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TECHNIQUES

Spinning organoids shed light on Zika

Human-derived induced pluripotent stem cells (iPSCs) — when cultured under specific conditions — can self-assemble to form three-dimensional aggregates of neural cells, known as cerebral organoids, that resemble brain tissue and that can be used to model aspects of neurodevelopment. Qian *et al.* now describe a method to generate cerebral organoids that better model human cortical development. Using these organoids, they find evidence for the detrimental effects of Zika virus (ZIKV) on cortical development, findings that agree with those of a recently published study by Garcez *et al.*

The usefulness of cerebral organoids has been limited by the high variability of cell-type composition and organization among organoids generated using existing approaches and by difficulties in recapitulating some key features of human cortical development. A 'spinning bioreactor' approach has been shown to produce larger, more complex organoids but is associated with prohibitively high costs and space requirements. Qian *et al.* adapted this approach for use in a standard laboratory setting by engineering a miniaturized device — known as SpinΩ — to spin the cells within standard 12-well tissue culture plates. To further reduce variability between cultures, they exposed the iPSCs to a set of standard conditions that 'pre-patterned' the cells towards a forebrain fate before spinning.

To assess the efficacy of their approach, Qian *et al.* performed immunohistological analysis for cell-type markers at different stages of organoid growth. After 14 days of spinning, the organoids exhibited a well-defined ventricular zone (VZ)-like structure containing neural progenitor cells (NPCs) that was separated from a preplate-like layer. After longer periods in the SpinΩ, the authors observed the emergence of a subventricular zone (SVZ)-like structure that later split into an inner SVZ and an outer SVZ that contained cells that expressed markers of outer radial glia. The progression in the expression of neuronal subtype markers also mimicked cortical development: early-born neurons that expressed a marker of 'deep-layer' neurons formed a layer beneath the later-born neurons that expressed 'upper-layer' markers. After 70 days in the bioreactor, six differentially labelled layers and GABAergic neurons, similar to those in the cortex, were observed.

RNA-sequencing analysis showed a correspondence between the transcriptome of the organoids and that of fetal brain at the first and second trimester. Furthermore, electrophysiological analysis of neurons in acute slices of the organoids demonstrated functional connectivity and the recapitulation of features of neuronal maturation such as a switch from a depolarizing to a hyperpolarizing response to GABA. Importantly, there was little variability between the organoids, making the approach amenable for the testing of the effects of particular manipulations on organoid growth.

In light of recent proposed links between ZIKV infection and disrupted neurodevelopment, Qian *et al.* examined the effects of ZIKV infection on forebrain organoid growth. They found that ZIKV established a productive infection of NPCs in the organoids, resulting in deficits in NPC proliferation, increased cell death and decreased VZ and neuronal layer thickness (a feature associated with microcephaly). In another recent paper, Garcez *et al.* showed that ZIKV infection of neural stem cells generated from iPSCs reduced their viability and impaired the growth of neurospheres and cerebral organoids.

These findings provide support for the hypothesis that ZIKV infection can induce proliferation deficits that may cause microcephaly and illustrate the utility of cerebral organoid technology for the investigation of the mechanisms and disorders of human neurodevelopment.

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ORIGINAL ARTICLES Qian, X. *et al.* Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell* http://dx.doi.org/10.1016/j.cell.2016.04.032 (2016) | Garcez, P. P. *et al.* Zika virus impairs growth in human neurospheres and brain organoids. *Science* http://dx.doi.org/10.1126/science.aaf6116 (2016)

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