Characterizing Vascular Organoids with Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL)

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INTRODUCTION

CADASIL is a hereditary small vessel disease, which results in the thickening of blood vessels – which ultimately leads to the flow of blood vessels to the brain becoming blocked[1]. It is the most common genetic cause of stroke; however, the disorder often goes undiagnosed, making it difficult to determine how many are affected.

The Notch3 gene on chromosome 19 is known to be the primary contributor to the development of CADASIL when it is mutated. Notch3 is a type I transmembrane receptor, primarily seen in pericytes and vascular smooth muscle cells, which are crucial in vascular stability[2]; this is what led us to primarily focus on endothelial cells.

In this study, organoids were utilized to characterize CADASIL in 3D culture, alongside 2D culture to help with baseline characterization via immunostaining. RNA sequencing utilized as well.

METHODS & MATERIALS

ORGANOID CULTURE

Protocol for organoid generation was adopted from Wimmer et al [3].

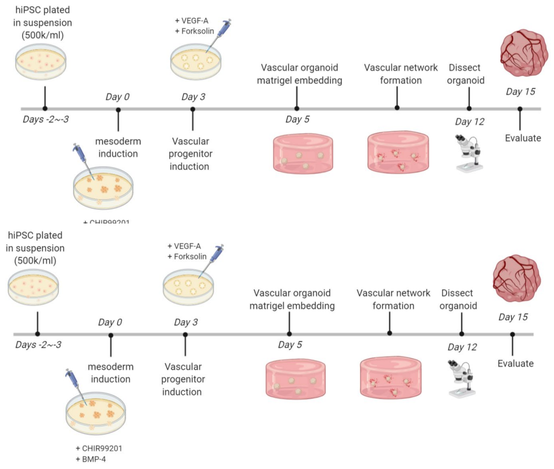

Figure 1: organoid culture, adapted from Wimmer et al [3]. Schematic depicting timeline of organoid differentiation from hiPSCs, to day 15 organoids.

Following an adapted protocol from Wimmer et al., organoids are differentiated from human induced pluripotent stem cells (hiPSCs) using various growth factors.

2D CULTURE

2D cultures are grown on glass sides, resulting in a monolayer of cells. A divider is placed upon the glass slide (figure 2).

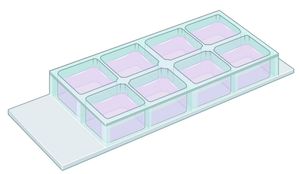

Figure 2: Eight chamber divider on a glass slide.

RESULTS

2D CULTURE

Immunostaining was used in order to obtain an initial baseline for what Notch1, Notch3, and VECAD should look like in WTC cells (figure 3). All but Notch3 seem to have results from other studies that can be used to help determine whether what we found is 'normal', and to verify our baseline, but it was found that there is, so far as we are able to see, no other similar staining that has been done in endothelial cells (ECs) for Notch3. Continued culture is being done with more WTC cells, as well as CADASIL.

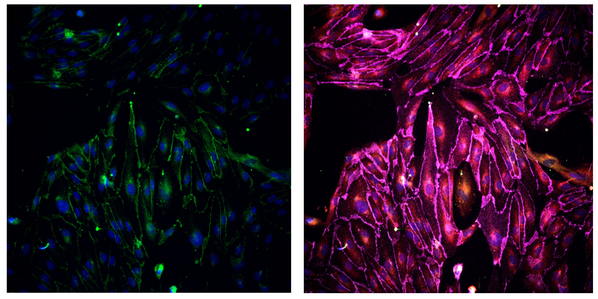

Figure 3: Immunostaining of 2D culture of WTC, with staining done for Dapi, Notch3, Notch1, Vecad.

RNA sequencing done of the 2D CADASIL cultures showed increased levels of Notch1, Notch3, and Jag1 in CADASIL iPSC-ECs as compared to the 2D culture WTCs. Hence, we have continued to explore this avenue in both 2D culture as well as 3D culture via organoids.

ORGANOID CULTURE

Imaging of WTC and CADASIL organoids stained for PDGFRB and CD31 found dislocalization of pericytes and endothelial cells.

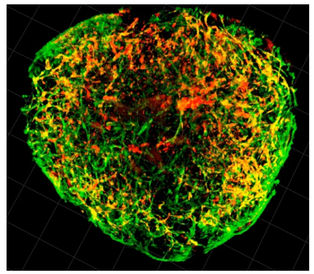

Figure 4: 3D imaging via IMARIS software of a WTC organoid. Immunostaining done with CD31 and PDGFRB.

Typically, overlap between pericytes and endothelial cells is expected to be seen, as pericytes line the abluminal side of blood vessels while endothelial cells are the inner lining of vessels. Between our WTC and CADASIL organoids, we have observed higher percent localization between where the PDGFRB shows and and the CD31 shows up in scans, and lower percent localization in CADASIL organoids (in one run, WTC was found to have 66% colocalization while one of the CADASIL organoids was found to have 18.56%).

CONCLUSION

Following results from RNA sequencing, we have imaged 2D and 3D cultures of WTC and multiple CADASIL lines. Analysis of these images has thus far shown dislocalization of PDGFRB and CD31 in CADASIL organoids compared to WTC organoids, suggesting dislocalization of pericytes and ECs in those with CADASIL. Plans are to continue to trial stainings in 2D and 3D culture, as well as comparing more lines of CADASIL.

REFERENCES

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