this problem, we are using the experimental autoimmune encephalomyelitis (EAE) mice, a mouse model for multiple sclerosis in which an increase in cell fusion has been reported in previous studies. Nevertheless, these studies are extremely time-consuming and differ in the methodologies used, mainly in experimental timings for transplant, EAE induction, and sacrifice. For this reason, we aimed to establish an optimal protocol for studying cell fusion after BMDC transplantation in EAE mice, reaching a good equilibrium between the experimental time window and the number of fused cells detected. We have observed fused Purkinje cells using different induction protocols and time windows. We also found a massive infiltration of various cell types, most of them macrophages/microglia and a few lymphocytes. In conclusion, our data demonstrate that our four months protocol is adequate to detect enough infiltrated and fused cells in the cerebellum of the EAE mice. This will allow us to study cell fusion in half the time of previous studies and better understand fusion mechanisms. Support: MIU (FPU2020); MICINN (PID2019-106943RB-100); JCyL (SA129P20), USAL. Contact: pabgonses@usal.es, jorgevalero@usal.es, jralonso@usal.es.

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Topic: AS03 Stem Cells, Organoids, Neural Injury Neurotoxicity and Repair

HIGHLY EFFICIENT GENERATION OF HUMAN CEREBRAL ORGANOIDS BYPASSING EMBRYOID BODY STAGE

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Human cerebral organoids (hCOs) are a promising in vitro model that may overcome some of the limitations that currently exist when studying human brain development and disease. Since Lancaster et al. first generated hCOs, efforts have been made to better recapitulate the physiology of the human brain and improve the efficiency and reproducibility of protocols. Different groups employed dual-SMAD inhibition (double inhibition of the transforming growth factor-β (TGF-β) and bone morphogenetic proteins (BMPs) pathways) to achieve rapid neural induction. The method developed here, outlines the generation of homogeneous organoids by rapid neuroepithelial induction, avoiding the Embryoid Body (EB) stage. The efficiency of this protocol to form neuroepithelial structures and subsequently organoids is almost 100% due to the use of dual-SMAD inhibition in combination with CHIR99021 (a GSK3β inhibitor/Wnt activator) at the neural induction stage. This is a simple and reproducible protocol as we do not need to use Matrigel or bioreactors which standardizes the methodology. It is also a robust protocol as we have successfully performed it on human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs). We performed IHC and Q-RT-PCR assays for cell cycle, neural precursors, neuronal and synaptic vesicle markers. The generated hCOs are highly homogeneous and show ventricular zones (VZs) with radial glia in the center that differentiate to give rise to neurons located around these VZs. These neurons acquire a mature state and are able to form synapses. In addition, we also performed IHC and Q-RT-PCR assays for different markers of oligodendrocytes, astrocytes, microglial cells and vasculature obtaining promising results. We believe that this protocol will be a breakthrough in the generation of organoids for use as a model for the study of neurodevelopmental and neurological diseases, as well as for drug testing.

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REGULATION OF THE TRANSCRIPTIONAL AND CELLULAR LANDSCAPE OF THE ADULT MOUSE SUBVENTRICULAR ZONE BY THYROID HORMONE SIGNALING

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In the adult mammalian subventricular zone (SVZ), activated neural stem cells (NSCs) generate new neuroblasts and oligodendrocyte precursor cells (OPCs). These neuroblasts migrate following the rostral migratory stream (RMS) to integrate into the olfactory bulbs (OB) while OPCs migrate radially to integrate into the overlaying white matter. This complex process is regulated by several cues, among which thyroid hormones (TH) are crucial. Increased intracellular levels of the genomically active TH T3 maintain NSC and progenitor renewal and promote neuronal lineage commitment. However, how regulators of TH availability modulate T3 content in the SVZ cells and how this regulation affects neurogliogenic processes, is less understood. Here, we used adult *Mct8/Dio2* double knockout (*Mct8/Dio2* KO) mice to assess the role of TH transporter monocarboxylate transporter 8 (MCT8), the only TH specific transmembrane transporter, and deiodinase type 2 (DIO2), the enzyme responsible for local generation of the majority of T3. We performed single-cell RNAseq on dissected SVZs. This analysis revealed that the absence of MCT8 and DIO2 dysregulated the SVZ cells' transcriptome and increased the proportion of neuroblasts. Consistently, immunofluorescence assays also revealed increased neuroblast/OPC ratios in the adult SVZ. Reduced OPC differentiation was also observed, consistently with the hypomyelination phenotype previously described in *Mct8/Dio2* KO mice. *Ex vivo* neurosphere assays and *in vivo* experiments also showed increased progenitor proliferation and hindered neuronal migration, which led to fewer SVZ-generated neuroblasts supplied to the OB. Lowered neuronal integration into the OB was observed and associated with compromised short-term olfactory memory and discrimination in *Mct8/Dio2* KO mice, showing that the absence of both regulators functionally impaired SVZ-neurogenesis. In conclusion, MCT8 and DIO2 play a crucial role in adult murine SVZ homeostasis and function. Further