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Research Advance

Vascularizing the brain organoids

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The recently developed brain organoids are self-organized neural tissues derived from pluripotent stem cells (PSCs) that could recapitulate the architectural and cellular characteristics of certain brain regions. Although brain organoid models have been used in the study of human brain development and neurological diseases, the lack of non-neuronal parts, including vascular structures and immune cells, in the prevailing brain organoid models limits the applications of the system (Mansour et al., 2021). A recent study published in *eLife* by Sun et al. (2022) reported a new strategy of vascularizing the brain organoids by fusing together the blood vessel and brain organoids (Figure 1).

Since the blood vessels and neurons in the brain are derived from mesodermal and ectodermic origins, respectively, it is difficult to simultaneously induce cell fates from distinct germ layers, which causes a barrier for the generation of vascularized brain organoids. Previously, several studies have tried to vascularize the brain organoids. For example, co-culturing brain organoids with endothelial cells (ECs) or their progenitors enabled some vasculatures wrapping or invading into the brain organoids (Pham et al., 2018; Shi et al., 2020). Brain organoids transplanted into the cerebral cortex of immunodeficient mice have been shown to be invaded by host blood vessels with perfusion of active blood flow (Mansour et al., 2018). In another study, endogenous ECs were generated by overexpression of the transcription factor human ETS variant 2, which induced differentiation of a subset of PSCs into ECs during brain organoid induction (Cakir et al., 2019). Nevertheless, the formation of integrated vasculatures composed of an entire repertoire of major cell types, including ECs, mural cells, and immune cells such as microglia (MG), in brain organoids *in vitro* is still challenging.

Considering the mesodermal origin of blood vessels and the cerebrovascular characteristics, Sun et al. (2022) first generated the vessel organoids (VOrs) following the steps of transient mesodermal induction, sequential vascular progenitor (VP) and EC induction, and finally incubation with neurotrophic reagents (Figure 1). The VOrs were derived from human embryonic stem cells with stable expression of green fluorescent protein and embedded with Matrigel at the VP stage. Vessels sprouted out and finally formed the integrated and complex vasculature networks, which are patent to allow fluid flow when pressurized fluid is delivered to the vascular tube. Single-cell RNA sequencing revealed the presence of multiple cell types in VOrs, including almost all of the vascular cells resembling their counterparts *in vivo*. The ECs in VOrs assembled the vessel lumens, with mural cells (pericytes and smooth muscle cells) sticking around and forming the vessel walls. These two kinds of cells in VOrs showed a higher correlation with human brain vessels than mouse brain vessels, indicating the transcriptomic differences between species. Surprisingly, extensive amounts of MG, the resident immune cells in the brain, were observed in the VOrs, as reflected by single-cell RNA sequencing and immunostaining results.

Next, the authors fused the VOrs with the brain organoids, which were induced according to the methods reported previously (Lancaster et al., 2017; Hou et al., 2021), with some modifications (Figure 1). Intriguingly, the fused vascularized brain organoids (fVBOrs) formed structures similar to the blood–brain barrier (BBB), a complex intercellular tight junction that prevents harmful substances of the blood from entering the brain. The BBB-like structures in the fVBOrs appeared to be functional as reflected by the selective permeability of angiopep-2, a peptide ligand of the low-density lipoprotein receptor-related protein-1 receptor (Bergmann et al., 2018). As the major immune cell type in the brain, MG interacts with neurons and blood vessels to maintain nervous system homeostasis. The fVBOrs also contained extensive amounts of MG-like cells, which were not observed in brain organoids. These cells were able to engulf synaptic proteins, suggesting their role in mediating synaptic elimination. Furthermore, these cells actively responded to immune stimuli such as lipopolysaccharide, an endotoxin that can activate cells to initiate an immune response. In line with the previous conclusion that ECs can promote neuronal stem cell proliferation (Shen et al., 2004), the neural progenitors and cortical thickness in fVBOrs markedly increased, while the number of apoptotic cells was markedly decreased compared

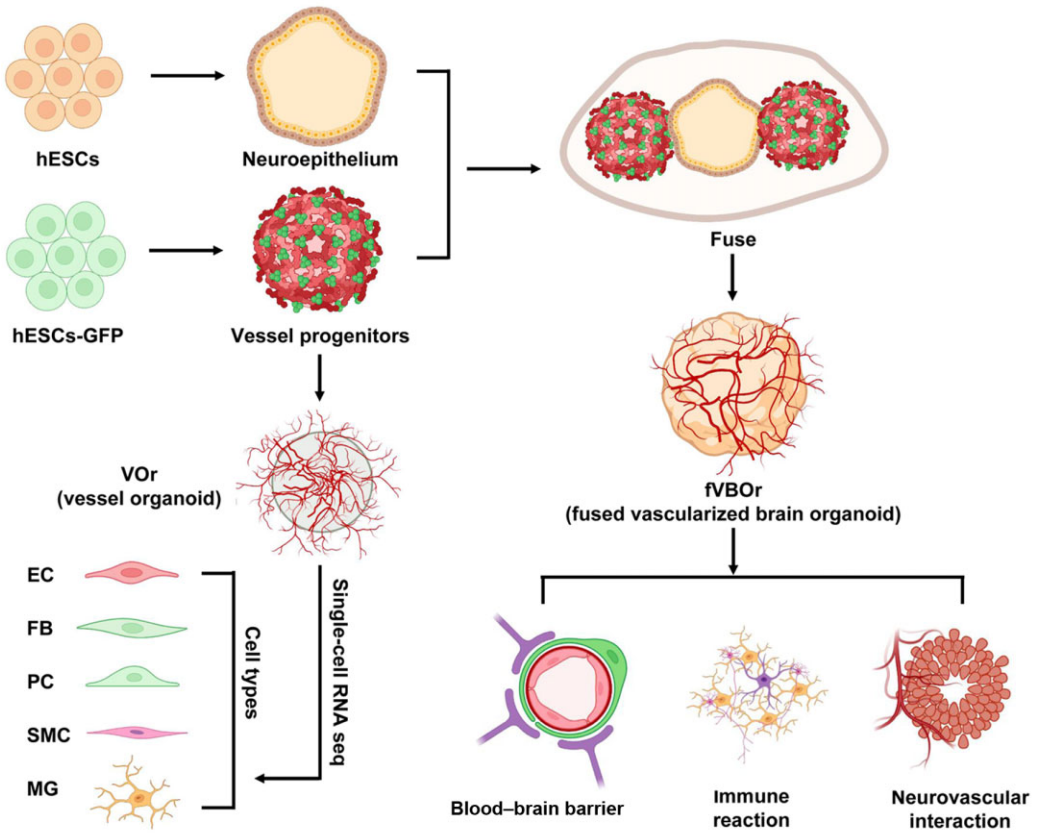

Figure 1 The strategy for the generation of vascularized brain organoids. The brain organoids and blood VOrs are developed independently and then fused together to obtain vascularized brain organoids. hESCs, human embryonic stem cells; FB, fibroblast; PC, pericyte; SMC, smooth muscle cell.

to that in brain organoids. In addition, neurons in fVBOrs showed accelerated functional maturation. The diffusion or production of some trophic factors by vascular structures may contribute to these effects.

In summary, Sun et al. (2022) have developed a new strategy that allows simultaneous production of vessel-like networks and brain-resident MG immune cells in a single brain organoid, making it possible to mimic and investigate interactions among multiple cell types under normal and disease conditions. The BBB-like structure in the system could provide an opportunity for the screening of drugs that could cross the barrier. For further applications of the vascularized brain organoids, there are still challenges that need to be solved: (i) astrocytes and oligodendrocytes have not been widely detected in the fVBOr model, which may require longer culture and maturation process; (ii) the cortical layers in fVBOrs do not fully mimic that *in vivo*, and vasculatures may contribute to the proper assembly of cortical lamina structures; (iii) blood cells are difficult to be induced *in vitro*, and thus adding blood flow to the system using approaches like microfluidic system will be the next step to optimize the system; and (iv) stem cell transplantation is one of the promising strategy for the treatment of neuronal injury or degenerative diseases, and thus fVBOrs containing vasculatures may help integrate with the host vessels for better therapeutic repairing.

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