Randy Maysaud – EN.585.685.81 – Module 11 Case Study – Models in Neurobiology

Background Information:

Regarding nerve regeneration the Central Nervous System (CNS) nerves cannot regenerate after injury while the Peripheral Nervous System (PNS) can readily regenerate.[1] It is shown that the two proteins PTEN and SOC3 that are found in the CNS neurons inhibit AKT and JAK/STAT signaling which play a role in promoting growth.[2] The signaling of AKT and JAK/STAT3 help activate the Regeneration-Associated Genes (RAGs), view figure 1 for a visual representation.

Aim and Overview:

The aim for this research project is to gauge if removing proteins PTEN and SOC3 in CNS nerves of a human iPSC 3D human culture can regenerate the nerve from an Axonotmesis.

The reason for removing PTEN and SOC3 is because these proteins inhibit the signaling of genes that promote growth of the axon. Therefore, removing the proteins can allow for increased signaling to activate RAGs. The reason for using human iPSC 3D human culture as the test is because it is the closest model to represent humans and is a good model for In Vitro modeling. The injury we will test is Axonotmesis because it will not require surgical repair and allows us to view natural regeneration properties.

Procedure:

1. We will create two 3D cultures of CNS for the project (one is the control). Obtain fibroblast cells from a volunteer (healthy individual), then incorporate pluripotency genes (KLF4, SOX2, c-Myc, and Oct-3/4) to create the induced pluripotent stem cells (iPSCs). Next, we will create a cerebral organoid by separating the iPSCs to single cells that will create embryoid bodies, then embed the organoid in Matrigel, once complete put the organoids in the spinning bioreactor[3], view figure 2 for more information. Organoid A will be control and Organoid B will have the removal of PTEN and SOC3.

2. We will perform PTEN and SOC3 deletion using adeno-associated virus (AAV) injection on organoid B. Perform the injection to the CNS nerve that will be damaged, this will remove the inhibiting proteins of the nerve.[4]

3. Using oculomotor nerve injury forceps, crush the injected CNS nerves from the AAV injection on Organoid B and crush a similar CNS nerve on Organoid A.[5]

4. Using a magnetic resonance neurography (MRN) to view the broke nerves, we will be comparing the healing process between the damaged nerves in organoid A and B for one week.

Conclusion:

I believe that the results of this research project will show that the broken nerve in organoid B will show some healing (not like PNS) and organoid A will remain injured.

References:

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[4] Moses, C., Hodgetts, S. I., Nugent, F., Ben-Ary, G., Park, K. K., Blancafort, P., & Harvey, A. R. (2020, July 9). *Transcriptional repression of PTEN in neural cells using CRISPR/dcas9 epigenetic editing*. Nature News. Retrieved November 14, 2021, from https://www.nature.com/articles/s41598-020-68257-y.

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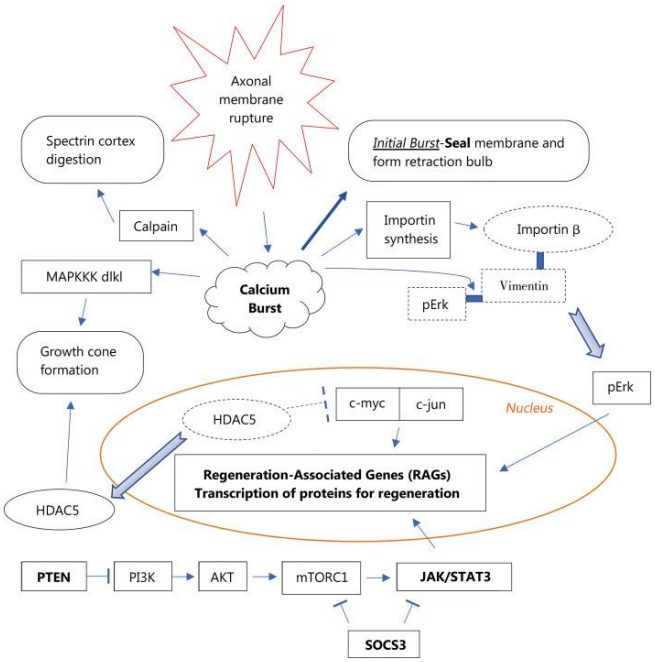

Figure 1: RAGs activation and signaling [2]

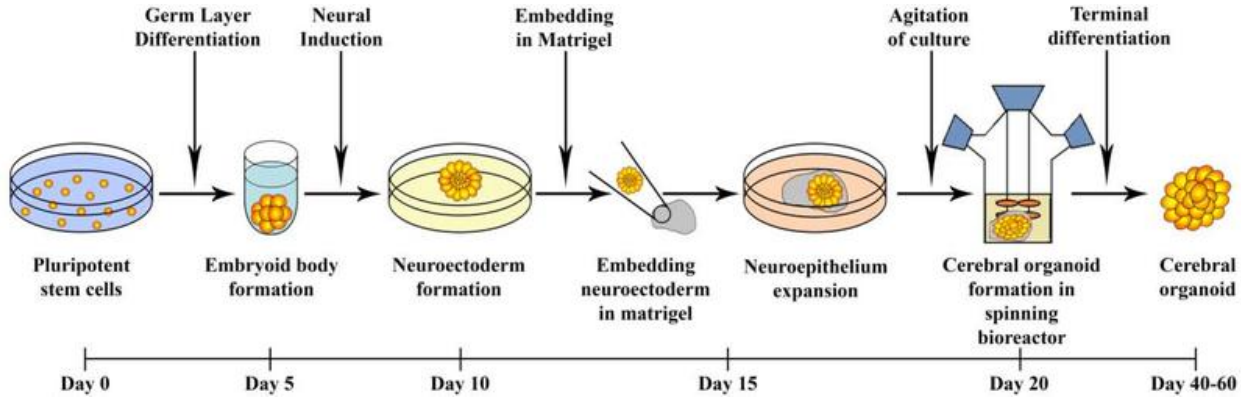

Figure 2: Cerebral Organoid from iPSCs [3]