.

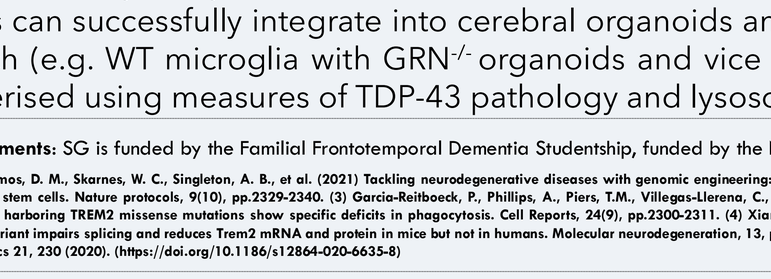


Figure 2. iPSC lines were sequenced using Source Bioscience sequencing services. The red box highlights the site of the mutation for each iPSC lines. Chromatograms produced using Gear Genomics (5).

Cerebral Organoid Model

We successfully established and characterised a cerebral organoid model of PGRN-associated FTD using immunocytochemistry to assess neuronal differentiation, (see *Figure 3).*

Results *continued*

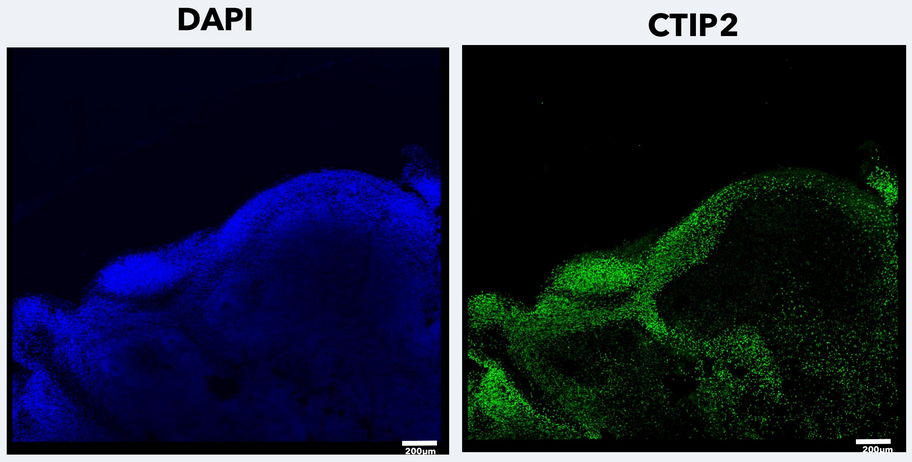


Figure 3. To confirm successful neuronal differentiation imm-unocytochemistry was performed on organoid sections at 80 DIV. DAPI (blue), CTIP2 (green) was used as a marker of deep layer neurons. Scale bar show 200µm.

IPSC derived microglia

As seen in *Figure 4* below, we characterised our iMGs using ICC, staining for Iba1 and TREM2.

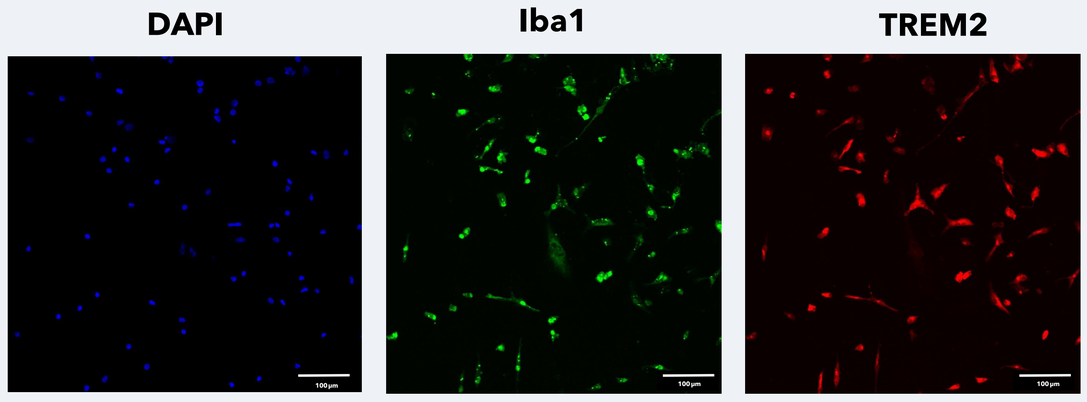


Figure 4. Characterisation of iPSC derived microglia (iMGs) using immunocytochemistry. IMGs were stained with DAPI (blue), IBA1 (green) and TREM2 (red) to confirm microglial like identity. Scale bar show 100µm.

Cerebral Organoid – Microglia Co-Culture

As seen in *Figure 5* below, after staining for Iba1 and MAP2 we saw successful integration of these iMGs into the organoids however further work is required to quantify and assess further how successfully they integrate.

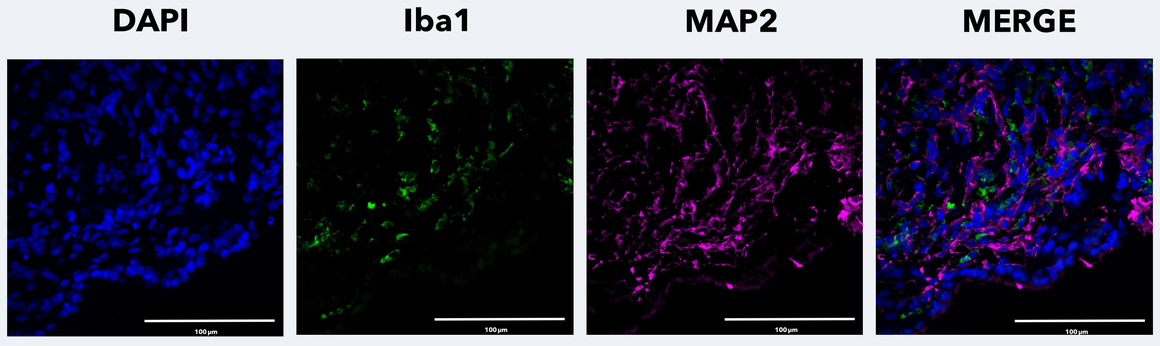
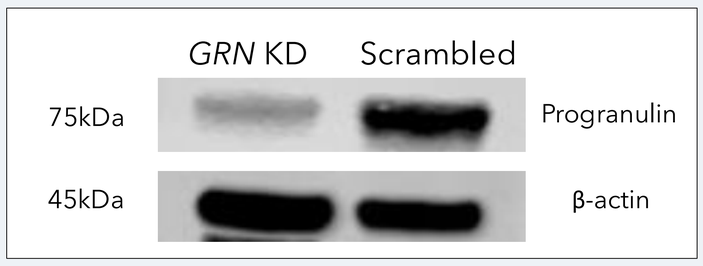


Figure 5. To assess whether iMGs successfully integrated into from iPSCs to neural tissue, immunocytochemistry was performed on co-culture sections 7 days after adding $1x10^5$ iMGs per CO to the media. DAPI (blue), Iba1 (green) was used as a marker of microglia and MAP2 (far-red) as a neuronal marker. Scale bar shows 100µm.



Finally, we have started progranulin antibody validation using siRNA knockdown in iMGs. (See *Figure 6*).

Figure 6. Progranulin antibody validation for PGRN Sigma Anti-Granulin (SAB4200310) used at 1:1000. This blot shows iPSC derived microglia samples after GRN siRNA knockdown (left) compared with scrambled (SCR) siRNA control (right). β-actin was used as a loading control.

Conclusion

In this preliminary study, we established and characterised a cerebral organoid model of progranulin associated FTD. As part of our second aim, we saw that microglia like cells can successfully integrate into cerebral organoids and this will provide the basis of our work going forward. For the next steps, we plan to do a mix-matched approach (e.g. WT microglia with $GRN^{-/-}$ organoids and vice versa) to assess potential non-cell autonomous mechanisms underlying fFTD pathology. Pathology will characterised using measures of TDP-43 pathology and lysosomal dysfunction.

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References: (1) Ramos, D. M., Skarnes, W. C., Singleton, A. B., et al. (2021) Tackling neurodegenerative diseases with genomic engineering: A new stem cell initiative from the NIH. Neuron, 109, 1080– 1083 (2) Lancaster, M.A. and Knoblich, J.A., 2014. Generation of cerebral organoids from human pluripotent stem cells. Nature protocols, 9(10), pp.2329-2340. (3) Garcia-Reitboeck, P., Phillips, A., Piers, T.M., Villegas-Llerena, C., Butler, M., Mallach, A., Rodrigues, C., Arber, C.E., Heslegrave, A., Zetterberg, H. and Neumann, H., 2018. Human induced pluripotent stem cell-derived microglia-like cells harboring TREM2 missense mutations show specific deficits in phagocytosis. Cell Reports, 24(9), pp.2300-2311. (4) Xiang, X., Piers, T.M., Wefers, B., Zhu, K., Mallach, A., Brunner, B., Kleinberger, G., Song, W., Colonna, M., Herms, J. and Wurst, W., 2018. The Trem2 R47H Alzheimer's risk variant impairs splicing and reduces Trem2 mRNA and protein in mice but not in humans. Molecular neurodegeneration, 13, pp.1-14. (5) Rausch, T., Fritz, M.H., Untergasser, A. and Benes, V. Tracy: basecalling, alignment, assembly and deconvolution of sanger chromatogram trace files. BMC Genomics 21, 230 (2020). (https://doi.org/10.1186/s12864-020-6635-8)