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Cryopreservation Preserves Cellular Integrity and Innate Immunity in Human iPSC-Derived Brain Organoids

Human iPSC-derived brain organoids are increasingly used for neurobiological research; however, their widespread application is limited by long-term cultivation requirements, contamination risk, and high cost. Therefore, reliable storage of brain organoids is essential for biobanking to ensure reproducible studies in disease modeling. Here, we investigated whether cryopreservation preserves cellular composition and innate immune function in human iPSC-derived brain organoids containing microglia and BBB endothelial cells. We demonstrate that cryopreservation preserves the cellular composition and molecular identity of these organoids, as evidenced by comparable mRNA and protein expression levels of major brain cell markers (GFAP, IBA-1, TUJ-1, MAP2, TMEM119, and OLIG2) between cryopreserved and control organoids. Importantly, cryopreserved and subsequently revived organoids retained immunological functionality. Both freshly cultured and cryopreserved-revived organoids mounted robust innate immune responses following stimulation with the TLR3 ligand Poly(I:C), as demonstrated by significant transcriptional upregulation of IFN- β , Viperin, and ISG56. Collectively, these findings indicate that cryopreservation preserves structural and immunological integrity of human iPSC-derived brain organoids, supporting its use as a reliable biobanking strategy for scalable and reproducible neurobiological research.

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