Modeling 22q11.2 Deletion Syndrome Vasculopathy with Blood Vessel Organoids

Siyu He[1,8], Cong Xu[1], Yeh-Hsing Lao[1], Shradha Chauhan[2], Yang Xiao[1], Moshe J. Willner[1], Yinuo Jin[1,8], Shannon McElroy[1], Sneha B. Rao[7], Joseph A. Gogos[3,4,5,7], Raju Tomer[2], Elham Azizi[1,8], Bin Xu[3*], and Kam W. Leong[1, 6*]

[1] Departments of Biomedical Engineering, [2] Biological Sciences, [3] Psychiatry, [4] Physiology and Cellular Biophysics, [5] Neuroscience
[6] Systems Biology, Columbia University, New York, NY 10027, USA
[7] The Mortimer B. Zuckerman Mind Brain Behavior Institute, Columbia University, New York, NY, USA
[8] Irving Institute for Cancer Dynamics, Columbia University, New York, NY, USA

22q11.2 deletion syndrome (22q11.2 DS), is a genetic disorder caused by microdeletions in chromosome 22, impairing the function of endothelial cells (EC) and/or mural cells and leading to deficits in blood vessel development such as abnormal aortic arch morphology, tortuous retinal vessels, and tetralogy of Fallot. The mechanism by which dysfunctional endothelial cells and pericytes contribute to the vasculopathy, however, remains unknown. In this study, we used human blood vessel organoids (VOs) generated from iPSC of 22q11.2 DS patients to model the vascular malformations and genetic dysfunctions. We combined high-resolution lightsheet imaging and single-cell transcriptome analysis to link the genetic profile and vascular phenotype at the single-cell level. We developed a comprehensive analytical methodology by integrating deep learning-mediated blood vessel segmentation, network graph construction, and tessellation analysis for automated morphology characterization. We report that 22q11.2DS VOs demonstrate a smaller size with increased angiogenesis/sprouting, suggesting a less stable vascular network. Overall, clinical presentations of smaller vascular diameter, less connected vasculature, and increased branch points were recapitulated in 22q11.2DS VOs. Single-cell transcriptome profiling showed heterogeneity in both 22q11.2DS and control VOs, but the former demonstrated alterations in endothelial characteristics that are organ-specific and suggest a perturbation in the vascular developmental process. Intercellular communication analysis indicated that the vascular dysfunctions in 22q11.2 deletion were due to a lower cell-cell contact and upregulated extracellular matrix organization involving collagen and fibronectin. Voronoi diagram-based tessellation analysis also indicated that the colocalization of endothelial tubes and mural cells was different between control and 22q11.2 VOs, indicating that alterations in EC and mural interactions might contribute to the deficits in vascular network formation. This study illustrates the utility of VO in revealing the pathogenesis of 22q11.2DS vasculopathy.

Statement of Purpose

22q11.2 DS is a genetic disorder caused by microdeletions in the chromosome 22. Patients experience variable developmental dysfunction, such as abnormal vasculature, congenital heart disease, and skeletal abnormalities. However, how dysfunction endothelial cells and pericytes contribute to the vasculopathy remains unknown. In this study, we used the 3D self-assembled human blood vessel organoids (VOs) generated from 22q11.2 patients induced pluripotent stem cells (iPSCs) to model the vascular malformation and genetic dysfunctions. We found that the VOs with 22q11.2 have smaller VO size. The alterations in 22q11.2DS VO size is the results of instability in vasculature network formation while increased angiogenesis/sprouting. Comprehensive methodologies combining deep learning and single cell transcriptomics also indicated that alterations in EC and mural interactions might contribute to the deficits in vascular network formation. Our study verified the ability of VOs modeling vascular diseases and offered a powerful patient specific biomaterial for studying disease mechanisms and screening countermeasure drugs.

METHODS

Generation and characterization of VO model.

We followed the previously established protocols to generate VOs (**Fig. 1ab**). To visualize vasculature structure with high resolution, we performed immunostaining of endothelial cells and pericytes with CD31 and PDGFRb, respectively. The whole VOs were imaged by CLARITY-optimized confocal light sheet microscopy (**Fig. 1cd**). Then we modified the fully convolutional networks (FCNs) base deep learning architecture4 to recognize and segment the EC tubes in our VOs, followed by a method for extracting the parameters of blood vessels, including tubular diameter, bifurcation, and density (**Fig. 2**).

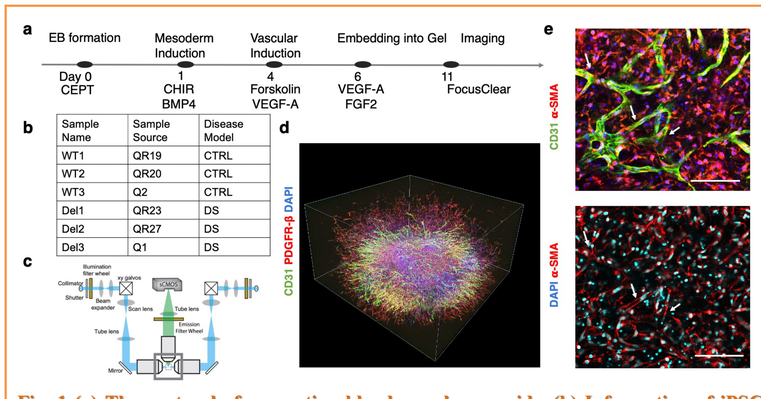

Fig. 1 (a) The protocol of generating blood vessel organoids. (b) Information of iPSC lines in this study. Three lines WT1, WT2, and WT3 were control. Del1, Del2, Del3 were corresponding 22q11.2 patient lines. (c) Diagram of COLM. (d) 3D rendering of one exemplary blood vessel organoid, with the staining of CD31, PDGFR-β, and DAPI. (e) Zoom in of EC and mural cells in VO (Scale bar: 300um).

Genetic analysis at the single-cell level

single cell RNA sequencing was performed to reveal transcriptome defect in 22q11.2DS. Using multiple analysis, such as Scanpy and scVelo, we identified the cell type, RNA velocity and highly variable genes in 22q11.2DS. Then by implementing CellChat, we inferred the intercellular communications on vascular related signaling pathways.

RESULTS AND DISCUSSION

Colocalization study of ECs and mural cells in 22q11.2DS VO

To understand the potential physical interaction between these two cell types at the pixel level, we implemented a colocalization analysis based on the Voronoi algorithm and utilized the two colocalization metrics Manders and Spearman's coefficients, to evaluate the interactions of EC and mural cells in our VOs. The 22q11.2 DS VOs had significant decreases in Manders and Spearman Rank coefficients in both EC colocalization and mural cell colocalization. These findings indicated an abnormality of EC and mural co-localization in the 22q11.2 DS samples, suggesting a misalignment of their localization, and decreased cell-cell contacts between EC and perivascular cells (**Fig. 3**).

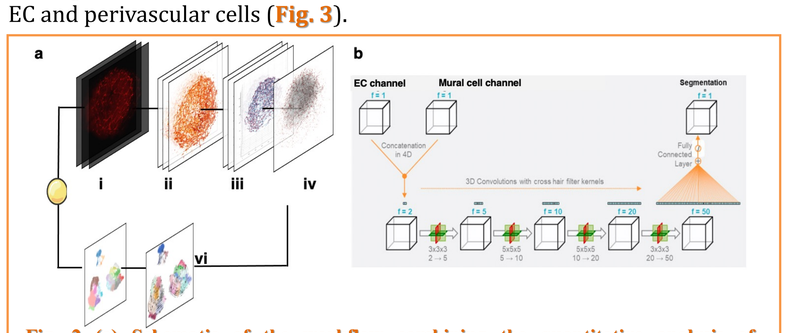

Fig. 2 (a) Schematic of the workflow combining the quantitative analysis of vasculature phenotype and single cell transcriptome. i: COLM imaging; ii: 3D segmentation by deep learning; iii: Morphology features; iv: topology features; vi: single cell analysis including trajectory inference and intercellular communications. (b) Modified DeepVesselNet to segment the vasculature in VOs.

Morphological and topological alterations in 22q11.2 DS

Through staining of the endothelial cell (EC) tubes and mural cells, the VOs showed an architecture similar to what was reported in the literature. We found the significant difference in size, vascular morphology, vascular density distribution, and network graph configuration in 22q11.2 DS VOs. We found the VO size derived from three 22q11.2 DS patient iPSC lines was smaller than their control counterparts (**Fig. 4**). The vascular density distribution calculated by Gaussian kernel density estimation has demonstrated that the 22q11.2DS VOs had a significant higher value compared with their control counterpart. The network graph of VOs demonstrated the connectivity of the nodes in the vascular network. The calculation demonstrated the significantly higher connectivity in 22q11.2DS VO.

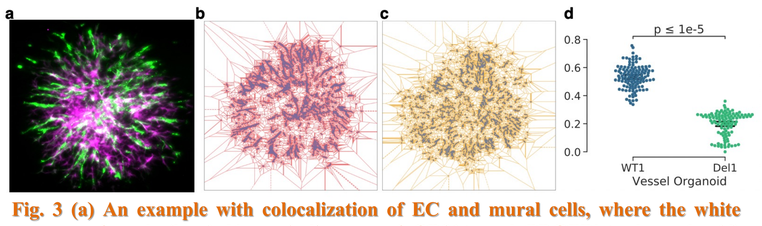

Fig. 3 (a) An example with colocalization of EC and mural cells, where the white arrows referred. (b, c) Voronoi diagram of CD31+ and PDGFRb+ channels. (d) Manders coefficients of mural cell colocalization corresponding to ECs in VO.

KEY REFERENCES

1. Kobrynski, L.J. & Sullivan, K.E. The Lancet 370, 1443-1452 (2007).
2. Wimmer, R.A., Leopoldi, A., Aichinger, M., Kerjaschki, D. & Penninger, J.M. Nature Protocols 14, 3082-3100 (2019).
3. Tomer, R., Ye, L., Hsueh, B. & Deisseroth, K. Nature protocols 9, 1682 (2014).
4. Tetteh, G. et al. Frontiers in Neuroscience 14 (2020).

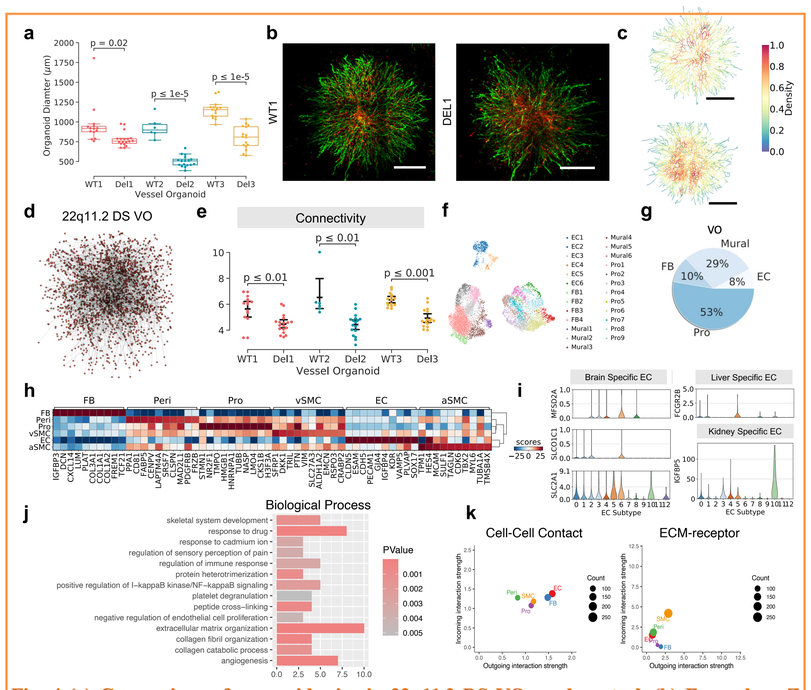

Fig. 4 (a) Comparison of organoids size in 22q11.2 DS VOs and control. (b) Exemplary Z projection of VOs derived from one paired iPSC line (WT1 and Del1, Scale bar: 200 um). (c) Density distribution of control and 22q11.2 DS VO. (d) Network graph of 22q11.2 DS VO. (e) Connectivity of 22q11.2 DS and control VO. (f) UMAP of sequenced cells in VO. (g) Pie plot of cell types in VO. (h) Differential genes cluster map for cell types in VO. (i) Organ specific gene expression in endothelial cells. (j) Gene ontology analysis. (k) Relationship between incoming and outgoing interaction strength in the signaling pathways

Alterations in the cell components and functional signaling pathways in 22q11.2DS VOs

Moreover, our results of single-cell RNA sequencing of blood vessel organoids have demonstrated the heterogeneous vascular associated cell types existed in the organoids with the percentages, including endothelial cells (PECAM1, CDH5, CLDN5, ERG), fibroblasts (COL1A1), and mural cells such such as smooth muscle cells (ACTA2) and pericytes (TAGLN, PDGFRB). However, the percentages of EC and fibroblast (FB) in diseased VO were higher than those in the control. The investigation of organ-specific endothelial markers indicates the heterogeneity of VOs is not only among the types of vascular cells but also among the organ resources. The gene ontology analysis showed upregulated genes were associated with immune response, positive regulation of vasoconstriction, extracellular matrix organization, negative regulation of endothelial cell proliferation and collagen fibril organization (**Fig. 4**).

SUMMARY

Our vessel organoids successfully modeled 22q11.2DS vasculopathy, with quantitative suggestion in morphology characterization and transcriptomics profiling. In conclusion, this study aspires to innovate the integration of stem cell engineering and novel analysis on modeling disease and screening drugs preclinically.

ACKNOWLEDGMENT

This work was supported by NIH UH3TR002151 and the Columbia Facility Core..