Final Report

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Title of Project: Modelling cortical dysplasia in epilepsy patients

Summary:

This project used stem cells from epilepsy patients with cortical dysplasia to create brain organoids, and investigated whether these brain organoids recapitulated abnormal cortical structures (Aim 1), and whether they demonstrated seizure-like electrical activity (Aim 2).

Aim 1: Demonstrate that 3D brain organoids can capture a cortical dysplasia phenotype

We began with iPSCs from an epilepsy patient clinically diagnosed with cortical dysplasia and who carries a variant in the DCX gene, and planned to use CRISPR-Cas9 technology to genetically correct the mutation. We discovered that the patient's DCX mutation was somatic and thus not present in every cell in their blood. We re-examined all iPSC clones originally generated from the patient using Sanger sequencing, and were able to identify different clones that were positive and negative for the mutation in DCX. This provided us with the isogenic controls we need to best compare effects of a DCX variant, and avoids the confounding effects of comparing to unrelated control iPSC lines.

We generated 3D brain organoids from two epilepsy patients; the DCX variant and a second patient with a variant in the NPRL3 gene. We compared to the DCX isogenic control, as well as two additional unrelated control iPSC lines. We stained for neural progenitor cell markers and for markers of cortical layer identity and quantified numbers of positive cells and numbers of neural rosette centres, as an indicator of structural organisation. We found significant dysregulation of cortical layer organisation in the two cortical dysplasia lines compared to the two unrelated controls and the DCX isogenic line, both in terms of number of cortical layer neurons and number of progenitor zones.

We concluded that organoids derived from these two patients displayed some features of impaired cortical development; indicating that brain organoids as a model can capture at least some elements of a cortical dysplasia phenotype.

Aim 2: Compare neuronal hyperexcitability between cortical dysplasia and controls

Multi-electrode array (MEA) technology enables recording of a neurons' electrical activity *in-vitro*. We set out to determine if brain organoids from cortical dysplasia patients could capture seizure-like activity, and if this activity was amenable to modulation with pro-convulsants or anti-seizure medication (ASMs). We cultured organoids for at least 3 months to ensure neuronal maturity, and up to 6 months of age before adhering them to MEA chips and recording electrical activity.

We identified spontaneous activity (spiking and synchronous network activity) in control and cortical dysplasia organoids and confirmed that we could modulate this activity with 4AP, a pro-convulsant commonly used in epilepsy research. 4AP induced hyperexcitability was observed by an increased frequency of spike and burst activity. This result confirmed that brain organoids display electrical activity that can be modulated by a chemical stimulus and

can display neuronal hyperexcitability. We next compared epilepsy organoids to controls and found distinct increases in firing (spike) rates and burst (5< spikes with maximum inter-spike intervals of 100ms) at 100 to 150 days in culture. Interestingly, the differences appeared to reduce over time; 200 day old organoids no longer displayed clear differences. We hypothesise that this may indicate a strengthening of neuronal connectivity over time in control organoids, while epilepsy organoids may suffer detrimental effects from increased and/or persistent neuronal firing.

Finally, we wished to determine if neuronal hyperexcitability in cortical dysplasia organoids could be modulated by administration of anti-seizure medication (ASMs). Activity was measured before treatment, and organoids were then incubated with the ASMs for 2hrs before activity was measured again. ASM treatment (Lamotrigine and Topiramate) significantly reduced both firing rate and frequency of burst activity. Interestingly, application of Levetiracetam did not have the same effect of reducing neuronal hyperactivity.

Hypothesis vs Findings and Unanswered Questions

Aim 1: Demonstrate that 3D brain organoids can capture a cortical dysplasia phenotype

We hypothesised that brain organoids would be a good model for cortical dysplasia as they can recapitulate aspects of human cortical development, with mature brain organoids most closely resembling a foetal brain. We found that organoids from cortical dysplasia patients indeed showed reduced cortical layer neuron markers and lacked structural organisation of progenitor zones. It remains unclear how DCX and NPRL3 variants contribute to a reduction of progenitor zoning; traditionally DCX is considered to regulate the migration of immature neurons, and is relatively lowly expressed in highly proliferative progenitor cells.

We will next expand our characterisation of these organoids, by in depth quantifications of further markers of progenitor and cortical layer identity, and will also explore the timing of emergence of these populations. In the long term, effects of DCX and/or NPRL3 mutations can be explored in progenitor populations specifically, to determine any effects on proliferation or maturation, both key aspects of cortical development.

Aim 2: Compare neuronal hyperexcitability between cortical dysplasia and controls

We hypothesised that brain organoids from epilepsy patients would display neuronal hyperactivity on an MEA platform when compared to controls; we confirmed this hypothesis when we observed a seizure-like phenotype in cortical dysplasia organoids with increased burst and firing rates. However, we noted variability between organoids in terms of how much electrical activity they possessed, as well as how they responded to 4AP and ASMs. This is a well acknowledged draw back of the organoid model, though can also serve to generate more robust data by capturing natural variability, and more repeats will be necessary. Further, we found that some ASM treatment did not always reduce electrical activity as expected. This may be due to the specific mode of action of the ASM, or an absence of the specific cell type or receptor that ASM acts at. We have now completed recordings of the effects of ASMs on control organoids, and the completion of that analysis will inform on how an epilepsy genotype may contribute to the response observed.

It is also well recognised that ASM effectiveness can differ drastically between epilepsy patients, and thus our findings may be indicative of a patient specific drug response. How well organoids capture the specific patient drug response profile remains to be determined, as in

this instance the patients have not been trialled on the specific drugs used in this project. More work remains to be completed in the ASM-resistance space, by specifically comparing organoid response profiles with a patient's clinical response profile.

What these research outcomes mean

This project has been among the first to examine cortical dysplasias in an epilepsy patient derived 3D brain organoid model. These are the first steps to examining cortical layer formation and neuronal composition in a patient specific and clinically relevant model. Further, comparisons of patient specific drug response signatures in organoid models *in-vitro* with the clinical data will validate the potential of a patient specific and personalised drug selection platform for epilepsy patients. This project has demonstrated that while 3D brain organoids are in their infancy, we are transitioning epilepsy research to a point where the power of 3D organoid cultures can be used to advance our understanding of epilepsy and for use as a drug screening platform, offering a personalised *in vitro* model to facilitate care for epilepsy patients.

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