

Fumao Sun, Thesis abstract:

Synaptic pruning is the selective elimination of synapses in the central nervous system (CNS) during early postnatal development. Aberrant synaptic elimination has been associated with neurodevelopmental and neurodegenerative disorders. Microglia, the resident macrophages of the CNS, participate in brain development and homeostasis of the adult CNS. Previous studies suggest that microglia also play an essential role in synaptic elimination, which is necessary for normal brain development and synaptic plasticity. C1Q, the initiator of classical complement cascade, is predominately expressed by microglia in CNS and tags synapses for elimination. Multiple studies indicate that complement signalling drive microglia engulfment of synapses in various conditions and disorders. However, we have limited understanding of how microglia and complement signalling contribute to synaptic elimination. Differences between rodent and human immune and neurological functions limit translating knowledge about synaptic elimination from animal models to humans.

Human induced pluripotent stem cell (iPSC) -derived 3D co-culture can bridge the gap between in vitro human studies and in vivo studies. To understand the role of microglia in synaptic elimination under healthy and neuroinflammatory conditions, I generated an all-human neural microphysiological system (MPS) including human iPSC-derived neurons, astrocytes and microglia-like cells (MLCs). I evaluated the MLC-mediated synaptic elimination under normal or LPS-induced neuroinflammatory condition. I found that MLC co-culturing can reduce the density of synapses in MPS, while LPS treatment showed a context-dependent effect on synapses.

As proof-of-concept, I investigated the function of complement C1Q in microglia-mediated synaptic elimination by creating C1QA knockout iPSC lines using CRISPR-Cas9 and generating C1Q-deficient MLCs. I examined the C1Q-deficient MLCs by assessing their phagocytic ability and inflammatory responses to LPS-challenge through the quantification of relevant gene expressions, but there were no strong genotype related effects. I also generated 3D MPS containing C1Q-deficient MLCs to investigate their changes in phagocytosing and eliminating synapses. Under LPS-treated condition, C1Q deficiency in MLCs partially recovered the MLC-dependent synapse loss phenotype, but did not cause a significant impairment on microglial engulfment of synapses. This project provided a novel system and extended the toolbox of to study human-specific features in synaptic eliminating during normal brain development and pathological situations.